THE NEXT STEP: DISENTANGLING THE ROLE OF PLANT-SOIL FEEDBACKS IN PLANT PERFORMANCE AND SPECIES COEXISTENCE UNDER NATURAL CONDITIONS

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EDITORIAL
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Editorial: The Next Step: Disentangling the Role of Plant-Soil Feedbacks in Plant Performance and Species Coexistence Under Natural Conditions

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Editorial on the Research Topic

The Next Step: Disentangling the Role of Plant-Soil Feedbacks in Plant Performance and Species Coexistence Under Natural Conditions

Effects of plant-induced changes in soil properties, which impact subsequent plant growth, have received increasing attention in plant ecology (e.g., Smith-Ramesh and Reynolds, 2017). These plant-soil feedbacks (PSFs) are considered to be important for plant performance and plant-community composition in many terrestrial ecosystems (e.g., Van der Putten et al., 2013). However, so far most conclusions on the importance of PSFs in natural systems have been drawn from experiments performed under highly controlled and artificial conditions. Under natural conditions, the growth and development of plants as well as that of soil organisms is influenced by many more abiotic and biotic interactions than in the greenhouse. Hence, there is an urgent need to investigate PSFs under more natural conditions and to better understand the interactions between PSFs and environmental drivers (DeLong et al., 2019). This Research Topic comprises 14 articles—ranging from Original research articles, meta-analytical Reviews and Perspectives—that aim to advance our understanding of the contribution of PSFs to plant growth and plant community composition in different environmental contexts.

BASELINE

Forero et al. provide an overview of why many PSF studies have been performed under controlled conditions and show that field-based PSF studies are generally scarce. They furthermore present additional empirical evidence that PSFs differ between greenhouse and field conditions, thus highlighting the need to consider effects of environmental conditions in PSF research (see e.g., Heinze et al., 2016; Van der Putten et al., 2016).

ENVIRONMENTAL CONDITIONS AND PSFs IN GREENHOUSE STUDIES

Although greenhouse conditions typically shelter plants from being exposed to many of the abiotic effects and biotic interactions that occur outdoors (Heinze et al., 2016), they are ideal to test effects of environmental factors on PSFs in isolation. For instance, using field-collected soil in a greenhouse study McCarthy-Neumann and Kobe observed that nutrient and light conditions impact the magnitude and direction of PSFs of tree species. These altered PSF effects could explain species abundance in the field. Furthermore, comparing effects of ambient and elevated temperatures in the conditioning and the feedback phase, the study of Duell et al. revealed that year-to-year variation in temperature reversed negative PSF effects especially for non-native grass species. This might enhance competitive strength compared to native grass species. Several studies have shown that PSFs can influence competition between native and non-native plant species (e.g., Van Grunsven et al., 2007; Callaway et al., 2013). In this context, Bennett et al. investigated an important biotic factor—aboveground herbivory—that was reported to affect PSFs (e.g., Bezemer et al., 2013; Heinze and Joshi, 2018; Heinze et al., 2019). Under greenhouse conditions, they found that simulated aboveground herbivory changed PSFs in native and non-native plants when grown in a competitive mixture. This suggests that interactions between PSFs and herbivory might influence invasion processes.

Interactions between different plant species that are mediated by PSFs are also impacted by differences in soil biota *per-se*. In a nature restoration context Wubs et al. show that inoculating nutrient-rich soil collected from a former arable field, with soils from grassland and heathland suppressed positive PSFs for early successional ruderal plant species. This enhanced the competitive success of more late-successional species.

These greenhouse studies show that PSFs are driven and affected by abiotic and biotic environmental factors (Bennett and Klironomos, 2019; DeLong et al., 2019) as well as by the composition of soil microbial communities. They suggest that PSFs might be an important factor influencing plant-community dynamics in the field.

LINKING GREENHOUSE AND FIELD STUDIES

Kostenko and Bezemer link plant performance in greenhouse experiments with tests in the field. Under controlled conditions they investigated the response of a focal species to abiotic and biotic soil legacy effects that were created in field plots with different levels of plant diversity. They compared these results to the performance of individuals of this focal species when planted in the field plots. The authors found that plant diversity had a weak impact on soil legacy effects. However, these effects could not be explained by differences in soil community composition because responses of the plant in the greenhouse and field considerably differed.

FIELD STUDIES AND APPLIED ASPECTS

Under natural conditions abiotic and biotic factors act together in driving PSFs (Bennett and Klironomos, 2019; DeLong et al., 2019) and several studies have indicated that such PSF effects can be relevant for plant-community dynamics in the field. For instance, in their observational study Vukicevich et al. report that management practices in vineyards influence ground-cover vegetation and that these differences in ground-cover vegetation affect soil fungal communities involved in PSFs. Kulmatiski performed a classical two-phase experiment directly within a long-term field experiment and showed that PSFs are critical for species abundance in plant communities. Lance et al. tested PSF effects of 10 different tree species native for eastern North America with field-collected conspecific and heterospecific soil as inoculum in a field experiment. They report that PSF effects impact soil fungal communities, but that these effects play a rather minor role for tree growth in the field.

Similar results were obtained by Kirchhoff et al. in an PSF experiment in grasslands. Their experiment investigated intraspecific variation in plant responses to soil biota and how these responses are shaped by aboveground insect herbivory. They found that the PSFs mediated by soil biota alone play a minor role in influencing plant performance. However, their results support the theory that interactions between plants and soil biota can be mediated through aboveground-herbivores and the responses they induce in plants.

That effects of PSFs are modulated by environmental factors under field conditions was also observed by Dietterich et al.. In a grassland-to-forest transition experiment they planted tree seedlings into tree or grass dominated plots and manipulated levels of competition between experimental plants and neighboring vegetation. Their study shows that most PSF effects are overpowered by biotic interactions such as competition and herbivory and by abiotic soil factors. Using similar field manipulations, Collins et al. investigated competition and PSF effects of a range-expanding species on two resident plant species. Although the soil of the range-expanding species negatively affected growth of the resident species, facilitation effects of the range-expanding species ameliorated the negative PSFs. This indicates that PSFs have the potential to influence plant community composition but must be examined within the context of other ecological processes.

GENERAL FINDINGS AND FUTURE ASPECTS

In a meta-analysis Beals et al. address how environmental context (competition, stress, disturbance) impact the direction and strength of PSFs. By analyzing data from 76 studies they provide broad evidence that environmental context can change PSF effects.

Furthermore, to enhance our understanding of PSFs effects, researchers should also consider plant-litter feedbacks, as pointed out in the perspectives article by Veen et al..

CONCLUSION

The papers in this Research Topic indicate that PSF effects are shaped, but mostly overwhelmed in the field, by environmental factors. Therefore, PSFs need to be investigated in combination with environmental factors—preferably directly in the field. This is particularly important when the goal of PSF research is to understand its contribution to plant growth under field conditions. How PSFs are influenced by environmental factors in a changing world will improve our understanding of the importance of PSFs for plant growth and plant community composition in the future.

AUTHOR CONTRIBUTIONS

JH, TB, and JJ co-edited the Research Topic. All authors wrote, edited, and approved the final version of the Editorial.

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Influence of Groundcover Vegetation, Soil Physicochemical Properties, and Irrigation Practices on Soil Fungi in Semi-arid Vineyards

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Although plants are known to have a strong influence on soil biota, the effect of groundcover vegetation in perennial cropping systems on soil fungi has been little explored. We surveyed extensively managed vineyards to determine how plant community functional characteristics, soil factors, and irrigation management related to the abundance of two guilds of soil fungi that may play a role in plant-soil feedback (entomopathogenic fungi represented by *Beauveria bassiana,* and the pathogenic species complex, *Ilyonectria* spp.). We found that plant community characteristics were related to fungal abundance for both fungi assayed. *Beauveria bassiana* increased with native species, annual plants, and legumes consistently across sampling periods. *Ilyonectria* spp. increased with the abundance of forbs and exotic species, though only the relationship with forbs was consistent across sampling periods. Both fungal guilds increased with increasing soil organic matter. The use of dual or sprinkler irrigation systems also increased *B. bassiana* and *Ilyonectria* spp. in vineyard soils. Overall, groundcover vegetation played a significant role in driving abundance of these important groups of soil fungi. Groundcover management may therefore be a viable tool to manipulate soil fungi with the potential for improving ecosystem services such as conservation biological control of soil dwelling insect pests and deterring pathogens in perennial cropping systems.

Keywords: cover crops, entomopathogenic fungi, black foot disease, conservation biocontrol, vineyards

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INTRODUCTION

Perennial agriculture is characterized by crop rows alternating with drive rows to facilitate field and tractor work. This means that much of the land area in a perennial cropping system is not actually planted to crop plants, but subjected to floor management practices. Depending on regional climate patterns, pest pressure, nutrient challenges, and aesthetics, different strategies are used to manage drive rows. Perhaps the most common approach is the maintenance and management of vegetation in the drive row using cover crops or groundcovers that provide a host of ecosystem services, such as improved carbon sequestration, pest control, and soil fertility (Winter, 2018). With increasing interest in the linkage between soil microbial diversity and ecosystem functioning (Bardgett and van der Putten, 2014) and the potential for groundcover vegetation to affect crop plant health

through plant-soil feedbacks (Vukicevich et al., 2018), a logical question is: how does groundcover vegetation affect key groups of soil biota?

Plants alter the spatial distribution of soil resources through rhizodeposition (Badri and Vivanco, 2009) and litter decomposition (Fanin et al., 2014), creating unique nutrient rich patches that vary with plant species (Broeckling et al., 2008; De Deyn et al., 2011). In fact, specific plants have been used by farmers for millennia to affect changes in populations of soil microorganisms, e.g., through crop rotations (Bullock, 1992). Because rotation of the crop plant is not possible in perennial vineyards and orchards, the drive row then provides an opportunity to introduce plant diversity and subsequently soil microbial diversity to the system (Vukicevich et al., 2016). Cover crops or permanent groundcovers could have particularly pronounced effects on important crop pathogens and mutualists as evidenced by the strong effects of plant identity on soil fungi (De Bellis et al., 2007; De Deyn et al., 2011; Corneo et al., 2013; Lankau and Lankau, 2014; Detheridge et al., 2016).

One beneficial group of soil fungi in agriculture is the entomopathogenic (EP) fungi. EP fungi, typified by the well-studied and commercially sold *Beauveria bassiana* and *Metarhizium anisopliae*, are naturally common in soils where they are responsible for regulation of insect pest communities and appreciated as biocontrol agents (Shah and Pell, 2003; Pell et al., 2010). Living in close association with plants (Moonjely et al., 2016), they are even able to transfer N from infected insects to a plant host in return for plant carbon (Behie et al., 2012, 2017). They have also been implicated in plant protection from soilborne disease (Ownley et al., 2010). Because they may show rhizosphere specificity to some degree (Hu and St. Leger, 2002; Behie et al., 2015) and are preferentially associated with certain types of habitats (Meyling et al., 2009), vineyard groundcover management might also affect the abundance of these beneficial fungi thereby promoting positive plant-soil feedback through regulation of soil dwelling insect herbivores and decreases in plant disease.

In addition to beneficial soil fungi, generalist soil borne plant pathogens that harm woody perennial crops may build up on certain alternate host plants. For example, Agustí-Brisach et al. (2011) found *Ilyonectria* spp., the causal agent of black foot disease of grape, living in various asymptomatic common vineyard weeds. Because some groundcover plants may be good hosts for these pathogens, there is potential for spillover onto grapevine roots that occupy the same soil space. The perceived benefit of increased microbial diversity through enhanced vegetative diversity may be negated if these generalist pathogens accumulate in non-crop vegetation and promote establishment of disease in vines. On the other hand, certain groundcover plants have been seen to decrease the prevalence of these fungi and improve replant outcomes in crops such as apple (Manici et al., 2015). The choice of groundcover may therefore contribute to negative plant-soil feedback on vines if it promotes pathogens like *Ilyonectria* spp. or minimize negative feedbacks if it deters these fungi.

Understanding how to manage groundcover vegetation for positive plant-soil feedback via increasing beneficial fungi while deterring pathogenic fungi could improve the sustainability of perennial crop production (Vukicevich et al., 2016). Though some work has shown that vegetation management can increase overall microbial biomass and activity in vineyard soils (Ingels et al., 2005; Whitelaw-Weckert et al., 2007; Steenwerth and Belina, 2008), how groundcovers may change key fungal guilds over time remains largely unknown.

We sampled groundcover plant communities and associated soils from vineyards in the Okanagan valley, British Columbia. This provided a variety of groundcover management practices that already exist in vineyards in this region to test for effects on soil microbes in real world cropping scenarios. Across these vineyards, we studied how groundcover vegetation affects the abundance of the common EP fungus, *B. bassiana*, and plant pathogenic *Ilyonectria* spp. We used a model-selection approach to identify which plant community characteristics, irrigation techniques, and soil physicochemical factors affected the abundance of each of these fungal guilds as measured by digital droplet PCR (ddPCR) assays. Although there are no published studies that look specifically at how plant functional characteristics affect these groups of soil fungi, we made some predictions based on a review of related literature (Vukicevich et al., 2016). Specifically, we predicted that native plants might increase the abundance of EP fungi (Meyling et al., 2009). Legumes in groundcovers might also be expected to increase EP fungi (Shapiro-Ilan et al., 2012). We expected that *Ilyonectria* spp. might accumulate under legumes (Benitez et al., 2016), but might be less abundant with grasses as grasses seem to be less suitable hosts compared to other vineyard floor vegetation (Agustí-Brisach et al., 2011).

METHODS

Field Sites

All five field sites were located in the southern Okanagan Valley (British Columbia, Canada) (from 49°33′52.44″N, 119°38′19.55″W south to 49° 4′44.02″N, 119°30′41.78″W). This region receives on average ∼320 mm of precipitation annually in the form of snow in winter months and occasional rainfall in spring, summer, and fall. We chose vineyard sites that had different vegetation management schemes within the same vineyard block, i.e., alternating rows or randomized complete block designs. Sites were chosen independent of soil type or management practices, which were controlled for statistically. Site characteristics, including groundcover schemes and irrigation types, are given in **Table 1**.

Plant Community Assessment

We quantified plant communities using quadrats measuring 25 × 50 cm. Four quadrats were evenly spaced throughout four rows of each management scheme (in the case of alternating rows at sites BM, MH, and BC) or four replicate plots (in the case of randomized complete block designs at sites PME and PCH) making for 16 quadrats per management scheme at each site at each sampling period. Nearly 800 plant communities and concomitant soil samples were analyzed in total over the course of this study. Details of sample collection at each site

TABLE 1 | Characteristics of vineyard sites sampled during this project.

Site	Location	Soil series	Pest/fertility management	Groundcover types[a]	Irrigation[a]	Year GC established	Dates sampled	# Samples taken each date[b]
PME	Summerland, BC	Osoyoos sandy loam	"Integrated"	Exotic grass mix; Exotic grass mix plus legumes; Native grass mix; Native grass mix plus forbs	Sprinkler Dual Drip Dual	2014	su 2015; sp 2016; su 2016; sp 2017	64
PCH	Summerland, BC	Osoyoos sandy loam	"Integrated"	Exotic grass mix; Buffalo grass; Grass plus legumes; Clean cultivation	Dual	2011	su 2015; sp 2016; su 2016; sp 2017	60
BM	Okanagan Falls, BC	Rutland sandy loam	"Organic"	Sheep fescue; Annual mix	Sprinkler	2010; 2015	su 2015; sp 2016	32
MH	Oliver, BC	Ratnip sandy loam	"Organic"	Pollinator mix; Resident vegetation	Dual; drip	2009	su 2015; sp 2016; su 2016; sp 2017	32
BC	Osoyoos, BC	Osoyoos sandy loam	"Integrated"	Fescue mix; Alfalfa + perennial ryegrass; Yarrow	Sprinkler	2009	su 2016; sp 2017	64

Inherent differences among sites were controlled for statistically by the inclusion of site as a random factor in the mixed models.

[a] *If more than one irrigation type in place at a site, irrigation type is listed in corresponding order to groundcover type with which it is coupled, except for MH, which transitioned from dual to drip between summer 2016 and spring 2017.*

[b] *Equal numbers of samples were taken from each ground cover type at each site and situated in a consistent spatial pattern (e.g., four alternating vineyard rows with four samples per row with each sample occurring every 10 m). Sample location was accounted for in the mixed model.*

are given in **Supplementary Materials**. Vines adjacent to quadrat placement were marked to facilitate sample collection from the same location at each sampling event. Visual estimation of percent coverage of the quadrat by each plant species was used as a proxy for relative abundance.

Soil Collection and Processing

We collected three soil cores (2.5 × 20 cm) per quadrat, pooled them in sealed plastic bags and kept them on ice for transport to a −20°C freezer. Samples were then weighed, oven dried at 60°C for 72 h to ensure DNA extraction from equal quantities of soil in each sample, and sieved to 2 mm to remove most roots and rocks and homogenize samples. Dried samples were stored at −20°C until use.

Soil Physicochemical Factors

As soil abiotic factors may also be important determinants of fungal abundance in soils, a composite sample of post-processed (after drying and sieving) soil for each treatment was sent to Zenalytic Laboratories (Kelowna, BC) for analysis of organic matter (by loss on ignition) (Davies, 1974), Total N (Kjeldahl, 1883), Extractable P (Mehlich-3 ICP)(Mehlich, 1984), and pH (1:1 in water) (Jackson, 1956).

Molecular Analysis

A 0.5 g subsample was then taken for DNA extraction and quantification of fungi. Genomic DNA was extracted from bulk soil using the FastPrep spin kit for soils (MP Biomedical, Carlsbad, CA) following the manufacturer's instructions. Quantification of target fungal groups was then performed using digital droplet PCR (ddPCR). Each protocol was optimized through the use of dilution series and melt curve analysis in qPCR and annealing/extension temperature gradients with both positive pure culture controls and positive environmental samples in ddPCR.

To quantify the EP fungus we used the primer set BB.fw/BB.rv (Landa et al., 2013), which targets *B. bassiana* at the species level. The following was used in a 20 μL final reaction volume: 10 μL QX200 ddPCR EvaGreen supermix (BioRad, Livermore, CA), 250 nM each primer, 2 μL DNA template, and 7 μL nuclease-free water. Droplets were prepared using BioRad droplet generation cartridges and a QX100 droplet generator. Reaction conditions consisted of initial denaturing at 95°C for 10 min followed by 40 cycles of denaturing at 95°C for 30 s, annealing/extension at 56°C for 2 min, 4°C for 5 min, and 90°C for 5 min. To quantify the plant pathogenic *Ilyonectria* spp. we used the primer set YT2F (Tewoldemedhin et al., 2011) and CYLR (Dubrovsky and Fabritius, 2007), which targets *Ilyonectria* spp. (species complex) that cause black foot disease of grape. This primer set has been used to evaluate abundance of *Ilyonectria* spp. (including *I. macrodidyma* and *I. liriodendri*) in nursery soils (Agusti-Brisach et al., 2014) as well as *I. macrodidyma, I. pauciseptatum, Cylindrocarpon destructans,* and *I. liriodendri* in diseased apple roots (Tewoldemedhin et al., 2011). PCR trials using this primer set in our lab also indicate positive amplification for *I. torresensis, I. europaea, I. ianthothele, I. gamsii, Dactylonectria pauciseptum*, *Clyindrocarpon cylindroides,* and *C. olidum,* all of which are known to cause black foot disease of grape (Úrbez-Torres et al.,

2015). The same recipe and reaction conditions were used as described above except for the annealing/extension step was 60°C for 1 min.

After PCR, droplets were read for fluorescence in a QX200 droplet reader (BioRad, Livermore, CA). Only samples with >10,000 droplets were used for analysis. Raw amplitude and cluster data was exported from Quantasoft version 1.7 (BioRad, Livermore, CA) and the open source software "ddPCRquant" (Trypsteen et al., 2015) was used to determine amplicon concentration of each sample.

Data generated during this project can be viewed on the Open Science Framework, following this link: https://osf.io/cxesk/?view_only=f513573264b141389af7cf23e67200b0.

Data Analysis

Quantification of Plant Community Characteristics

To assess the effect of groundcover vegetation on abundance of our two fungal guilds, we first calculated the abundance and species richness of the plant community, as well as the abundance of different functional traits within the community. Plant abundance was calculated as the total % cover of all vascular plants, whereas species richness was the sum of the number of species. For functional traits, we focused on life history strategy (annual, annual/biennial, biennial, biennial/perennial, and perennial), origin (native/exotic), mycorrhizal status (±) and plant functional group (grass, forb, or legume). For the life history strategies, we coded the different strategies ordinally by increasing length (1 = annual, 2 = annual/biennial, etc.), and calculated the community weighted mean using the R package "FD" (Laliberté and Legendre, 2010). We also coded origin and mycorrhizal status ordinally (0 = native, 1 = exotic, and 0 = non-mycorrhizal, 1 = mycorrhizal) and calculated community weighted means, resulting in indices representing the weighted abundance of exotic and mycorrhizal plants within each quadrat. Exotic plants included both seeded exotic groundcover species as well as exotic weedy species. For plant functional groups, we did not recode the groups as there was no obvious order among them. Instead, we calculated the community weighted mean of % cover of each category; however, forb abundance was not used in subsequent models to avoid extreme collinearity among the indicators.

Determination of Effects Using Model Selection

To determine which factors affect the abundance of fungal taxa, we used mixed models in the R package lme4 (Bates et al., 2015) along with model selection. We used separate mixed models for each fungal group (*B. bassiana,* and *Ilyonectria* spp.). Within the mixed models, we included irrigation type, soil characteristics (phosphorus, pH, and organic matter) and plant community characteristics (total cover, species richness, plant life history strategy, mycorrhizal status, origin, and functional groups) as fixed effects. Given that microbial abundance was quantified in multiple seasons and years, we also included interaction terms between each of these predictors and the sampling period. As random effects, we included block nested within site and quadrat nested within block to account for spatial structuring of samples and inherent site differences.

To reduce the complexity of these models, we used a combination of model and variable selection. First, we ran all possible combinations of the models using the *dredge* function in the R package MuMIn (Barton, 2017). These models were then ranked by their AICc score relative to the most parsimonious model (ΔAICc). Models with a ΔAICc score > 2 were considered uninformative and not considered further (Burnham and Anderson, 2002). Using this subset of models, we weighted each variable using the sums of the ΔAICc scores for the models in which they were included using the *model.avg* function in "MuMIn." Variables with a weight > 0.7 were considered important and included in the final model. This procedure was repeated separately for each microbial group. Outputs from the *model.avg* function listing the average importance of all variables tested across all models run using the *dredge* function are given in **Tables S1**, **S2**. For the final models, we also estimated R^2 values, partitioned between the fixed effects and fixed plus random (Nakagawa and Schielzeth, 2013), as implemented in MuMIn.

To aid the interpretation of the effects of sampling periods, we calculated the estimated marginal means for each sampling period using the R package "emmeans" (Lenth, 2018). Additionally, we used the "emtrends" function within this package to compare the slopes between sampling periods in cases where there were significant interactions with the continuous predictors. To enable comparison among indicators, each indicator variable was scaled to a mean of zero and divided by the standard deviation prior to calculating the trends.

Results are organized into two categories based on the two individual models (*B. bassiana,* and *Ilyonectria* spp.) and then based on three subcategories ("plant effects," "soil effects," and "irrigation and time effects") for ease of interpretation and discussion. Full results for each model can be obtained from the Open Science Framework by following this link: https://osf.io/7qctx/?view_only=e0c567cad4f74a57a81185640b93d62a.

RESULTS

Beauveria bassiana

The final model for *B. bassiana* included sample period, irrigation type, plant life history strategy, exotic species, legumes, grasses, organic matter, and soil P, as well as sampling period interaction terms with irrigation type, grasses, legumes, and soil P (**Table 2**). The fixed effects in this model explained 19% of the variation in *B. bassiana* abundance, while 54% of the variation was explained by fixed plus random effects (vineyard site and spatial structuring of sampling). Sampling period affected the abundance of *B. bassiana* [$F_{(3,717)} = 21.93$, $P < 0.001$], with abundance increasing in spring 2017 compared to spring 2016.

Plant Effects on *Beauveria bassiana*

The abundance of *B. bassiana* was consistently related to plant life history strategy, proportion of exotic species, and legumes (**Table 2**). *B. bassiana* decreased with average plant lifespan [$F_{(1,586)} = 3.91$, $P = 0.048$], increased with the proportion of native plant species [$F_{(1,670)}$=11.57, $P < 0.001$], and increased with legume cover [$F_{(1,667)} = 4.84$, $P = 0.03$] (**Figure 1A**). These plant effects were consistent across the experiment, i.e., did not

TABLE 2 | Effect of sampling date and biotic and abiotic factors in the model of *Beauveria bassiana* abundance as tested using Satterwaite type III approximation for degrees of freedom (Model AIC: 2,166, $R^2_{fixed} = 0.19$).

Category	Factor	*F*-value	*P*-value
Time	Sample period[a]	21.93	**<0.001**
Abiotic factors	Irrigation type[b]	7.5333	**<0.001**
	Organic matter	9.70	**0.002**
	Soil P	0.01	0.90
Biotic factors	Exotics[c]	11.57	**<0.001**
	Legumes[c]	4.84	**0.028**
	Grasses	0.18	0.67
	Life history strategy[d]	3.91	**0.048**
Interactions	Sample period × Irrigation type	8.01	**<0.001**
	Sample period × Soil P	3.49	**0.015**
	Sample period × Grasses	8.50	**<0.001**
	Sample period × Legumes	2.51	0.058

Significant ($P < 0.05$) P-values are in bold.

[a] *"Sample period" indicates when the samples were collected (summer 2015, spring 2016, summer 2016, and spring 2017).*

[b] *"Irrigation type" includes drip (no supplemental irrigation applied to groundcover), dual (occasional watering of groundcover), and sprinkler (frequent watering of groundcover when vines are irrigated).*

[c] *"Exotics" and "Legumes" refers to % quadrat covered by: exotic (non-native) species and legume species, respectively.*

[d] *Plant "Life history strategy" was dummy coded along a continuum from annual (1) to perennial (5).*

depend on sampling period. The effect of grass cover was not significant overall, but was positively associated with *B. bassiana* abundance in spring of 2017.

Soil and Irrigation Effects on *Beauveria bassiana*

Soil organic matter had a consistently positive effect on *B. bassiana* [$F_{(1,662)} = 9.70$, $P = 0.002$] (**Figure 1B**). Soil P had no effect overall, but depended on sampling period [$F_{(3,733)} = 3.49$, $P = 0.015$] with a positive relationship seen in spring 2017 (**Figure 1B**).

Irrigation type was also related to *B. bassiana* abundance with dual and sprinkler irrigation increasing *Beauveria* compared with drip irrigation [$F_{(2,689)} = 7.53$, $P < 0.001$], but effects were inconsistent among sampling periods [$F_{(6,719)} = 8.01$, $P < 0.001$] (**Figure 2**).

Ilyonectria spp.

The final model for *Ilyonectria* spp. included sample period, irrigation type, organic matter, soil P, total plant cover, exotic plant cover, and grass cover, as well as interaction terms with sample period for all factors except grass cover and soil P. Fixed effects in this model explained 38% of the variation, with fixed plus random effects explaining 45%. *Ilyonectria* spp. abundance varied with sampling period [$F_{(3,568)} = 12.21$, $P < 0.001$], with a lower amount of *Ilyonectria* spp. detected in spring 2016 compared to the other sampling periods (**Table 3**).

Plant Effects on *Ilyonectria* spp.

The abundance of *Ilyonectria* spp. was consistently negatively associated with grass cover [$F_{(1,531)} = 22.38$, $P < 0.001$]

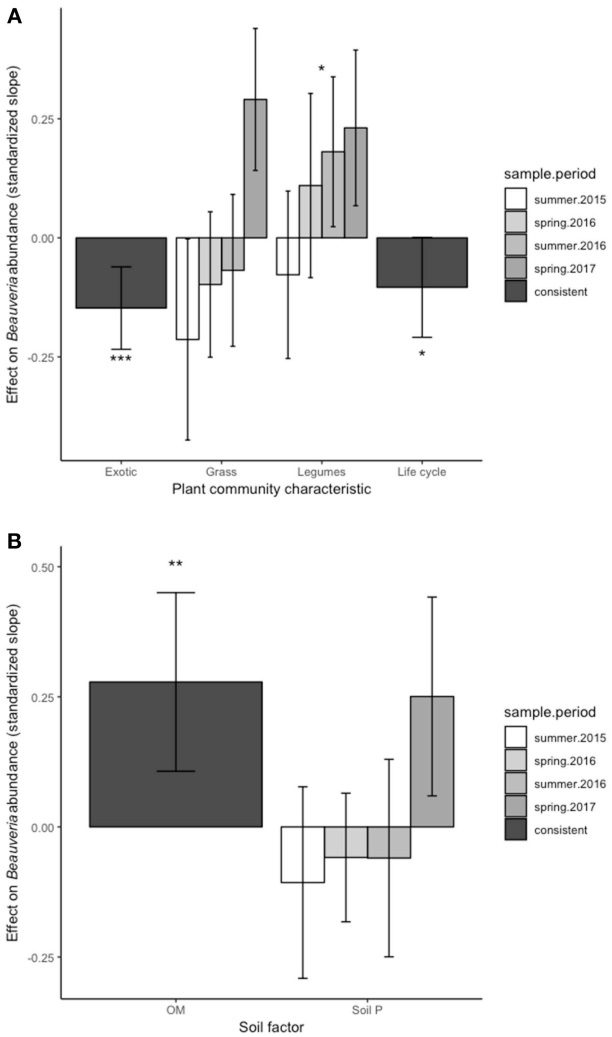

FIGURE 1 | Effect of plant community characteristics **(A)** and soil properties **(B)** on *Beauveria bassisana* abundance. Positive values indicate a positive relationship (standardized slope) between a given factor and *B.bassiana* at that sampling point while negative values represent a negative relationship. A single bar for a given factor means that sampling time did not change the relationship between *B. bassiana* and that factor, i.e., effects were consistent across time. The presence of bars for each sampling time indicates that the relationship between *B. bassiana* and a given factor depended on sampling time. Height of the bar indicates relative strength of each effect. Error bars represent 95% confidence limits. Error bars that do not cross 0 on the y-axis indicate significantly positive or negative relationships between soil factors and *B. bassiana* abundance at a given sample period. "*," "**," and "***" indicate overall significant ($P < 0.05$, 0.01, and 0.001, respectively) positive or negative effects of a factor on *B. bassiana* abundance.

(**Figure 3A**). Overall, *Ilyonectria* spp. increased with exotic plant cover [$F_{(1,675)} = 11.25$, $P < 0.001$], but this relationship varied with sampling period (**Figure 3A**).

Soil and Irrigation Effects on *Ilyonectria* spp.

Soil organic matter was positively related [$F_{(1,47)} = 27.02$, $P < 0.001$] at all sampling periods despite a weak interaction with sampling period [$F_{(3,751)} = 3.06$, $P = 0.03$] (**Figure 3B**).

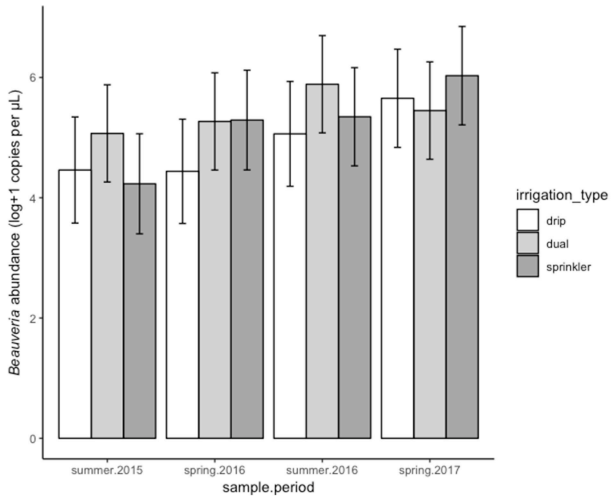

FIGURE 2 | Effect of irrigation type (shades) on *Beauveria bassiana* abundance across all sites at four sampling periods. Error bars indicate 95% confidence limits based on likelihood of these estimates within the final model, i.e., accounting for all other factors included in the model.

TABLE 3 | Effects of sampling date and biotic and abiotic factors in the model of *Ilyonectria* spp. abundance as tested using Satterwaite type III approximation for degrees of freedom (Model AIC: 2,168, $R^2_{fixed} = 0.38$).

Category	Factor	*F*-value	*P*-value
Time	Sample period[a]	12.21	**<0.001**
Abiotic factors	Irrigation type[b]	9.51	**<0.001**
	Organic matter	27.02	**<0.001**
	Soil P	1.77	0.184
Biotic factors	Grasses	22.38	**<0.001**
	Exotics[c]	11.25	**<0.001**
	Total plant cover[c]	3.09	0.079
Interactions	Sample period x Irrigation type	5.02	**<0.001**
	Sample period x Exotics	3.27	**0.021**
	Sample period x Total plant cover	5.14	**0.002**
	Sample period x Organic matter	3.06	**0.027**

Significant ($P < 0.05$) P-values are in bold.

[a] *"Sample period" indicates when the samples were collected (summer 2015, spring 2016, summer 2016, and spring 2017).*

[b] *"Irrigation type" includes drip (no supplemental irrigation applied to groundcover), dual (occasional watering of groundcover), and sprinkler (frequent watering of groundcover when vines are irrigated).*

[c] *"Exotics" and "Total plant cover" refers to % quadrat covered by exotic (non-native) species and all plant species, respectively.*

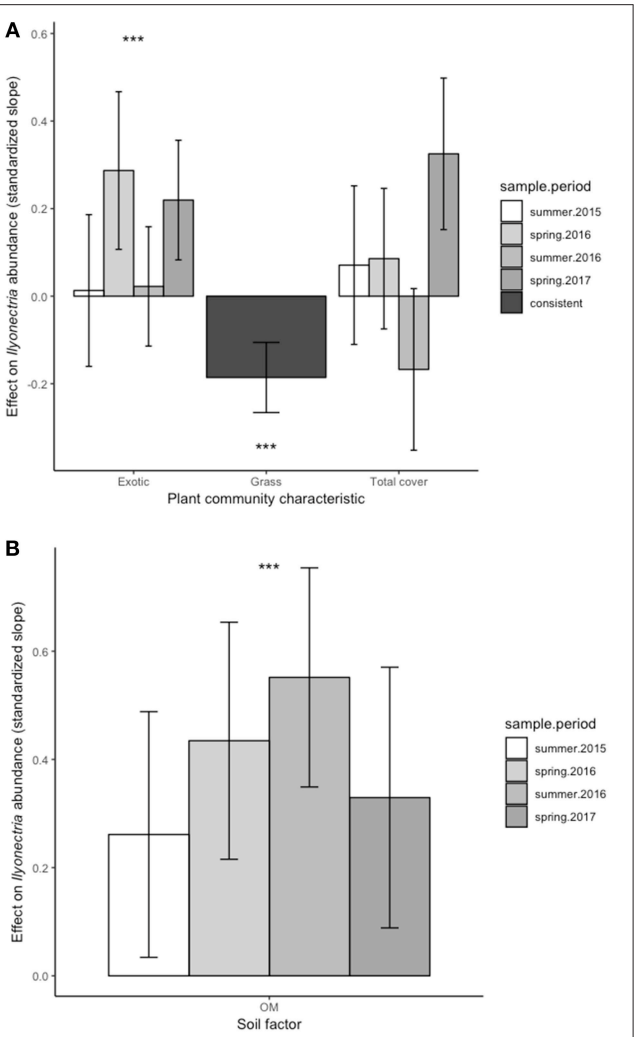

FIGURE 3 | Effect of plant community characteristics **(A)** and soil properties **(B)** on *Ilyonectria* spp. abundance. Positive values indicate a positive relationship (slope) between a given factor and *Ilyonectria* spp. abundance at that sampling point while negative values represent a negative relationship. A single bar for a given factor means that sampling time did not change the relationship between *Ilyonectria* spp. and that factor, i.e., effects were consistent across time. The presence of bars for each sampling time indicates that the relationship between *Ilyonectria* spp. and a given factor depended on sampling time. Height of the bar indicates relative strength of each effect. Error bars represent 95% confidence limits. Error bars that do not cross 0 on the y-axis indicate significantly positive or negative relationships between soil factors and *Ilyonectria* spp. at a given sample period. ""***" indicate overall significant (0.001, respectively) positive or negative effects of a factor on *Ilyonectria* spp. abundance.

Irrigation type also affected *Ilyonectria* spp. abundance [$F_{(2,115)} = 9.51$, $P < 0.001$], with both dual and sprinkler irrigation leading to greater abundance overall compared to drip irrigation, though the strength of this effect depended on sampling period (**Figure 4**).

DISCUSSION

Groundcover vegetation was related to the abundance of both soil fungal guilds studied. As expected, native plants were associated with greater amounts of EP fungi, suggesting that coadaptation may promote positive plant soil feedback through improved herbivore control and plant protection. The positive effect of legumes on EP fungi also matched our predictions, though there were no consistent effects of legumes on *Ilyonectria* spp. Instead, *Ilyonectria* spp. were mostly deterred by the presence of grasses and native species, further suggesting that native species could play a role in promoting positive plant-soil feedbacks

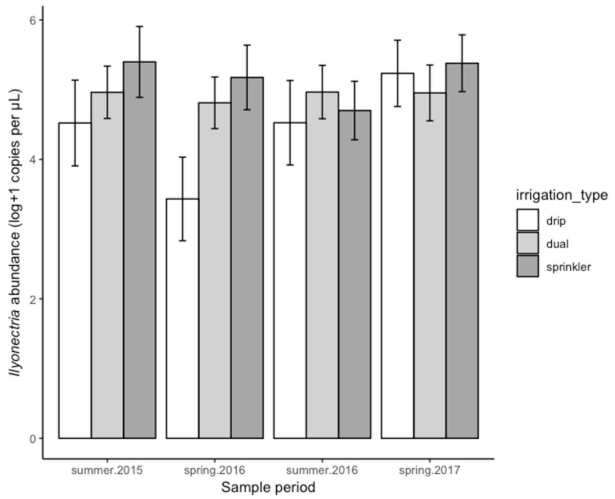

FIGURE 4 | Effect of irrigation type (shades) on *Ilyonectria* spp. abundance across all sites at four sampling periods. Error bars indicate 95% confidence limits based on likelihood of these estimates within the final model, i.e., accounting for all other factors included in the model.

in these vineyards by limiting this soil borne pathogen in this semi-arid region.

Beauveria bassiana

The increase in *B. bassiana* with locally adapted plant communities as seen in this study may have a basis in co-adaptation, as *B. bassiana* is now known to have diverged from fungi with purely endophytic lifestyles (Moonjely et al., 2016). This is, to our knowledge, the first report of a preferential association between EP fungi and native plant communities within an agricultural field. The inclusion of native species may encourage positive plant-soil feedback for crop plants such as grapevines if the larger EP fungal populations help control soil-dwelling herbivores such as climbing cutworm, mealybug, or phylloxera. To date there are no studies that specifically include EP fungi as an important player in plant-soil feedbacks, but regulation of herbivory as well as pathogen protection offered by these fungi (Ownley et al., 2010) warrant further investigation, e.g., in the context of conservation biological control (Pell et al., 2010).

The positive effect of annual species on *B. bassiana* could be because this EP fungus can persist in annually cropped fields, unlike some other EP fungi that prefer undisturbed habitats (Meyling and Eilenberg, 2006; Meyling et al., 2009; Medo and Cagan, 2011). Randhawa et al. (2018) recently showed that another common EP fungus, *Metarhizium robertsii*, occurs in higher numbers soon after disturbance and then declines with time since disturbance. Perhaps common EP fungi such as *B. bassiana* and *Metarhizium* spp. are most dominant in annual agricultural fields due to some unknown adaptation to physical disturbance, annual and weedy plant species, or the insect communities that occur in these disturbed habitats (e.g., ground-dwelling decomposers).

The increase in *B. bassiana* with legumes as seen here is consistent with a previous finding that *B. bassiana* persists well in legume cover crops in orchards (Shapiro-Ilan et al., 2012), although that study only compared legumes to the absence of a cover crop. Because legumes had a positive influence on *B. bassiana* in our large dataset looking at many different plant traits, this hints at some functional attribute of legumes that is especially beneficial for the success of *B. bassiana*. Most EP fungi including *B. bassiana* are poor competitors as saprotrophs (Meyling and Eilenberg, 2007), ruling out the effect of high litter quality of legumes. Most likely the benefits of a legume for EP fungi lie either in the attractiveness of the legume roots to soil herbivores (Schallhart et al., 2012), their suitability for endophytic colonization by EP fungi (Behie et al., 2015), protection from environmental stresses (Shapiro-Ilan et al., 2012) or some combination of these. The higher litter N quality of legumes may also attract more soil-dwelling insects (House and Alzugaray, 1989), thus indirectly increasing EP fungi.

Organic matter was positively associated with *B. bassiana* abundance at most sampling periods. A positive correlation between organic matter and the organisms that contribute to its formation can be expected (Kallenbach et al., 2016). There are several studies that show greater EP fungal isolation associated with higher organic matter soils (Ali-Shtayeh et al., 2003; Medo and Cagan, 2011) and organic fertilization (Clifton et al., 2015). Because *Beauveria* are generally poor competitors as saprotrophs, it is unlikely that there is a direct effect of organic matter on their populations. Instead, higher organic matter is likely associated with greater biological activity in general, including more plant roots and insects, both of which are hosts for these fungi.

Ilyonectria spp.

The decrease in *Ilyonectria* spp. with grass cover is consistent with the small amount of peripheral work on the effect of non-crop vegetation on these pathogens. For example, although a survey of vineyard weeds by Agustí-Brisach et al. (2011) found *Ilyonectria* spp. in many common weeds, only six species of grass were included in that study, of which only two hosted *Ilyonectria* spp. In studies of replant disease of apple, Mazzola et al. (2004) were able to reduce damage caused by pathogens such as *Ilyonectria* spp. through stimulation of an antagonistic rhizobacteria population using a wheat cover crop. Other grasses, such as *Lolium perenne*, have also been implicated in promoting bacteria with fungistatic genes (Latz et al., 2015), suggesting that perhaps some grasses deter these generalist pathogens by culturing an antagonistic rhizosphere community and thus are not good hosts. Although these potential mechanisms are speculative, the consistency of our results suggest that grasses are somehow poorer hosts for *Ilyonectria* spp. in these vineyards than broadleaves and might then promote positive plant-soil feedback by deterring generalist pathogens.

Exotic plants tended to increase *Ilyonectria* spp. abundance overall, but this was due to the strong effects seen only during spring sampling periods. It could be that this pathogen thrives in cultivated plants and associated weedy species, which are mostly exotic species in the studied region. Work on invasive plant species has shown that generalist pathogens can build up on

exotic species with negligible effects on those plants (Mangla and Callaway, 2008). If *Ilyonectria* spp. accumulates on exotic plants, this may lead to negative feedback on vines sharing the same soil through a spillover effect. A possible explanation for the springtime effects of exotic plants on *Ilyonectria* spp. abundance could be that many of the exotic weedy species may be more active in the spring.

As with *B. bassiana*, *Ilyonectria* spp. was positively related to soil organic matter, perhaps relating to the general microbial contribution to stable organic matter (Kallenbach et al., 2016). This increase in *Ilyonectria* spp. with greater amounts of organic matter is not necessarily an indication of increased disease pressure for crop plants occupying this soil because increased microbial competition and antagonism also occurs with the use of organic amendments(Bonanomi et al., 2007; Watson et al., 2017).

The increase in *Ilyonectria* spp. with supplemental irrigation could be expected given that *Ilyonectria* spp.-related diseases such as black foot disease of grape tend to be more problematic with prolonged periods of excessive soil moisture (Halleen et al., 2006). It is also likely that the use of sprinkler irrigation leads to broader distribution of grapevine roots throughout the vineyard floor. As grapevines are good hosts for these fungi, the proximity of vine roots to the drive row sample plots could have also contributed to this effect in addition to the increased frequency of wetting.

CONCLUSION

This study is the first to investigate the relationships between groundcover plant community characteristics as well as soil and irrigation factors on the abundance of the entomopathogenic *B. bassiana,* and the plant pathogenic *Ilyonectria* spp.. We conclude that native species may play an important role in managing plant-soil feedbacks in perennial agroecosystems as they promoted the plant-beneficial fungi *B. bassiana* but deterred the plant-pathogenic *Ilyonectria* spp. Practical application of this work will require further studies linking these plant-induced changes to soil fungi with measurable plant-soil feedbacks along with continued field trials to find locally adapted species best suited for use in perennial agricultural groundcovers.

AUTHOR CONTRIBUTIONS

EV performed all data collection, laboratory analysis, and manuscript preparation. DL maintained experimental field plots, identified potential sample sites, and gave feedback on the manuscript. JB performed statistical analysis. MH provided laboratory resources, extra student help, and feedback on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00118/full#supplementary-material

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Plant-Soil Feedbacks Predict Native but Not Non-native Plant Community Composition: A 7-Year Common-Garden Experiment

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Plant-soil feedbacks (PSFs) have gained attention as a potential mechanism of plant growth and coexistence, however, because they are typically measured using plant monocultures in greenhouse conditions, the link between PSFs and plant growth in field communities remains poorly tested. Here, PSFs for six native and four non-native species were measured in a 7-year, common-garden experiment. A plant community growth model was then parameterized either with PSF data (PSF model) or without PSF data (Null model). PSF and Null model predictions were compared to plant ground cover in experimental and natural communities. For eight of 10 species, plant cover at the end of the experiment differed among soils cultivated by different species. For native plants, the Null model incorrectly predicted rank-order abundance for three of four experimental communities and Null model predictions were not correlated with observed plant growth. In contrast, when PSF data were added to the same model, the model correctly predicted rank-order abundance for all four experimental communities and PSF model predictions were well-correlated with plant cover in experimental communities and on the landscape (R^2 = 0.62). For non-native species, predictions from both models were correlated with observed species cover (R^2 = 0.37 and 0.35, respectively), but there was no difference between PSF and Null model predictions. Previous studies at the study site have shown that PSF maintains alternate-state native and non-native plant communities. Here, it was shown that PSF is also critical for determining species composition within native plant communities, but that other mechanisms appear to be necessary to simulate the rapidly-fluctuating abundances of the short-lived, non-native species in this system. Using a relatively long-term field experiment, this study provided unusually direct evidence for the role of PSF in determining plant abundance in plant communities in field conditions, at least for long-lived native plants.

Keywords: plant soil feedback, field experiment, invasive, native, plant community, model, factorial, prediction

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INTRODUCTION

Plant-soil feedbacks (PSFs) have gained attention for their potential to determine plant growth, succession, coexistence, and invasion (van der Putten et al., 2013; Bailey and Schweitzer, 2016; Zhang et al., 2019). PSFs are typically measured by comparing the growth of a plant on soils cultivated by conspecifics ("self" or "home" soils) to soils not cultivated by conspecifics ("other"

or "away" soils; Bever, 1994; Bever et al., 1997). While there are many variations, PSF experiments are often performed by growing plant monocultures in a greenhouse for a roughly 3-month training phase and a 3-month test phase (Kulmatiski and Kardol, 2008; Brinkman et al., 2010). Plants that grow better on "self" than "other" soils have positive PSF, while plants that grow better on "other" than "self" soils have a negative PSF (Brinkman et al., 2010; Reinhart and Rinella, 2016; Teste et al., 2019). Mathematical models suggest that positive PSF encourages competitive exclusion while negative PSF encourages species coexistence through replacement (Bever, 2003; Eppinga et al., 2018; Mack et al., 2019).

There are, however, many reasons why PSFs measured in monocultures in greenhouse experiments may not be important to plant communities in the field (Poorter et al., 2016; Schittko et al., 2016). Greenhouse conditions may create PSFs that are not relevant to field communities. The sterilized soils that are often used in greenhouse PSF experiments are likely to encourage the growth of microbes that may not be common in field conditions (Hawkes et al., 2013; Kardol et al., 2013; Bergmann et al., 2016). Larger soil organisms may be almost completely excluded from greenhouse experiments (Cesarz et al., 2018). Moderate climate conditions in the greenhouse are also likely to change plant growth, soil organism growth, and their interactions relative to field soils (Heinze et al., 2016; Schittko et al., 2016; van der Putten et al., 2016; Fry et al., 2018). In addition to the effects of greenhouse conditions, plant monocultures may create conditions that are not common in the field. For example, diverse plant communities may create soil microbial communities that have greater disease suppressiveness than soil communities associated with plant monocultures (Compant et al., 2005; Latz et al., 2012). As a result, PSFs measured in plant monocultures may or may not be relevant to PSFs realized in plant communities (Poorter et al., 2016; Smith-Ramesh and Reynolds, 2017; Wubs and Bezemer, 2018).

The mathematical models used to infer PSF effects in communities may also be misleading. PSF models often make the unrealistic assumption that plant species are competitively equivalent (Crawford and Knight, 2017; Vincenot et al., 2017; Bezemer et al., 2018). However, to have large effects on plant abundance in communities, PSFs should be of a similar magnitude as differences in intrinsic growth rates among species (Revilla et al., 2013; Kulmatiski, 2016; Kulmatiski et al., 2016; Lekberg et al., 2018). Published data suggest that PSFs are sometimes large enough to overcome competitive inequality among species, suggesting that PSF, at least in some cases, will be an important driver of plant coexistence, though it is not known how commonly this occurs (Crawford and Knight, 2017; Lekberg et al., 2018; Mack et al., 2019).

Testing whether or not PSF determines plant growth in communities remains a central goal in PSF research (van der Putten et al., 2016; Smith-Ramesh and Reynolds, 2017; Lekberg et al., 2018). One way to test whether or not PSF effects are large enough to overcome the effects of other plant growth factors is to use PSF effects in plant growth models and compare model predictions to plant growth observed in plant communities (Mangan et al., 2010; Kulmatiski, 2018). This approach, however, has not been widely used (Bennett and Cahill, 2016; Chung and Rudgers, 2016; Schittko et al., 2016; Bennett et al., 2017; Teste et al., 2017).

The goal of this research was to test whether or not PSF data could be used to improve predictions of plant growth in communities. To meet this objective, PSFs were measured for six native and four non-native species in a 7-year, common-garden experiment. To test whether or not these measured PSFs were important to plant growth in communities, a plant growth model was parameterized either with PSF effects (PSF model) or without PSF effects (Null model) and model predictions were compared to plant growth in experimental plant communities and to plant growth on the landscape [as reported in Kulmatiski and Beard (2019)].

MATERIALS AND METHODS

Research was conducted on the Newbon soil series (coarse-loamy, mixed mesic Typic Haploxerolls; Lenfesty, 1980), Winthrop, Washington (48.481 N, −120.117 W; elevation 780 m). Annual precipitation (380 mm) falls mostly as snow in the winter (November through March). There are two common plant community types on the landscape. Fields that have never been tilled represent most of the land in the hilly landscape and are dominated by native plants (62.4 ± 2%) with non-native plants less common (6.6 ± 0.8%; Kulmatiski and Beard, 2019). Fields that have been tilled and used for agriculture, primarily valley bottoms, and benches, are dominated by non-native plants (39.6 ± 2.1% absolute cover [mean ± std. dev.]) with native plants less common (19.2 ± 1.8%; Kulmatiski and Beard, 2019). Because these two plant community types (native and non-native dominated) are largely separated on the landscape, PSF effects on plant communities were determined for native and non-native plant communities separately.

Plant-Soil Feedback Experiment

A roughly 1-ha area in an abandoned agricultural field, previously used to grow alfalfa (*Medicago sativa*), was used to establish a two-phase, "self" vs. "other" PSF experiment (Bever, 1994; Brinkman et al., 2010). In this experimental approach, target species were maintained for a 4-year Phase I to create different soil treatments. Plants were then removed with herbicide and re-planted with either the same species (i.e., self plots) or different species (i.e., other plots). A full factorial design was used for three native species and for three non-native species. In this factorial design each plant was grown on soils cultivated by each of the other plants in the community. Because of limited space, PSFs for the remaining four species were assessed using a "self vs. control" PSF approach which requires fewer replicates (Kulmatiski, 2016). This "self vs. control" portion of the experiment and resultant PSF values were reported previously (Kulmatiski et al., 2017), but the factorial portion of this experiment, use of all PSF values in a plant growth model, and comparison of model predictions to plant growth in experimental plant communities are new to this manuscript.

Dominant species on the landscape were selected for this experiment with some exceptions. Native species included an

annual forb [*Collomia grandiflora* (COGR)], two perennial forbs [*Lomatium dissectum* (LODI) and *Lupinus sericeus* (LUSE)] and three perennial grasses [*Festuca idahoensis* (FEID), *Koeleria cristata* (KOCR), and *Pseudoroegneria spicata* Pursh A. Love (PSSP)]. Non-native species included an annual grass [*Bromus tectorum* (BRTE)], an annual forb [*Lactuca* serriola (LASE)], and two annual/perennial forbs [*Centaurea diffusa* Lam. (CEDI) and *Sissymbrium loeselii* (SILO)]. Because the experiment was conducted in 1.5 m^2 experimental plots, the dominant, but large native shrubs, *Purshia tridentata* and *Artemisia tridentata* and the rhizomatous non-native forb, *Cardaria draba* were not used. The native annual forb COGR is a widespread but not dominant species but it was used to provide inference to the PSF of a common native annual. *Poa bulbosa* is a dominant non-native, but we were unable to establish it. Unless otherwise noted, species naming follows that of Hitchcock and Cronquest (1973).

Prior to Phase I of the experiment, the top 10 cm of soil, and presumably much of the weed seed bank was removed by bulldozer, 7.6 m^3 of A-horizon soil from a native plant dominated field were mixed with sand from a nearby landslide, and all soils were disc-plowed to create a mixed agricultural-native-sand growth medium. A grid of 1.2 m-wide geotextile cloth was secured to the ground creating 750 1.5 m^2 plots. Of these, 250 plots were used to create self-cultivated soil treatments (25 plots for each species). These plots were replanted with the same species in Phase II of the experiment providing a measurement of plant growth on "self" soils. An additional 250 plots were maintained free of vegetation to be used as "control" plots. In Phase II, each species was planted in 25 replicate "control" plots to provide a measure of plant growth on "non-self" or "other" soils. The remaining 250 plots were used for the factorial PSF experiment. For the three native species in N3 (**Table 2**) and the three non-native species in X3 (**Table 2**), each plant was grown on "self" soils as well as on soils cultivated by each of the other plant species in the community. Because these factorial designs require many more plots than the "self" vs. "control" approach, the factorial design was used only for three native plant species and three non-native plant species. All treatments were replicated 25 times, though in a few cases, more than 25 replicate "self" plots were used because additional plots had been created for a related experiment in the same field.

Each fall from 2006 to 2009, 12 g of seed from the target species was added to each plot. Each spring and summer from 2007 to 2010, non-target species were removed from each plot by hand weeding. In May 2010, all plots were surveyed. Plots where the target species did not represent 65% or more of standing vegetation were removed from the experiment. Beginning June 2010, all remaining quadrats were treated with a broad-spectrum herbicide application (30 ml of Roundup® herbicide, 0.2 kg active ingredient ha^{-1}). Two weeks later, standing vegetation was clipped by hand and left in the plot. Plots were revisited over the next several months and additional herbicide spot-treatments and hand-pulling were used in quadrats where regrowth was observed. This created replicate plots with soils cultivated by target plant species.

Phase II began October 2010. Each species was replanted by seed in "self," "other," or "control" plots as appropriate. Non-target species were removed from all plots during the 2011, 2012, and 2013 growing seasons. In June 2013, percent cover of each plant was measured in each plot using visual estimation.

Plant-Community Experiment

Data from the PSF experiment were used in a plant growth model to predict the percent ground cover of plant species in communities. To test model predictions, three-species plant communities were grown in the field (**Table 1**). Three communities composed of native species and three communities composed of non-native species were grown for 4 years (2007–2010). Communities for which factorial PSF were available were grown again from 2010 to 2013 (i.e., N3 and X3 in **Table 1**). More specifically, in October 2006, six different three-species communities were established by seed (**Table 1**). Plots were the same size and randomly located among the plots used for the PSF experiment. The communities for which full factorial PSF data were available (i.e., N3 and X3) were replicated 120 times. The remaining four communities were replicated 50 times. As in the PSF experiment, between 2007 and 2010 all plots were seeded and weeded by hand. Plots in which target species did not represent 65% of total plant cover prior to the final weeding were removed from the experiment. Percent cover of each target species was determined June 2010. Also in June 2010, 60 plots that had grown the dominant native plant, *P. spicata,* were treated with herbicide and planted with either N3 or X3. This was done on *P. spicata* plots because this provided more inference into how these communities, for which factorial PSF data were available, grow on a common soil treatment and during a different time period and not just on control soils between 2007 and 2010.

The Plant Community Growth Model

The best-performing of five plant growth models described by Kulmatiski et al. (2016) was used to simulate plant community composition (i.e., the Logistic Pot-level-K model). Briefly, in this

TABLE 1 | Species compositions and the year of measurement for the six plant communities used in the "plant-community experiment."

Community name	Species in the community	Year measured	
		2007–2010	2013
NATIVE			
N1	COGR, KOCR, PSSP	X	
N2	LODI, LUSE, PSSP	X	
N3	FEID, KOCR, PSSP	X	X
NON-NATIVE			
X1	CEDI, LASE, SILO	X	
X2	BRTE, LASE, SILO	X	
X3	BRTE, CEDI, SILO	X	X

All plant communities were grown and observed for 4 years from 2007 to 2010. Communities N3 and X3 were grown for an additional 3 years from 2011 to 2013 because more precise "factorial" plant-soil feedback data were available to predict species abundances in these two communities. The remaining communities were used to predict plant abundance using "self" vs. "control" plant-soil feedback values.

TABLE 2 | Ground cover (%) of six native plants on different soil treatments.

Plant	Soil treatment						
	CONTROL	COGR	FEID	KOCR	LODI	LUSE	PSSP
COGR	0.71 ± 0.1a (14)	0.3 ± 0.3b (37)	NA*	NA	NA	NA	NA
FEID	11.7 ± 2.3a (24)	NA	7.6 ± 1.6ab (20)	2.7 ± 1.9bc (17)	NA	NA	1.6 ± 0.4c (30)
KOCR	13.6 ± 2.4a (21)	NA	15.3 ± 1.8a (16)	8.0 ± 3.2ab (19)	NA	NA	2.7 ± 0.9b (30)
LODI	0.5 ± 0.2a (13)	NA	NA	NA	0.1 ± 0.1b (18)	NA	NA
LUSE	3.9 ± 1.6 (15)	NA	NA	NA	NA	7.2 ± 2.0 (25)	NA
PSSP	5.4 ± 1.1b (21)	NA	5.9 ± 0.8b (19)	4.8 ± 3.0b (17)	NA	NA	11.6 ± 1.4a (82)

*Soil treatments were cultivated by the target plant for 4 years. Plant growth on each soil treatment was measured after 3 years of growth. Mean values ± 1 SE reported. Different lower case letters indicate differences in the growth of a plant species on the different soil treatments at the α = 0.05 level. Values in parentheses are sample sizes. *NA, Not available.*

continuous-time, logistic growth model, each plant's growth is a function of the proportional abundances of the soils cultivated by different plant species (Bever, 2003; Levine et al., 2006; Eppstein and Molofsky, 2007). The model assumes each plant's growth is limited by a community-level carrying capacity and total plant growth in the community. Carrying capacity was defined as the mean cover observed in native and non-native plant communities in the plant community experiment (i.e., 43 and 38%, respectively). Plants were assumed to start growth as seed (0.002 g) and time-step-specific growth rates were calculated for 40 time steps as $(\sqrt[40]{F/I}) - 1$, where F = final cover and I = initial cover. Final cover for each species on each soil treatment was determined from the cover observed at the end of the PSF experiment.

The model was parameterized with two datasets: Null and PSF. In the Null parameterization, only cover data from "control" soils was used. Control soils were soils that were maintained free of vegetation during Phase I. Plant growth on control soils was selected because this represents a standard method of measuring plant growth in a common-garden experiment (e.g., in a plant competition experiment). In the PSF parameterization, plant growth data from "self" and "other" soils was used. "Other" soils were species-specific when possible, or "control" soils when species-specific data were not available.

To better simulate multi-year plant growth, two changes to the model were made. First, to simulate annual senescence, after every 40 time steps, plant growth was decreased to equal 1% of the value in the previous timestep. Second, it was assumed that plants were affected more by "self" than "other" soils since plant roots grow within their own rhizosphere. To calculate this effect, "self" soils were calculated as the proportion of "self" plant abundance in the previous timestep plus an arbitrarily-selected 25% of the remainder of soil treatments. For example, if a plant represented 30% of a three-species plant community, that plant was estimated to grow on 30% + 0.25*(1–30%) = 47.5% "self" soils. The proportion of "other" soils was then down-weighted to account for the larger proportion of "self" soils. Null and PSF models were executed for 120 time steps to simulate growth of plant communities grown for 3 years (2011–2013), for 160 time steps for communities grown for 4 years (2007–2010) and for 400 time steps to simulate plant growth on the landscape.

Statistical Analyses

Plant growth data were primarily used to parameterize plant community growth models, but to determine if biomass differed by soil treatment, a one-factor GLMM was used with soil treatment as the fixed effect; analyses were performed by species because species by soil treatment comparisons were not of interest. Transformations to meet assumptions of homogeneity and normality were used as necessary. For all tests, a *post-hoc* Tukey–Kramer method was used to adjust for Type I error and determine pairwise differences among least square means. Means from raw data are reported.

To determine if PSF data improved Null model predictions across species, a Student's *t*-test on the absolute difference between observed and predicted values for the Null and PSF models was conducted. To determine the goodness-of-fit between observed and predicted values, a Pearson correlation coefficient was calculated and reported as an R^2 value. Correlations were performed first for plant cover values at the end of the experiment, then again for annual and final cover values. Similarly, correlations were first performed for the experimental communities then also for a combined dataset that included species abundances from the experimental communities as well as species abundances from the landscape. Correlation P values are reported and considered significant when $P < 0.05$. Plant cover on the landscape was reported by Kulmatiski and Beard (2019).

RESULTS

For all species except *L. sericeus* and *L. serriola*, ground cover differed among soil treatments (**Tables 2**, **3**). For native plant cover in experimental communities, 10 of 12 PSF model predictions were closer to observed values than Null model predictions. More specifically, seven of nine predictions of species abundance at the end of the 4-year community experiment (**Figure 1**) and three of three predictions of species abundance at the end of the 3-year community experiment (**Figure 2**). A T-test of the absolute difference between predicted and observed species cover in experimental communities indicated that PSF predictions were closer to observed values than Null values ($T_{(1,11)} = 5.95$, $P = 0.006$). Similarly, PSF model predictions were correlated with native species cover in

TABLE 3 | Ground cover (%) of four non-native plants on different soil treatments.

Plant	Soil treatment				
	CONTROL	**BRTE**	**CEDI**	**LASE**	**SILO**
BRTE	2.4 ± 0.5b (24)	5.2 ± 1.5ab (56)	2.4 ± 0.6b (29)	NA	6.3 ± 1.8a (29)
CEDI	14.9 ± 2.8ab (24)	23.5 ± 3.4a (24)	14.0 ± 1.7b (54)	NA	15.4 ± 2.5ab (29)
LASE	0.1 ± 0.1 (16)	NA	NA	0.1 ±0.1 (30)	NA
SILO	0.6 ± 0.4ab (21)	2.4 ± 0.1a (25)	0.9 ± 0.3ab (28)	NA	0.6 ± 0.1b (25)

*Soil treatments were cultivated by the target plant for 4 years. Plant growth on each soil treatment was measured after 3 years of growth. Mean values ± 1 SE reported. Different lower case letters indicate differences in the growth of a plant species on the different soil treatments at the α = 0.05 level. Values in parentheses are sample sizes. *NA, Not available.*

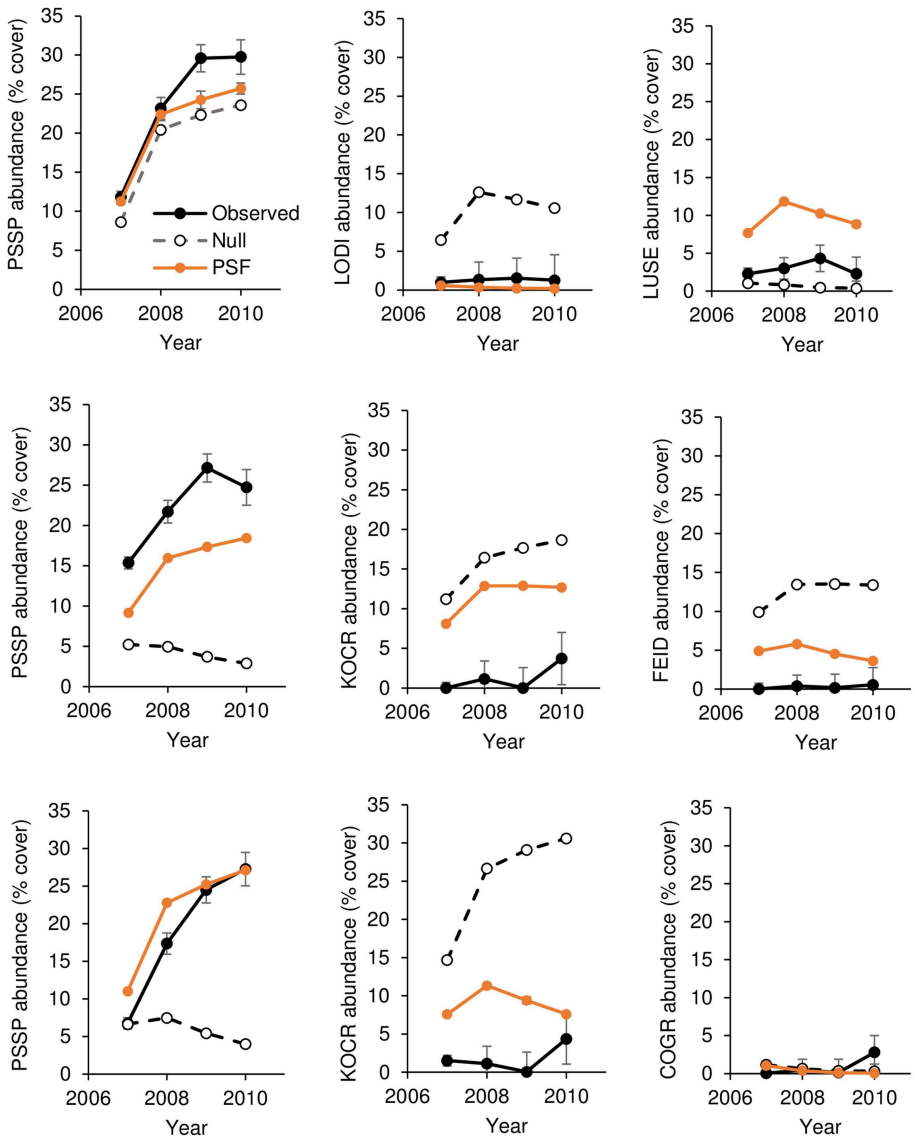

FIGURE 1 | Observed and predicted abundance of native plant species in three-species experimental plant communities grown for 4 years (2007–2010). Species in a row are from the same three-species community. Model predictions were made either with plant-soil feedback effects (PSF) or without plant-soil feedback effects (Null). PSSP, *Pseudoroegneria spicata*; LODI, *Lomatium dissectum*; LUSE, *Lupinus sericeus*; COGR, *Collomia grandifolia*; FEID, *Festuca idahoensis*; KOCR, *Koeleria cristata*.

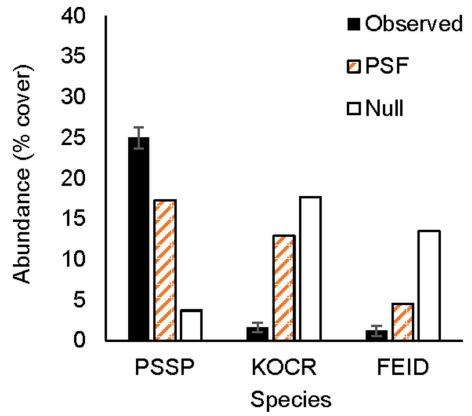

FIGURE 2 | Observed and predicted abundance of native plant species in a three-species experimental plant community grown for 3 years (2011–2013). Model predictions were made either with plant-soil feedback effects (PSF) or without plant-soil feedback effects (Null). Species codes listed in the **Figure 1**.

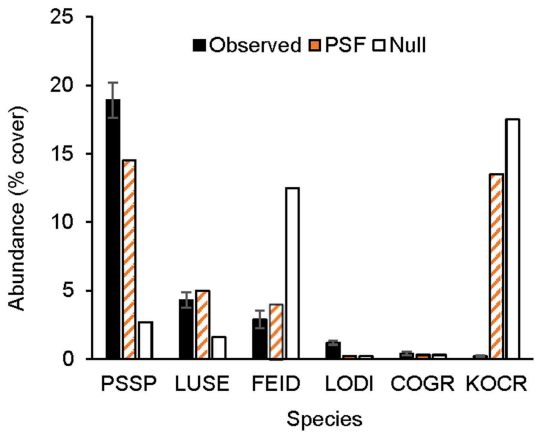

FIGURE 3 | Observed and predicted native plant abundance. Observed plant abundance was recorded in 25 ex-arable fields over 13 years (Kulmatiski and Beard, 2019). Model simulations of plant community growth for these six common native species were performed for a 10-year period either with plant-soil feedback data (PSF; i.e., with information on plant growth rates on different soil treatments) or with a null model (Null) which was parameterized only with plant growth rates on control soils. PSSP, *Pseudoroegneria spicata*; LODI, *Lomatium dissectum*; LUSE, *Lupinus sericeus*; COGR, *Collomia grandifolia*; FEID, *Festuca idahoensis*; KOCR, *Koeleria cristata*.

experimental communities ($F_{(1,10)} = 32.6$, $P < 0.001$, $R^2 = 0.77$) but Null model predictions were not ($F_{(1,10)} = 0.17$, $P = 0.686$). Results were similar when annual predictions of species cover were correlated with annual observations. PSF model predictions were correlated with species cover ($F_{(1,34)} = 90.3$, $P < 0.001$, $R^2 = 0.73$) but Null model predictions were not ($F_{(1,34)} = 0.00$, $P = 0.992$). PSF data also improved Null model predictions of species cover on the landscape (**Figure 3**). When data from the four experimental communities and plant cover on the landscape were combined, a T-test of the absolute difference between predicted and observed species cover indicated that PSF predictions were closer to observed values than Null values ($T_{(1,17)} = 3.70$, $P = 0.010$). Further, PSF model predictions of species cover in experimental communities and landscape communities were correlated with observed species cover ($F_{(1,16)} = 26.6$, $P < 0.001$, $R^2 = 0.62$) but Null model predictions were not ($F_{(1,16)} = 0.08$, $P = 0.78$).

For non-native plants, a T-test of the absolute difference between predicted and observed species cover in experimental communities indicated that PSF predictions were not different from Null values ($T_{(1,11)} = 1.16$, $P = 0.81$). Further, neither PSF model predictions ($F_{(1,10)} = 4.71$, $P = 0.055$) nor Null model predictions ($F_{(1,10)} = 4.42$, $P = 0.061$) were correlated with species cover in experimental communities (**Figures 4**, **5**). Results were similar when annual predictions of species cover in experimental communities were compared to annual observations: neither PSF model predictions ($F_{(1,34)} = 0.39$, $P = 0.536$) nor Null model predictions ($F_{(1,34)} = 0.06$, $P = 0.801$) were correlated with observed annual species cover (**Figure 4**). Model predictions of non-native species cover on the landscape were better (**Figure 6**). When species cover data from experimental communities and the landscape were combined, both PSF model predictions ($F_{(1,14)} = 8.17$, $P = 0.013$, $R^2 = 0.37$) and Null model predictions ($F_{(1,14)} = 7.62$, $P = 0.015$, $R^2 = 0.35$) were correlated with non-native species cover in experimental and landscape communities. However, a T-test of the absolute difference between predicted and observed values indicated that PSF predictions of species cover were not closer to observed values than Null values ($T_{(1,15)} = 1.15$, $P = 0.793$).

DISCUSSION

Results from this 7-year field experiment provided uncommonly direct evidence for the role of PSFs in plant communities. As is commonly reported, plant growth differed among soil treatments for eight of 10 species (i.e., PSF; **Tables 2**, **3**). More importantly, here it was shown that these PSFs were critical for predicting plant abundance, at least in native plant communities. Null model predictions, which only used plant growth data from common-garden or "control" soils, were not correlated with native plant cover in experimental plant communities, but when plant growth data from different soil treatments was included in this same model, predictions were well-correlated with plant cover in experimental plant communities (**Figures 1**, **2**). Further, Null model predictions of rank-order abundance were incorrect for three out of four native experimental communities, but PSF model predictions were correct for all four communities. Further still, PSFs improved predictions of native plant growth on the landscape relative to Null model predictions (**Figure 3**). Across the experimental and landscape communities, PSF model predictions of native cover were well-correlated with observations ($R^2 = 0.62$) while Null model predictions were not. In short, field-measured PSFs were critical for understanding native plant growth in plant communities in this system. This result is important because while PSFs are widely believed to be important in determining plant abundance, most evidence for the role of PSFs is derived from greenhouse experiments (Lekberg

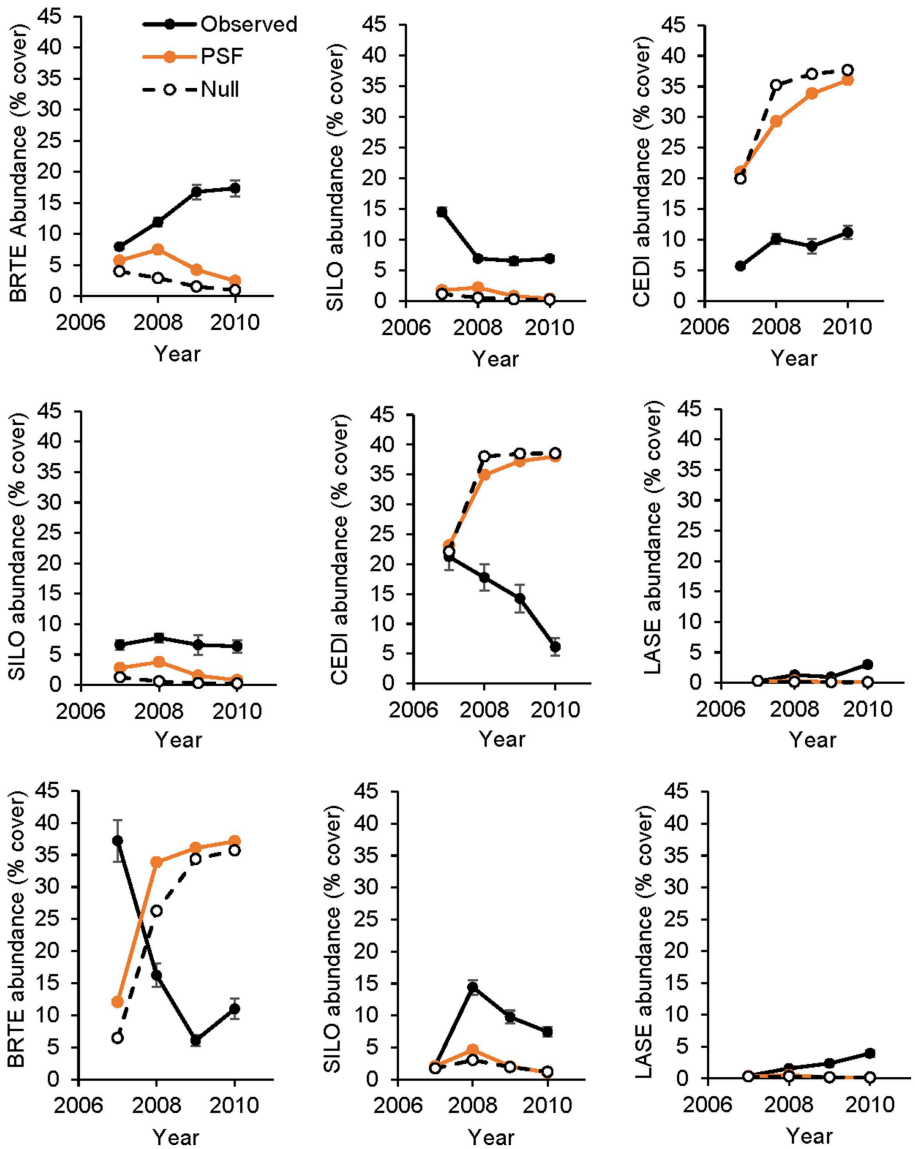

FIGURE 4 | Observed and predicted abundance of non-native plant species in three-species experimental plant communities over time. Species in a row are from the same three-species community. Model predictions were made either with plant-soil feedback effects (PSF) or without plant-soil feedback effects (Null). BRTE, *Bromus tectorum*; CEDI, *Centaurea diffusa*; SILO, *Syssymbrium loeselii*; LASE, *Lactuca serriola*.

et al., 2018; Chung et al., 2019; Mack et al., 2019). Results from this study demonstrate that PSF measured in the field can help predict plant abundance in the field.

A logistic growth plant competition model in which competition coefficients are equal to a value of one was used in this study. In this type of model, the plant with the fastest growth rate will dominate the plant community. Among native species, the Null model incorrectly predicted *K. cristata* dominance because *K. cristata* attained the greatest cover on control soils. In contrast, the PSF model correctly predicted less *K. cristata* growth than the Null model because *K. cristata* grew poorly on "self" soils (i.e., negative PSF). The PSF model also correctly predicted *P. spicata* dominance because *P. spicata* grew well on "self" soils (i.e., a positive PSF). Thus, both positive and negative PSF were important for improving Null model predictions of plant growth in communities. This result is important because it provides an example of PSF effects in the context of intrinsic growth differences among species (Lekberg et al., 2018).

Because they are difficult to execute, factorial PSF experiments are uncommon (Reinhart and Rinella, 2016; Mack et al., 2019; Teste et al., 2019). As a result, little data is available to determine whether or not factorial experiments are needed to understand PSF effects in plant communities (Kulmatiski, 2016; Reinhart and Rinella, 2016; Teste et al., 2019). Results from the factorial portion of this study provided an example where factorial PSF data were needed to correctly predict plant community composition.

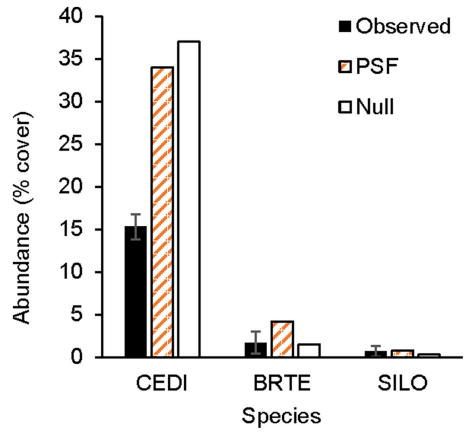

FIGURE 5 | Observed and predicted abundance of non-native plant species in a three-species experimental plant community grown for 3 years (2011–2013). Model predictions were made either with plant-soil feedback effects (PSF) or without plant-soil feedback effects (Null). Species codes listed in the **Figure 4**.

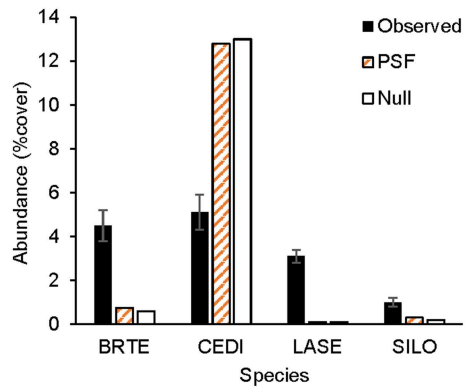

FIGURE 6 | Observed and predicted non-native plant abundance. Observed plant abundance was recorded in 25 ex-arable fields over 13 years (Kulmatiski and Beard, 2019). Model simulations of plant community growth for these four common non-native species were performed for a 10-year period either with plant-soil feedback data (PSF; i.e., with information on plant growth rates on different soil treatments) or with a null model (Null) which was parameterized only with plant growth rates on control soils. BRTE, *Bromus tectorum*; CEDI, *Centaurea diffusa*; SILO, *Syssymbrium loeselii*; LASE, *Lactuca serriola*.

K. cristata grew very poorly on *P. spicata* soils and well on *F. idahoensis* soils. As a result, *K. cristata* had a positive PSF with *P. spicata* soils and a negative PSF with *F. idahoensis* soils. These species-specific PSF values were critical for correct predictions of *K. cristata* and *P. spicata* cover. If *K. cristata* growth were predicted only from "self" and "control" soils, *K. cristata* would have been incorrectly predicted by a "self" vs. "other" PSF model to outcompete *P. spicata*.

While PSFs improved predictions of native plant abundance in communities, it is likely that other factors were also important to plant growth in this field experiment. It is possible, for example, that *K. cristata* may grow quickly in relatively high-resource monoculture plots, but that it is competitively suppressed in communities (Tsialtas et al., 2001; Fargione and Tilman, 2006; Lekberg et al., 2018). PSF effects and competitive suppression are not mutually exclusive, but it was not possible to determine the relative importance of these effects in this study. The fact that both the Null and PSF models overestimated *K. cristata* cover in experimental communities and in the field suggested that additional forms of negative density dependence may be needed to fully explain this species' abundance in native communities (Adler et al., 2007; Lekberg et al., 2018). Integration of PSF effects with other plant growth factors in conceptual and mathematical models has been widely recommended, but remains underdeveloped (van der Putten et al., 2013; Lekberg et al., 2018).

PSFs did not improve predictions for all species. Null and PSF model predictions of non-native cover did not differ and predictions from both models were poor relative to PSF model predictions of native cover. Null model predictions of non-native cover were poor because *B. tectorum* grew better in community plots than could be predicted from monoculture plots. Additionally, *C. diffusa* grew more poorly in community plots than could be predicted from monoculture plots. PSFs were either not large enough or not in the correct "direction" to improve Null model predictions. For example, because *B. tectorum* demonstrated a large positive PSF, the PSF model incorrectly predicted less *B. tectorum* cover in communities than the Null model.

Null and PSF model predictions of landscape-level non-native species cover were better than predictions of experimental species cover. Both models correctly predicted that *C. diffusa* would dominate and *B. tectorum* would be a subdominant with lower abundances of *L. serriola* and *S. loeselii.* A potential explanation for why landscape-level predictions of non-native cover where better than experimental-plot-level predictions is that landscape cover was determined from 25 fields that had been abandoned from agriculture between 1950 and 1999 (Kulmatiski and Beard, 2019). Averaging cover across these fields removes the large inter-annual variation seen in cover of the short-lived plants that dominate in these communities (Kulmatiski and Beard, 2019). For example, in experimental communities of native plants, rank-order abundance remained largely the same across 4 years of observations, while in non-native communities, rank-order abundance changed over time (**Appendix Figure 1**). It is likely that it is more difficult to predict the volatile dynamics of fast-growing, short-lived non-native species (Fukami and Nakajima, 2013; Suding et al., 2013).

A large body of research has suggested that PSF may help explain non-native and range-expanding plant success (Reinhart and Callaway, 2006; Eppstein and Molofsky, 2007; Suding et al., 2013). This study found that PSF improved predictions of native but not non-native plant community composition. These results are not mutually exclusive. Two previous studies in the study system explicitly examined the effects of native soils on non-native plants and non-native soils on native plants and found that PSFs can explain the presence of two alternate-state communities on the landscape (Kulmatiski et al., 2006; Kulmatiski, 2018). The current study focused on dynamics within native communities and within non-native plant communities. Here, it was found

that PSFs were important in explaining growth within native plant communities but not within non-native plant communities.

Understanding the context under which PSF are important to plant community development remains a critical direction for future research (Bailey and Schweitzer, 2016; Smith-Ramesh and Reynolds, 2017; Fry et al., 2018). In addition to distinguishing the role of PSF in native vs. non-native plant communities, the context of study site and experimental design used in this study likely affected results. Relatively little is known about how PSF varies among ecosystems, but a literature review has suggested that they may be larger in the type of semi-arid system used in this study relative to more mesic (e.g., forested) systems (Kulmatiski et al., 2008). The duration of this study is unusual for PSF experiments and may also have been important (Kardol et al., 2013; Bezemer et al., 2018). It is possible that longer PSF experiments create PSF values that are more relevant to plant growth in communities due to co-selection or co-evolution in plant-microbial interactions that may take years to develop (Zuppinger-Dingley et al., 2016; van Moorsel et al., 2018). Determining how PSF develop over time is important for measuring and modeling plant communities but has rarely been addressed (Kardol et al., 2013; Bailey and Schweitzer, 2016; van Moorsel et al., 2018).

There is both strong evidence that plants change soils in ways that affect subsequent plant growth and also conceptual and mathematical evidence that these PSFs can maintain species diversity (Adler et al., 2007; Revilla et al., 2013; van der Putten et al., 2013). There are also a handful of studies that have found correlations between PSF and species abundance (Klironomos, 2002; Heinze et al., 2015), and a few studies have used observed PSF values in simulation models to explore potential PSF effects on plant coexistence and abundance (Chung and Rudgers, 2016; Bennett et al., 2017; Teste et al., 2017; Eppinga et al., 2018). Very few studies have attempted to explicitly predict plant growth using PSF data and simulation models (Mangan et al., 2010; Kulmatiski et al., 2016). This link is important because it is reasonable to expect that PSFs measured in monocultures may not reflect plant-plant or plant-soil-plant interactions in plant communities (Eisenhauer et al., 2012; Latz et al., 2012; Crawford and Knight, 2017; Lekberg et al., 2018). This study, therefore, provides an important link between classic PSF two-phase experimental data and plant growth in communities in the field (Poorter et al., 2016), and revealed that PSFs were critical for understanding native plant abundance in plant communities in the field.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AK performed all aspects of this research.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00326/full#supplementary-material

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Site Soil-Fertility and Light Availability Influence Plant-Soil Feedback

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Negative plant-soil feedback (PSF), where plant performance is reduced in soils conditioned by conspecifics, is widely documented in plant communities. However, the strength and sometimes direction of PSF can vary widely, presumably not only due to the plant species within the community but also to environmental context. We hypothesized that soil fertility and light availability influence the direction and strength of plant-soil feedback experienced by tree seedlings. We conducted a 10-week greenhouse experiment and assessed survivorship of Northern red oak (*Quercus rubra*) and black cherry (*Prunus serotina*) in low (∼1% full sun) vs. high (∼18% full sun) light availability and in non-sterile vs. sterile soils collected under the canopy of conspecific vs. heterospecific adult trees at five sites that vary in nutrient availability in Manistee National Forest, Michigan, USA. *Q. rubra* seedlings experienced neutral plant-soil feedback regardless of light level or site as only one seedling died during the course of the experiment. *Prunus serotina* seedlings experienced microbe-mediated negative PSF in low fertility sites but positive feedback at high fertility sites, but these feedbacks occurred only under high light availability. Consistent with these results, microbe-mediated negative PSF increased with soil Fe^{3+} and C:N ratios and decreased with NH4+. Our results demonstrate the important role of environmental context, specifically light and soil nutrient availability, on the magnitude and direction of conspecific plant soil feedback, particularly in *P. serotina*. Since *Q. rubra* experienced neutral microbial PSF, *P. serotina* has a relative disadvantage to *Q. rubra* under lower site fertility, but a relative advantage under higher site fertility. These results are consistent with these species relative abundances in the field and thus PSF could be an important driver of plant community dynamics.

Keywords: C:N, light availability, NH^{4+}, plant-soil feedback, *Prunus serotina*, *Quercus rubra*, soil fertility, temperate forest

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INTRODUCTION

A long-standing challenge in ecology is to identify factors regulating plant abundance, co-existence, and community composition. For plant communities, the seedling-establishment phase is a major demographic bottleneck, and thus a critical stage for future community dynamics (Gurevitch et al., 2006). There has been a vast array of hypotheses proposed for how dominance by the most competitive species can be precluded (Palmer, 1994). Janzen (1970) and Connell (1971) proposed that host specific natural enemies could maintain high tree diversity by reducing seed

and/or seedling survivorship near conspecific adults and/or at high conspecific densities. Such NDD seedling mortality would favor establishment of heterospecific individuals under an adult tree, and thus promote species coexistence (Mangan et al., 2010; Alvarez-Loayza and Terborgh, 2011). Widespread patterns of plant demography consistent with NDD have been identified (e.g., HilleRisLambers et al., 2002; Comita et al., 2010; Swamy et al., 2011; Johnson et al., 2012). Interactions between adult plants and juveniles that are mediated by root-associated fungi and other symbiotic microorganisms ("biotic-mediated plant soil feedback") (Bever et al., 2012; Terborgh, 2012) is likely a prime mechanism creating these NDD patterns since plant-soil feedback can reduce seedling survival near conspecific adults while favoring heterospecific seedlings (Mangan et al., 2010; McCarthy-Neumann and Kobe, 2010; Alvarez-Loayza and Terborgh, 2011; McCarthy-Neumann and Ibáñez, 2012).

Plant-soil feedback (PSF) is created when a plant's presence alters soil conditions (biotic, physical, and/or chemical), which in turn affects its own and other plants' performance (Bever, 2003). A key component for these feedbacks to influence community dynamics is that there are species-specific responses to altered soil conditions; for biotic-mediated feedbacks this would result from plants culturing divergent microbial communities surrounding their root systems that exhibit some degree of host-preference (Benítez et al., 2013). Comparisons between the performance of plants in soil conditioned by conspecifics vs. heterospecifics assess the specificity of PSF effects (Brinkman et al., 2010), and have relevance to understanding cases of conspecific inhibition and facilitation due to forms of density-, distance-, and/or frequency-dependent effects. Negative PSF may stabilize species coexistence if a plant modifies its soil biota in a way that inhibits conspecific more than heterospecific recruits, thereby preventing that plant species from dominating the community (Bever et al., 1997). Positive PSF occurs when soil influenced by conspecifics has positive effects (Dickie et al., 2014; Bennett et al., 2017) and may contribute to either clumped distributions or monodominance.

Plant-soil feedback is often found to be widespread and often driven by soil biota in studies with many species (Klironomos, 2002; Petermann et al., 2008; Bennett et al., 2017), and in meta-analysis of published research (Kulmatiski et al., 2008; Crawford et al., 2019). However, there is strong variation in the strength of PSF. For instance, several studies have found strong positive correlation between local adult abundance for a species and the strength of PSF on seedling performance (Klironomos, 2002; Mangan et al., 2010; MacDougall et al., 2011; McCarthy-Neumann and Ibáñez, 2013; Xu et al., 2015; LaManna et al., 2016). However, other empirical studies report locally common species have greater negative PSF (Liu et al., 2015; Zhu et al., 2015), or that PSFs are pervasive across all species regardless of local abundance (Petermann et al., 2008). Variation in direction and strength of PSF has also been linked to mycorrhizal symbiont type, with arbuscular mycorrhizal fungi associated species experiencing negative and ectomycorrhizal fungi species positive feedback (Bennett et al., 2017). A recent meta-analysis found greater negative PSF when species shared the same mycorrhizal guild, were phylogenetically distant and native (Crawford et al., 2019). Species functional traits can also be a strong predictor with shade intolerant tree species experiencing stronger negative feedback than shade tolerant species (McCarthy-Neumann and Kobe, 2008; McCarthy-Neumann and Ibáñez, 2013).

The environmental context in which plants are establishing and growing likely impacts the occurrence and strength of these feedback as well. This influence can act through changes to the soil microbial community (e.g., abundance or composition) or plant response to soil micro-organisms under those environmental conditions. For instance, negative PSF can be stronger or occur only in low light conditions (McCarthy-Neumann and Kobe, 2010; McCarthy-Neumann and Ibáñez, 2013; but see Smith and Reynolds, 2015 which found the opposite pattern). Stronger negative PSF in low light may be due to either increased abundance of soil pathogens (Reinhart et al., 2010; Hersh et al., 2012), mycorrhizal fungi acting parasitically (Ibáñez and McCarthy-Neumann, 2016), and/or a reduction in the plant's physical (Augspurger, 1990) or chemical (Ichihara and Yamaji, 2009) defenses, or ability to re-grow lost tissue (Myers and Kitajima, 2007; Kobe et al., 2010) in low light.

Soil fertility may also influence the soil microbial community or plant response to soil micro-organisms. For instance, soil pathogens are more abundant under high soil resources such as with increased soil moisture (Hersh et al., 2012), and mycorrhizal fungi are beneficial under low-soil resources, but detrimental at high soil resources (Johnson et al., 1997; Grman et al., 2012). These soil biotic responses suggest increased negative PSF as soil fertility increases (as postulated by Revillini et al., 2016; Lekberg et al., 2018). However, in greenhouse experiments, fertilization reduced negative PSF (Petermann et al., 2008) or neutralized either positive or negative PSF (in 't Zandt et al., 2019). As outlined by Smith-Ramesh and Reynolds (2017), negative PSF may be expected to weaken due to a decline in nutrient stress and strengthened physical or chemical defenses (especially those that are N-based) against soil pathogens, and/or ability to re-grow lost tissue from non-structural carbohydrates.

The recent recognition of the importance of environmental context for PSFs [e.g., light and nutrients as described above, herbivory (Bezemer et al., 2013; Heinze and Joshi, 2018) or competition (Casper and Castelli, 2007; Bezemer et al., 2018; Lekberg et al., 2018)], will enable more robust predictions of how PSF regulates plant abundance, promotes co-existence, alters community composition, and impacts ecosystem function. Specifically, understanding the role of light availability on PSF will help us better understand seedling dynamics from small gap formation to large stand disturbance. Understanding the role of soil fertility on PSF will help us better predict how global environmental change (e.g., precipitation and nutrient enrichment) may influence plant coexistence and ecosystem function.

To test effects of environmental context on PSF, we conducted a greenhouse plant-soil feedback experiment with black cherry and red oak seedlings grown in soils from five forest sites (which vary in species local abundance, productivity and fertility), and at low and high light. We asked: (1) Does the direction and strength of biotic-mediated PSF vary across multiple sites? (2) Which factors at the site level (e.g., species local abundance,

productivity, and fertility) best correlate with species PSF? (3) Does light environment interact with soil factors in determining the direction and strength of PSFs? We predicted that increasing soil fertility will decrease biotic-mediated negative PSF. We also predicted that increasing light will decrease biotic-mediated negative PSF or even shift the direction of biotic-mediated PSF toward a positive feedback. Both predictions stem from the assumption that robust plants under high resources are better able to survive negative PSF and to take advantage of positive PSF than stressed plants under low resources.

METHODS

To assess context dependence for the existence and strength of PSF, we assessed survivorship for *Prunus serotina* and *Quercus rubra* seedlings to non-sterile vs. sterile soils collected under the canopy of conspecific vs. heterospecific adult trees at 5 different forest sites that range in species composition, productivity and soil fertility (**Tables 1**, **2**). The seedlings were grown at low and high light in a greenhouse experiment for 10-weeks starting in April 2013.

Soil Sources and Adult Tree Density Measures

In the first week of April (2013), we collected soil (top 15-cm) within 1 m from the bole of two mature adults for each of our study species at five different mixed hardwood stands located in Manistee National Forest in the lower peninsula of Michigan, USA. This is a region with well-defined associations among glacial landforms, soil fertility, and forest community composition. All sites were within an area of 960 km^2 (average distance 15.76 km) and are expected to have very similar climate. Mapped stands were established in 1999 at each of these sites and the most recent census (e.g., species identification, location, measurement of size, and classification of health) of all trees $\geq$10 cm diameter occurred in Fall of 2011.

To minimize the potential for multiple tree species influencing the soil, we sampled under trees that were at least two crown diameters away from adults of other study species. We diced roots and sifted soil through a 1-cm mesh sieve. To control for individual tree effects, soil from each adult was kept separate throughout the course of the experiment (e.g., Reinhart and Rinella, 2016). We sterilized half of the field and all of the potting soil (Fafard Mix #2, Conrad Fafard Inc., Agawam, MA USA) by gamma irradiation prior to the start of the experiment ($\sim$40 KGray; Sterigenics International, Inc., Schaumburg, IL, USA). Sterilization of soil biota through gamma irradiation appeared to be effective as we found no mycorrhizal fungal colonization in a sub-sample of harvested seedling in the sterile treatments.

Planting Methods

Prunus serotina seeds were collected from southeastern Michigan forests, and *Q. rubra* seeds were purchased from Sheffield's Seed Co. (Locke, NY, USA). To minimize diseases from non-experimental soil sources (e.g., from the collection sites), seeds were surface sterilized (0.6% NaOCl solution) prior to stratification and again prior to germinating in perlite. Seeds with newly emerged radicles were weighed and then planted into 6.4-cm diameter $\times$ 25-cm depth pots and grown in greenhouse facilities at Michigan State University's Tree Research Center.

The experiment consisted of a total of 6 replicate seedling pots per experimental treatment (e.g., soil collected from 2 individual adult trees $\times$ 3 replicate pots). Experimental treatments were site (5 sites spanning soil fertility) $\times$ soil source [conspecific vs. heterospecific soil] x soil status [sterile vs. non-sterile soil] x light availability [1 vs. 18% full sun]). Soil status consisted of a "non-sterile treatment," a 1:1:2 mixture of non-sterile field soil, sterile field soil, and sterile potting soil, and a "sterile treatment," a 1:1 mixture of sterile field soil and sterile potting soil. The sterile field soil was always fully composed of either conspecific or heterospecific soil depending upon the soil source treatment. The light availability treatment mimicked conditions from understory (1% full sun) to moderately sized tree-fall gaps (18% full sun) encountered in the Michigan forests at which the soil for this experiment was collected (Schreeg et al., 2005). The lowlight treatment was created by covering benches with an inner layer of black shade cloth and an outer layer of reflective knitted poly-aluminum shade cloth. Light availability as a percentage of full sun levels was confirmed at each bench by calculating the percentage of sunlight measured in the open and with paired photosynthetic active radiation (PAR) measurements at each bench in the greenhouse. PAR was measured on a uniformly overcast day with a LI-COR250A quantum sensor (LI-COR, Lincoln, NE, USA).

Individual pots were set up on two different benches (one low and high light), where all combinations of site, soil source, and soil treatments were represented, and were watered ($\sim$50 ml of deionized water) by hand every 3 days for 10 weeks. Emergence and survival were recorded twice weekly, and date of death was assigned as the first census with total leaf and/or stem tissue necrosis.

We used cation and anion PRS^{TM} probes that contain 17.5 cm^2 ion-exchange resins (Western Ag Innovations, Saskatoon, Saskatchewan) to assess the effect of site, soil source, sterilization and light on nutrient supply rates in the experiment (**Table 3**). We measured nutrient supply rates from 40 samples in high light (5 sites $\times$ 2 soil source $\times$ 2 soil status $\times$ 2 replicates) and 20 samples in low light [5 sites $\times$ 2 soil source x non-sterile $\times$ 2 replicates). We did not assess the effect of species of seedling growing in the soil on nutrient supply rates so replicates are derived from 4 *P. serotina* and 4 *Q. rubra* seedlings per treatment combination and in each pot paired cation and anion probes were inserted into the pots starting at week 2 after seedling planting and ending at week 5. Upon removal, the probes were washed with de-ionized water and sent to Western Ag Innovations for analysis of available nitrogen (N, sum of NO_3^- and NH^{4+}), Ca^{2+}, Mg^{2+}, K^+, $H_2PO_4^-$, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, $B(OH)_4$ $^{3+}$, SO_4^-, Pb^{2+}, and Al^{3+}.

Analytical Approach

We analyzed the survival data of seedlings grown in conspecific vs. heterospecific soil from five different forest stands, under sterile vs. non-sterile, and at low and high light levels. Data for each seedling i and each time t, N_{it}, were coded as 0 until the

TABLE 1 | Community productivity, structure and composition attributes for forest sites where soil was collected for the plant-soil feedback greenhouse experiment.

Site[a]	ANPP[bc]	Basal area[bc]	Density[bc]	Prse RA[bd]	Quru RA[bd]	Age[e]	Location
I	6.53	30.23	486	0.2	19	114	44°16′N, 85°53′W
mI	7.50	36.46	623	0.2	29	93	44°12′N, 85°48′W
pM	8.13	33.1	568	1	39	82	44°11′N, 85°45′W
M_1	9.22	33.59	506	3	5	95	44°13′N, 85°45′W
M_2	8.39	39.97	642	2	5	87	44°15′N, 85°45′W

Data modified from Baribault et al. (2010).
[a] *Landform codes: I, ice contact; mI, mesic ice contact; pM, poor moraine; M, Moraine.*
[b] *Attributes that are significantly different between sites.*
[c] *ANPP (Mg ha^{-1}) was calculated between 1999 and 2007. Basal area units are in m^2 ha^{-1} and Density in (stems ha^{-1}).*
[d] *Relative abundance (%) determined from 2011 mapped stand census. Species code: Prse, Prunus serotina; Quru, Quercus rubra.*
[e] *Stand age (years) should be interpreted as elapsed time since significant, stand-replacing disturbance.*

TABLE 2 | Mean soil fertility attributes for forest sites where soil was collected for the plant-soil feedback greenhouse experiment.

Site[a]	Soil water	Texture (silt + clay)	NO_3^-	NH_4^+	Nitrification	Total N	Total C	C:N	Ca^{2+}
I	0.107	12.3 ± 2.4	0.60 ± 0.10	1.57 ± 0.85	0.10 ± 0.01	0.05 ± 0.01	1.72 ± 0.25	32.49	0.43 ± 2.19
mI	0.128	12.7 ± 2.1	0.50 ± 0.16	1.07 ± 0.48	0.11 ± 0.01	0.06 ± 0.04	1.64 ± 0.73	25.59	0.69 ± 2.70
pM	0.100	8.8 ± 0.8	0.54 ± 0.04	2.94 ± 0.44	0.17 ± 0.05	0.09 ± 0.02	1.77 ± 0.08	20.86	0.73 ± 3.55
M_1	0.137	11.5 ± 5.0	2.28 ± 0.81	2.54 ± 0.98	0.79 ± 0.22	0.17 ± 0.04	2.81 ± 0.81	16.13	5.45 ± 2.76
M_2	0.113	11.5 ± 1.8	0.82 ± 0.32	2.66 ± 1.34	0.43 ± 0.20	0.12 ± 0.03	1.47 ± 0.84	12.14	4.51 ± 1.52

Data modified from Baribault et al. (2010). Only soil fertility attributes that were significantly different between these forests sites are shown.
[a] *Landform codes: I, ice contact; mI, mesic ice contact; pM, poor moraine; M, Moraine.*
Soil fertility attributes measurement units are in the following: soil water volume (m^3 m^{-3}), soil texture %, NO_3^- and NH_4^+ (mg kg^{-1}), Nitrification (mg kg^{-1} day^{-1}), Total N and C (%), and Ca^{2+} ($cmol_{charge-}$ kg^{-1} soil).

seedling was found dead, $N_{it} = 1$. We used a count process (Poisson distribution) to model the number of failures, in this case death of a seedling at a particular time (N_{it}), we then estimated the probability of mortality in a Cox survival model that included an intrinsic rate of mortality and an extrinsic risk (Andersen and Gil, 1982). We modeled the likelihood as:

$$N_{it} \sim Poisson\,(\lambda_{it})$$

and process model:

$$\lambda_{it} = h_t e^{(\mu_t)}$$

where parameter λ is then estimated as a function of the intrinsic rate of mortality (age or time dependent mortality), or hazard h, and the extrinsic risk of mortality or risk μ (mortality due to external factors like site of soil collection, light level, etc.):

Parameters in the model were then estimated at the species level, following a Bayesian approach that allowed us to consider the different sources of uncertainty associated with the data (Clark, 2005). The hazard was estimated for each time step, h_t, from a gamma distribution with non-informative parameter values, $h_t \sim Gamma$ (0.01, 0.01). The risk, μ_{it}, was estimated as a function of the covariates included in the analysis, $\mu_i = X_i B$. X_i is the matrix of covariates associated to each seedling. B is the vector of fixed effect coefficients associated to each covariate. These coefficients were estimated from normal distributions with non-informative parameter values, $B_k \sim Normal$ (0, 10,000). Covariates included site of soil collection, soil source (conspecific vs. heterospecific), soil sterilization (non-sterile vs. sterile), light level (low vs. high), and standardized seed size. We did not assess effect of individual tree for each species in this analysis as we were interested in the overall effect of conspecific vs. a heterospecific cultured soil on seedling survival and we did not have large enough replicate size to robustly assess any variation that might exist between individual trees that had cultured the soil.

Models were run only for *P. serotina* since only one *Q. rubra* seedling died during the course of the experiment. Models were run in OpenBUGS 1.4 (Thomas et al., 2006). Simulations (three chains) were run until convergence of the parameters was ensured (∼50,000 iterations) and then run for another 25,000 iterations from which posterior parameter values and predicted survival were estimated. Using model parameters, means, variances and covariances, we estimated survival (**Tables S1**, **S2**), and used these predicted survival values to explicitly assess whether there were differences in how species responded to soil site (five forest stands varying in site fertility), soil source (conspecific vs. heterospecific) and soil treatments (non-sterile vs. sterile) at low *vs.* high light levels (**Tables S3**, **S4**). We also assessed how species responded to microbial mediated PSF [measured by subtracting the PSF effect (survival in conspecific vs. heterospecific soil) on predicted survival in sterile soil from the PSF effect on predicted survival in non-sterile soil] across the five sites

TABLE 3 | Soil nutrient supply rates (μg ion/PRS® Probe/3 week) in the experimental pots across forest site treatments.

Source of variation[b]	Total N	Mg^{2+}	K^{+}	$H_2PO_4^-$	Fe^{3+}	Mn^{2+}	Al^{3+}
Site							
I	214.2^{a}	884.6^{b}	199.3^{ab}	11.0^{ab}	47.1^{b}	33.3^{a}	54.1^{ab}
mI	288.6^{ab}	858.1^{b}	258.1^{b}	9.1^{ab}	33.7^{ab}	19.0^{a}	64.2^{b}
pM	397.8^{b}	822.7^{ab}	163.5^{ab}	11.6^{ab}	32.5^{ab}	62.3^{b}	50.2^{ab}
M_1	560.5^{c}	697.4^{a}	112.8^{a}	7.0^{a}	24.8^{a}	26.6^{a}	43.6^{a}
M_2	456.9^{b}	746.9^{ab}	148.3^{ab}	23.6^{b}	21.7^{a}	25.2^{a}	42.7^{a}
SE	36.7	29.4	26.4	3.0	3.7	5.1	3.9
***P*(site)**	<0.001	0.04	0.01	0.01	0.02	0.00	0.01

Soils are from non-sterile treatments, combining soil (conspecific vs. heterospecific adults) at high light. Means, SE and P-values of an ANOVA test on the effects of site are shown. Within columns, means followed by the same letter are not significantly different ($P < 0.05$) based on a Tukey HSD test[a]. Only attributes that were significantly different between these forests sites are shown.

[a] Tests were performed using the Type III sum of squares from SPSS version 24. 20 PRS™ probe samples in model.

[b] Landform codes: I, ice contact; mI, mesic ice contact; pM, poor moraine; M, Moraine.

and at low vs. high light levels. Differences that did not include zero in their 95% credible intervals were considered statistically significant.

We determined whether nutrient supply rates within the experiment differed between site, soil source, soil status and light availability using ANOVA (SPSS version 24.0; SPSS, Chicago, Illinois, USA). Whether soil was from conspecific vs. heterospecific adults had little impact on nutrient supply rates (**Table S5**), and there was no significant difference in any nutrient supply rates between low vs. high light in non-sterile soil treatments (we did not have samples to compare light treatment effects in sterile soil; **Table S6**). What we consider to be microbial mediated PSF, difference between predicted PSF effects on survival in sterile soil vs. non-sterile soil, could be due to both the micro-organisms in the non-sterile soil as well as some other abiotic factor that was altered during sterilization (e.g., Troelstra et al., 2001). However, sterilization had only some relatively minor impact on nutrient supply rates in the experiment, resulting in greater supply rate for $H_2PO_4^-$, Mn^{2+}, Zn^{2+}, and Pb^{2+} (**Table S7**), and there were no sign of nutrient deficiency in seedlings planted in the sterile treatments. We then used multiple step-wise regressions (SPSS) to determine which site factors at the stand level (e.g., species composition, productivity and fertility, **Tables 1**, **2**) and at the experimental level (e.g., nutrient supply rates from the PRS probes, **Table 3**) correlate with PSF in both low and high light environments. Only factors (**Tables 1**–**3**) that were significantly different among the sites were included in the analysis.

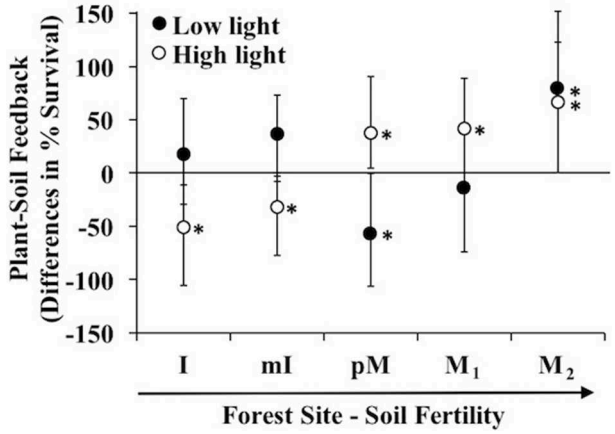

FIGURE 1 | Differences in predicted survival due to microbe mediated PSF (survival in conspecific vs. heterospecific non-sterile soil—survival in conspecific vs. heterospecific sterile soil) in a greenhouse experiment of *Prunus serotina* seedlings across 5 sites that range in soil fertility and community composition (**Tables 1**, **2**) conducted under low and high light. Sites are in rank order of soil fertility from left (low) to right. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant. *$P < 0.05$.

RESULTS

Environmental Context (i.e., Site Fertility and Light Availability) Alters the Occurrence and Direction of PSFs

In general as soil fertility increased, biotic-mediated plant soil feedbacks in high light went from negative to positive; while effects were less pronounced in low light, there were consistent results with a negative biotic-mediated PSF at the intermediate fertility site and a positive PSF at the highest fertility site (lowest C:N ratio). In low fertility sites at high light, *Prunus serotina* seedlings experienced a microbe-mediated negative feedback (i.e., the negative effect of conspecific vs. heterospecific soil was greater in non-sterile than sterile soil). In contrast in high fertility sites at high light, the feedback flipped to positive (**Figure 1**). In low light, seedlings showed a negative feedback at a moderate fertility site (poor Moraine) and positive feedback at higher fertility (Moraine) (**Figure 1**).

The above microbe-mediated PSF values remove abiotic effects of the soil (survival response to sterile soils cultured by conspecific vs. heterospecific adult trees). In the non-sterile soil treatment, where biotic and abiotic effects both occurred, *Prunus*

serotina seedlings grown at high light experienced negative PSF at low fertility sites, and positive PSF at the intermediate and one of the high fertility sites (**Figure 2A**). In contrast, under abiotic effects only (results from the sterile soil), *P. serotina* seedlings grown at high light in low fertility sites experienced positive PSF, but negative PSF at the intermediate and the other high fertility site (M_2) (**Figure 2B**). Thus, under high light, the negative microbe-mediated PSF in low fertility sites and positive microbe-mediated PSF at high fertility sites (**Figure 1**) is enhanced when abiotic-mediated PSF are explicitly considered and removed in the analysis. In particular, at one of the moraine sites (M_2) there was not a significant PSF when comparing just non-sterile soils (**Figure 2A**). The increased survival in conspecific soils due to soil biota was only apparent at this site (**Figure 1**) when the large negative abiotic effect of conspecific soil was removed (**Figure 2B**). In low light, only seedlings at the intermediate fertility site (pM) experienced negative PSF in the non-sterile treatment (**Figure 2A**), and abiotic-mediated PSF occurred at one of the most fertile sites (M_1). Lastly, only at the intermediate fertility site (pM) was there a significant difference in direction of PSF with negative PSF at low light and positive PSF at high light, but this only occurred when soil micro-organisms were included in the feedback (**Figures 1**, **2A**).

Negative microbe-mediated feedback could derive solely from detrimental micro-organisms in conspecific soil, beneficial micro-organisms in heterospecific soil or some combination of the two. At high light, *P. serotina* seedlings experienced negative microbe-mediated PSF in low fertility sites (**Figure 1**) due to detrimental micro-organisms in conspecific soil (**Figure 2C**), and beneficial micro-organisms in heterospecific soils (**Figure 2D**). The positive PSF at higher fertility sites for seedlings grown in high light environments (**Figure 1**) was due to beneficial micro-organisms in conspecific soil at each of the sites (**Figure 2C**). In addition, at the intermediate fertility site (pM) micro-organisms in the heterospecific soil were also detrimental (**Figure 2D**). The light environment affected *P. serotina's* seedling survival response to soil microbes from conspecific soil with a beneficial response in high light and a detrimental response in low light at the intermediate fertility site (pM) (**Figure 2C**). For all other sites and for all heterospecific soils, the light level did not influence how seedlings responded to soil microbes (**Figures 2C,D**).

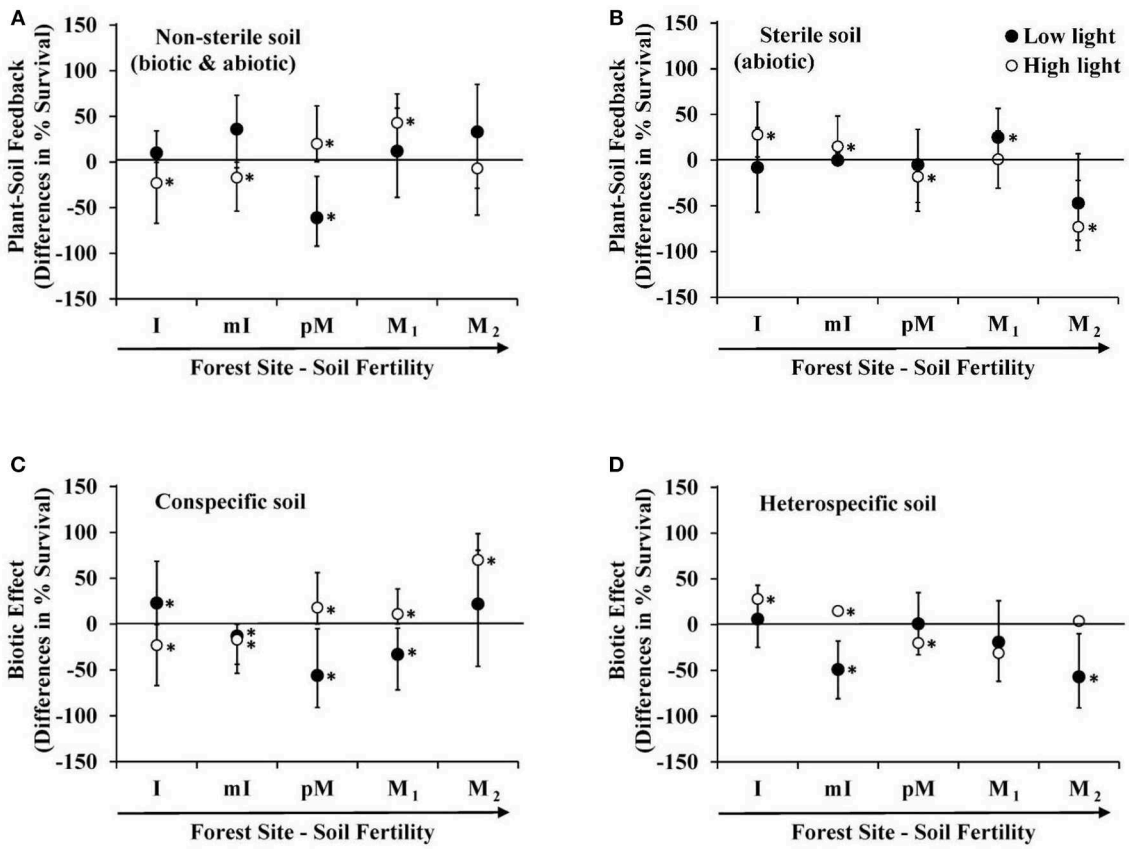

FIGURE 2 | Differences in predicted survival due to: **(A)** microbe + abiotic mediated PSF (survival in conspecific vs. heterospecific non-sterile soil), **(B)** abiotic mediated PSF (survival in conspecific vs. heterospecific sterile soil), **(C)** biotic effect in conspecific soil (survival in non-sterile vs. sterile conspecific soil), and **(D)** biotic effect in heterospecific soil (survival in non-sterile vs. sterile heterospecific soil) in a greenhouse experiment of *Prunus serotina* seedlings across 5 sites that range in soil fertility and community composition (**Tables 1**, **2**) conducted under low and high light. Sites are in rank order of soil fertility from left (low) to right. Data are means with 95% credible intervals; credible intervals that do not overlap with the zero line are statistically significant. *$P < 0.05$.

C:N, NH^{4+}, and Fe^{3+} Are Correlated With the Occurrence and Direction of PSF, but Only at High Light

A variety of community attributes (**Table 1**), and soil resource conditions (**Tables 2**, **3**) significantly varied across sites. Mean stand level soil C:N and NH^{4+} at the sites where soil was collected best predicted microbe-mediated PSF at high light [$F_{(2,4)} = 468.8$, $p = 0.002$, $R^2 = 0.99$]; sites with higher C:N ratios and lower NH^{4+} experienced more negative PSF. Similarly, microbe-mediated negative PSF at high light strengthened (became more negative) as average Fe^{3+} increased [$F_{(1,3)} = 11.2$, $p = 0.04$ with an R^2 of 0.76]. No site or experimental factors correlated with predicted microbe-mediated negative PSF in low light.

Age and Seed Size Influenced Seedling Survival

While soil fertility and light strongly influenced plant soil feedback over the entire experiment, there were multiple peaks (weeks 4, 6, and 8) of relatively higher seedling mortality (**Figure S1**). Seed weight significantly affected survival with smaller seeded individuals more likely to die (**Table S1**).

DISCUSSION

While light and soil fertility interact to influence seedling survivorship and growth (e.g., Kobe, 1996; Peltzer and Wardle, 2016), light and soil fertility also could influence PSF which in turn has an effect on seedling performance. We found that the direction of microbe-mediated PSF varied across sites with highly negative PSF in low fertility sites, but highly positive PSF in higher fertility sites for *P. serotina* seedlings. High C:N and Fe^{3+}availability and low NH^{4+} in soils were the factors associated with strong negative PSF. This correlation of PSF going from highly negative to highly positive as soil fertility increased only occurred in high light conditions. In low light, results were idiosyncratic with seedlings experiencing negative PSF (vs. positive PSF in high light) at the intermediate fertility site to positive PSF (found in both low and high light) at the site with the lowest C:N. These findings suggest that PSF is a widespread, important factor in *P. serotina* seedling recruitment, but its effects are dynamic and can be altered by the soil fertility of the site and the light availability that germinating seedlings experience. Seedling survival in soils cultured by conspecific adults can result in widely diverging outcomes (i.e., from highly detrimental, neutral to highly beneficial) depending upon the local environmental context.

Moreover, these results suggest that the plasticity of microbe-mediated PSF in response to environmental context can shift relative advantages to different species, thereby contributing to species coexistence. In the present study, *Prunus serotina* has a relative disadvantage to *Q. rubra* under low fertility but a relative advantage under high fertility. Of course, numerous other factors, such as direct resource effects and herbivory as well as abiotic feedbacks, also will influence growth and survival and the outcome of which species "wins" under a given set of environmental conditions. Nevertheless, because *Q rubra* had a neutral PSF (same microbe-mediated PSF across all soil fertility levels), the microbial PSF-mediated advantage shifts toward *P. serotina*'s favor relative to *Q. rubra*, as soil fertility increases. The feedbacks in the field would be an integration of both the biotic and abiotic mechanisms. However, the predictions based on microbe-mediated PSF from our greenhouse experiment are in fact consistent with the actual distribution of these species in the field (see **Table 1**).

Our findings are the first showing the importance of soil fertility on PSF in a tree species and are mostly consistent with the conceptual framework for PSF context dependence proposed by Smith-Ramesh and Reynolds (2017), and experimental work with herbaceous grassland species that shows stronger negative PSF in low fertility conditions (Petermann et al., 2008; in 't Zandt et al., 2019; but see Harrison and Bardgett, 2010 which found no effect of soil fertility on PSF). However, instead of the PSF becoming less negative (Petermann et al., 2008), absent (in 't Zandt et al., 2019), or weakly positive (Smith-Ramesh and Reynolds, 2017) we found PSF in higher fertility conditions to be equally strong, but in the opposite direction (i.e., seedling survival switching from worse to better in conspecific relative to heterospecific cultured soil). Although, pathogens are often found to be more abundant as nutrients, such as nitrogen, increase (Wei et al., 2018) their impact on seedling survival may be mitigated. For instance, based upon our results we hypothesize that plants with increased overall vigor (i.e., growing in favorable conditions with high levels of nutrients and light) are either better able to defend themselves or tolerate soil-borne pathogens in these environments. Increased survival in conspecific soil in these high resource conditions were due to soil biota. We speculate that mycorrhizal fungi were the biotic agents underlying this result and may have both protected seedlings against species-specific pathogens (Jung et al., 2012) as well as increased nutrient and water uptake.

We found that biotic-mediated PSF was correlated with soil C:N ratio; seedling survival due to soil biota decreased in conspecific soil as soil C:N increased. We are not aware of studies in non-agricultural systems that have examined the effects of organic matter and soil C:N ratio on disease dynamics. In agriculture and horticulture, however, it is common practice to add organic matter to soil to suppress damping-off (Bonanomi et al., 2018). While the importance of C:N ratio of organic matter for disease suppression is contested (Bonanomi et al., 2018), there is some evidence that organic matter additions with low C:N can ameliorate damping-off (Pane et al., 2011). Also, fertilizers with low C:N result in lowered mortality from *Pythium*-induced damping-off (Al-Azizi et al., 2013). Strong competition by soil microbes in low C:N soils may limit carbon availability to germinating fungal spores or invading hyphae, resulting in stagnation of fungal growth (Bonanomi et al., 2013).

Contrary to our prediction, only at one site (pM) did an increase in light shift biotic-mediated PSF from negative to positive. In fact, biotic-mediated PSF were prevalent across

all sites in high light conditions, although the direction of PSF varied based on site fertility. Our results on PSF and light interactions appears to be highly sensitive to timing of soil collection based on comparisons with our earlier work (McCarthy-Neumann and Kobe, 2010; McCarthy-Neumann and Ibáñez, 2012, 2013), when soil was collected during winter when the fungal community was more likely to be present as spores, which enables higher microbe survival during transport and cold storage (Reinhart et al., 2005), which found PSF prevalent in low light rather than high light (Appendix S8). Methodology for soil biota inoculum may also be important since the use of microbial wash (McCarthy-Neumann and Kobe, 2010), which likely reduced or eliminated some pathogenic microbes and excluded AMF spores vs. use of whole soil, resulted in the majority of PSF being abiotic-mediated (**Table S8**).

Plant-soil feedback is a complex process that incorporates the sum effect of biotic (natural enemies, mutualists, and self-DNA Mazzoleni et al., 2015), and abiotic (nutrient availability and secondary chemicals) interactions between a plant and the soil it is growing in (Bennett and Klrionomos, 2019). We found strong biotic-mediated PSF for *P. serotina* grown at high light that went from highly negative in low fertility, due to detrimental micro-organisms in conspecific vs. beneficial micro-organisms in heterospecific soils, to highly positive in high fertility sites, due to beneficial micro-organisms in conspecific soils. Interestingly, abiotic-mediated PSF was opposite in nature relative to biotic-mediated PSF with positive effects in low fertility and negative effects in high fertility sites. Thus, if we had solely focused on PSF derived from combined biotic and abiotic mechanisms (e.g., non-sterile treatment), we would have concluded that there was no PSF at the highest fertility site). It is unlikely that the abiotic effects we found in our study are due to nutrient availability since there was rarely any instances where nutrient levels were significantly different between conspecific and heterospecific soils at a site. Thus, secondary chemicals are a likely primary mechanism for abiotic-mediated PSF found in our study. Consistent with this, allelopathic chemicals in heterospecific soil could cause the positive abiotic-mediated PSF at low fertility sites. For example, condensed tannins in *Q. rubra* leaves increase in low N conditions (Kinney et al., 1997). In contrast, autotoxic chemicals in conspecific soils could be the cause for the negative abiotic-mediated PSF at high fertility sites, but what autotoxic chemicals may be operating for *P. serotina* in high fertility sites is unknown.

CONCLUSION

Our results illustrate interactions among PSF (mediated by both biotic and abiotic agents), soil fertility and irradiance. The overall effect of these environmental factors on seedlings appears to be complex, especially since these factors operate at both fine (e.g., irradiance) and coarse (e.g., soil fertility across sites) spatial scales. Our results demonstrate that PSF can be an important driver in plant community dynamics, but their effects could be either promote species coexistence when PSF is negative or promote monodominance when PSF is positive, which depends upon environmental context. A broad understanding of the environmental dependency of PSF is needed to both understand the dynamics of communities and to apply that understanding to ecological challenges such as invasive species management, native species restoration, and climate change adaptation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

SM-N performed all data collection, statistical analysis, and manuscript preparation. RK provided greenhouse and laboratory resources, extra student help, and feedback on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00383/full#supplementary-material

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The Role of Plant Litter in Driving Plant-Soil Feedbacks

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Most studies focusing on plant-soil feedbacks (PSFs) have considered direct interactions between plants, abiotic conditions (e. g., soil nutrients) and rhizosphere communities (e.g., pathogens, mutualists). However, few studies have addressed the role of indirect interactions mediated by plant litter inputs. This is problematic because it has left a major gap in our understanding of PSFs in natural ecosystems, where plant litter is a key component of feedback effects. Here, we propose a new conceptual framework that integrates rhizosphere- and litter-mediated PSF effects. Our framework provides insights into the relative contribution of direct effects mediated by interactions between plants and soil rhizosphere organisms, and indirect effects between plants and decomposer organisms mediated by plant root and shoot litter. We distinguish between three pathways through which senesced root and shoot litter may influence PSFs. Specifically, we examine: (1) physical effects of litter (layer) traits on seed germination, soil structure, and plant growth; (2) chemical effects of litter on concentrations of soil nutrients and secondary metabolites (e.g., allelopathic chemicals); and (3) biotic effects of saprotrophic soil communities that can perform different functional roles in the soil food web, or that may have specialized interactions with litter types, thereby altering soil nutrient cycling. We assess the role of litter in PSF effects via physical, chemical and biotic pathways to address how litter-mediated feedbacks may play out relative to, and in interaction with, feedbacks mediated through the plant rhizosphere. We also present one of the first experimental studies to show the occurrence and species-specificity of litter-mediated feedbacks and we identify critical research gaps. By formally incorporating the plant-litter feedback pathway into PSF experiments, we will further our understanding of PSFs under natural conditions.

Keywords: decomposition, indirect plant-soil feedback effects, allelopathy, home-field advantage, rhizosphere-mediated feedback, litter-mediated feedback

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INTRODUCTION

Plants modify their biotic and abiotic soil environment, which in turn has a major influence on subsequent plant growth, also referred to as plant-soil feedback (PSF) (Bever et al., 1997; Wardle et al., 2004). Plant-soil feedbacks are key drivers of plant physiology, growth and community composition (Kulmatiski et al., 2008; van der Putten et al., 2013; Teste et al., 2017) and thereby underlie ecosystem functioning (Bennett et al., 2017; Mariotte et al., 2018). Therefore, managing

PSFs can be of importance for improving sustainability in agro-ecosystems (Mariotte et al., 2018), for restoration of natural ecosystems (Kardol and Wardle, 2010 review TREE, Wubs et al., 2016) and for enhancing the resilience of ecosystems under global change and species invasions (van der Putten et al., 2016). For example, exploiting positive PSF effects may allow us to improve yield and reduce the use of artificial fertilizer and pesticides in agricultural fields (Mariotte et al., 2018; Veen et al., 2019). However, to be able to manage PSFs it is crucial to unravel how PSFs operate.

It has been long recognized that PSFs can be mediated via direct interactions between plants and rhizosphere communities, as well as indirect interactions between plants and decomposer communities driven by litter inputs (**Figure 1**) (Wardle et al., 2004; Ehrenfeld et al., 2005; Kardol et al., 2015). However, most studies focusing on PSFs have considered direct interactions (e.g., pathogens, mutualists), while largely neglecting the role of plant litter and decomposer communities (Elgersma et al., 2012; van der Putten et al., 2016). In natural ecosystems, litter may leave physical, chemical and biotic legacies in the soil that have a strong impact on soil functioning and plant growth (Ehrenfeld et al., 2005; Elgersma et al., 2012). In addition, litter-mediated PSFs may modify rhizosphere-mediated PSFs (Kardol et al., 2015; Ke et al., 2015; Zhang et al., 2016), for example, because saprotrophic soil organisms may suppress plant pathogens as a result of competition for space and nutrients (Rodríguez et al., 2016). Therefore, to understand the importance of PSFs under natural conditions, it will be essential to unravel the role of plant litter and decomposer communities in PSFs (Ke et al., 2015; Veen et al., 2018).

Plant-soil feedback effects mediated via the biotic community in the rhizosphere are known to be driven by species-specific relationships between plant species and organisms inhabiting the rhizosphere. Sugars and plant signaling compounds excreted from plant roots attract or repel soil biota inhabiting the rhizosphere (Philippot et al., 2013; el Zahar Haichar et al., 2014; Kaiser et al., 2015), thus creating a plant-specific rhizosphere microbiome (Raaijmakers et al., 2009). Depending on the balance between mutualists and pathogens, this community will directly stimulate or inhibit plant growth (Raaijmakers et al., 2009; van der Putten et al., 2016). Importantly, such species-specific PSFs may also be mediated via plant litter pathways (Elgersma et al., 2012; Mazzoleni et al., 2015). For example, we showed in a greenhouse experiment that the biomass of the grass *Festuca rubra* was higher on soils previously incubated with conspecific litter (litter-mediated feedback), while biomass of the tree *Betula pendula* was not affected by litter, but was increased on soils where conspecific plants were grown previously (rhizosphere-mediated feedback) (**Box 1**; **Figures 2A,B**). Recent work has indicated multiple mechanisms that may explain such specific litter-mediated PSFs. For example, decomposer communities may have a strong affinity for plant litter types from the plant with which they are associated (Austin et al., 2014; Palozzi and Lindo, 2018), resulting in accelerated litter breakdown (Freschet et al., 2012a; Veen et al., 2015) and plant-specific patterns of nutrient release (Perez et al., 2013). In addition, the release of DNA and toxic compounds from decomposing plant litter can inhibit the growth of the plant from which the litter originates (Mazzoleni et al., 2015; Cartenì et al., 2016). How the negative feedback of self-DNA works is not fully understood, but it is hypothesized that plants have mechanisms to recognize their own DNA (Mazzoleni et al., 2015; Cartenì et al., 2016). Understanding the specificity of litter-mediated PSFs is crucial to elucidate how PSFs control plant growth and coexistence in natural systems (Kardol et al., 2015; van der Putten et al., 2016).

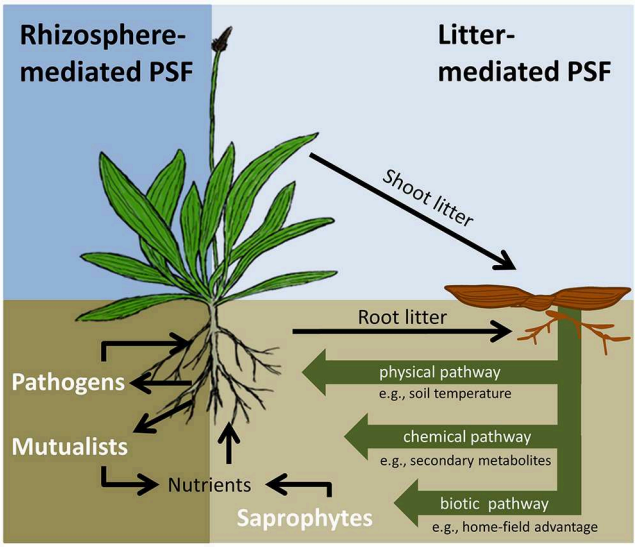

FIGURE 1 | Overview of the plant-soil feedback (PSF) framework integrating rhizosphere- and litter-mediated PSF effects. Rhizosphere-mediated PSFs will be driven by direct interactions between living plant roots and pathogens or mutualists. Litter-mediated PSFs will run via physical (e.g., litter layer thickness; litter physical traits), chemical (e.g., soil nutrient availability; secondary metabolites; allelopathy) or biotic (e.g., soil community composition; biotic interactions; home-field advantage effects) pathways. Litter-mediated PSFs via the physical pathway may be driven by species-specific impacts of e.g., litter layer thickness or light availability underneath litter layers on seedling germination or plant growth (Facelli and Pickett, 1991a). Litter-mediated effects via the chemical pathway may be driven by plant growth responses to the release of primary of secondary chemicals from leaf litter during decomposition (Facelli and Pickett, 1991a). Finally, litter-mediated PSFs via the biotic pathway may be driven by local variation in the composition and activity of decomposer communities and hence the rates at which they control the recycling of plant litter. Rhizosphere-mediated and litter-mediated PSFs interact, for example, through direct competition for space and nutrients between pathogens, mutualists and saprotrophs, the immobilization of nutrients released from litter by biota in the rhizosphere, or responses of pathogens and mutualists to physical and chemical changes in the soil induced by litter. Also, it is important to note that, physical, chemical, and biotic pathways of litter-mediated PSFs interact, for example, because plant-induced changes in the saprotrophic community will affect the rate at which nutrients and chemical compounds from litter are released into the soil. Interactions between the different pathways of litter-mediated PSFs are not specifically depicted in the figure.

Here, we propose a novel framework that integrates rhizosphere- and litter-mediated PSF effects (**Figure 1**). In this paper we will focus on the role of litter-mediated PSF effects as there are already detailed reviews and meta-analyses focussing on rhizosphere-mediated PSFs (e.g., Kulmatiski et al., 2008; van der Putten et al., 2013). We explore three main pathways via which plant litter can drive PSFs: physical, chemical and biological (Ehrenfeld et al., 2005). For each of these pathways,

BOX 1 | Description of set up and results of greenhouse experiment. The aim of this experiment is to disentangle rhizosphere- and litter-mediated plant-soil feedback effects for the tree *Betula pendula* and the grass *Festuca rubra*.

To test the role of plant litter inputs in driving plant-soil feedback effects, we set up a reciprocal litter transplant experiment in the greenhouse with soils and leaf litters from two plant species, i.e., the tree *Betula pendula* and the grass *Festuca rubra*. Soils and litters were collected from six replicate ex-arable grasslands and adjacent forests along a well-established chronosequence, in the nature reserve "the Veluwe" in the Netherlands; located between Ede (52°04′20″N, 5°44′12″) and Wolfheze (52°00′77″ N, 5°48′58″) (Kardol et al., 2006; Morriën et al., 2017; Veen et al., 2018). At each site, soil samples and associated plant litter were collected in October 2014 underneath *Betula* and *Festuca* plants (see Veen et al., 2018 for details on sample collection). For each site, soil samples were sieved over 10 mm to remove large roots and stones. Litter subsamples were also homogenized per site, air-dried, cut into 1-cm fragments and sterilized using gamma-irradiation (25 K Gray). We used the soils and litters to set up a two-phase experiment, with a litter incubation phase where we created "litter-specific soils" and a feedback phase where we tested the biomass response of *Betula* and *Festuca* seedlings to the incubated soils.

For the litter incubation phase, we used the soils to fill two 0.5-L pots per site, with each pot receiving 160 g of soil on a dry weight basis. Then, 2.0 g of litter was added to each pot following a full-factorial design. This resulted in two soil sources (*Betula*, *Festuca*) × 2 litter types (*Betula*, *Festuca*) × 6 replicates = 24 pots (**Figure 2C**, incubation phase). After 3 and 6 months of litter incubation, we added an additional 2.0 g of fresh litter to each of the pots, resulting in a 9-month litter incubation period in total. The amount of litter used was representative of the amount of natural litter fall in temperate grasslands (Penuelas et al., 2007). During incubation, pots were kept at 18°C and 65% water holding capacity (WHC). For the feedback phase, we collected two 32-g subsamples of soil (dry weight basis) from each pot, which we inoculated into two pots with 128 g of sterilized soil (dry weight basis) to test the biomass response of *Betula* and *Festuca*, respectively, resulting in 48 pots (**Figure 2C**, feedback phase). Soil for sterilization was collected from one of the ex-arable fields (van der Putten et al., 2000). We used this approach, which is common in plant-soil feedback research, to reduce the effect of abiotic differences between soil sources on plant growth in the feedback phase (Brinkman et al., 2010). During the feedback phase pots received 2.0 g of the same litter type as what they had received during the incubation phase and were put in the greenhouse for 5 months before growing *Betula* and *Festuca* seedlings. Seedlings were grown for 6 weeks according to a full-factorial design with each plant species growing on each combination of soil and litter sources. During the feedback phase pots were kept at day/night temperatures of 21/16°C, relative humidity of 60% and a day length of 16 h and 70% soil WHC. At the end of the experiment, we determined plant shoot and root biomass. Data were analyzed in R Core Team (2013) using general linear mixed models (Bates and Maechler, 2009) with soil source, litter source and plant species as fixed factors and replicate site as a random factor, followed by Tukey HSD tests. We have checked for a normal distribution of the residuals and homogeneity of variances before proceeding with our analyses.

Both shoot ($F_{1,39} = 75.70$, $P < 0.001$) and root ($F_{1,39} = 183.03$, $P < 0.001$) biomass were higher for *Festuca* than for *Betula* seedlings (**Figures 2A,B**). In addition, there was a main effect of soil source, indicating that shoot ($F_{1,39} = 11.03$, $P = 0.002$) and root ($F_{1,39} = 16.65$, $P < 0.001$) biomass were higher in *Betula* soils than in *Festuca* soils (**Figures 2A,B**). Finally, the interaction between litter source and plant species affected root biomass ($F_{1,39} = 13.04$, $P < 0.001$) and tended to affect shoot biomass ($F_{1,39} = 3.64$, $P = 0.064$), indicating that *Festuca* plants had more root biomass on soils incubated with *Festuca* litter, while there was no effect of litter incubation treatment on *Betula* (**Figures 2A,B**). Our findings show that litter inputs can have species-specific feedback effects to plant growth. As a result, litter feedbacks have the potential to contribute to or modify rhizosphere-mediated feedbacks. From this experiment we cannot disentangle to what extent feedback effects were mediated via litter-induced changes in soil chemistry or via changes in the composition and functioning of the saprotrophic community. However, our findings indicate that we need to integrate litter-feedback effects into the framework of plant-soil feedbacks.

we describe how they may contribute to explain PSF effects. It is important to note that even though we separate the litter-mediated PSFs via the three different pathways, under natural conditions, effects are often hard to disentangle as PSF effects will be mediated by interactions between the pathways. In addition, we identify ways forward in PSF research to increasing our understanding of interactions between litter-mediated and rhizosphere-mediated PSFs, which will advance our knowledge of PSFs in natural ecosystems.

PHYSICAL PATHWAYS

Physical pathways have received little attention in research on litter-mediated PSFs, but it has long been known that plant litter has strong effects on the physical soil environment (Facelli and Pickett, 1991a), both in natural (Facelli and Pickett, 1991b) and agricultural ecosystems, i.e., via crop residues (Teasdale et al., 1991; Walia and Dick, 2018). The effects of plant litter on the physical soil environment have strong potential to feed back to plant performance through effects on seed germination, seedling establishment, and initial plant growth (Olson and Wallander, 2002; Asplund et al., 2018).

A layer of leaf litter may improve the microclimatic conditions for seed germination through moisture retention and buffering against temperature extremes. In laboratory studies, it has been shown that under conditions of strong desiccation, acorns covered by a layer of leaves suffered lower water losses and, therefore, had higher rates of germination (García et al., 2002). However, under field conditions the evidence for improved germination rates in the presence of a litter layer is mixed (e.g., Barrett, 1931; Shaw, 1968; García et al., 2002; Kremer et al., 2019), probably because the extent to which the litter layer improves germination depends on ambient moisture conditions. Further, soil temperature is generally higher under a layer of plant litter (Sharratt, 2002), because the build-up of litter on the ground surface affects the transfer of heat between the soil and the atmosphere, which in turn can lead to increased seed germination rates (Paul et al., 2004). In addition, plant litter may physically protect seeds against predation (García et al., 2002). In contrast, the litter layer can also have negative impacts on seed germination and plant growth, because it can reduce the amount of light reaching the soil surface (Facelli and Pickett, 1991a) and it may act as a physical barrier to seedling emergence (Barrett, 1931). Finally, incorporation of litter into the soil matrix may modify soil structure, which may modify plant growth responses. Although most evidence for physical effects of litter on plant performance comes from studies on leaf litter, there may also be important effects mediated via root litter (Bardgett et al., 2014), but these remain to be tested. As root litter is already incorporated into the soil matrix, it may not form a litter

layer and therefore may have different physical PSF effects than leaf litter.

Litter-mediated PSFs via the physical pathway have not been studied extensively, but may be plant species-specific. The retention of soil moisture underneath leaf litter layers is strongly related to the traits of the plant litter species, for example specific leaf area (SLA), three-dimensionality of the litter, or tensile strength (Swift et al., 1979; Makkonen et al., 2013). Also, plant litter may have species-specific effects on soil water repellency, depending on the presence of organic hydrophobic compounds (Cesarano et al., 2016). Similarly, light extinction curves in litter layers differ between litter species (Facelli and Pickett, 1991b). The empirical support is scarce, but these litter-specific effects on environmental conditions strongly suggest that litter-mediated PSFs via the physical pathway have species-specific effects on seed germination and seedling growth.

Plant responses to litter-induced changes in environmental conditions via the physical pathway may also be species-specific (e.g., Olson and Wallander, 2002). In many empirical experiments it remains hard to untangle physical litter PSF effects, from chemical or biological effects. However, measurements on e.g., light penetration or mechanical barriers by litter in pot and field experiments indicate that physical litter-mediated PSF effects may play a role in driving species-specific plant responses. For example, using a controlled pot experiment testing effects of litter on seedling emergence, Donath and Eckstein (2008) found significant interactions between litter type and plant species origin at least partly driven through mechanical effects. Woodland species produced more biomass in presence of oak litter than in presence of grass litter, indicating a positive litter-mediated PSF effect which was explained by oak litter consisting of individual leaves, while grass litter typically forms dense interwoven mats which might be difficult to penetrate and could inhibit shoot emergence. For mechanical inhibition, seed size and seed position can be important in determining the strength of physical litter feedbacks (Donath and Eckstein, 2010). Zhang et al. (2017) found that a moderate amount of litter was beneficial for seedling emergence of small-seeded species, while large-seeded species were more tolerant to high amounts of litter input than small-seeded species. Also, litter accumulation by an exotic plant species *Avena fatua* reduced the germination of small-sized seeds of native species via increased litter depth and light reduction, thereby facilitating invasion of *A. fatua* in Californian grasslands (Mariotte et al., 2017). The impact of light reduction suggests that at least physical pathways may play role, but to what extent other pathways also play a role remains to be tested.

Together, these plant-specific impacts on and responses to the litter layer show that the creation of physical barriers and alteration of the abiotic environment by plant litter may play a key role in PSF in natural ecosystems. Understanding how shoot and root litter traits drive such physical litter-mediated PSFs may offer a promising avenue for further exploring the role of litter in PSF (Bardgett et al., 2014; Cortois et al., 2016). However, only few empirical studies have specifically focused on the role of physical effects in driving PSF. This is experimentally

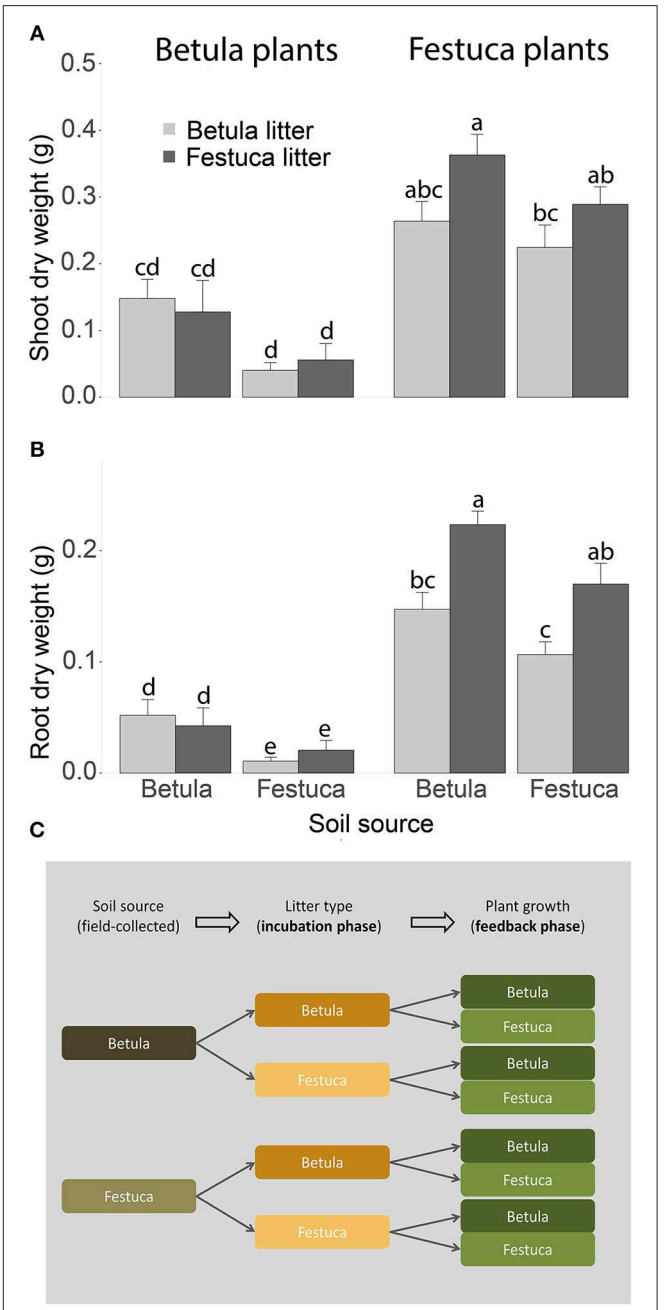

FIGURE 2 | Shoot dry weight **(A)** and root dry weight **(B)** of *Betula pendula* and *Festuca rubra* plants in response to soil source (the plant type underneath which the soil was collected in the field) and litter incubation treatments (the litter type that was incubated in the soil during the experiment). Different letters above the bars indicate statistical differences at $P > 0.05$ indicated by Tukey HSD *post-hoc* tests. **(C)** Schematic overview of the experimental set up. Soil samples used in this experiment were collected in the field underneath *Betula* and *Festuca* plants (left column). Then soil samples were incubated under controlled conditions with plant litter from both plant species using a full-factorial reciprocal transplant design (middle column). Finally, we grew seedlings of both plant species on each soil in a full-factorial design (right column). This resulted in 8 pots for each replicate ($n = 6$) where each plant species (*Betula* and *Festuca*) was grown on each type of litter incubation (*Betula* and *Festuca*) and each type of field soil (*Betula* and *Festuca*).

challenging, but may provide insights in plant growth responses to litter inputs. It is important to mention that the level of specificity of physical litter-mediated PSF may be lower than for rhizosphere-mediated PSF, because the strength and direction of physical litter feedbacks may be largely determined by generic leaf and seeds traits; however, this warrants further investigation.

CHEMICAL PATHWAYS

During decomposition a wide range of chemical compounds is released from plant litter which can be beneficial or detrimental to subsequent generations of plants (Facelli and Pickett, 1991a). Litter-mediated PSFs via the chemical pathway can be driven by the liberation of plant nutrients, secondary metabolites or DNA from decomposing litter (Facelli and Pickett, 1991a; Mazzoleni et al., 2015). Soil nutrients can be made available through rapid decomposition of nutrient-rich litter. This will increase plant nutrient availability in the next generation. In contrast, litter with a high concentration of structural carbohydrates, such as cellulose, decomposes slower and may produce less positive or even negative PSFs (Vahdat et al., 2011). Although litter-mediated PSF via nutrients may be less species-specific than PSFs mediated via other chemicals, plant species responses to litter-mediated changes in soil nutrient cycling often differ between plant growth strategies. Generally, fast-growing, exploitative plant species will benefit most from positive litter-mediated PSFs via nutrient availability, while plant species with conservative resource-use strategies may be less hampered by slow recycling of nutrients (Wardle et al., 2004). Also, positive litter-mediated PSFs may favor the invasion of exotic plants, that are often better competitors for nutrients released from plant litter (Eppinga et al., 2011). However, generalizing litter-mediated PSFs through nutrients may be complicated because of confounding effects of other chemical compounds or biotic interactions, which may result in unexpected effects on future generations of plants (Hobbie, 2015).

Plant litter also contains a range of secondary metabolites, including alkaloids, phenolic compounds and terpenes, which are often used as a defense mechanism against herbivores and pathogens and therefore play a key role in plant-microbe and plant-herbivore interactions (Chomel et al., 2016). These secondary metabolites are important for determining decomposition rates. For example, tannins may slow rates of litter decomposition (Hättenschwiler and Vitousek, 2000) and phenolic compounds can delay the colonization of litter by decomposer organisms (Ormeno et al., 2006; Chomel et al., 2014). Yet, it is now increasingly acknowledged that some complex compounds, such as lignin, can be degraded more quickly than previously assumed (Lehmann and Kleber, 2015), but that this may depend on the presence of specialized microbial communities, such as certain lignin-degrading fungi (van der Wal et al., 2015). As a result, secondary metabolites can contribute to litter-mediated PSFs via impeding nutrient cycling (Wardle et al., 2012). Secondary metabolites can also have direct impacts on plant growth when released into the soil, and their impact may strongly depend on the residence time in the soil (Chomel et al., 2016). Many chemical compounds that are present in the living plant, such as alkaloids, are quickly metabolized in litter and are thought to have limited effects on soil processes and plant responses (Siegrist et al., 2010). However, other chemicals liberated from plant litter can persist in the soil after senescence and may inhibit the growth or germination of neighboring and next-generation plants (Bonanomi et al., 2011), a phenomenon referred to as allelopathy (Muller, 1966). For example, the growth of tree seedlings may be inhibited by phenol-rich litter (Hättenschwiler and Vitousek, 2000). Also, flavonoids, which are known to play a role in attracting beneficial microbes, such as *Rhizobia*, may remain in plant tissue after senescence and affect plant growth by scavenging free radicals and improving stress tolerance (Barazani and Friedman, 2001). In addition to secondary metabolites, DNA released from decomposing material may also hamper plant growth of next generations of plants, and this negative feedback is specifically targeted toward congeneric plant species (Mazzoleni et al., 2015).

Litter-mediated PSFs via chemical compounds may strongly differ between above- and belowground plant organs. Although decomposition rates of shoots, stems and roots broadly correlate across large-scale fertility gradients, at the level of sites or individual plant species decomposition rates may differ between plant organs (Hobbie et al., 2010). This means that above- and belowground plant organs, as well as different types of roots, may play a different role in ecosystem processes (Freschet et al., 2012b). For example, leaf litter decomposes often more rapidly than root litter (Freschet et al., 2013), and finer roots, often richer in nitrogen (Pregitzer et al., 1997), faster than coarser roots. Although this may affect litter-mediated PSF, only a few studies have focused on the impacts of root litter on PSF (e.g., Mazzoleni et al., 2015; Zhang et al., 2016), and none of them compare how PSFs induced by root and shoot litter differ. Yet, root decomposition is likely to play a key role in subsurface soil layers and in ecosystems, such as tundra and grasslands, where half to three quarters of plant biomass is produced belowground as roots (Poorter et al., 2012). Therefore, it is essential to start disentangling the role of root and shoot litter driving chemical litter-mediated PSF effects.

BIOTIC PATHWAYS

Local variation in decomposer community composition and activity drives variation in decomposition rates (Hattenschwiler and Gasser, 2005; Vos et al., 2011; Bradford et al., 2014, 2017). Variation in decomposer composition may be tightly linked to plant composition (Bezemer et al., 2010), indicating that decomposer composition and activity may have an important role in driving litter-mediated PSFs. The litter-fragmenting community (or detritivores), including earthworms, millipedes, myriapods, diplopods, and various insect larvae, transforms a large part of plant litter into feces, thereby increasing the surface area for microbial decomposition, which often results in accelerated litter breakdown (Hattenschwiler and Gasser, 2005; David, 2014; Joly et al., 2018). The impact of litter shredders on decomposition may be strongly affected by decomposers

actively selecting certain litter types or compounds. For example, earthworms often prefer palatable residues, such as litter with low carbon:nitrogen ratios and tend to avoid ingestion of root litter (Curry and Schmidt, 2007; Vidal et al., 2017). In addition, the functioning of the gut microbiome of litter shredders, which is likely to be picked up from the soil (Hannula et al., 2019), may affect decomposition processes. Saprotrophic fungi and bacteria in the soil are involved in the mineralization of nutrients into inorganic forms that can be taken up by plants. How this affects plant species may be strongly determined by the stoichiometric constraints of both plants and the microorganisms involved in soil nutrient cycling (Capek et al., 2018). Depending on how plants drive shifts in the composition and activity of detritivores, saprotrophs, and gut microbes, variation in the soil community will contribute to litter-mediated PSFs by altering soil nutrient availability (Joly et al., 2018) and liberating secondary metabolites from plant litter. Also, interactions between decomposers and other soil organisms (e.g., predators in the soil) may have a large impact on litter-mediated PSFs. For example, consumption of detritivores and microbial decomposers by predators in the soil could inhibit decomposition (Liu et al., 2014) and thereby short-circuit litter-mediated PSFs.

Litter-mediated PSFs may also operate via home-field advantage effects, which is the process that litter decomposition is accelerated near the plant where the litter originates from relative to litter decomposition further away from that plant (see Gholz et al., 2000; Ayres et al., 2009; Veen et al., 2015; Palozzi and Lindo, 2018). The hypothesis is that home-field advantage effects are driven by species-specific associations between plants and decomposer communities (Freschet et al., 2012a; Austin et al., 2014). Recent experimental work showed that different litter types can indeed harbor specific litter microbiomes (Keiser et al., 2011; Lin et al., 2019) and that variation in abundant litter fungal groups can contribute to home-field advantage effects (Veen et al., 2019). This results in plant-specific patterns in nutrient release (Perez et al., 2013) and, hence, may contribute to litter-mediated PSFs. In general, home-field advantage effects may be expected to favor the plants where the litter originated from Zhang et al. (2016), but nutrients released from litter may also be taken up by neighboring plant species (Hood et al., 2000; Collins et al., 2007). Therefore, the extent of plant specificity in litter-induced feedbacks via home-field advantage remains to be tested (van der Putten et al., 2016; Zhang et al., 2016).

Most studies focus on shoot litter decomposition, but decomposition of root litter may be equally or more important in driving biotic litter-mediated PSFs (Li et al., 2015; Zhang et al., 2016), because it takes place in or near the rhizosphere of plants. As a result, saprotrophic communities involved in root litter breakdown closely interact with mutualists- and pathogens located in the rhizosphere, via competition for carbon and nutrients (Rousk and Bååth, 2011; Boddy, 2016; Money, 2016; Sokol and Bradford, 2019). The extent of these interactions depends on the availability of easily available sugars (Rousk and Bååth, 2011; Ballhausen and de Boer, 2016), the stoichiometry of plant litter (e.g., how much nitrogen is available) (Zhang and Elser, 2017), the chemical composition of the litter (Gartner et al., 2012) and accessibility of other soil organic material to saprotrophs (Rillig and Mummey, 2006). In turn, the mutualistic, pathogenic, and saprotrophic components of the microbial community are consumed by organisms, such as collembola that graze on fungal mycelium (Scheu and Schulz, 1996) or protists that feed on unicellular bacteria and fungi (Radosa et al., 2019). In addition, saprotrophs can compete for root exudates with plant parasites or ectomycorrhiza in the rhizosphere. As a result, both rhizosphere- and litter-mediated PSFs can be controlled by similar trophic top-down and bottom-up factors (De Long et al., 2019). It is therefore important not to regard them as separate units, but as intertwined operating pathways imbedded in the same food-web context.

THE WAY FORWARD

Here, we demonstrated how root and shoot litter can affect PSFs through physical, chemical and biotic pathways. Although an emerging body of literature supports the potential importance of litter-mediated PSFs, many critical knowledge gaps remain which require further investigation. Here we identify five important avenues for further research into the role of litter-mediated PSFs:

(1) Disentangle the interactions between litter-mediated vs. rhizosphere-mediated PSFs. We already know that rhizosphere pathogens or symbionts play a strong role in mediating PSFs (Bennett et al., 2017; Semchenko et al., 2018) and litter-mediated PSFs are gaining more attention (Freschet et al., 2013; Hobbie, 2015; Zhang et al., 2016; Manrubia et al., 2019), but interactions and their relative hierarchy of importance needs to be elucidated. For example, modeling work showed that rhizosphere-mediated PSFs may modify or override litter-mediated PSFs (Ke et al., 2015). Also, microbes in the rhizosphere can immobilize the nutrients liberated from plant litter, thereby buffering litter-mediated PSFs via nutrient cycling (Miki et al., 2010). These findings indicate the need to study rhizosphere- and litter-mediated PSFs in combination (see **Box 1** for an example) to disentangle their interactions and relative importance. This will require full-factorial experiments where rhizosphere- and litter-mediated PSFs are manipulated. Within such studies it will be important to carefully consider spatial and temporal scales at which litter- and rhizosphere-mediated PSF effects occur, as these are not necessarily the same. In addition (stage-structured), models could be powerful to further explore how litter-mediated PSFs could operate (Ke et al., 2015; Campbell et al., 2016).

(2) Gain a better understanding of interactions between physical, chemical and biotic pathways of litter-mediated PSFs. For example, litter cover (i.e., physical) may enhance seedling establishment by creating favorable microclimatic conditions (Facelli et al., 1999), but it may have negative impacts on seedling survival due to allelopathy (i.e., chemical) (Wardle et al., 1998) or damage by litter fungal pathogens (i.e., biotic) (Beckstead et al., 2012). Integrating research across all three pathways will help us to untangle the key mechanisms driving litter-mediated PSFs. This will require experiments that specifically manipulate the physical,

chemical or biotic pathway, for example by using sterilized litter to rule our biotic effects or fake (e.g., plastic) litter to identify physical effects. Alternatively, experiments using a more "holistic" approach including physical, chemical and biotic effects of litter without specifically disentangling them, may provide a better understanding of the overall impact of plant litter on PSFs. For example, plant roots from the first generation of plants can be left in the soil intact to allow for physical, chemical and biotic legacy effects of root litter on next generations of plants.

(3) Elucidate the species-specificity of litter-mediated PSF effects. Evidence is accumulating that plant litters build up unique decomposer communities (Lin et al., 2019; Veen et al., 2019), which may drive litter-mediated PSF via the biotic plant pathway. This may however generate relatively unspecific feedback effects, because different plant species may all profit from nutrients liberated from litter by specialized decomposer communities, however this is not tested. Future work should disentangle to what extent litter-mediated PSFs via the physical, chemical or biotic pathways result in species-specific effects on the growth and performance of next-generation plants.

(4) More emphasis is needed on the role of root litter decomposition in driving PSFs. So far, most work has focused on aboveground litter pathways, but root decomposition may be key in driving litter-mediated PSFs and, importantly, in modifying rhizosphere-mediated PSFs. Future studies should build on established concepts originating from work on shoot litter decomposition (Wardle et al., 2004; Austin et al., 2014), but they should also focus on the fundamental differences between root and shoot litter, such as impacts of continuous vs. seasonal litter inputs, or the depth of the roots in the soil.

(5) Litter-mediated PSFs need to be investigated under natural conditions, with a particular emphasis on the contexts under which such effects are most important. As PSF research moves out of the glasshouse and into the field (Kulmatiski and Kardol, 2008; De Long et al., 2019), it will be imperative to include litter-mediated PSFs alongside the more traditional rhizosphere-mediated PSFs when designing and executing experiments. Only via inclusion of litter-mediated PSFs will we be able to generate a more comprehensive understanding of PSFs and the ecological processes they control under natural conditions. Also, field PSF experiments allow for testing litter- and rhizosphere-mediated PSFs across larger spatial (i.e., at the level of plant communities) and temporal scales (i.e., multiple plant generations).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author and will be stored in the data archive of NIOO-KNAW.

AUTHOR CONTRIBUTIONS

All authors have contributed to develop the ideas presented in this manuscript. GV and FH designed and performed the experiment (**Box 1**). GV has led the writing of the manuscript and all authors have contributed to individual sections. All authors have read and approved the final version of this manuscript.

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Climate Affects Plant-Soil Feedback of Native and Invasive Grasses: Negative Feedbacks in Stable but Not in Variable Environments

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The plant-soil feedback framework allows researchers to target the interaction of plants and root-associated microbes and to determine its interplay on plant-plant interactions. Plant-soil feedbacks in terrestrial ecology are well-documented, but the strength and direction of feedbacks as influenced by abiotic environmental factors, such as temperature and soil moisture, has not been fully explored. In our study, we examined plant-soil feedback responses of both cool- and warm-season native and non-native grasses to elevated temperatures (ambient and +5°C) and soil moisture (100 and 75% field capacity). In a previous experiment, grasses were grown under temperature and soil moisture conditions similar to our current study. The resultant trained soil communities served as the inoculum sources for our current experiment. We found that consistent training and experimental temperatures resulted in negative PSF, where plants produced greater biomass in soils conditioned by heterospecifics. However, the direction of PSF was reversed when training and experimental conditions were mismatched. That is, when training and experimental temperatures mirrored one another, negative PSF occurred, suggesting coexistence between the two species is likely under these conditions. However, when only training or testing temperatures were elevated, positive PSF were detected, favoring the non-native species. These alterations in plant-soil feedbacks were relatively consistent across pairings of warm- and cool-season grasses. Overall, our results indicate inconsistent year-to-year environmental conditions, such as extreme temperatures, may undermine the stabilizing forces of negative PSF and favor of non-native grasses.

Keywords: ***Bothriochloa ischaemum***, ***Bromus inermis***, **climate, invasive species,** ***Pascopyrum smithii***, **plant-soil feedback,** ***Schizachyrium scoparium***, **soil training**

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INTRODUCTION

Interactions between plants and their associated soils play an important role in the formation of plant communities and maintenance of biodiversity (Mangan et al., 2010; Bauer et al., 2015; Bever et al., 2015). Plants affect the biotic and abiotic conditions of associated soils, with subsequent reciprocal interactions known as plant-soil feedbacks (PSFs)

(Kulmatiski et al., 2008; Bever et al., 2010). These feedbacks range from positive to negative, and the direction of the feedback is largely driven by the presence and abundance of certain soil biota. Negative PSFs occur when a plant performs better in soils conditioned by heterospecifics, thus promoting community diversity, whereas positive PSFs are created when a plant's growth is increased in conspecific-conditioned soil, often resulting in monotypic stands (Bever et al., 1997; van der Putten et al., 2013). Generally, PSFs are negative, particularly between native plant species and species that are phylogenetically unrelated (Meiners et al., 2017; Crawford et al., 2019), suggesting that soil microbes are likely to contribute to coexistence of native and phylogenetically diverse plant species. In recent years, the role of PSFs in the success of non-native invasive plant species has been the focus of a growing body of research (Inderjit and van der Putten, 2010; van der Putten et al., 2013; Kulmatiski, 2018).

Previous research has shown the presence of invasive plant species can alter the density and composition of arbuscular mycorrhizal (AM) fungal communities, which may influence the feedback interactions that affect subsequent growth and establishment of both native and invasive species (Reinhart and Callaway, 2006; Vogelsang and Bever, 2009; Allen et al., 2018). Plant invasion can disrupt mutualistic interactions between native plants and soil microbial communities, further increasing ecosystem susceptibility to invasion. Past and current empirical studies suggest that invasive plants often create positive PSFs, thus suppressing native biodiversity and promoting growth of conspecifics (Reinhart and Callaway, 2006; Crawford and Knight, 2017; Crawford et al., 2019). This can occur through a number of mechanisms. Non-native plant species are often less dependent on native AM fungi, compared to native species, decreasing AM fungal densities with a concomitant decrease of native plant growth rates (Pringle et al., 2009; Vogelsang and Bever, 2009; Zubek et al., 2016; Grove et al., 2017). Alternatively, invasive species can be highly dependent on AM fungal associations, yet alter local soil microbial community composition, resulting in a loss of native plant growth and survival (Wilson et al., 2012; Zubek et al., 2016; Ba et al., 2018; Zhang et al., 2019).

Current climate models predict warmer, drier conditions across much of North America (IPCC, 2014), and these conditions, coupled with continued pressure by invasive plant species, will likely exacerbate losses of native biodiversity. Alterations in climatic conditions will also likely have dramatic effects on the interactions of plants, their associated soil microbiota, and subsequent strength and direction of PSF, as soil microbes can mediate plant responses to drought (Kivlin et al., 2013; Xi et al., 2018). Warmer, drier conditions have been shown to reduce AM biomass (Duell et al., 2016; Yu et al., 2018), which likely alter PSFs. Drought has been shown to shift PSF in co-existing plants from negative or positive to neutral, depending on the species, suggesting that drought may neutralize PSF (Heinze et al., 2017; Fry et al., 2018), and these alterations in PSFs may be persistent (Kaisermann et al., 2017). While drought and invasive species individually alter the strength and direction of PSFs, there has been very little research linking the two and assessing the coupled effects on native plant growth.

Plant-microbe interactions can also mediate plant adaptation to perturbations in climate. Mycorrhizal fungi, for example, can increase drought tolerance of their host (Delavaux et al., 2017; Bowles et al., 2018). Even for plants that do not associate with mycorrhizal fungi such as mustards, changes in their microbiome can mediate tolerance to drought. For example, Lau and Lennon (2011) found that plants that were associated with more diverse microbial communities and subjected to drought exhibited greater growth and changes in phenological traits, compared to plants grown with less diverse microbial communities. Additional work by Lau and Lennon (2012) suggests that plant productivity and fitness is greatest when previous and contemporary environmental conditions were similar, as opposed to mismatched conditions. This suggests that plants may benefit from soil microbial communities trained under particular environmental conditions, and that variation in climate and weather patterns will affect certain species' abilities to persist.

To assess potential effects of climate change on PSF dynamics and the potential for microbes to mediate plant response to changing climate, a greenhouse experiment was conducted to: (1) assess the strength and direction of native and non-native grass PSFs under ambient conditions (well-watered and moderate temperatures) and (2) examine the strength and direction of native and non-native grass PSFs under projected climate scenarios (drought conditions and elevated temperatures). Based on results from Duell et al. (2016), we hypothesized 1a) soil microbial alterations resulting from non-native species will result in positive PSF under ambient conditions and 1b) soil microbial alterations resulting from native species will result in negative PSF under ambient conditions. Further, we hypothesized (2) PSFs of both non-native and native species will be exacerbated under elevated temperatures and drought conditions, relative to ambient conditions. Finally, we hypothesized (3) plants will perform best when grown in soils of matching environments as their current environment.

MATERIALS AND METHODS

Plant Species and Soil Collection

In our experiment, we used paired native and non-native warm- and cool-season perennial grasses to test the effects of soil moisture and temperature on PSF dynamics of these species. *Schizachyrium scoparium* (Michx.) Nash is a native, warm-season perennial bunchgrass found throughout the North America, especially in temperate, grass-dominated ecosystems. *Bothriochloa ischaemum* (L.) Keng is a non-native, warm-season perennial grass found throughout southern and central North American grasslands. Native to Europe, Asia, and northern Africa, *B. ischaemum* was introduced into North American grasslands in the early 1900's as a fast-growing livestock forage (Celarier and Harlan, 1955). *Pascopyrum smithii* (Rydb.) Á Löve is a native, cool-season perennial grass found throughout the Great Plains region of North America, and is a dominant component of northern grassland plant communities. *Bromus inermis* Leyss. is a non-native, cool-season perennial grass that can be found throughout North America. *B. inermis* was

introduced into North America in the late 1800's from Eurasia as livestock forage and for its role in soil stabilization on degraded landscapes (Larson et al., 2001). Both *B. ischaemum* and *B. inermis* are widely-considered as invasive plants, often forming monocultures and decreasing biodiversity at many trophic levels (Hickman et al., 2006; Gabbard and Fowler, 2007; Dillemuth et al., 2009; Stotz et al., 2017).

Native tallgrass prairie soil was collected from the Konza Prairie Biological Station, Manhattan, KS, USA, where all four species used in this experiment can be commonly found. Soil was sieved through a 10 mm sieve to remove rocks and coarse plant material. Soil was steam-pasteurized at 80°C for 2 h and transported to Oklahoma State University greenhouse facilities.

Training of Soil Microbes for Inoculum

To assess the consequences of alterations in soil microbial communities, including AM fungal communities, soil inoculum was collected from a previous climate perturbation experiment (Duell et al., 2016) which investigated the effects of elevated temperatures and reduced soil moisture on both native and non-native grasses. In Duell et al. (2016), we conducted two experiments, one used two warm-season grass species [one native (*S. scoparium*) and one invasive (*B. ischaemum*)] and a second that used two cool-season grass species [one native (*P. smithii*) and one invasive (*B. inermis*)] grown under two climatic regimes to "train" microbial communities for inocula. Warm-season species were maintained at ambient (24°C) and elevated (29°C) temperatures and cool-season species were grown at ambient (17°C) and elevated temperatures (22°C). Temperature treatments were combined with two levels of soil moisture [field capacity and drought (35% less than field capacity)]. Temperature and soil moisture treatments were initiated following seedling establishment. Temperatures represent the mean daily high temperature for each respective treatment for the entirety of the experiment. Once plants were established, soil moisture was monitored twice per week using the gravimetric water content of each pot. The complete experimental design that produced our inoculum consisted of 16 treatment combinations: 4 plant species × 2 temperature treatments × 2 soil moisture treatments, arranged in a complete block design with 6 replications for a total of 96 pots (Duell et al., 2016). Training phase conditions will hereafter be referred to as source conditions (e.g., source temperature).

Determination of Plant-Soil Feedback

The experimental design of feedback test experiment was based on the feedback approach described in Bever (1994). Separate feedback test experiments were conducted for the warm-season grasses and the cool-season grasses (Zaiger, 2016). Warm- and cool-season grasses were germinated in vermiculite. After 14–21 days (second-leaf stage), individual seedlings were transplanted into pots (6 cm diameter × 25 cm deep: DeePots; stuewe.com, Tangent, Oregon), filled with 600 g (dry weight) of soil partitioned into three layers: 400 g of steam-pasteurized soil [80°C for 2 h and allowed to cool for 72 h to eliminate biotic communities but retain abiotic soil traits (Hetrick et al., 1990; Wilson and Hartnett, 1997, Johnson et al., 2010)], followed by 100 g of soil inoculum (inoculum described above), followed by 100 g of steam-pasteurized soil to protect cross-contamination during the growing period. One seedling was planted per pot

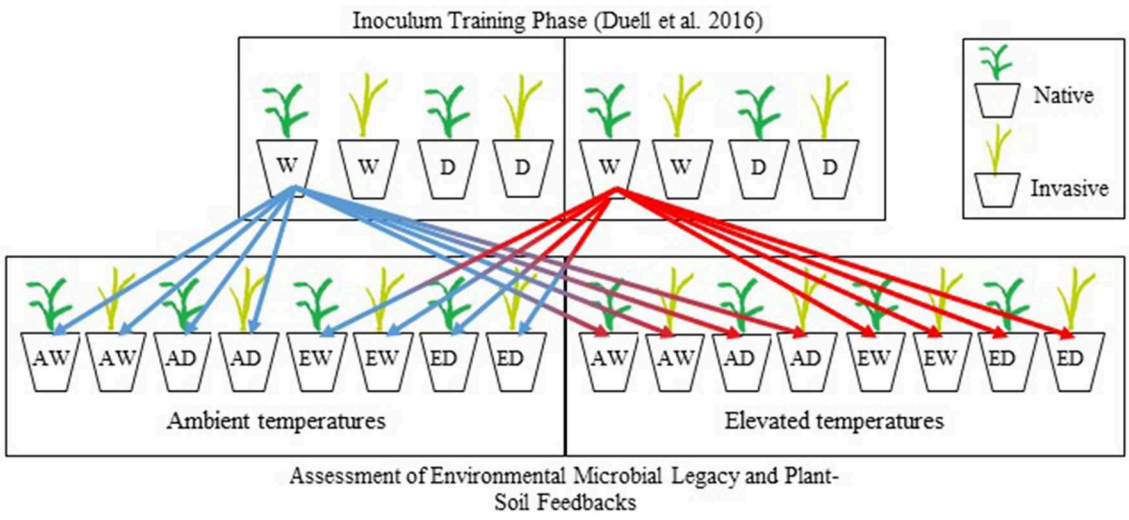

FIGURE 1 | Schematic diagram for the experimental design of the training and experimental testing phases of this research. In the training phase, two warm-season grasses (*Schizachyrium scoparium, Bothriochloa ischaemum*) were grown under four combinations of soil moisture and temperature, replicated 6 times for a complete experimental design of 48 pots. Eight different soil communities conditioned in the training phase were used as inocula in the testing of PSF. The schematic shows only one paired native and invasive grass. As shown by our schematic, each soil treatment from the training phase was divided eight times and used as inoculum for native or invasive plant species of the same functional group, maintained at two soil moistures (well-watered, drought), and 2 temperatures (ambient, elevated). This experimental was replicated using cool-season grasses (*Pascopyrum smithii*, *Bromus inermis*). W and D, well-watered and drought, respectively; A and E, ambient and elevated temperatures, respectively.

and inoculated with soil trained by the non-native or native grass under all combinations of temperature and soil moisture treatments in Duell et al. (2016) (described above). Each of two feedback test experiments consisted of a full factorial design with three factors (plant species, temperature, and soil moisture) each consisting of the same temperature and soil moisture treatments used in the training phase (**Figure 1**). In total, both the cool-season and warm-season experimental studies consisted of 392 pots [8 inocula × 2 plants × 2 temperature treatment levels × 2 soil moisture treatment levels × 6 replications + 8 sterile controls (no inoculum)], for a total of 784 pots. Sterile controls consisted of 600 g of steam-pasteurized soil. Environmental conditions tested during this phase of the experiment will hereafter be referred to as experimental conditions (e.g., experimental temperature). After 16 weeks, prior to shoot senescence, plants were harvested, and root and shoot biomass was separated. Roots were washed free of soil, and all biomass was dried at 60°C for 48 h, and weighed.

Statistical Analyses

Feedbacks were calculated for total biomass. Interaction coefficients were calculated to quantify PSF between native and non-native plants grown with inoculum trained by either conspecific or heterospecific plants. We used the following equation (Equation 1):$I_s = G(A)_\alpha - G(A)_\beta - G(B)_\alpha + G(B)_\beta$, where I_s is the feedback interaction coefficient, $G(A)_\alpha$ is growth of plant species A inoculated with conspecific soil, $G(A)_\beta$ is growth of plant species A inoculated with heterospecific soil, $G(B)_\alpha$ is growth of plant species B inoculated with heterospecific soil and, $G(B)_\beta$ is growth of plant species B inoculated with conspecific soil (Bever et al., 1997). When I_s values are positive ($I_s > 0$), a net positive feedback on plant growth is generated by the soil community, and coexistence between plant species does not occur. Conversely, when $I_s$values are negative ($I_s < 0$), a net negative feedback on plant growth is generated by the soil community, and coexistence between plant species does occur (Bever, 2003). Interaction coefficient values were calculated for each temperature and drought combination of both inoculum training and experimental conditions.

Using PROC-GLM in SAS, we constructed a general linear model using log-transformed (for normalization of biomass data due to extreme values caused by drought) biomass and percent colonization as the dependent variables. Species identity, drought, and temperature treatments from the inoculum source and from the feedback study (6 total) were used as factors with all possible interactions. Analyses of these experiments were split by drought treatments due to low survival of water-limited warm-season plants. In the experiment with warm-season grasses, mortality under drought prevented analysis of growth, but in the experiment with cool-season grasses, analyses of growth responses was possible under well-watered and drought conditions. Therefore, the model included total of 5 factors (experimental temperature, experimental plant identity, source temperature, source soil moisture (source water) treatment, and source plant identity) examined in the analysis. For each treatment combination, three of the six replicates were scored for mycorrhizal root colonization. Pairwise feedback was tested within the "plant_species*source_plant_species" interaction where source_plant_species represents the plant species that trained the soil (Bever, 1994). In fact, for full factorial experiments with two plant species training (source) and experimental as are ours, significance of pairwise feedback is tested directly as the "species* source species" interaction and the dependence of pairwise feedback on environmental conditions (either training or experimental conditions or their interaction) is tested directly as the interaction of "species*source species*environmental condition." For example, a significant "species*source species*source temperature" interaction indicates that the strength of pairwise feedback depends upon the experimental temperature, while a significant "species*source species*source temperature*temperature" interaction indicates that pairwise feedback varies significantly with the interaction of source and experimental temperature. For all significant pairwise interactions between current plant 8 treatments and source treatments interaction coefficients were calculated using the formula (Equation 1, Bever et al., 1997). Differences in biomass under either soil moisture or temperature treatments with significant feedback interactions were assessed using a two-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) in R version 3.2.3 (R Core Team, 2015). Biomass and colonization were analyzed using the PROC GLM procedure in (SAS Institute, Cary, NC, U.S.A.), version 9.4 of the SAS System for Windows.

RESULTS

Warm- and Cool-Season Biomass Production

Regardless of species, plants subjected to reduced soil moisture exhibited high mortality, and therefore were removed from analyses. When subjected to well-watered experimental conditions, the main effects of plant species [$F_{(1, 94)} = 30.87$, $p \leq 0.001$], experimental temperature [$F_{(1, 94)} = 23.81$, $p \leq 0.001$], and plant species of the training phase (source species) [$F_{(1, 94)} = 9.62$, $p = 0.002$] were significant for our warm-season grasses. The non-native species, *B. ischaemum*, produced greater total biomass in all environmental treatments, compared to the native species, *S. scoparium* (**Figures 2A–D**). In addition, plants grown under elevated temperatures produced greater biomass, compared to plants subjected to ambient temperatures.

When subjected to well-watered experimental conditions, cool-season biomass production of native *P. smithii* was significantly greater as compared to invasive *B. inermis* [$F_{(1, 149)} = 4.89$, $p = 0.03$]; however, no differences were found between native and non-native plant species when analyzed by combinations of experimental and source conditions (**Figure 3**). Overall, cool-season grasses subjected to elevated experimental temperatures produced greater total biomass, relative to individuals grown at ambient experimental temperatures [$F_{(1, 149)} = 5.31$, $p = 0.02$]. Regardless of species, plants grown with inoculum trained under elevated temperatures produced consistently greater biomass than plants inoculated with microbes trained under ambient experimental temperatures

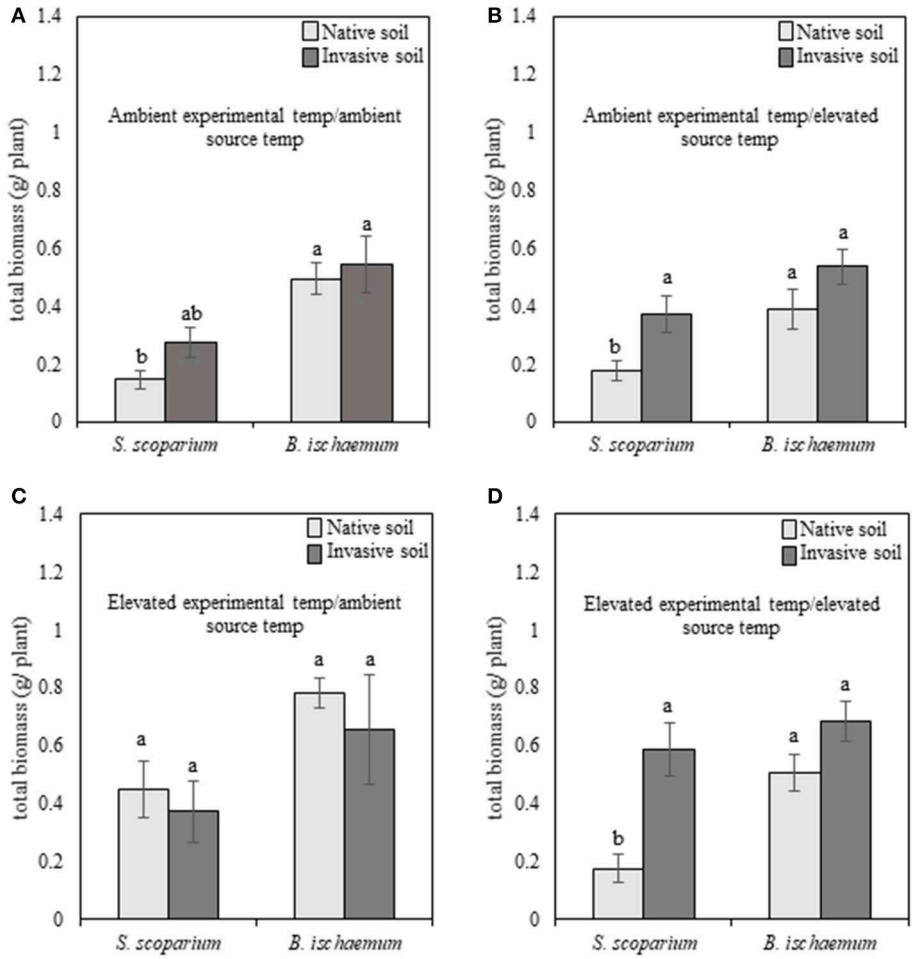

FIGURE 2 | Total biomass production of native (*Schizachyrium scoparium*) and invasive (*Bothriochloa ischaemum*) warm-season grasses grown under well-watered conditions in response to source soil plant identity and source soil temperature. Light bars indicate inoculum derived from native *Schizachyrium scoparium* and dark gray bars represent inoculum derived from invasive *Bothriochloa ischaemum*. In each panel, pairings of bars on the left represent native *S. scoparium*, and bars on the right represent invasive *B. ischaemum*. Panels represent the following treatment combinations: **(A)** ambient experimental temperatures, ambient source temperatures, **(B)** ambient experimental temperatures, elevated source temperatures, **(C)** elevated experimental temperatures, ambient source temperatures, and **(D)** elevated experimental temperatures, elevated source temperatures. Different letters indicate significant differences within panel ($p \leq 0.05$).

$[F_{(1, 149)} = 5.26, p = 0.02]$. Under well-watered experimental conditions none of the interactions and no feedback effects were significant (**Supplementary Table 2**).

Warm- and Cool-Season Species Pairwise Feedbacks

In the warm-season experiment, we observed a significant interaction $[F_{(1, 94)} = 3.93, p = 0.05]$ between native and non-native plant species, source plant species (i.e., plant species that trained the soil), experimental temperature, and source temperature (i.e., temperature at which the soil was trained) for our warm-season pairing, indicating that pairwise PSF varies with the interaction of source and experimental temperature. We illustrate this significant interaction in **Figure 4A**. When the source and experimental temperatures were consistent, PSF was negative, but when source and experimental temperatures were reversed, PSF was neutral or positive, and this reversal is significant (**Supplementary Table 1**). Specifically, total biomass of warm-season grasses grown under ambient temperatures was characterized by negative (ambient source soil temperature) and neutral (elevated source soil temperature) PSF (**Figure 4A**). When subjected to elevated experimental temperatures, the direction of PSF reversed, resulting in positive (ambient inoculum) or negative (elevated inoculum) PSF (**Figure 4A**). The negative PSF detected in warm-season total biomass under elevated temperatures with inoculum trained under elevated conditions was driven by significantly greater *S. scoparium* biomass production in *B. ischaemum* inoculum, compared to inoculum trained by the conspecific; whereas no difference was observed in *B. ischaemum* production with inoculum trained by either plant species.

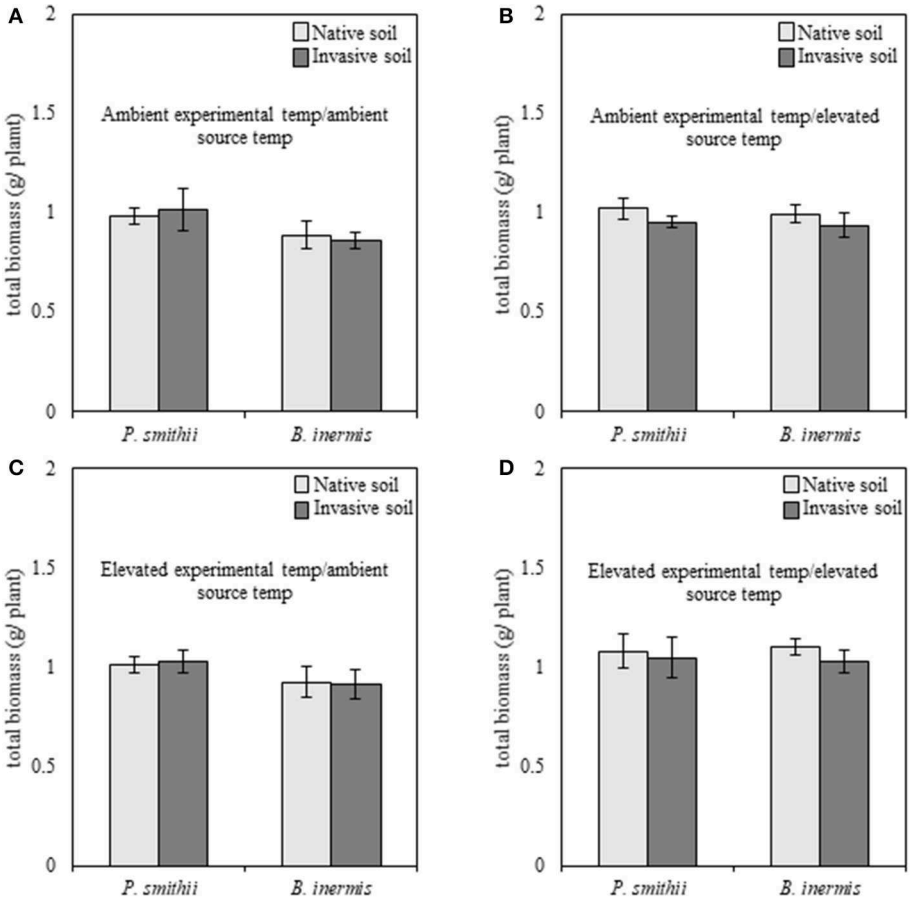

FIGURE 3 | Total biomass production of native (*Pascopyrum smithii*) and invasive (*Bromus inermis*) cool-season grasses grown under well-watered conditions in response to source soil plant identity and source soil temperature. Light bars indicate inoculum derived from native *Pascopyrum smithii* and dark gray bars represent inoculum derived from invasive *Bromus inermis*. In each panel, pairings of bars on the left represent native *P. smithii*, and bars on the right represent invasive *B. inermis*. Panels represent the following treatment combinations: **(A)** ambient experimental temperatures, ambient source temperatures, **(B)** ambient experimental temperatures, elevated source temperatures, **(C)** elevated experimental temperatures, ambient source temperatures, and **(D)** elevated experimental temperatures, elevated source temperatures.

In our cool-season experiment, the main effects were not significant under drought conditions (**Supplementary Table 3**). However, under drought conditions, we observe a significant interaction of feedback with source temperature and experimental temperature [$F_{(1, 60)} = 4.97$, $p = 0.03$] (**Figure 4B**). When the source and experimental temperature was consistent, PSF was negative, but when source and experimental temperature were reversed, PSF was neutral or positive. Specifically, under ambient experimental temperatures, PSF were negative when inoculum soil had also been subjected to ambient temperatures (**Figure 4B**). However, the direction of the PSF was reversed when ambient inoculum soil had been subjected to elevated temperatures (**Figure 4B**). When grown under elevated temperatures, PSF were positive when grown with source soils subjected to ambient temperatures, and negative when plants were grown with source soil trained at elevated temperatures (**Figure 4B**).

Legacy Effects of Soil Environment

We hypothesized that training soil microbial communities would result in environmental stress mitigation; however, our data do not support this *a priori* hypothesis. No evidence was observed for microbial mediation of drought stress, as we did not detect significant interactions between experimental soil moisture and source soil moisture, though this test was weak because of low survivorship in drought experimental conditions. Furthermore, we did not observe plants growing better when experimental and source temperatures were matched (**Supplementary Tables 1–3**).

DISCUSSION

There is increasing evidence suggesting that a rapidly changing climate will impact soil microbial communities (Rillig et al., 2002; Johnson et al., 2013; Schmidt et al., 2018), though little is known about strength or direction of PSF in response to extreme weather events, such as severe drought (Singh et al., 2010; Johnson et al.,

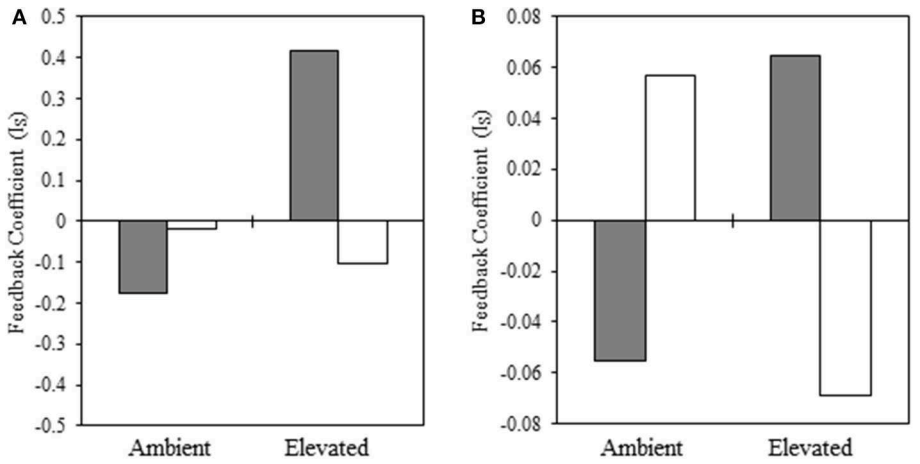

FIGURE 4 | Interaction coefficient for PSF related to biomass production of native and non-native **(A)** warm-season and **(B)** cool-season grasses. Filled bars indicate inoculum from ambient temperatures and open bars represent inoculum conditioned in elevated temperatures. In each panel, the left pair of bars represent plants grown at ambient temperatures, while the right-hand pair of bars represent plants grown at elevated temperatures (Note: y-axes differ in scale).

2013). Abiotic factors, such as light availability, can influence the strength and direction of feedback interactions (Smith and Reynolds, 2015), but the effects of environmental drivers, such as precipitation and temperature, on the strength and direction of PSF interactions is far less certain. In general, it is thought that elevated temperatures will result in more negative PSF, as increased temperatures are expected to result in increased pathogen prevalence, as well as reduced AM fungal activity (Mohan et al., 2014). However, the results are likely context-dependent, and more research is needed to elucidate any patterns and processes that may exist. In addition, many current and past PSF studies that have tested climate effects have used native species pairings and agricultural crops (Hendriks et al., 2015; Wang et al., 2017; Fry et al., 2018). Consequently, far less research has explored PSF dynamics surrounding climate and non-native invasive species. Our study is one of the first of its kind to test PSF theory with combined environmental factors and non-native invasive plant species.

We found negative feedbacks when the temperature is stable over the training and testing phase of the feedback experiment, but positive or neutral feedback when the training and testing temperature were not matched. As negative pairwise plant soil feedback is a necessary condition for soil microbial dynamics mediating plant species coexistence (Bever et al., 1997; Eppinga et al., 2018), our results suggest soil microbial dynamics can be stabilizing in either ambient or elevated temperatures, but this dynamic is disrupted in variable environments. However, as pairwise negative feedback is not a complete description of the coexistence conditions (Bever et al., 1997; Bever, 2003, Eppinga et al., 2018, Kandlikar et al., 2019), further work is necessary to evaluate whether soil microbial dynamics do determine variation in coexistence patterns across climate stability. We found that elevated temperatures, combined with a soil legacy of elevated temperatures, led to strongly negative PSF between native and non-native warm-season grasses. This was in contrast to the slightly negative to positive feedback exhibited in other experimental temperature and altered inoculum temperature combinations. We observed that PSF was also significantly more negative when the training and testing temperatures were constant in the cool-season grasses when tested under drought conditions. Our findings suggest that changes in environmental drivers can impact the strength and direction of PSFs. Both warm- and cool-season pairings produced negative PSF when experimental temperatures mirrored training phase temperatures. However, the direction of PSF was reversed when experimental and training temperatures were mismatched. These results suggest that coexistence is likely when environmental conditions are similar from year to year, and homogeneity may be promoted when growing conditions are dramatically different than the previous year. These results are in contrast with previous results of van Grunsven et al. (2010), in which direction of PSF detected between pairs of European congeners was largely unaffected by variation in testing temperature, though this study did not manipulate temperature in the testing environment. Our results suggest that across year variation in climate may be one reason why plant-soil feedbacks have been observed to be variable (De Long et al., 2019).

Despite our hypothesis that experimental drought conditions would result in strong positive feedbacks, we found the opposite occurred. Our hypothesis was based on a combination of observations that non-native *B. ischaemum* currently invades into grasslands, and Duell et al. (2016) reported elevated temperatures and reduced soil moisture did not affect biomass production of the species. We observed the alternative scenario, in that the biomass of the non-native was not influenced, while the biomass of *S. scoparium* was greater in non-native soil compared to when grown in conspecific soil. While we do not suggest that the non-native soil generally promotes native growth, we propose that these results may result from two mechanisms. The first mechanism is that the changes in

the AM fungal community contributed to the negative PSF observed (Bever, 2002). In the event of elevated temperatures, AM fungi may decrease activity (Mohan et al., 2014), which in turn weakens positive PSF (De Long et al., 2019), which could further explain our observed negative feedbacks. Changes to the fungal community were likely more pronounced due to the greater growth of the non-native grass relative to the native grass in the training phase of the experiment (Duell et al., 2016) under elevated temperatures (**Supplementary Tables 4–6**; **Supplementary Figure 1**). Warm-season grasses, such as *B. ischaemum*, readily associate with AM fungi (Wilson and Hartnett, 1998) and can alter the soil community. Native *S. scoparium* might have taken advantage of the changes in the fungal community composition more effectively than the non-native species. Alternatively, while not assessed in our current study, the accumulation of host specific pathogens could explain the increase in *S. scoparium* biomass in *B. ischaemum* soil in elevated temperatures with soil from elevated temperature. Plants in their native communities can accumulate host specific pathogens that contribute to negative feedback and to community succession (Bauer et al., 2015; Wang et al., 2017, Crawford et al., 2019). These host specific pathogens inhibit the growth of the host paving the way for colonization of other plant species. The release of *S. scoparium* from its host-specific pathogens would also result in the increase in S. scoparium growth in the non-native soil that led to the observed negative PSF. Either mechanism indicates that the native grass is able to utilize soil communities altered by the non-native more effectively relative to the non-native grass, when grown under elevated temperatures or following a soil legacy of elevated temperatures. We observed similar reversal of PSF direction in our cool-season species. The weaker PSF in cool-season grasses was driven by smaller, but consistent improvements in growth of both plant species in each other's soil communities. Given that these species are not strongly responsive to AM fungi (Wilson and Hartnett, 1998), we expect that these feedbacks are likely due to other soil biota, as pathogens and rhizobacteria are known to affect plant performance (Bever et al., 2012, Pineda et al., 2013; Rubin et al., 2017, Crawford et al., 2019). The impacts of pathogens, in particular, are likely to depend upon climate (Bever et al., 2015). This is supported by the consistent growth in each other's soils, as *B. inermis* was introduced into North America in the 1800's, and this length of time may be sufficient to adapt to local soil pathogens.

Similar to findings by Duell et al. (2016), various combinations of soil moisture and temperature did not affect biomass production of non-native *B. ischaemum*, and it consistently produced significantly greater biomass compared to native *S. scoparium*. This is not surprising, as *B. ischaemum* was introduced into the Great Plains as an improved forage, producing substantially greater biomass than many native grasses of similar stature. Additionally, while native *P. smithii* produced overall greater total biomass relative to non-native *B. inermis*, no differences were detected when analyzing by source training species, source temperature, and experimental temperature when grown under well-watered conditions. While we expected that both plant species would perform best when the climate legacy of a soil was matched with the current environment, but we did not see any evidence of microbial mediation of plant adaptation to the environment.

Our findings suggest that plant responses to warming temperatures and drought will be species-specific, and some invasives, such as *B. ischaemum*, will continue to produce large amounts of biomass relative to native species. We suspect that the presence of native plants will have a greater influence on the inhibition of non-native growth and establishment under predicted climate change scenarios. More research is required to confirm the extent to which soil environmental legacy affects the following year's PSF, especially in the context of moderate and severe drought. Furthermore, our study consisted of two pairings of functionally-similar native and non-native invasive prairie grasses, and additional species should be assessed to further our knowledge of the role of environmental soil training on invasive species PSF dynamics. There are still many questions surrounding plant invasion dynamics, and research such as our current study provide key insight into plant-soil-microbial interactions under projected climate regimes. Nevertheless, results from our two experiments suggest that when climate is consistent across years, soil microbes can contribute to coexistence of native and non-native plant species, while this does not occur when climate is variable across years. Further work on other plant species pairs and other environmental dimensions is required to test whether this is a general result.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

KZ, JB, and GW conceived and designed the research. ED conducted Experiment 1 and KZ conducted Experiment 2. KZ and JB analyzed the results. KZ and ED wrote the manuscript. All authors contributed the editing and preparation of the final product.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00419/full#supplementary-material

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Plant-Soil Feedbacks of *Plantago lanceolata* in the Field Depend on Plant Origin and Herbivory

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Soil biota involved in plant-soil feedbacks (PSFs) have an impact on the growth of plant individuals. So far, studies investigating the role of soil-biota mediated PSFs in plant performance were mostly performed in greenhouses and focused predominantly on species differences, whereas the contribution of PSFs to plant performance under field conditions and intraspecific variation in PSFs among plant populations remain poorly investigated. Here, we performed a PSF pot experiment under field conditions to study intraspecific variation in plant responses to soil biota. We used seeds from multiple seed families of *Plantago lanceolata* L. together with *Plantago*-conditioned soils from contrasting habitats (three non-fertilized pastures vs. three fertilized mown pastures) to test whether plants show a positive or negative response to their parental soil biota. We furthermore tested whether these PSFs depend on abiotic habitat factors and insect herbivory. To this end, we reciprocally transplanted plants and their soil biota between the two habitat types and excluded aboveground herbivores from half of the plants, respectively. When grown without herbivores, plants from both habitat types showed similar and neutral PSFs independently of the transplant site. In contrast, in the presence of herbivores, PSFs for plants from non-fertilized pastures were negative in both habitats (i.e., plants performed better when they grew with foreign soil biota), whereas PSFs for plants from fertilized mown-pastures remained neutral. Our results suggest that soil biota alone might only play a minor role for performance of *P. lanceolata* and that the outcome of soil-biota mediated PSFs is modulated by effects of herbivores in different habitats.

Keywords: aboveground-insect herbivory, eco-evolutionary feedback, intraspecific variation, land use, plant-soil feedback, reciprocal transplant experiment, soil biota

INTRODUCTION

For a better understanding of the interplay between ecosystem dynamics and evolution, we not only need to understand how species interact, but also how populations evolve in response to environmental drivers (Van Nuland et al., 2016; Ware et al., 2019a). The concept of eco-evolutionary feedbacks looks at the interaction and adaptation between plants and their biotic and abiotic environment (Bailey et al., 2009). So-called plant-soil feedbacks (PSFs; Bever et al., 1997) are an ideal system to investigate such eco-evolutionary dynamics, as soil microbiota have short

life-cycles and can quickly adapt while plant fitness is very closely linked to its interaction with soil biota (TerHorst and Zee, 2016). PSFs influence plant biomass production and performance and thus competitiveness and coexistence of species in plant communities (Klironomos, 2002; Van der Putten et al., 2013; Heinze et al., 2015a). On top of that, other environmental players, such as herbivory or habitat, can alter PSF responses (Heinze and Joshi, 2018; Pfennigwerth et al., 2018; Ware et al., 2019b). Simultaneously, PSFs may depend on the plant genotype, and different plant populations may evolve diverging PSF responses (Bukowski and Petermann, 2014; Wagg et al., 2015; Luo et al., 2016; Allen et al., 2018; Hawkins and Crawford, 2018). Given the ecological impact of PSFs on plant communities and the substantial intraspecific variation in PSFs, there is a need for an integrated approach that considers evolutionary- and ecological-scale processes simultaneously in order to improve our understanding of PSFs.

To measure PSF effects, experiments typically compare plant growth on "home" soils (i.e., self-cultivated) to plant growth on "away" soils (i.e., non-self-cultivated; Bever et al., 1997; Brinkman et al., 2010; Van der Putten et al., 2013). PSFs are positive when plant growth is greater on home than on away soils and negative when plant growth is greater on away than on home soils (Bever, 1994). Soil biota play an important role in PSFs (Ehrenfeld et al., 2005; Brinkman et al., 2010). Negative PSFs mostly result from the harmful effects of soil pathogens and herbivores such as fungal pathogens, insect larvae or nematodes, whereas positive PSFs follow from symbioses with mycorrhizal fungi and other microorganisms such as decomposers involved in nutrient cycling (Van der Putten et al., 2016). Across the literature PSFs are predominantly negative (e.g., Kulmatiski et al., 2008) suggesting that pathogenic or parasitic soil biota accumulate over time in "home" soils. Subsequently, these negative PSFs would lead to a decrease in competitive ability (Kardol et al., 2007) and would cause local rarity (Klironomos, 2002) or even extinction in local communities when competing with species that gain positive PSFs from soil biota. Similar to species in a community, populations within a species can respond differently to their soil biota as a result of evolutionary processes (Bukowski and Petermann, 2014; Wagg et al., 2015).

Agriculturally used grasslands provide an ideal system to study intraspecific variation in PSFs. Agricultural land use affects environmental conditions belowground by influencing abiotic soil properties (Alt et al., 2011; Birkhofer et al., 2012), which in turn influences the composition of soil biota (Herold et al., 2014). Plant roots, representing a large part of a plant's biomass (Yang et al., 2010), are exposed to these soil biota and interact with them. Research on such interactions has shown that land-use mediated changes in soil biota affect plant performance and coexistence (Heinze et al., 2015a,b). Plants also alter abiotic soil properties and soil biota through litter production, nutrient-uptake and exudation processes and hence create soil-legacy effects (Ehrenfeld et al., 2005; Van der Putten et al., 2013). Such initial plant-induced changes of soil properties can then influence the establishment and growth of subsequently establishing plants (Bever, 1994; Bever et al., 1997; Kardol et al., 2007). Interestingly, long-term land-use management may elicit evolutionary responses in soil biota, plants and their interaction, which has been well investigated for plants (Turesson, 1922; Warwick and Briggs, 1979; Silvertown et al., 2006). Moreover, additional factors, such as herbivores and habitat, can be influenced by land use as well (Gardiner and Hassall, 2009; Gossner et al., 2014; Simons et al., 2014) and may have evolutionary implications for PSFs. Therefore, including multiple environmental variables may help to understand the eco-evolutionary dynamics of PSFs under realistic environmental conditions.

The majority of PSF experiments have been performed in greenhouse conditions that fail to place PSFs in the context of environmental conditions that are likely to affect the role of plant growth in communities (Heinze et al., 2016). Relative to PSFs measured in greenhouse experiments, measurements of PSFs under field conditions have been found to vary mainly due to the diverse abiotic and biotic interactions that plants and soils receive under natural field conditions (Casper et al., 2008; Heinze et al., 2016). Hence, there is a need to perform PSF experiments under field conditions (Van der Putten et al., 2016), where biotic interactions, such as herbivory, are present. Under such natural conditions recent research found, for example, that aboveground insect herbivory modulated the outcome of PSFs (Heinze and Joshi, 2018) and that the PSF response increased with the intensity of herbivory (Heinze et al., 2019).

We performed a reciprocal pot transplant experiment in the field and investigated intraspecific variation in biotic PSFs (i.e., using inoculated standardized soils) in the widespread plant species, *Plantago lanceolata* L. We included six *P. lanceolata* populations, three each from two contrasting habitats with contrasting land-use intensity (non-fertilized pastures vs. fertilized mown pastures). In this experiment we also tested the effect of abiotic habitat factors by reciprocally transplanting plants into the two habitat types, and the effect of herbivore presence versus absence, using a herbivory-exclosure treatment, on the outcome of intraspecific biotic PSFs.

Specifically, we asked:

1. Do *P. lanceolata* populations differ in biotic PSFs between the two contrasting plant origins?

2. Are biotic PSFs of *P. lanceolata* affected by aboveground-insect herbivory and habitat?

3. Does herbivory damage to plants vary with respect to plant and soil origin?

4. What is the relative impact of soil biota (i.e., biotic PSFs) compared to aboveground-insect herbivory and habitat on the overall biomass variation?

MATERIALS AND METHODS

Study Site and Species

To test whether populations of *P. lanceolata* from two habitat types of contrasting land use show PSFs to local soil biota and how PSFs are affected by herbivory and abiotic habitat factors, we performed an experiment under field conditions in the UNESCO Biosphere Reserve Schorfheide-Chorin embedded in a glacial landscape in the lowlands of north-eastern Germany (**Figure S1A**). The experiment was conducted within

the framework of the Biodiversity Exploratories, a large-scale and long-term project investigating the effects of land use on biodiversity in Germany (Fischer et al., 2010). The two contrasting habitat types—non-fertilized pastures and fertilized mown pastures—were chosen, because the factors fertilization and mowing were shown to affect the composition of soil biota (Herold et al., 2014) as well as aboveground phenotypic trait differentiation of plants (Völler et al., 2013) on the sites of the Biodiversity Exploratories. For each habitat type, three sites were chosen resulting in three pairs of non-fertilized pasture vs. fertilized mown pasture (mean geographic distance between pairs: 1.3 km, **Figure S1B**).

The perennial herbaceous plant species, *Plantago lanceolata* L., was chosen as a model organism because it is widespread in both habitat types (Joshi et al., 2001). *P. lanceolata* is an outbreeding (Ross, 1973) and mostly wind-pollinated (Clifford, 1962) grassland species. In previous studies *P. lanceolata* has been shown to exhibit strong trait differentiation and adaptation to specific land use practices (e.g. Wolff and Delden, 1987; Van Tienderen and van der Toorn, 1991; Joshi et al., 2001). For instance, in a reciprocal transplant experiment using three populations from sites with contrasting land use, fitness was always higher in the home site and fitness-related traits such as flowering time varied (Van Tienderen and van der Toorn, 1991).

Seed and Soil Collection and Preparation

Seeds of *P. lanceolata* were collected in summer 2017 in all six sites from five randomly chosen individual plants, which were growing at least 1 m apart from each other. Seeds collected from a single plant individual were considered a seed family. To avoid microbial contaminations, seeds were surface-sterilized using 7% sodium hypochlorite (NaClO) solution (Heinze et al., 2017). Afterwards seeds were washed with autoclaved water (20 min, 121°C), germinated in sterilized (5-times in 24 h; 20 min, 121°C) sand (grain size: 2 mm; Brun and Böhm, Potsdam, Germany) in petri dishes (diameter: 9 cm) and placed in sterile plastic chambers (32 cm × 50 cm × 14 cm; Meyer; Germany) in a greenhouse at the University of Potsdam.

To investigate the effect of local soil biota on plants from different origins we used species-specific field-conditioned soils of *P. lanceolata* in accordance with the "natural experiment" approach (Kulmatiski and Kardol, 2008). Previous results from the Biodiversity Exploratories have shown that the land-use treatments affected abiotic soil properties (Alt et al., 2011; Birkhofer et al., 2012) and the composition of soil biota (Herold et al., 2014) on the chosen sites. In spring 2018 on the respective sites, similar to Heinze and Joshi (2018), soil material was collected from below *P. lanceolata* individuals located in the center of larger patches (diameter >20 cm) of *P. lanceolata* to make sure that the soil was conditioned by our target species and over longer time periods in both habitats. Following Brandt et al. (2014) we collected rhizosphere soil and soil directly adjacent to the rhizosphere from 20 individuals per site. After sampling, the soil was stored for 3 weeks at 4°C in the dark until use in the experiment.

PSF Experiment

Seeds and soils of plants from six study sites were used in an experiment to investigate intraspecific variation in PSFs and how effects of land use modulate PSF responses. Within the three habitat pairs (i.e., a non-fertilized pasture and a fertilized mown pasture), rhizosphere soils from the same habitat served as "home" soil, whereas soils from the contrasting habitat served as "away" soil. As we were interested in site-specific PSFs and not within-site variation in PSFs we mixed the 20 individual soil samples to one bulk sample per site. Although this mixing procedure has been criticized for its potential to increase the likelihood of falsely detecting PSFs by decreasing variance in plant responses among individual soil samples (Reinhart and Rinella, 2016) this procedure was appropriate for our specific research question. Furthermore, several studies reported that soil mixtures produce similar PSFs compared to independent soil samples and suggest that soil handling methods should be dependent on specific research questions and feasibility (e.g., Cahill et al., 2016; Kulmatiski, 2016; Gundale et al., 2019; Teste et al., 2019). To reduce potential differences in soil nutrient availability among soils and to focus on effects of soil biota we used the collected rhizosphere soils as inoculum (10%) into an autoclaved soil:sand mixture (Brinkman et al., 2010). The soil:sand mixture consisted of a 1:1 mixture of sieved (mesh size: 7 mm) field soil collected from a meadow at the field site of the University of Potsdam (N52° 24′ 29.76″, E13° 1′ 13.74″, Brandenburg, Germany) and purchased sand (grain size: 2 mm; Brun and Böhm; Potsdam, Germany).

Pots (Deepots D25: volume 0.41 L; height 25 cm; diameter 5 cm; Stuewe and Sons; USA) were prepared with an autoclaved fleece strip (6 cm × 25 cm) covering 10 cm of the pots' inside and extending 15 cm below the pot to enable continuous watering from below. The pots were subsequently filled with the inoculated soils. To limit cross-contamination of soil biota between the pots, each pot was placed in a separate plastic cup (volume 0.3 L; height 15.2 cm; diameter 5.9 cm) and received an additional layer (1 cm) of sterilized sand on top.

In May 2018, 1-week old seedlings were transplanted into the prepared pots. The planting scheme followed a reciprocal transplant design: per habitat pair, seedlings of both habitat types were planted in their home and away soils. Each plant origin × soil origin × herbivory treatment (see below and **Figure 1**) combination was replicated 10 times in each site, by including two offspring each from the five randomly chosen seed families per plant origin.

After planting, seedlings were transported to a protected outdoor location on the field site of the University of Potsdam and were allowed to acclimatize for 2 weeks.

Herbivory Treatment

To investigate the impact of aboveground insect herbivory on PSFs of the different plant origins we performed a herbivory-exclusion treatment in accordance with Heinze and Joshi (2018) and Heinze et al. (2019). This herbivore-exclusion treatment was applied on all six experimental sites. In each site we established two plots (120 cm × 160 cm) that were spaced 80 cm apart. Each plot was equipped with a cage (length 160 cm × width

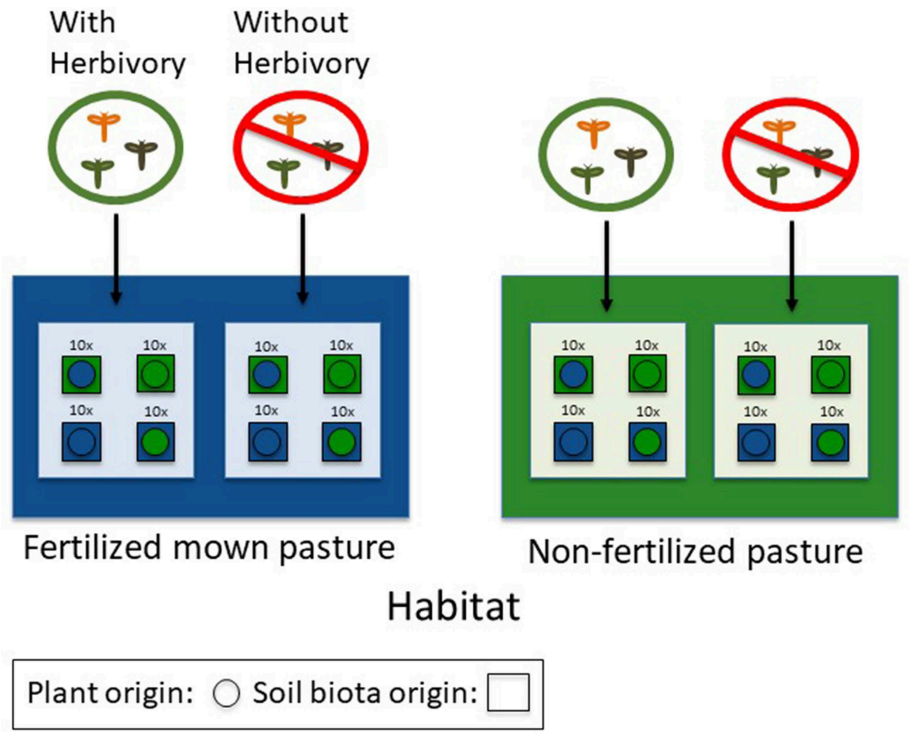

FIGURE 1 | Conceptual diagram of the experimental design. In three pairs of contrasting habitats (non-fertilized pastures vs. fertilized mown pastures) plants (circles) of different origin were reciprocally transplanted and grown in their home and away soils (squares) in the presence and absence of aboveground insect herbivores.

120 cm × height 100 cm) that was either completely covered with fly mesh (mesh size: 1.3 mm; Meyer; Germany) or only shaded by fly mesh on top. The fully covered cages excluded herbivorous insects (MacDonald and Kotanen, 2010), whereas the shaded cages allowed aboveground herbivorous insects to reach the experimental plants while providing the same levels of shade and precipitation as the cage treatment (Heinze and Joshi, 2018). In the fully covered plots, we removed the aboveground vegetation with the top 5 cm of the soil to exclude non-developed aboveground-herbivorous insects whose eggs might be attached to plants or buried in the soil. Furthermore, in the fully covered plots the fly screen was buried into the soil and one side was prepared as a door to water the plants. The fully covered plots that excluded aboveground herbivorous insects (>1.3 mm) are referred to as "without herbivory," whereas the shaded plots are referred to as "with herbivory" treatment throughout the manuscript.

Experimental Set-Up and Measurements

After 2 weeks of acclimatization in early-June, the prepared pots were brought to the six prepared sites. At each site, plants from both habitat types were grown in their home and away soil in the presence vs. absence of aboveground herbivory (**Figure 1**) resulting in a total of 480 experimental plants (3 habitat pairs × 2 sites × 2 plant origins × 2 soil origins × 2 herbivory treatments × 5 seed families × 2 replicates).

As we wanted to exclude direct competition between experimental plants and neighboring plants and as we were interested in the effects of aboveground invertebrates (excluding slugs) the experimental plants were placed in boxes (78 cm × 50 cm × 30 cm). The two replicates per treatment combination were divided between the two boxes at each plot and placed at random positions within those boxes. In that way each box contained one replicate for every treatment combination, either in the with herbivory treatment or the without herbivory treatment. At all sites the experimental set-up was fenced (3 m × 3 m) to prevent damages by cattle.

During the experiment, plants were watered every third week. After 12 weeks in the field, damage by aboveground chewing insects without further discrimination of feeding guilds was visually estimated for whole plants. Afterwards shoot biomass was harvested and roots were washed. Root and shoot biomass were dried to constant weight (minimum 48 h at 80°C) and kept in the drying oven until it was weighed.

Statistical Analysis

To compare plant performance on their home vs. away soil, PSFs were calculated for each seed family individually. PSF values were calculated using total plant biomass (shoot + root). To obtain a quantitative measure of PSFs, where positive values show positive PSF and negative values negative PSF, respectively, the log ratio of home vs. away biomass was calculated (Brinkman et al., 2010). Home biomass was the biomass of a certain seed family on its own soil and away biomass was the biomass of the same seed family on the soil from the contrasting site.

Due to the random distribution of the pots in the boxes a block-wise analysis was not appropriate. Therefore, in accordance with Heinze et al. (2016) we calculated PSFs using all possible combinations of seed family-specific biomass production of one seed family in home soil in comparison to biomass production of this seed family in away soils (Equation 1).

$$PSF_{A\,1} = \frac{1}{n}\sum_{i=1}^{n}\log\left(\frac{home_{A\,1}}{away_{B\,i}}\right) \quad (1)$$

For each replicate of the six plant origins, the home/away ratio in multiple comparisons with all 10 replicates in the same away group was calculated.

The PSF values were used in a linear mixed-effects model, testing for effects of the experimental treatments. Since the factor soil origin was already incorporated in our calculation of the PSFs, the factors plant origin (non-fertilized pasture vs. fertilized mown pasture), habitat (idem) and herbivory (with vs. without) and all possible interactions were included as fixed factors whereas habitat pair was included as a random factor.

To test for the effect of the herbivory treatment, a linear mixed-effects model was performed using the estimated percentage of feeding damage on aboveground biomass as response variable against all the factors included in our design, i.e., plant origin, habitat, soil origin and herbivory, and all possible two-way interactions. We included habitat pair and seed family as random factors in this model.

To determine the amount of variance in total plant biomass explained by the experimental treatments, a variance component analysis was performed using a linear mixed-effects model in which the same explanatory factors were included as in the model for feeding damage, but now all factors were treated as random factors.

The residuals of all models were checked for normality of distribution and homogeneity of variance. Data analysis was performed using R version 3.4.2 (R Core Team, 2017) and the lme4 package (Bates et al., 2014).

RESULTS

Biotic PSFs Between Contrasting Plant Origins in the Absence or Presence of Aboveground Insect Herbivory

In our PSF model, plant origin as well as its interaction with the herbivory treatment had a significant influence on PSFs, while other factors remained non-significant (**Table 1**). To explore the environmental interactions in more detail, we performed two separate models, one using data for plants excluded from herbivory and the other on data of plants exposed to natural herbivory.

When grown without herbivores, biotic PSFs of plants from both habitat types were neutral, independently of transplant site (**Table 2**; **Figure 2A**). In the presence of aboveground insect herbivores, however, the plants from non-fertilized pastures showed negative biotic PSFs in both habitat types, whereas the PSFs for plants from fertilized mown pastures remained neutral (**Table 2**; **Figure 2B**).

TABLE 1 | Results of linear mixed-effects model for PSFs.

Source of variation	NumDF	DenDF	*F*	*P*
Plant origin	1	232	7.73	**0.0059**
Habitat	1	232	0.60	0.4379
Herbivory	1	232	0.73	0.3949
Plant origin × Habitat	1	232	0.10	0.7582
Plant origin × Herbivory	1	232	5.17	**0.0239**
Habitat × Herbivory	1	232	1.86	0.1735
Habitat × Plant origin x Herbivory	1	232	1.17	0.2803

The model tested for all the factors included in our study design, except for soil origin which is already incorporated in the calculation for the PSF quantification. Those are plant origin, habitat (non-fertilized pastures vs. fertilized mown pastures) and herbivory (with vs. without). PSF was calculated using log(biomass on home soil / biomass on away soil). Significant P-values (P < 0.05) are indicated in bold typeface.

TABLE 2 | Results of linear mixed-effects models for PSFs separated by herbivory treatment.

			Without herbivory		With herbivory	
Source of variation	**NumDF**	**DenDF**	***F***	***P***	***F***	***P***
Plant origin	1	114	0.14	0.7124	12.30	**0.0007**
Habitat	1	114	2.45	0.1201	0.17	0.6841
Plant origin × Habitat	1	114	0.32	0.5727	0.93	0.3367

The model incorporated the factors plant origin, habitat (non-fertilized pastures vs. fertilized mown pastures) as well as their interaction. PSF was calculated using log(biomass on home soil/biomass on away soil). Significant P-values (P < 0.05) are indicated in bold typeface.

Herbivory Damage

In general, damage by aboveground herbivorous insects on experimental plants was low (1.02% ± 0.07, **Figure 3**) and was unaffected by soil biota [$F_{(1,\,340)} = 0.07$; $P > 0.5$; **Table S1**]. Although some feeding damage was observed in the without-herbivory plots, the percentage of aboveground feeding damage was significantly higher in the with-herbivory plots [$F_{(340,\,7)} = 13.79$; $P = 0.0002$; **Table S1**; **Figure 3**], but only in the fertilized mown pastures (**Figure 3**). Damage in the with- and without-herbivory plots was similar in the non-fertilized pastures (**Figure 3**). These different herbivory effects had, however, no significant effect on plant biomass in this experiment (see below and **Table 3**).

Relative Impact of Treatments on Plant Performance

Overall, the factors –and their interactions– included in the study design together explained less than 10% of the variation in biomass of the experimental plants (**Table 3**). Out of these factors the origin of soil biota accounted for most of the variance in biomass production (4.9%). The interaction between plant origin and habitat accounted for 2.0% of the variance whereas all other factors and their interactions explained less than 1% (**Table 3**).

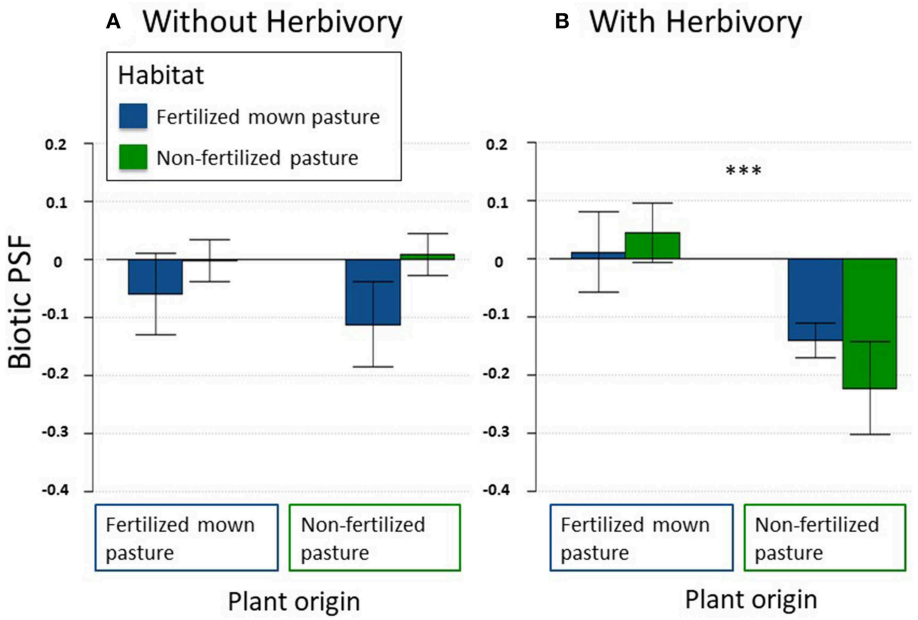

FIGURE 2 | Biotic plant-soil feedback [PSF; log(biomass on home soil/biomass on away soil)] of *P. lanceolata* grown without **(A)** and with **(B)** aboveground herbivorous insects. Within graphs, left bars represent plants that originated from fertilized mown pastures, whereas right bars represent plants from non-fertilized pastures. The habitat the plants grew in during the experiment is indicated by colors (blue: fertilized mown pastures, green: non-fertilized pastures). Data represent mean ± SE ($n = 30$). Asteriks between bars represent signifigance, ***$P < 0.001$.

DISCUSSION

PSFs Without Aboveground Herbivores

We found a main effect of plant origin on PSF as well as an interaction between plant origin and herbivory. When focusing on the results when natural aboveground insect herbivory was excluded, i.e., the sole effect of soil biota, no differences in intraspecific PSFs for populations of *P. lanceolata* were observed. Although only scarcely treated in the literature, there are examples of studies that found intraspecific variation in PSFs (Wagg et al., 2015; Luo et al., 2016; Eck et al., 2019). For example, Smith et al. (2012) found seedling survival of *Populus angustifolia* to be 2.5 times higher in soil biota from the rhizosphere of their mother tree, but neutral PSFs as well have been found (Bukowski and Petermann, 2014; Allen et al., 2018). However, our study did not test for individual effects of maternal soil, but for land-use effects (i.e., fertilization etc.) on soil biota and how different intensities of land use might influence PSFs in a population.

Fertilization is known to influence the composition of soil biota (Herold et al., 2014) and thus the relative abundance of fungi and bacteria in soils (van der Heijden et al., 2008). Although we used soils from non-fertilized vs. fertilized sites in our experiment, we did not observe differences in PSFs when aboveground insect herbivores were excluded. Either the contrasting land-use regimes did not create as much differentiation in local soil biota as expected or there were indeed differences in local soil biota, but the plants respond neutrally to them. We do not know the effects of soil biota *per se*, since a soil inoculum was used without an unconditioned or sterilized control soil to test against the effect of soil biota. Although the inoculation technique is optimal to investigate effects of soil biota (e.g., biotic PSFs; Brinkman et al., 2010) there are also several disadvantages. For example, inoculation into sterilized background soil might dilute the soil biota to an extent where effects, actually present in nature, are not detected anymore (Brinkman et al., 2010). Furthermore, since populations of bacteria grow much faster than those of fungi, the latter would decrease in their ratio when adding the inoculum to standardized soil. This effect could further minimize the effect of fungi in our experimental set-up. However, in contrast to other studies working on intraspecific PSFs using experimental conditioning, we used field-conditioned soil according to the "natural experiment" approach (Kulmatiski and Kardol, 2008) that is suggested to better reflect the soil communities in the field (Brinkman et al., 2010). Furthermore, with regard to our questions, the approach appeared favorable, as land use was shown to create substantial and long-lasting changes to the microbial soil community (Jangid et al., 2011; Dupouey et al., 2013) and thus soil legacies that cannot be changed via short-term conditioning with the targeted plants under artificial experimental conditions (Kulmatiski and Beard, 2011).

In general, experimental growth conditions –controlled conditions in greenhouses or climate chambers vs. field conditions– were shown to affect plant physiology (Ferreira et al., 1995) and growth (Mishra et al., 2012) as well as the development and composition of soil biota (Ge et al., 2011). In the field, harsh abiotic conditions (e.g., wind; Whitehead and Luti, 1962; Nicoll and Ray, 1996), fluctuating light regimes (Hunt and Burnett, 1973) as well as temperature (thermoperiodicity; Went, 1974) may increase biomass allocation to roots which are suggested

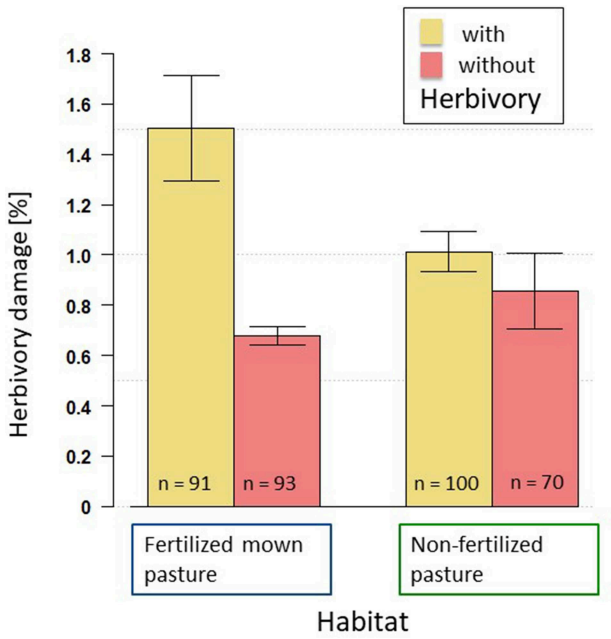

FIGURE 3 | Visually estimated damage by aboveground insect herbivores on experimental plants in the with-herbivory plots (yellow) and without-herbivory plots (orange) grown in fertilized mown pastures (left bars) and non-fertilized pastures (right bars).

TABLE 3 | Results of a variance component analysis for total plant biomass using a linear mixed-effects model, testing all factors included in our study design (plant origin, habitat, soil origin, and herbivory) and all possible two-way interactions.

Source of variation	Variance component (%)
Plant origin	<0.01
Habitat	0.69
Soil origin	4.90
Herbivory	0.00
Habitat × Plant origin	2.00
Soil origin × Plant origin	0.44
Habitat × Soil origin	<0.01
Plant origin × Herbivory	<0.01
Habitat × Herbivory	0.00
Soil origin × Herbivory	0.85
Residuals	91.10

The percentage shows the amount of total variance explained.

to modulate interactions between plants and soil biota (Bardgett et al., 1998). Hence, our field experiment testing for intraspecific PSF effects fulfilled the need to consider PSFs under more natural conditions (Van der Putten et al., 2016).

The absence of a response to local soil biota under natural conditions –but without aboveground insect herbivory– does not imply that other environmental factors do not affect the performance of plants (see below). Further studies on PSFs might incorporate other important factors affecting plant growth and soil biota such as abiotic soil conditions that are influenced by land use (Alt et al., 2011; Birkhofer et al., 2012). However, we included one important environmental factor—aboveground insect herbivory—in our experiment that was shown to affect the outcome of PSFs under field conditions (Heinze and Joshi, 2018), depending on the intensity of herbivory (Heinze et al., 2019).

PSFs With Aboveground Insect Herbivory

When experimental plants were exposed to natural aboveground insect herbivory, PSFs became negative for plants from the non-fertilized pastures but remained neutral for plants from the fertilized mown pastures. Such neutral to negative PSFs were often found in grasslands (Ehrenfeld et al., 2005; Kulmatiski et al., 2008; Van der Putten et al., 2016). In our study on intraspecific variation in PSF, the performance of *P. lanceolata* from non-fertilized pastures increased when growing on away soils, but only when insect herbivores were present. This result indicates that *P. lanceolata* harbors intraspecific variation in PSF between populations with contrasting land use. This is a remarkable result given that the six study sites were located in a geographically small area and that gene flow is thus likely to be substantial among the study populations of this wind-pollinated outcrossing species. We thus propose that natural selection for differential PSF effects in the respective sites is stronger than the counteracting effects of gene flow. Simultaneously, it may be that gene flow allows the plant populations to evolve resistance against rapidly evolving antagonistic soil organisms. Both processes suggest a strong ecological significance for the observed intraspecific differences in PSF and implies that eco-evolutionary feedbacks play a role in shaping these grassland ecosystems.

The mechanism behind the negative PSF effects remains unclear. Since aboveground insect herbivory obviously has a direct effect on shoot biomass, it is important to assess whether our results could be driven purely by shoot biomass loss. When testing for an effect of soil origin under the presence of insect herbivory on shoot and root biomass separately, we found that root biomass was more strongly affected than shoot biomass (**Figure S2**). Moreover, aboveground herbivory itself significantly affected root biomass but not shoot biomass (**Table S2**). Since root biomass can only be indirectly influenced by aboveground herbivory, the observed negative PSFs on total biomass under the influence of herbivory is thus—at least in part—indirectly caused by aboveground herbivory and seems to involve changes in biomass allocation by the plant as the integrative result of both soil biota and insect herbivory.

Similarly, Mursinoff and Tack (2017) found that the response of *P. lanceolata* to local vs. foreign soil differed under the presence of a specialist leaf pathogen. Also Zhu et al. (2018) found similar effects in these plant-soil-herbivore interactions in *P. lanceolata*. Interestingly, some studies revealed that herbivory can change plant-soil-fungi interactions (Bardgett and Wardle, 2003; Bennett et al., 2009; Kostenko et al., 2012), while other studies demonstrated effects of plant-soil fungi interactions on herbivory (Fontana et al., 2009). From our results we cannot infer the exact mechanisms acting here, i.e., how the three parties are influencing each other. Further experiments are

needed to elucidate the causal directions between herbivory and soil biota through *P. lanceolata* in our system and to understand the evolutionary drivers shaping this intraspecific variation in PSF. We speculate that the negative relationship between insect abundance and land-use intensity (Simons et al., 2017) as well as the negative relationship between symbiotic soil fungi and land-use intensity (de Vries et al., 2006) may act as selection pressures causing the observed intraspecific variation in PSF.

An important result is that insect herbivory was needed to elicit differential responses to soil origin between plants from different habitats. Although strictly speaking PSF solely involves plants and their soil biota and is ideally measured under controlled experimental conditions such as a greenhouse, it is clear that such approaches take plants and their associated soil biota out of their natural ecological context. By conducting PSF experiments under more natural conditions, we may be better able to assess the significance of various ecological drivers and their interactions, although the complexity of such experiments increases as well.

Relative Effect of Treatments on Plant Performance

With 4.9%, soil differences explained a small, but nevertheless the biggest part of total variance in our variance component analysis. Interestingly, differences in habitat only accounted for 0.7% of explained intraspecific variation in *P. lanceolata*. Assuming that land use is the primary driver of the differences in soil biota, this would imply that results of reciprocal transplant experiments may be driven substantially by soil biota rather than abiotic habitat effects. This is interesting given that data on the importance of climate vs. soil in plant local adaptation is scarce (Macel et al., 2007). However, it should be noted that the six study sites were located in a geographically small area and variation in microclimate among the habitats is therefore presumably small and solely influenced by land-use effects on biotic factors such as vegetation height. The interaction between habitat and plant origin accounted for 2% of the explained variation. Although such an interaction indicates variable responses of plants from different origins when transplanted to different habitats and may suggest local (mal-)adaptation (Kawecki and Ebert, 2004), this factor was not significant in our PSF model (**Table 1**) and does not reflect a consistent pattern among habitat pairs.

CONCLUSION

Our experiment investigated the effects of soil biota on intraspecific variation among plant populations under natural conditions. We did not consider plants and their soils in isolation, but included their interaction with other biotic and abiotic factors. Our study therefore contributes to the growing literature considering eco-evolutionary feedbacks to explain how complex interactions between multiple parties influence ecological and evolutionary dynamics (Van Nuland et al., 2016; Ware et al., 2019a).

The most interesting finding of our study is that intraspecific differences between populations of *P. lanceolata* from sites with contrasting land-use intensity only became apparent when plants were exposed to insect herbivory. Thus, our study offers support to the theory that interactions between plants and soil-microbiota can be mediated through aboveground-herbivores and the responses they induce in plants. Additionally, our study is among the first to show such complex interactions under field conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AK, JJ, JS, and JH designed the study. LK, AK, JS, and JH collected seeds and soils, performed the experiment, and measured the data. LK, JS, and JH analyzed the data and wrote the first draft of the manuscript. LK, AK, JJ, OB, JS, and JH contributed to later versions of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00422/full#supplementary-material

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Plant-Soil Feedbacks and Facilitation Influence the Demography of Herbaceous Alpine Species in Response to Woody Plant Range Expansion

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Plant species migrations, or range shifts, in response to changing climate are one of many interacting factors influencing plant population and community dynamics in an era of global change. Range shifts may cause novel assemblages of competing species because species may respond to changing climate at different rates. Range-expanding species may directly influence resident species through resource competition or indirectly by modifying the local environment both aboveground and belowground. Further, range-expanding plant species can create novel plant-soil feedbacks (PSFs) by altering soil microbial community structure and function and the interactions of resident plant species with microbial symbionts. These changes can have important implications for resident plant population dynamics and their ability to coexist with novel competitors. Here we test the impacts of competitive interactions and plant-soil feedbacks (PSFs) of a range-expanding sagebrush species (*Artemisia rothrockii*) on the demography and population growth rates of two resident alpine plant species (*Koeleria macrantha* and *Eriogonum ovalifolium*). We use an experimental, multi-year field approach combined with integral projection modeling to determine how PSFs and competition influence species coexistence in both the historic and range expansion zone of *A. rothrockii*. We find that sagebrush has an overall net negative effect on herbaceous plant demography, primarily due to negative PSFs for plants growing in sagebrush-conditioned soil. However, these negative soil effects are partially buffered via facilitation effects for herbs growing under or nearby sagebrush canopies. In general, population growth rates were more sensitive to survival than other demographic rates, furthermore this sensitivity to survival was higher for herbaceous species in sagebrush soils. Identifying the major drivers of plant population dynamics and species interactions remains an important and unresolved question in ecology. PSFs are a central mechanism influencing plant species interactions, yet the majority of PSF research has made little direct connection between plant population dynamics and PSFs *in situ.* We believe that utilizing a field-based approach, focusing on multiple components of plant demography, is an important next step in understanding the role of PSFs and species interactions in a changing world.

Keywords: plant-soil (belowground) feedbacks, range expansion, alpine, global change, demography, population dynamics, woody plant encroachment, integral projection model (IPM)

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INTRODUCTION

Changes in climate and land use are creating novel communities of organisms around the globe (Tylianakis et al., 2008; Lurgi et al., 2012). In terrestrial ecosystems, species migrations or range shifts, often upwards in latitude or elevation, are an important mechanism driving these changes (Parmesan et al., 2003; Valéry et al., 2008). Species ranges may become larger (expansion), smaller (contraction), or simply shift in their distribution along a climate or land use gradient (Sexton et al., 2009; Chen et al., 2011). Range shifts are limited by both environmental and biotic filters as well as species dispersal abilities (HilleRisLambers et al., 2012) and many species are unable to migrate (Zhu et al., 2012). Together, these changes in species distributions are "reshuffling" the composition of plant communities, and often have significant community and ecosystem consequences, such as altered nutrient cycling and net primary production (Wardle et al., 2011; Alexander et al., 2016; Manrubia et al., 2019).

Competitive interactions between local and range-expanding plant species will influence both the ability of the range-expanding species to successfully establish and the capacity of resident plant species to persist within their historic distribution (Körner et al., 2008; Alexander et al., 2016; Fadrique and Feeley, 2016). Successful range shifts may require strong competitive abilities, while lack thereof may limit a species' ability to colonize a new area (Krapek and Buma, 2018; Neuschulz et al., 2018). In fact, novel plant competitors were equally or more influential than warming on plant performance in plant community transplants across an alpine elevation gradient (Alexander et al., 2015). The outcomes of species interactions are determined by coexistence mechanisms including the balance between inter- and intraspecific competition and negative density dependence (Callaway et al., 1997; Chesson, 2000; Mangan et al., 2010; Piao et al., 2014). Assessing the influence of range-expanding competitors on the demography and population dynamics of resident plant species will be critical to predicting whether resident and range-expanding species will successfully coexist.

In addition to altered competitive interactions, species range shifts may have indirect effects on resident plants through altering the local environment or trophic interactions. For example, range-expanding plant species can modify local resource pools, microclimate conditions, densities of species-specific herbivores or pollinators and interactions with soil organisms (Tylianakis et al., 2008; Metcalfe et al., 2011). Plant-soil-feedbacks (PSFs) are plant-induced changes to the soil which feedback to affect plant performance (Van der Putten et al., 2013). PSFs can play an important role in shaping plant species interactions and promoting species coexistence (Bever et al., 1997, 2012; Bever, 2003). For example, PSFs can help maintain species diversity by enhancing negative soil feedbacks on conspecific individuals via the accumulation species-specific soil pathogens (Bever, 2003). On the other hand, positive PSFs can lead to competitive exclusion and species dominance, thereby reducing overall diversity (Bever, 2003). Non-native invasive species often create PSFs which further promote their invasion, including reducing the diversity of mycorrhizal fungi or soil mutualists of resident species (Hawkes et al., 2006), enhancing native soil pathogens (Eppinga et al., 2006), or selecting for microbes which preferentially degrade their own litter (Austin et al., 2014). Range-shifting plant species can also influence resident plant species via PSFs (Dostálek et al., 2016) and changes in mycorrhizal dominance (Williams et al., 2013), however further information is necessary to determine under which range-expansion scenarios this will occur (Tomiolo and Ward, 2018).

Range-expanding species that are functionally dissimilar to the native plant community may create strong PSFs, as plant origin alone (native vs. range expanding) does not necessarily predict impacts on soil microbial communities (Manrubia et al., 2019; Ramirez et al., 2019). These PSFs may arise through multiple mechanisms, including changes in the quantity or chemistry of leaf and root litter entering soil organic matter pools, changes to soil hydrology via rooting depth and structure, or association with novel microbial mutualists or pathogens (Klironomos, 2002; Wardle et al., 2004). For example, Mesquite trees expanding into desert grasslands associate with N-fixing bacteria and have deep taproots, thus altering soil nutrient pools, microbial communities and water availability for resident grasses (Wilson et al., 2001). Novel secondary compounds in litter of range-expanding species can also alter interactions of other plants with mycorrhizal fungi and free-living soil microbes (Weaver and Klarich, 1977; Nilsson et al., 1993; Wardle et al., 1998), creating potentially positive or negative PSFs.

Finally, PSFs can alter many components of the plant life cycle, including growth, survival, and reproduction, however the majority of PSF research has only considered effects on plant growth or biomass (Hovatter et al., 2013; Dudenhöffer et al., 2017). For example, seed germination may be limited by species-specific pathogens, particularly in close proximity to conspecific individuals (Mangan et al., 2010) and flower production can be enhanced by spatial heterogeneity of PSFs (Burns et al., 2017). Additionally, PSFs may cause contrasting responses across distinct phases of the plant life cycle, such as increased growth or vegetative biomass but decreased seed germination or flowering (Mehrabi et al., 2015; Dudenhöffer et al., 2017) creating an overall neutral effect on plant fitness. Therefore, all demographic life stages need to be simultaneously considered for a complete picture of how PSFs influence plant population dynamics (Dudenhöffer et al., 2017).

Woody plant range shifts are occurring in mountainous regions globally due to a variety of global change drivers including warming temperatures, increased CO_2, altered precipitation, and changes in fire and grazing regimes (Myers-Smith et al., 2011). In the White Mountains of California, climate and land use change has led to an upward range expansion of a dominant subalpine shrub species, *Artemisia rothrockii* A. Gray (Rothrock sagebrush) into alpine grasslands over the last 60 years (Kopp and Cleland, 2014). This range expansion has coincided with decreased abundance of a native bunchgrass [*Koeleria macrantha* (Ledeb.) Schult] and cushion plant (*Eriogonum ovalifolium* Nutt.), however the mechanism(s) of these species' declines are unknown (Kopp and Cleland, 2014). We sought to determine the relative importance of direct competition with sagebrush vs. indirect soil effects, a form of

apparent competition, for driving the decline in abundance of *K. macrantha* and *E. ovalifolium* in the White Mountains.

Specifically, we asked: Does sagebrush range expansion influence the demography of native alpine plant species in the White Mountains? Are sagebrush influences on demography and population growth rates (lambdas) via direct competition and/or apparent competition via PSFs, and what are the relative strengths of these mechanisms? We hypothesized that sagebrush creates negative PSFs for *K. macrantha* and *E. ovalifolium,* which manifest in lower demographic and population growth rates for plants growing in sagebrush soil. Inducing negative PSFs is a common mechanism by which non-native invasive plants gain a competitive advantage over resident species (Suding et al., 2013), and we extend this line of reasoning to a native range-expanding species. We predicted that the negative effects of PSFs will be stronger than the effects of direct competition with sagebrush because competitive interactions can be weak or shift to facilitation in stressful abiotic conditions, such as alpine environments (Callaway et al., 2002; Maestre et al., 2009).

MATERIALS AND METHODS

Location

This study takes place in the subalpine to alpine zones of the White Mountains of California, which lie on the western edge of the Great Basin in the rain shadow of the Sierra Nevada range. The climate is cold and dry, receiving between 327 and 456 mm of precipitation annually and mean annual temperatures span from 0.9 to −1.7°C (Hall, 1991). These mountains have extremely diverse soil histories (Mooney and Zavaleta, 2016) but this study was confined only to granitic soils (Colluvium derived from granite) and east-/south-east-facing slopes to control for edaphic and topographic variation. Abiotic soil characteristics across the elevation gradient and plant communities of this area are summarized in Collins et al. (2016), but in general, soils have low levels of organic matter (∼1.7–2.6 mg/L TOC, 0.8–0.34 mg/L TON) and low soil moisture (∼1.9–10.3% VWC), which both increase with elevation. Soil pH is slightly acidic (∼6) across the study area.

Study Species

We conducted this study across a gradient of *A. rothrockii* range expansion from subalpine (<3,500 m) to alpine (>3,500 m) zones that has been documented in the White Mountains over the last 50 years (Kopp and Cleland, 2014). In 1961, *A. rothrockii* was not present at the 3,800 m site, was found in moderate to low densities at the 3,500 m site, and high densities at the 3,200 m site (Mooney et al., 1962; Kopp and Cleland, 2014). Currently, it is found at high densities, but with more spacing between individuals (∼1–2 m) at the low and middle elevation sites, and in isolated circular patches ∼10 m wide but with shrubs more closely clustered at high elevation sites (Kopp and Cleland, 2018, personal observation). Therefore, this gradient spans from the "historic range" of A. rothrockii at low elevations to the leading edge of the "expansion range" at high elevations where *A. rothrockii* transitions from an almost continuous population to isolated patches.

Artemisia rothrockii is a California endemic shrub, while *K. macrantha* and *E. ovalifolium* are more widespread throughout the western United States and span a wide habitat range from pinyon juniper woodlands to subalpine forests, and alpine fellfield (Calflora, 2019). Sagebrush, primarily *A. tridentata*, is known to strongly compete with herbaceous plant species for water and nutrients, particularly phosphorus, across the inter-mountain west (Robertson, 1947; Caldwell et al., 1985; Fowler, 1986; Ryel et al., 2004). Sagebrush also directly alters the abiotic soil environment including enhancing water and nutrients below its canopies as compared to herbaceous soils (Collins et al., 2016). Additionally, sagebrush alters the soil microbial community structure and function, including the diversity and community composition of soil bacteria and fungi, substrate induced respiration (CO_2 flux) and extracellular enzyme activity (Collins et al., 2016, 2018, and unpublished data).

These changes are likely to have important feedbacks on herbaceous plant species by altering the relative abundances of microbial taxa, such as species-specific soil mutualists and pathogens. In addition, secondary compounds in sagebrush litter may alter how herbaceous plants (grasses, forbs etc.) interact with mycorrhizal fungi and free-living soil microbes (Weaver and Klarich, 1977; Nilsson et al., 1993; Wardle et al., 1998). Aboveground sagebrush removal led to the re-establishment of herbaceous cover (including *K. macrantha* and *E. ovalifolium)* after 4 years in the White Mountains suggesting potentially high levels of interspecific competition (Kopp and Cleland, 2018). However, indirect soil effects of sagebrush on alpine plant growth, a form of apparent competition, may be as strong or stronger than the direct effects of competition with sagebrush (Allen et al., 2018).

Experimental Design

We monitored populations of *K. macrantha* and *E. ovalifolium* at three elevations described previously: 3,200, 3,500, and 3,800 m (3,200 m site: UTM: 396148 E; 4151156 N; 3,500 m site: UTM: 390629 E124157248 N; 3,800 m site: UTM: 390445 E; 4159559 N-UTM Zone 11). In July 2015, we established 30 experimental blocks, each with four 0.5 × 0.5 m plots (treatments) and each block was repeated 5 times for each species at each elevation (site). Each plot has one of the following 4 treatments: shrub competition, shrub removal, herbaceous competition and herbaceous removal (**Figure 1**). Shrub plots (competition and removal) were selected where individuals of *K. macrantha* and/or *E. ovalifolium* were growing directly under or very nearby (<0.25 m) a sagebrush canopy. Herbaceous plots (competition and removal) were selected in the interspaces of sagebrush between 1 and 5 m away from the nearest shrub canopy, based on the sagebrush density at each site. For competition plots, the entire plant community was left intact. For shrub removal plots, aboveground sagebrush biomass was removed by cutting down stems at the base. For herb removal plots, aboveground biomass of all non-target herbaceous plant species was removed by manually clipping with scissors. For both removal plots, only aboveground biomass was removed to prevent significant disturbance to soil structure. All treatments were maintained annually, and any regrowth trimmed back.

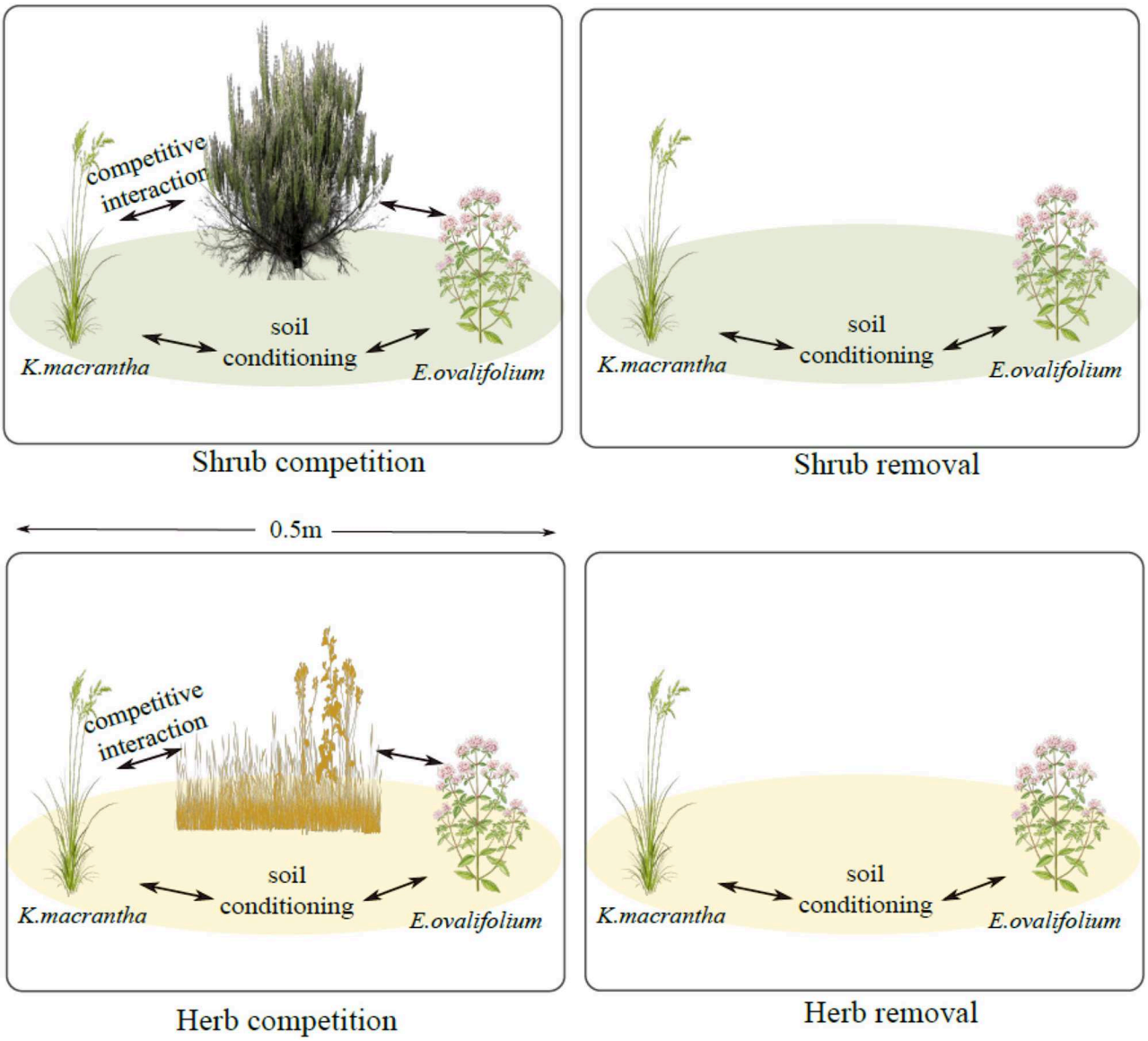

FIGURE 1 | Experimental plot design for field demographic measurements. Green ovals represent sagebrush soil conditioning and yellow ovals represent soil conditioning by the herbaceous community.

Our experiment was designed to disentangle above- and belowground influences of sagebrush on herbaceous plant demography under natural field conditions. Plants growing in removal plots are experiencing soil legacies (both biotic and abiotic) of either the shrub or the herbaceous community, but without aboveground competition from the removed species. In the non-removal plots (competition), plants are experiencing both the soil conditioning and competition from shrubs and/or herbs. By comparing performance of the focal species in the shrub plots and the shrub removal plots, we can therefore isolate the effect of shrub competition (**Figure 1**). Similarly, by comparing the herbaceous plots to the herb-removal plots, we estimated the effects of herb competition. To estimate the effects of shrub PSFs, in the absence of shrub competition, we compared the shrub removal plots to the herbaceous removal plots, because the primary difference between those plots was the identity of plants conditioning the soil. Sagebrush, soil conditioning overwhelms that of herbaceous plants in its litter chemistry and biomass, and creates a distinct soil environment underneath its crown (Welch, 2005; Collins et al., 2016) and therefore we attribute soil effects to sagebrush directly. However, we do not estimate PSFs of the herbaceous community, as the species composition was more variable across sites, and soil conditioning is much less concentrated than under shrub canopies.

Field-based approaches for measuring plant-soil feedbacks have been used successfully in other studies (Kulmatiski, 2006;

Mccarthy-Neumann and Ibáñez, 2012). Nonetheless, there are trade-offs to this approach relative to more traditional controlled experiments in a greenhouse or common garden. A strength of this approach is that soils are conditioned in the field, under natural climate conditions and over longer time periods, creating a more realistic soil environment (Kulmatiski and Kardol, 2008; Pernilla Brinkman et al., 2010). Additionally, this approach does not risk spurious effects that can occur with soil sterilization (Bonanomi et al., 2005). A limitation of a field-based approach, however, is that is not possible to disentangle the influences of soil microbial communities and the physical soil environment in the field (Kulmatiski and Kardol, 2008; Pernilla Brinkman et al., 2010). Thus, our estimated PSFs include all physio-chemical and microbial changes caused by sagebrush soil conditioning to be plant-soil feedbacks potentially influencing plant demography.

Demographic Measurements

Within each of the four plots (treatments) in all blocks, we tagged up to five adult individuals, depending on species density at the site, of either *K. macrantha* or *E. ovalifolium* and took initial demographic measurements in July 2015. For *K. macrantha*, plant area was calculated by multiplying height of the tallest leaf (cm) by width of the tussock (cm). For *E. ovalifolium*, plant area was calculated through digital image analysis. Photos of each individual plant were taken with a ruler for scale in the field and were then analyzed in ImageJ (Version 1.51 J8) based on the methodology of Jarou (2009). For both species, we measured flowering status (Y/N), and number of inflorescences of each flowering individual. Seed production per inflorescence was calculated as a single value for each species on 100 additional inflorescences which were counted in the laboratory using a dissecting microscope to ensure seed maturity/viability. Beginning in 2016, mortality was also recorded as alive (Y/N) for each individual. Plots were re-sampled yearly in mid-July to early August (depending on snow melt) and all measurements taken for three subsequent years, for a total of four years of measurements (2015, 2016, 2017, 2018).

Recruitment probabilities were estimated using seed germination trials for each species. In September 2017, mature seeds from both species were collected from 10 individuals at each elevation. Seeds were placed in 12 × 12 cm mesh bags and then deployed in the field by fixing them to the upper soil surface using metal stakes. Each bag contained 10 seeds and for each species, 12 bags were deployed at each elevation site, six under sagebrush canopies and six in shrub interspace. Bags were collected in mid-July 2018, and total number of germinated seeds in each bag were recorded. Probabilities were calculated as the total of germinated seeds/sum seeds deployed. Due to low overall germination, single probabilities were calculated for each species and were not elevation- or treatment-specific. In addition, due to low germination percentages and slow growth of alpine plants, we were unable to measure recruit sizes in the field. For *K. macrantha,* we estimated recruit size distribution from the seedling dataset of Chu and Adler (2014). For *E. ovalifolium,* due to the lack of available information on this species, we simulated seedling size data based on the smallest 2.5% of adults in the dataset, which produced a size distribution of 0.001–2.5 cm^2 and a mean of 0.6 cm^2. While this modeling choice could affect the magnitude of estimated population growth rates, it should not bias the analysis of treatment effects.

Population Modeling

We calculated size-dependent demographic rates (growth, survival probability, flowering probability, and seed production) using 229 and 224 individuals of *K. macrantha* and *E. ovalifolium*, respectively. Plant size was logged in all models for normality and seed number was logged to transform from count data to continuous. Germination probability was estimated as a single value for each species based on seed germination trials, and recruit size was estimated using an intercept only linear model using the dataset from Chu and Adler (2014) for *K. macrantha* and a simulated dataset of realistic recruit sizes for *E. ovalifolium* as described above.

We used mixed effects models for each demographic rate, including fixed effects of size, treatment and elevation, and a random effect of year. We fit these as Bayesian models using the brms package (Bürkner, 2018) in R (R Core Team, 2015), and using the default non-informative, improper priors for all models. We used a "nested" model structure with elevation effects nested within treatments. Importantly, we fit this model with elevation nested within treatments in order to allow for "partial pooling" of information across elevations within each treatment. Partial pooling allowed separate estimates of demographic rates at each treatment × elevation combination, but the data from different elevations, within a treatment, informed each other. This approach is therefore a compromise between complete pooling of data across elevations and independent estimates for each elevation × treatment. This was a conservative modeling decision based on the observation that mortality events in particular were sparse in the dataset; the partial pooling prevents biases from sparse data, such as the chance event that an elevation has no mortality (see **Figure S3** for comparison of the "partial pooling" model with a "no pooling" model). We tested for the treatment and elevation effects on each demographic rate by calculating pairwise contrasts using the posterior distributions and computing the probabilities that the difference between each pair was different from zero.

Using the posterior distributions from the demographic rate models, we constructed integral projection models (IPMs) to calculate population growth rates (lambdas) for each species within each treatment × elevation combination. These population models and estimated lambdas were used as a way to integrate the effects of sagebrush across multiple phases of the plant life cycle, rather than accurate projections of population growth rates. Thus, we consider lambda to be an estimate of the relative fitness of each species among the different plot treatments, and do not suggest they will accurately predict changes in population sizes over time. The effects of shrub and herbaceous competition, as well as shrub PSFs, on lambdas were calculated using a-priori contrasts between the lambdas estimated in the different treatments: Shrub Competition = (Shrub competition) – (Shrub removal); Herb Competition = (Herb competition) – (Herb removal); Shrub PSF = (Shrub removal) – (Herb removal) (**Figure 1**). These contrasts were

TABLE 1 | Contrasts among vital rates for *Koeleria macrantha* (KOMA) and *Eriogonom ovalifolium* (EROV). Pr(negative) represents the probability that the effect of Treatment 1 < Treatment 2, whereas Pr(positive) is the probability that the effect of Treatment 1 > Treatment 2 [and is equal to 1 – Pr(negative)]. Only contrasts with probabilities >0.75 are displayed.

Vital rate	Elevation	Spp.	Treatment 1	Treatment 2	Pr(negative)	Pr(positive)
Growth	3,200	KOMA	Herb	Shrub	0.16	0.84
		KOMA	Herb	Shrub removal	0.08	0.92
		KOMA	Herb removal	Shrub	0.13	0.87
		KOMA	Herb removal	Shrub removal	0.07	0.94
		KOMA	Herb removal	Shrub removal	0.14	0.86
	3,800	KOMA	Herb	Shrub	0.17	0.83
		KOMA	Herb	Shrub removal	0.10	0.90
		KOMA	Herb removal	Shrub	0.11	0.89
		KOMA	Herb removal	Shrub removal	0.06	0.94
Survival	3,200	KOMA	Herb	Herb removal	0.04	0.96
		KOMA	Herb	Shrub	0.22	0.78
		KOMA	Herb	Shrub removal	0.17	0.83
		KOMA	Herb removal	Shrub	0.81	0.19
		KOMA	Herb removal	Shrub removal	0.80	0.20
	3,500	KOMA	Herb	Herb removal	0.77	0.23
	3,800	KOMA	Herb	Herb removal	0.05	0.95
		KOMA	Herb	Shrub	0.23	0.77
		KOMA	Herb	Shrub removal	0.11	0.89
		KOMA	Herb removal	Shrub	0.84	0.16
Flowering	3,200	KOMA	Herb	Herb removal	0.85	0.15
		KOMA	Herb removal	Shrub	0.24	0.76
		KOMA	Herb removal	Shrub removal	0.12	0.88
	3,500	KOMA	Herb	Herb removal	0.84	0.16
		KOMA	Herb	Shrub	0.81	0.20
		KOMA	Herb	Shrub removal	0.80	0.20
	3,800	KOMA	Herb	Herb removal	0.13	0.87
		KOMA	Herb	Shrub	0.01	0.99
		KOMA	Herb	Shrub removal	0.05	0.95
		KOMA	Herb removal	Shrub	0.06	0.94
		KOMA	Shrub	Shrub removal	0.82	0.18
Seeds	3,200	KOMA	Herb	Shrub	0.08	0.92
		KOMA	Herb removal	Shrub	0.02	0.98
		KOMA	Shrub	Shrub removal	0.98	0.02
	3,500	KOMA	Herb removal	Shrub	0.80	0.20
	3,800	KOMA	Herb	Herb removal	0.23	0.77
		KOMA	Herb	Shrub	0.00	1.00
		KOMA	Herb	Shrub removal	0.01	0.99
		KOMA	Herb removal	Shrub	0.00	1.00
		KOMA	Herb removal	Shrub removal	0.03	0.97
		KOMA	Shrub	Shrub removal	0.86	0.14
Growth	3,500	EROV	Herb	Herb removal	0.15	0.85
		EROV	Herb	Shrub	0.13	0.87
		EROV	Herb	Shrub removal	0.12	0.88
	3,800	EROV	Herb	Herb removal	0.05	0.95
		EROV	Herb	Shrub	0.00	1.00
		EROV	Herb	Shrub removal	0.00	1.00
		EROV	Herb removal	Shrub	0.00	1.00
		EROV	Herb removal	Shrub removal	0.00	1.00
		EROV	Shrub	Shrub removal	0.23	0.77

(Continued)

TABLE 1 | Continued

Vital rate	Elevation	Spp.	Treatment 1	Treatment 2	Pr(negative)	Pr(positive)
Survival	3,200	EROV	Herb	Herb removal	0.80	0.20
		EROV	Herb	Shrub	0.96	0.04
		EROV	Herb	Shrub removal	0.91	0.09
		EROV	Herb removal	Shrub	0.88	0.12
	3,500	EROV	Herb	Shrub	0.91	0.09
		EROV	Herb removal	Shrub	0.86	0.14
		EROV	Shrub	Shrub removal	0.16	0.84
	3,800	EROV	Herb	Herb removal	0.22	0.78
		EROV	Herb	Shrub	0.16	0.84
		EROV	Herb	Shrub removal	0.09	0.91
		EROV	Herb removal	Shrub removal	0.21	0.79
Flowering	3,500	EROV	Herb	Herb removal	0.04	0.96
		EROV	Herb	Shrub	0.00	1.00
		EROV	Herb	Shrub removal	0.01	0.99
		EROV	Herb removal	Shrub	0.12	0.88
	3,800	EROV	Herb	Herb removal	0.15	0.85
		EROV	Herb	Shrub	0.00	1.00
		EROV	Herb	Shrub removal	0.00	1.00
		EROV	Herb removal	Shrub	0.00	1.00
		EROV	Herb removal	Shrub removal	0.00	1.00
Seeds	3,200	EROV	Herb	Shrub	0.03	0.97
		EROV	Herb removal	Shrub	0.04	0.96
		EROV	Shrub	Shrub removal	0.95	0.05
	3,500	EROV	Herb	Herb removal	0.83	0.17
		EROV	Herb	Shrub	0.06	0.94
		EROV	Herb removal	Shrub	0.01	0.99
		EROV	Herb removal	Shrub removal	0.24	0.76
		EROV	Shrub	Shrub removal	0.93	0.07
		EROV	Herb	Herb removal	0.92	0.08
	3,800	EROV	Herb	Shrub	0.04	0.96
		EROV	Herb removal	Shrub	0.00	1.00
		EROV	Herb removal	Shrub removal	0.20	0.80
		EROV	Shrub	Shrub removal	0.96	0.04

calculated from the posterior distributions of estimated lambdas within each treatment × elevation.

Finally, we conducted elasticity analyses to assess the contributions of different demographic rates to lambdas, and the effects of treatments on demographic rate sensitivities. The elasticity analyses on growth, survival, and probability of reproduction determine the sensitivity of lambda to changes in specific demographic rates. For all IPM analyses, we used modified R code from Ellner et al. (2016).

RESULTS

Demographic Rate Models

All demographic rates varied by treatment and elevation for both species. Pairwise contrasts for all demographic rates among plot treatments within each elevation and the probabilities that each treatment is greater (or less) than another treatment are listed in **Tables S1** and **S2**. Here we report contrasts where the probability was >0.75 (**Table 1**).

We find in general that demographic rates tended to be highest in herbaceous and herbaceous removal plots for both species, and this pattern was strongest at the high and low elevation sites. For *K. macrantha*, growth was highest in herbaceous plots at the low elevation site and in herbaceous removal plots at the middle and high elevation sites. Growth in herbaceous and herbaceous removal plots was higher in than in shrub and shrub removal plots which suggests that sagebrush has a negative effect on *Koeleria* growth (**Table 1**, **Figure S1**). Survival varied greatly by treatment and elevation and overall was highest herbaceous removal plots at the middle elevation site and herbaceous plots at the low and high elevation sites (**Table 1**, **Figure 2**, **Figure S1**). Probability of flowering was greatest in herbaceous and herbaceous removal plots particularly at high elevation, however was higher in shrub plots at the middle elevation site (**Table 1**, **Figure S1**). Similarly seed production was higher in

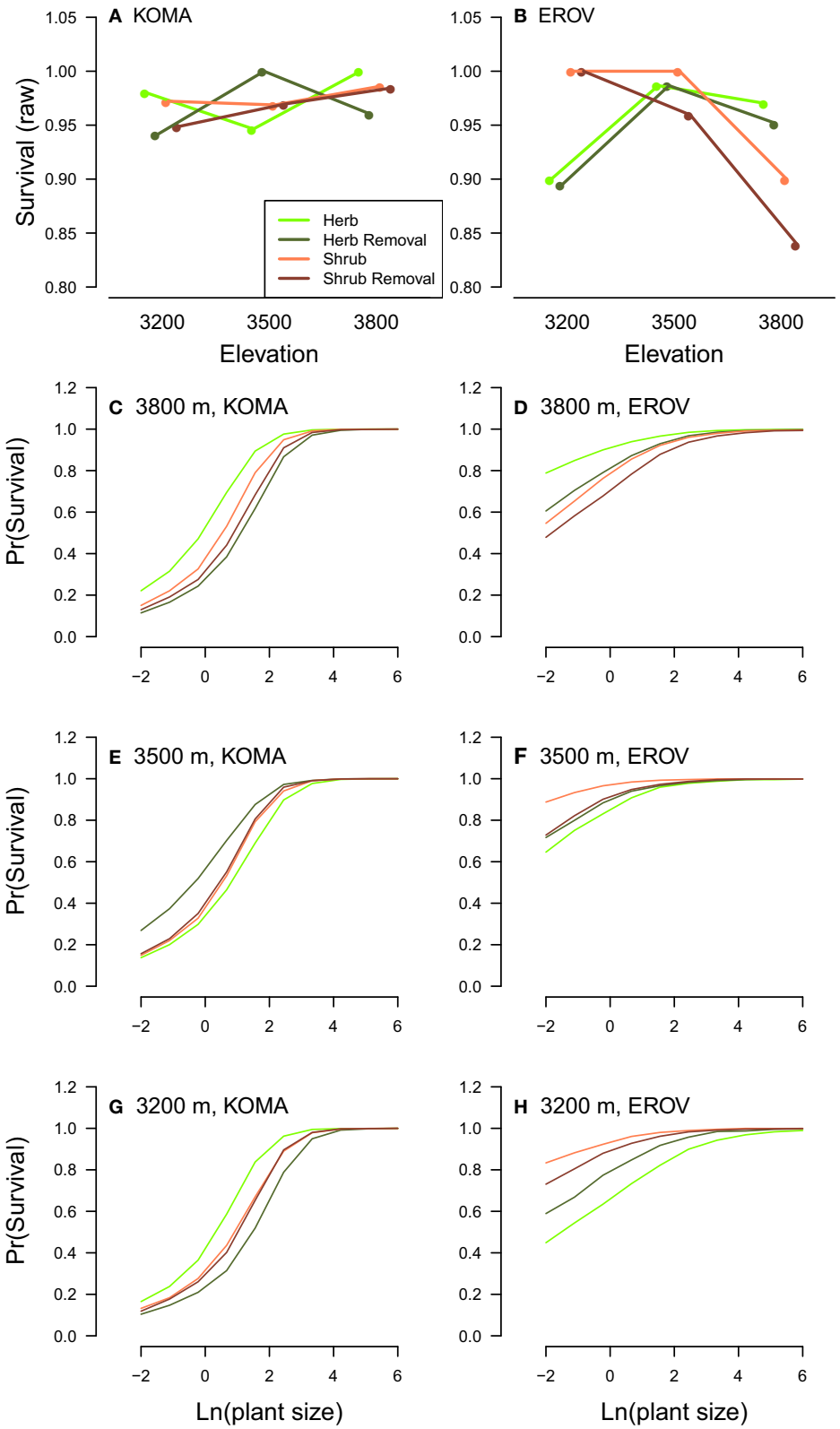

FIGURE 2 | Plot treatment effects on survival for *K. macrantha* (KOMA, left column) and *E. ovalifolium* (EROV, right column). Raw survival data is shown in the top row **(A,B)** plotted by elevation (m) on the x-axis and by plot treatments in different colors. Modeled probability of survival is shown in the bottom figures **(C–H)** and plotted separately for each elevation by species combination, with individual plant size on the x-axis and colored lines signifying different plot treatments.

shrub plots at the middle elevation site which suggests that sagebrush may have a positive effect on reproduction at this site which counters the negative effects on growth and survival (**Table 1**, **Figure S1**). At low and high elevations flowering and seed production were greater in herbaceous and herbaceous removal plots than in shrub and shrub removal plots, which follows the general pattern we observe for the other demographic rates (**Table 1**, **Figure S1**).

Many patterns observed in *K. macrantha* demographic rates were similar for *E. ovalifolium*. Growth was highest in herbaceous and herbaceous removal plots at the middle and high elevation sites and no treatments were different at the low elevation site. Growth in herbaceous and herbaceous removal plots was higher in than in shrub and shrub removal plots which suggests that sagebrush also has a negative effect on *Eriogonum* growth (**Table 1**, **Figure S2**). Survival also varied by treatment and elevation and overall was highest shrub and shrub removal plots at the low and middle elevation site, but then dropped significantly at the high elevation site, and fell below both herbaceous and herbaceous removal plots (**Table 1**, **Figure 2**, **Figure S2**). Probability of flowering was greatest in herbaceous and herbaceous removal plots, particularly at high elevation, and was higher than shrub and shrub removal plots except at low elevation where no treatments differed (**Table 1**, **Figure S2**). Seed production was highest in herbaceous and herbaceous removal plots across all elevations but especially at the high elevation site.

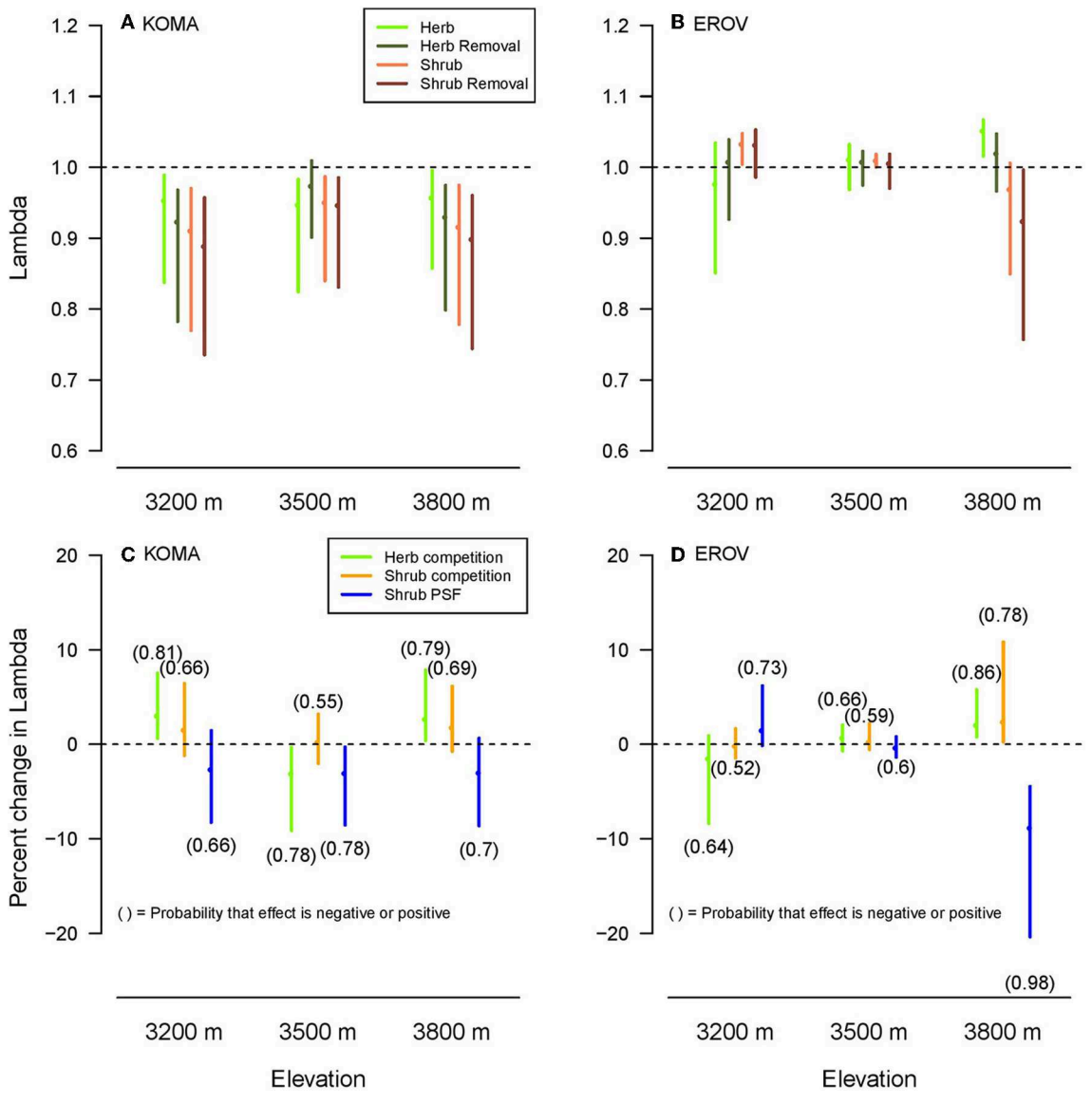

FIGURE 3 | Population lambdas **(A,B)** by plot treatment and elevation for *K. macrantha* (KOMA, left column) and *E. ovalifolium* (EROV, right column). Change in lambda **(C,D)** plotted for each a-priori contrast (Herb competition: Herb–Herb removal, Shrub competition: Shrub–Shrub removal, Shrub PSF: Shrub removal-Herb removal) shown in colors. Numbers above or below line, respectively signify the probability that the effect of the contrast on lambda is positive or negative.

This suggests that sagebrush has a more negative influence on *Eriogonum* than *Koeleria* reproduction, particularly in the range expansion zone (**Table 1**, **Figure S2**).

Population Growth Rates (Lambdas)

Lambda values were higher overall for *E. ovalifolium* than *K. macrantha*, while the differences among lambdas across treatments and elevations were distinct for each species. Herb and herb removal plots had higher median lambda values than shrub and shrub removal plots for *K. macrantha* at low and high elevations and for *E. ovalifolium* at high elevation (**Figures 3A,B**). This reflects the general pattern observed in the demographic rate models where negative effects of sagebrush were strongest at the two ends of the elevation gradient. At 3,500 m elevation, lambdas did not differ among the plot treatments except for a slight increase in herbaceous removal plots for *K. macrantha* (**Figure 3A**). Lambdas in shrub and shrub removal plots were higher than in herb and herb removal plots for *E. ovalifolium* at 3,200 m elevation (**Figure 3B**). This is likely due to the fact that survival was higher in shrub and shrub removal plots at this site, as survival has a very large contribution to lambda in this system (see elasticity analyses).

For the a-priori contrasts, Herb competition (herb competition-herb removal) had a positive effect (probability >0.75) on lambda for *K. macrantha* at 3,200 and 3,800 m elevations and *E. ovalifolium* at 3,800 m elevation, and a negative effect on lambda for *K. macrantha* at 3,500 m elevation (**Figures 3C,D**). Shrub competition (shrub competition – shrub removal) had a positive effect on lambda for *E. ovalifolium* at 3,800 m elevation, and slightly positive to neutral effect at other elevations (**Figures 3C,D**). Shrub PSFs (shrub removal – herb removal) had a negative effect on lambda for *E. ovalifolium* at 3,800 m elevation and *K. macrantha* at 3,500 m elevation and slightly negative to neutral effect on lambda at other elevations except for *E. ovalifolium* at 3,200 m where the effect was slightly positive (**Figures 3C,D**).

Elasticity Analyses

Survival had the highest impact on population lambdas of all demographic rates (max elasticity values ∼0.35–0.45) for both species however *E. ovalifolium* had slightly higher elasticity than *K. macrantha*, particularly at the high elevation site in shrub and shrub removal plots (**Figure S4**). Growth had the next highest impact on lambda (max elasticity values ∼0.16–0.25) for both species however *K. macrantha*, had higher elasticities than *E. ovalifolium,* which signifies that growth contributed more to population lambdas in the grass species. Additionally, the influence of plant growth on lambdas was more important at larger size class transitions [moving from size class ∼z(log) 4 to ∼z(log) 5] for *Koeleria* vs. smaller size class transitions [∼z(log) 2 to ∼z(log) 3] for *Eriogonum* (**Figure S5**). This shows that changes in the growth rate of larger, smaller individuals are more important for population lambdas in the grass, cushion plant, respectively. Growth elasticities did not vary noticeably by elevation or treatment. Probability of reproduction contributed the least to population lambdas, however had a much more significant contribution for Eriogonum (max elasticities ∼0.005–0.016) than Koeleria (max elasticities ∼0–0.0025) (**Figure S4**).

DISCUSSION

Understanding the broader implications of species range shifts will be crucial as climate change continues to promote differential species migration and novel species interactions (Midgley et al., 2007; Tomiolo and Ward, 2018). In this study, we sought to tease apart the potential above- and belowground mechanisms by which a range-expanding sagebrush species, *Artemisia rothrockii,* affected the demography of two herbaceous alpine plant species. We found that sagebrush most often had a net negative effect on the demographic rates and population lambdas of herbaceous species. Our results suggest that these effects were driven by negative PSFs for plants growing in sagebrush conditioned soil, counteracting facilitative effects of sagebrush aboveground. However, we found variation among species and across elevations suggesting that the effects of sagebrush PSFs vary based on abiotic conditions of the site and for herbaceous species identity. Overall these results show for the first time, using a manipulative field experiment and demographic modeling, that shrubs may have both positive and negative impacts on herbaceous plant demography due to distinct aboveground and belowground mechanisms.

Demographic Rates and Lambdas

Sagebrush affected the demography and population lambdas of both *E. ovalifolium* and *K. macrantha* across an elevation gradient in the White Mountains. Specifically, plants growing with sagebrush, or in its conditioned soils, tended to have slower growth, reduced probability of flowering and lower seed production than those growing in herbaceous dominated soils. Thus, population lambdas that were highest in herbaceous and herbaceous removal plots and lowest in shrub and shrub removal plots, respectively (**Figure 3**). This supports the hypothesis that sagebrush would have a negative effect on the demography of native alpine plants. These differences were particularly pronounced at the high elevation site and were consistent for both species of interest, suggesting the strong influence of sagebrush on herbaceous plant population dynamics in the range expansion zone.

Similar to our findings, an herbaceous forb species growing in association with four Mediterranean montane shrubs had reduced reproductive output including lower number of seeds and reproductive stems, and lower infructescence volume compared to individuals growing in open areas (Macek et al., 2016). In our study system, however, previous work showed that *A. rothrockii* slowed the phenology and reduced the flower production of a *Trifolium* cushion plant via shading, thus decreasing the benefits of climate warming on reproduction (Kopp and Cleland, 2015). This contrasts somewhat with our finding of reduced reproductive output (flowering and seeds) in both shrub and shrub removal plots, suggesting that the relative importance of shading vs. belowground effects may vary among herbaceous species.

Survival of *E. ovalifolium* was a notable exception to this overall pattern, being significantly higher in shrub and shrub removal plots at low and middle elevations. However, survival sharply declined in shrub and shrub removal plots at the high elevation site for *E. ovalifolium* (**Figure 3B**). For *K. macrantha*, survival was intermediate in shrub and shrub removal plots depending on elevation. These treatment effects on survival were critical because overall mortality was low across the observation period (∼2% *Koeleria*, ∼2.5% *Eriogonum*). Low mortality rates are common for slow-growing alpine species that are well-adapted to stressful abiotic conditions (Körner, 2003). Therefore, when mortality events do occur, they can strongly impact population growth rates (see Elasticity analyses).

The differences in how *E. ovalifolium* and K. *macrantha* responded to the experimental treatments reflect the large differences in their life history characteristics. *E. ovalifolium* is a slow growing, long lived, cushion plant while, *K. macrantha* is a/perennial bunchgrass with an average lifespan between 7 and 10 years (Dixon, 2000; Rundel et al., 2005; Anderson, 2006). *Koeleria* is solely wind pollinated, while *Eriogonum* is wind pollinated, but also largely insect and bird pollinated, and produces many fewer seeds per inflorescence. Finally, *Koeleria* senesces most of its aboveground biomass annually, while *Eriogonum* retains green leaves throughout the winter. Despite these strong differences, overall patterns in lambda were relatively similar, particularly at high elevation sites, where sagebrush establishment is most recent and therefore demographic rates will potentially be most responsive.

Elasticity Analyses

We used elasticity analyses to understand which demographic rates contributed most to the observed patterns in population lambdas and how robust lambda values were to changes in demographic rates. Survival had the largest contribution to population lambdas, followed by growth and then probability of reproduction. Elasticities also varied among the two species, treatments and by elevation. *Eriogonum* had higher elasticities in response to changes in survival, particularly at high elevation in shrub and shrub removal plots. This suggest that in areas of recent sagebrush establishment, population growth may be particularly affected by mortality events for this species, and more so than when growing in the herbaceous plant community.

Although survival was the most important demographic rate for both species, changes in growth were more important for *Koeleria* than *Eriogonum*, likely due to faster growth and a shorter lifespan of this grass species. Finally, the probability of reproduction had a very minor influence on lambda but was significantly more important for *E. ovalifolium* than *K. macrantha*. Again, this likely reflects differences in the life history characteristics of these species in that *Eriogonum* produces fewer seeds per inflorescence and has a more complex pollinator strategy, making reproduction a more important component of its overall population growth. Overall, lower lambda values suggest that K. macrantha is more likely than *E. ovalifolium* to decline in the future (**Figures 3A,B**), however, more years of data are needed to confirm this trend.

Above and Belowground Effects on Lambdas

We found support for the hypothesis that the effects of sagebrush PSFs on lambda would be more negative than the effects of sagebrush competition. The effects of sagebrush presence on lambda were neutral to slightly positive suggesting weak competition to facilitation, with the strongest positive effect for *E. ovalifolium* at the high elevation site. This supports the well-known hypothesis that species interactions will become more positive (facilitative) as abiotic stress increases (Callaway et al., 2002; Maestre et al., 2009).

Nurse plant facilitation of herbs commonly occurs through enhanced resources, such as water and nutrients and by buffering effects of extreme temperatures, wind or snow in the understory (Körner, 2003). Indeed, *A. rothrockii* has increased soil moisture and higher soil organic matter content below its canopies as compared to shrub interspace areas in the White Mountains (Collins et al., 2016); however, the effect of sagebrush facilitation on herbaceous demography was not consisitent across elevations and herbaceous species. Facilitation intensity can increase with functional dissimilarity among species at the cold and wet end of a stress gradient (Gallien et al., 2018) suggesting that shrubs may most strongly facilitate herbs at high elevations. Despite this, overall lambda values for shrub plots tended to be lower than herbaceous plots regardless of treatment, suggesting that the benefits do not outweigh the costs of growing in association with this shrub species.

As predicted, the effects of shrub PSFs on lambda were generally negative, implying that in the absence of competition, plants growing in shrub-conditioned soils had lower growth, survival and reproduction than those growing in herbaceous-conditioned soils. The one exception to this pattern was a slightly positive effect on lambda for *E. ovalifolium* at the low elevation. PSFs are therefore a potentially strong form of apparent competition by which sagebrush negatively impacts resident plant species. Many factors can determine the strength of PSFs of range expanding species on native communities and whether they are positive or negative. For example a range-expanding forb species had positive PSFs that enhanced the growth of a co-occurring native grass species, but only in the expansion zone (Dostálek et al., 2016). Here we find accordingly that sagebrush PSFs were stronger in the range expansion zone than in the native range (high vs. low elevation), but unlike the previous study, effects on resident plants were negative rather than positive. This may be due to differences in the time of soil conditioning between the historic and range expansion zones, as sagebrush is more recently established at high elevation sites. Range expanding species may also impose different PSFs depending on their relatedness to the resident community. Koorem et al. (2018) found that range expanders that were unrelated to resident plant species reduced the biomass production of the resident plant community, whereas related range expanding species did not. In our study, PSFs may be enhanced because sagebrush is not closely related (congeneric) with either herbaceous species.

Due to our experimental design however, we can only speculate whether the PSFs of sagebrush on resident plant species are due to changes in soil microbial communities, abiotic soil conditions, or both. One potential PSF mechanism is through secondary compounds (e.g., terpenes, jasmonic acid) in aromatic shrubs, such as *Artemisia* that can enter the soil through leaf litter and root exudates and have strong negative effects on plant growth, metabolism, and seed germination (Weaver and Klarich, 1977; Kelsey et al., 1978; Karban, 2007). These classes of chemicals can also strongly influence soil microbial community structure and function including microbial biomass C and N, respiration, nitrogen fixation, soil faunal substrate choice, and mycorrhizal networks of co-occurring plant species (Weston and Putnam, 1985; Wardle et al., 1998; Asensio et al., 2012; Austin et al., 2014). For example, organic compounds in the dwarf shrub *Empetrum hermaphroditum* greatly reduced Ectomycorrhizal infection of root tips and mycorrhizal uptake of soil Nitrogen for pine seedlings (Nilsson et al., 1993). Labile C in these compounds may also stimulate free-living (saprotrophic) microbial growth and nutrient immobilization, thus increasing resident plant-microbial competition for limiting soil nutrients. This was proposed as a mechanism by which *Betula*, *Empetrum*, and *Cassiope* shrub species inhibited the growth of nearby graminoid species in arctic soils (Michelsen et al., 1995). Therefore, via secondary chemicals, sagebrush may similarly alter plant-microbe competition in ways that enhance their own growth and nutrient acquisition to the detriment of co-occurring herbaceous plant species.

In previous work, we found that soils under sagebrush had higher bacterial diversity but lower fungal diversity than soils under herbaceous plants, and this corresponded with a decrease in both pathogenic and mutualistic fungi (Collins et al., 2016, 2018, and unpublished data). A change in soil mutualist to pathogen ratios has been shown to facilitate both inter- and intracontinental range expansions, as plants may benefit from decreased species-specific pathogens, while utilizing more generalist soil mutualists (van der Putten et al., 2016). However, the patterns we observe in this study suggest the opposite may be true for herbaceous plants growing in association with the range expander (sagebrush), which may still be experiencing negative effects of their own soil pathogens, but also a decreased abundance of soil mutualists. These effects may strengthen over time in the range expansion zone with more sagebrush soil conditioning, or they may attenuate as herbaceous plants become adapted to the sagebrush soil community (Rout and Callaway, 2012).

We acknowledge that the experimental plot treatments do not completely isolate the effects of sagebrush soil conditioning, and there may be other factors contributing to the changes in lambdas in the shrub PSF contrasts. It is possible, for example, that removal of shrub facilitation could negatively impact lambdas in shrub removal plots. However, we find that shrubs are weaker facilitators than the herbaceous community, and yet the difference between shrub removal and herbaceous removal plots is mostly negative, suggesting that lack of shrub facilitation is not driving the negative PSFs. It is also possible that some belowground competition may still occur between herbaceous and shrub roots after aboveground shrub removal, however we expect these interactions to be minor and short term, while soil legacy effects left by sagebrush can last many years after shrub removal or death (Collins et al., 2016, 2018). Therefore, the demographic patterns observed in shrub removal plots are very likely attributable to sagebrush soil conditioning, although we cannot rule out some remnant belowground competition. Additionally, our experiment lacks distinct "conditioning" and "feedbacks" phases, but instead measures feedbacks over a longer, continuous time frame. While this has the strength of being more relevant to population dynamics, which unfold over longer time periods than most discrete PSF experiments, it also may dampen the ability to disentangle PSFs during the transition period after aboveground biomass removal.

Overall the patterns we observed were variable and sometimes weak, however this is to be expected due to the due to slow growth, low mortality and high stress tolerance of plants in alpine environments, and the relatively short observation period (4 years) given these species' lifespans. Nonetheless, the data show that the effects of sagebrush on lambdas were more negative than the effects of the herbaceous community, particularly in the absence of aboveground competition and at middle and high elevation sites, providing a potential PSF mechanism for the observed declines in cover of *K. macrantha* and *E. ovalifolium* in areas of sagebrush expansion. While stronger effect sizes may have been observed in a more controlled greenhouse setting, estimating longer-term population dynamics in a field setting is more indicative of likely consequences for the species in nature.

CONCLUSIONS

Alpine landscapes are characterized by heterogeneous microclimates, resource availability and species interactions which can have large impacts on plant fitness (Körner, 2003). The movement of woody shrubs upwards in elevation, occurring in alpine ecosystems across the globe due to climate and land use change, may therefore alter these landscapes and affect the persistence of rare and endemic plant species. Here we find that shrubs can have both positive and negative impacts on herbaceous alpine plants simultaneously, and through both above and belowground mechanisms. Shrubs may facilitate herbs growing under or nearby their canopies particularly at high elevations, likely by shielding the effects of extreme temperatures, wind or snow. At the same time, shrubs may cause negative effects on herbaceous plants through PSFs predictably from changes in litter chemistry and their influences on plant growth and soil microbial community structure and function. These changes created by shrubs may impact multiple components of the plant life cycle, including growth, survival, and reproduction, and these components can have divergent responses which together determine the net outcomes for population growth.

The majority of PSF research has been carried out in controlled environments, particularly greenhouse experiments,

while little direct connection has been made between plant population dynamics and PSFs *in situ* (Kulmatiski and Kardol, 2008; Kulmatiski et al., 2008; Heinze et al., 2016). In addition, while conceptually well-developed, attempts to disentangle the effects of direct competition and PSFs on population dynamics and species coexistence have been rare (Bever et al., 1997; Revilla et al., 2013). The experimental field approach used here can help isolate direct competitive interactions vs. influences of soil conditioning on plant demography. By coupling these experiments to demographic modeling, it is possible to scale the aboveground and belowground effects of a range-expanding species to the effects on population dynamics and species coexistence over time. We believe this approach of combining estimates of PSFs in the field with species-specific population modeling is an important next step in understanding plant community dynamics in a changing world.

DATA AVAILABILITY STATEMENT

The datasets generated by this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CC developed the research questions, designed and set up the experimental plots, collected the field data, and wrote the first draft of the manuscript. JD and TB analyzed the data and ran the statistical models. All authors created the graphics, wrote the sections of the manuscript, contributed to the manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00417/full#supplementary-material

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ORIGINAL RESEARCH
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Soil Inoculation Steers Plant-Soil Feedback, Suppressing Ruderal Plant Species

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Plant-soil feedbacks (PSFs) are important drivers of vegetation composition in nature. Whole-soil inoculations can help to steer plant-community assembly for nature restoration success. However, it is unclear how subsequent colonization by ruderal and late-successional plant species influences PSFs. Here we test the direction and strength of the PSFs for ruderal and target plant species on differently inoculated soils. We hypothesize that inoculation with late-successional field soil promotes positive and negative PSFs for late-successional and ruderal plants, respectively. We conducted a glasshouse experiment with three plant-growth phases. First, we inoculated a common nutrient-rich ex-arable recipient soil with either a heathland, grassland or an arable soil, and grew mixtures of three ruderal, and three late-successional target species in all soils. Subsequently, we divided the experimental units over four new pots and planted half with mixtures of three ruderal and half with mixtures of three late-successional plant species, to simulate local colonization by these species groups. After 9 weeks, we removed the plants and replanted the pots with ruderal and late-successional species mixtures in a full factorial design to quantify the induced PSFs, after a further 9 weeks of growth. We found that ruderal plants developed positive PSF on soils inoculated with arable soil and neutral feedback when soils were inoculated with grassland or heathland inoculum. The positive PSF was most pronounced for *Myosotis arvensis*, although all ruderal species showed the most positive PSFs on arable-inoculated soils. There was no significant effect of inoculation on the PSF of late-successional species. As a result of changing PSFs, the relative performance of ruderal and late-successional target species became more similar on soils inoculated with grassland or heathland inoculum, leading to higher evenness. We conclude that soil inoculation on nutrient-rich ex-arable soils can affect PSFs. Inoculation with grassland or heathland inoculum prevented ruderal species from developing positive PSF and shifted the competitive balance in favor of late-successional plants. Future studies need to address the longer-term dynamics of soil inoculation-induced shifts in PSFs, particularly because these are expected to develop over longer timescales in the slow-growing late-successional target species, as well as the impact of competitive plant-plant interactions.

Keywords: colonization, nature restoration, plant-community assembly, plant-soil feedback, soil inoculation

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INTRODUCTION

Plants and their associated soil biota are continuously interacting and their interplay can lead to net positive and negative effects on plant performance (survival, growth, and reproduction). These phenomena are termed positive and negative plant-soil feedback (PSF), respectively (Bever, 1994; Van der Putten et al., 2013). It is clear that plant-soil feedback effects are important drivers of plant community dynamics, both in the lab (Kulmatiski et al., 2008; Lekberg et al., 2018) and in the field (Bennett et al., 2017; Teste et al., 2017). However, it is becoming increasingly clear that the realized plant-soil feedback is dependent on the local conditions. For instance, plant-soil feedback strengths change with soil type, fertilization and herbivory (Bezemer et al., 2006; Veen et al., 2014; Heinze and Joshi, 2017; Wubs and Bezemer, 2018b). The realized plant-soil feedback is not a property of the plant species *per se*, but arises through the interplay between plants and their soil biota within the local biotic and abiotic conditions. The latter is illustrated by the observation that PSFs change importantly with the successional stage of the plant as well as the soil (Kardol et al., 2006).

Recently, we applied this contextual knowledge on plant-soil interactions to improve nature restoration success through soil inoculation on sandy soils (Wubs et al., 2016). Previous studies show that late-successional plant species, the typical target species for restoration, develop positive PSF, measured as plant biomass (De Deyn et al., 2003; Carbajo et al., 2011) and this is particularly pronounced in late-successional soils (Kardol et al., 2006). In field experiments it has been shown that introduction of late-successional soil communities can facilitate the establishment and growth of late-successional plant species (Vécrin and Muller, 2003; Pywell et al., 2011; Buisson et al., 2018) and can determine the composition of the developing plant community for decades (Wubs et al., 2016, 2019). While this approach can thus be a successful intervention method, the areas to be restored are still open to potential colonization by plant species from elsewhere and it is unclear how soil inoculation affects the PSFs experienced by subsequently colonizing plants.

Here we test whether inoculation with soils from early- and late-successional systems affect the direction and strength of the PSFs, measured as plant biomass, of ruderal as well as restoration target plant species. We use the sandy glacial deposits in the central parts of the Netherlands as our model system (Kardol et al., 2006; Carbajo et al., 2011; Wubs et al., 2016). In the area, arable farming has been intensive in many places since World War 2, but many farms are no longer economically viable due to the low inherent soil fertility and limits on spatial expansion. Species-rich grasslands and dry heathlands are important national restoration targets for the region, harboring important flora and fauna typical of dry habitats. Both ecosystems are stages along the secondary succession on sandy soils, but under different management regimes (Kardol, 2007). The grasslands arise from the arable fields within a span of 10–30 years under mowing or grazing regimes. The dry heaths in this system are the result of, historically prolonged, sod cutting and grazing. We refer to the arable system as early-succession, and the grassland and heathland both as late-succession.

Plant-soil feedbacks are typically studied using two-phase experiments (Kulmatiski et al., 2008; Brinkman et al., 2010). In the first phase, a plant species or group of species is allowed to condition the soil by growing in it, which alters the local soil biotic and abiotic conditions (Ehrenfeld et al., 2005; Van der Putten et al., 2013): the conditioning phase. In the second phase, the test phase, the same (or a different) group of plant species is allowed to grow on that soil and metrics of their performance on conditioned and control soil are recorded (e.g., survival, biomass, reproduction). Based on the difference in plant performance on the conditioned and a control soil the net PSF is calculated (Brinkman et al., 2010), with positive PSF indicating better performance in the conditioned soil than in the control and negative PSF the converse. In this study, we couple a soil inoculation experiment (Phase 1) with a classical PSF experiment, with a conditioning phase (Phase 2) and a test phase (Phase 3). In the first phase we inoculated a common ex-arable soil with either arable, grassland or heathland soil and grew a common plant community of six species to simulate what would happen in a soil inoculation based restoration project (Wubs et al., 2018). After harvest, we then separately grew three ruderal and three target plant species on all the inoculated soils to allow soil conditioning by the ruderal and target species groups, respectively (Phase 2). Finally, after harvest, we again grew the same ruderal and target species groups on each of the conditioned soils in a full factorial design and quantified their performance as shoot biomass production (Phase 3). This design allows us to quantify net PSFs, by comparing performance of plants grown on soils conditioned by the same group of plants to performance on soils conditioned by the other group of plants, across the three different inoculation treatments. We hypothesize that late successional plants experience positive PSF, while ruderal species develop negative PSF (Kardol et al., 2006). Furthermore, when inoculated with late-successional field soil the positive and negative PSFs for late-successional and ruderal plants, respectively, are expected to be exacerbated. This would result in even higher and lower plant biomass for these groups.

MATERIALS AND METHODS

We conducted a glasshouse experiment with soils setup in a prior study (Wubs et al., 2018). More details on this part of the methods can be found there. Briefly, in the experiment inocula of three ecosystem types, arable, grassland, and heathland, were sourced from the field (January 2015), with three replicate fields in each type. Within each field an area of 5 × 5 m was selected at least 20 m from the edge of the field. At each corner of the selected area, 5 kg of soil was collected from the upper 10–15 cm. The soil was sieved over a 1 cm mesh to remove stones and large roots. Upon return to the lab, the four samples per field were pooled based on equal amounts of dry weight resulting in homogenized inoculum material of 20 kg per field. These inocula were introduced (1:9 w:w inoculum:soil ratio) into a common ex-arable field soil (total 4 kg/container). The common ex-arable soil was from a field that had been in intensive agricultural use at least since World War 2 until 2004. Then, it was used for extensive

wheat cultivation for 2 years prior to the implementation of large-scale nature restoration measures in 2006 (Wubs et al., 2016). The soil was collected from the central part of the field, where the only management consisted of cattle grazing (25–30 cows throughout the year, roaming freely in the entire 160 ha field) and removal of tree seedlings (particularly *Betula* spp. and *Prunus serotina*). We collected soil from the organic layer within 10–50 cm depth (∼1,300 kg), which was subsequently sieved over a 1 cm mesh to remove major roots and stones and homogenized. The common background soil was sterilized (>25 KGray gamma radiation, Isotron, Ede, the Netherlands) to eliminate the resident soil community. The inocula were not sterilized. The abiotic conditions in the inocula and the common ex-arable soil have been reported in Table S1 of Wubs et al. (2018). Likewise, soil biotic composition data reported previously by Wubs et al. (2016) are available on Figshare (doi: 10.6084/m9.figshare.3435404). The major difference were that bacterial and fungal biomass were lower on the arable soil compared to grassland and heathland (their Table S4) and the microbial and nematode community composition was strongly different (their Figures 1f,g).

Phase 1

The containers (17 × 17 × 17 cm) were planted with two seedlings each of three ruderal and three late-successional target species (12 plants per pot). Three species were early-successional ruderals: *Crepis capillaris* (L.) Wallr. (Asteraceae), *Lolium perenne* L. (Poaceae) and *Myosotis arvensis* (L.) Hill (Boraginaceae), and three were late-successional, conservation target species: *Arnica montana* L. (Asteraceae), *Festuca filiformis* Pourr. (Poaceae) and *Campanula rotundifolia* L. (Campanulaceae), with one grass and two forbs in each group. Seeds were obtained from commercial suppliers of wild plant seeds (Cruydthoeck, Assen, the Netherlands and B&T World Seeds, Paguignan, France) and germinated (sterilized 1 min. in 5% NaClO solution) on moistened glass beads in a climate chamber (12 h light/dark cycle, 20°C by day and 15°C at night). The mixed plant communities were allowed to grow for 7 weeks (Phase 1) and subsequently shoot biomass was harvested (oven-dried for 48 h at 75°C). In the prior experiment the three inocula types were mixed in different ratio's and the effects of the different ratio's were assessed (Wubs et al., 2018). Here we only used those experimental containers where 100% pure inoculum (i.e., from one field) was introduced. The present experiment used 36 containers (3 soil inoculum types x 3 replicate fields x 4 replicates per field) in Phase 1.

Phase 2

After the Phase 1 harvest, the soil from each container was sieved (4 mm mesh) to remove roots. From each original Phase 1 container, four smaller pots were filled with homogenized soil (900 g, 10 x 10 x 11 cm pots). For each Phase 1 container, two Phase 2 pots were randomly selected and planted with one individual of each of the three ruderal plant species. The other two pots per Phase 1 container were planted with one individual each of the three target species ($N = 36$ Phase 1 containers × 2 Phase 2 treatments in duplicate = 144). Seeds were germinated as before. Pots were placed in the greenhouse in a random spatial design under the same conditions as described before. Any seedlings that died in the first week were replaced. The pots were hand-weeded every week to remove seedlings emerging from the seedbank, and watered three times per week. The plants were allowed to grow for 9 weeks. Subsequently, shoot biomass of each species was cut and dried (48 h, 75°C), before weighing per species.

Phase 3

For the final phase (Phase 3), the soils of Phase 2 were again sieved (4 mm) to remove plant roots and put back in same size pots. Next, one of the two pots conditioned by ruderals, per Phase 1 container, was randomly selected and planted with ruderals and the other was planted with target plant species. The same was done for the two pots conditioned by target species, again per Phase 1 container. This led to a full factorial design of soil inoculum type with Phase 2 and Phase 3 plant species groups (ruderal or target; $N = 36$ Phase 1 containers × 2 Phase 2 × 2 Phase 3 treatments = 144) and the soils of independent replicates were kept separate throughout the three phases of the experiment. The same methods were used as above and the plants were allowed to grow for 9 weeks, after which shoot biomass of each species was determined.

Data Analysis

Differences in seedling mortality among treatments were analyzed using a generalized linear mixed model with a binomial error distribution. The analysis was conducted at Phase 3 pot level and in case any of the seedlings died this was scored as a case of mortality. Fixed effects included Phase 3 and 2 plant groups, soil inoculation and their interactions, while the random effects consisted of the sampling field of the inoculated soil and the original Phase 1 container.

Plant biomass responses were evaluated at two levels, plant group and individual plant species. The plant group level responses were analyzed using a linear mixed model with the same fixed and random effects as for the mortality model above. In addition, we tested a model where we included Phase 2 shoot biomass as a continuous predictor into the above model and we included all possible interactions. Plant-soil feedbacks were tested directly as planned contrast of biomass production on Phase 2-soils conditioned by ruderal and target species, respectively (Adbi and Williams, 2010). For the individual plant species model, PSF was calculated as the log-ratio of plant biomass in own plant group to the other plant group (Brinkman et al., 2010). The individual plant PSFs were analyzed using a linear mixed effects model, with the same random factors as before. As fixed effects plant species, soil inoculation and their interaction were included.

All analyses were conducted in R v3.5.2 (R Core Team, 2019) and model assumptions were checked graphically. Model heteroscedasticity was modeled explicitly using generalized least squares (Pinheiro and Bates, 2000; Zuur et al., 2009). Linear mixed models were analyzed using the *nlme* v3.1-137 package (Pinheiro et al., 2017) and the GLMM in package *MASS* v7.3-51.1 (Venables and Ripley, 2002).

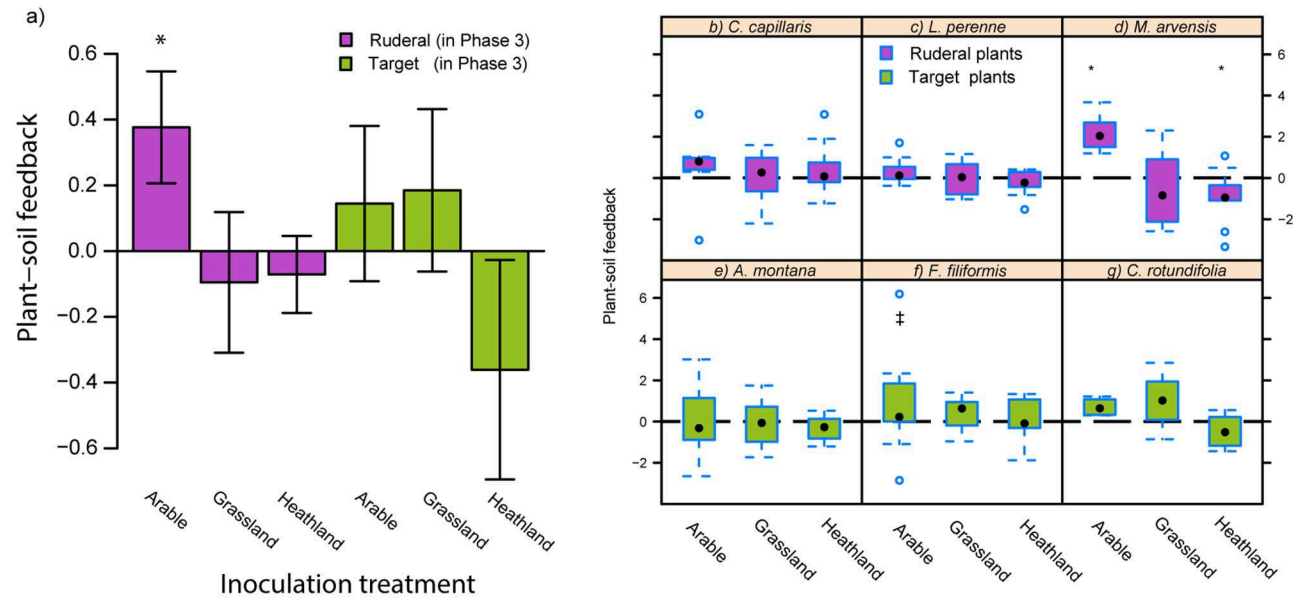

FIGURE 1 | Mean (± SE) plant species group PSFs in Phase 3 based on shoot biomass **(a)**, boxplots showing PSF per species **(b–g)**. The box extends from the lower to the upper quartile, while the thick dot represents the median. The whiskers represent the 5th and 95th percentile. Any data points beyond these cut-offs are individually presented. Plants grew in mixtures of ruderal and late-successional plant species, respectively, in pots in Phase 3. Significance of PSF effects are based on planned contrasts (*$p < 0.05$, $^{\ddagger}p = 0.0669$; **Tables S2, S3**).

RESULTS

Ruderal plant species developed positive feedback on soil inoculated with arable-soil inoculum, while on grassland and heathland inoculated soil they showed neutral PSFs (**Figure 1a**; **Tables S1, S2**). Plant species differed in their PSF responses (**Figures 1b–g**). The positive PSF in arable-inoculated soil was mostly associated with improved performance in *M. arvensis* (**Figure 1d**; **Table S3**). On heathland soil, *M. arvensis* developed a weakly negative PSF, which was unique among the tested species. As a group, the late-successional target species did not develop significant feedback in response to soil inoculation, although most negative values occurred on heathland-inoculated soil. In fact, *F. filliformis* showed a trend for positive PSF on arable-inoculated soil (**Figure 1f**; **Table S3**).

Overall, ruderal biomass was higher than that of the late-successional target species (**Figure 2**; **Table S1**). However, the difference in performance between ruderal and target species was smaller in heathland-inoculated soil than in arable-inoculated soil (**Figure 2A**; P3 × Inoc interaction; **Table S1**). There was no relationship between the pot biomass in Phase 2 and Phase 3 (**Figure 3**), and Phase 2 biomass did not interact with any experimental treatment (**Table S4**). The interaction between the two plant groups and inoculation (P3 plant × P2 plant x Inoculation) remained significant, indicating that the way the plant groups conditioned the soil (Phase 2) and affected the test plants (PSF, Phase 3) was dependent on the type of soil inoculated, also when accounting for Phase 2 plant biomass. Seedling mortality was overall low and was not affected by the experimental treatments (**Figure S1**; **Table S1**).

DISCUSSION

Our results show that soil inoculation can alter plant-soil feedbacks. However, contrary to our expectation, positive PSFs for target species were not strengthened by inoculation with late-successional soil. Instead, we found that ruderal species had positive PSFs on arable-inoculated soils, in terms of plant biomass. On soil inoculated with late-successional soil, PSFs of ruderal plants became less pronounced and not significantly different from no effect (neutral feedback; Van der Putten et al., 2013). As a group, the late-successional target species developed no significant PSF in this experiment. In the heathland-inoculated soil this led to the most even plant performance between these two groups. Since the positive PSF of ruderal species on arable-inoculated soil was suppressed in pots inoculated with late-successional soil, this suggests that these inoculated soils are robust to colonization by non-target ruderal species.

There was considerable variation in the responses of the individual plant species within the two species groups. For ruderals, the positive PSF on arable-inoculated soil was only clear in the biomass of *M. arvensis*. The other two ruderal species also had positive mean PSFs on those soils, but these effects were non-significant. Next, we found a trend for a positive PSF in one target species, *F. filliformis*, on arable-inoculated soils, while we found no other significant responses for late-successional target species. It is well-known that there is large variation in PSF strengths among plant species (Van de Voorde et al., 2011; Cortois et al., 2016) and among plant genotypes (Schweitzer et al., 2008; Evans et al., 2016; Semchenko et al.,

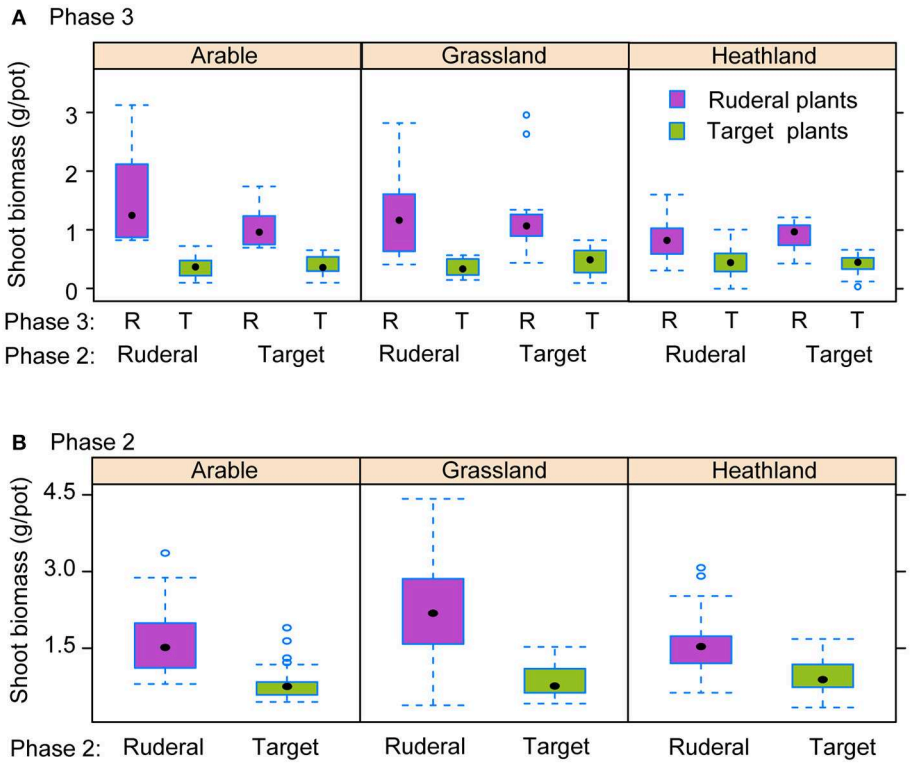

FIGURE 2 | Shoot biomass of ruderal and target plants in Phase 3 **(A)** and Phase 2 **(B)**, for statistical analyses see **Table S1**. Phase 2 and Phase 3 indicate the plants that had been grown in the soil, and Inoc indicates the soil type that was inoculated (Arable, Grassland, or Heathland soil). Other conventions follow **Figure 1**.

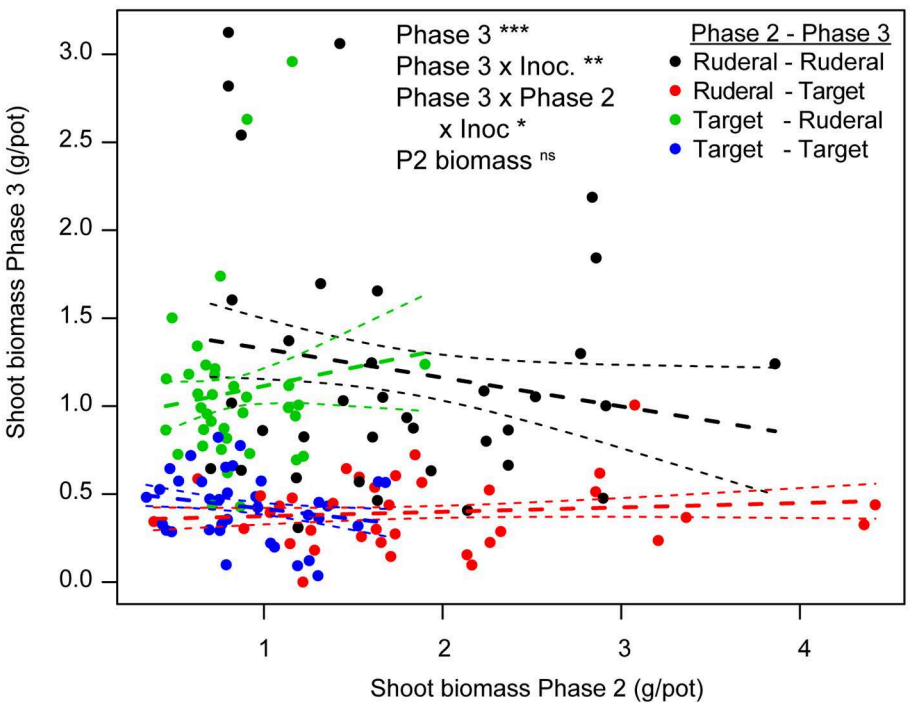

FIGURE 3 | Relationship between shoot biomass per pot in Phase 3 and Phase 2 for each of the four species groups. Significant effects in the mixed model with Phase 2 biomass as a covariate are indicated using stars (*$p < 0.05$, **$p < 0.025$, ***$p < 0.001$), for statistical analysis see **Table S4**. As a visual aid the fitted relationship of simple linear regressions per feedback group are given as dashed lines (mean ± SE). These analyses show that the effect of Phase 2 biomass was non-significant in all feedback groups.

2019). Broadly, plant traits associated with resource acquisition, such as specific root length and root diameter, may explain differences in plant-soil feedbacks among ecological groups, directly or *via* their interaction with soil biota (Lemmermeyer et al., 2015; Cortois et al., 2016; Semchenko et al., 2018). However, the variation in PSFs within plant functional groups and among genotypes of the same species are more likely to be associated with plant traits that regulate the co-evolutionary dynamics of plants and soil-organisms, e.g., *via* molecular signaling compounds in the roots (e.g., MAMPs and PAMPs; Jones and Dangl, 2006). The differences in these traits may lead to differential susceptibility of plant species and genotypes to the soil biota that are introduced *via* soil inoculation (Van der Putten et al., 2013; Bardgett et al., 2014; Wubs et al., 2016).

Earlier studies showed that late-successional species elicit positive PSFs on late-successional soils and ruderal species negative PSFs (De Deyn et al., 2003; Kardol et al., 2006; Kulmatiski et al., 2017). In contrast, our results show that for some species the PSF effects may act differently, i.e., via reduced positive PSFs for ruderal species. Interestingly, the selected target species are largely the same in our experiment and for De Deyn et al. (2003) and Kardol et al. (2006). As we used seeds from *ex-situ* cultivated populations, it could be that the plants established plant-soil interactions that are weaker than those produced by co-evolved plant and soil populations (Felker-Quinn et al., 2011; Evans et al., 2016) leading to different net PSFs. Alternatively, plant-soil feedbacks are known to vary across environmental gradients (De Deyn et al., 2004; Bezemer et al., 2006; Manning et al., 2008) and our common ex-arable soil had substantially higher P-Olsen (78.3 $\pm$ 6.71 mg P kg^{-1} soil) and soil organic matter (5.9 $\pm$ 0.2%) content than the soil used in these previous studies. At this point we can only speculate about the causes underlying the differences between these studies. Nevertheless, our results do highlight that even though changes in species abundance in response to soil inoculation (Carbajo et al., 2011; Wubs et al., 2016) may follow the expectations derived from greenhouse PSF experiments (De Deyn et al., 2003; Kardol et al., 2006; Kulmatiski et al., 2017), there may be a different combination of feedbacks in operation (Heinze et al., 2016).

The observed plant-soil feedbacks can in principle have been mediated by both abiotic and biotic changes induced in the soil through plant conditioning (Ehrenfeld et al., 2005; Van der Putten et al., 2013). Nevertheless, we suggest that the observed effects were biotically mediated for two reasons. First, we inoculated a common nutrient rich background soil with a limited amount of inoculum, so that differences in abiotic factors were diluted, thus limiting the scope for nutrient limitation. Secondly, Phase 2 plant biomass showed no correlation with Phase 3 biomass, directly nor in interaction with the experimental treatments, suggesting that abiotic factors such as nutrient limitation did not have an overriding role in our study (e.g., Kardol et al., 2006). The three ecosystem types used to inoculate the soil differ in their soil biotic community composition (Kardol et al., 2005; Van der Wal et al., 2006; Wubs et al., 2016): the grassland and heathland have higher microbial biomass than the arable soil, the grassland has more arbuscular mycorrhizae than the heathland, and the species composition of microbes and nematodes is distinct. Furthermore, data from the same study system showed that soil fungi better explain plant responses than soil abiotic factors (Wubs and Bezemer, 2018a). This is in line with results from other systems showing that the soil biota, and particularly fungi, play a prominent role in determining plant-soil feedback strengths (e.g., Kulmatiski et al., 2017; Mommer et al., 2018; Semchenko et al., 2018). Based on these arguments, we suggest that the effects observed in this experiment are due to plant-induced differences in the different inoculated soil communities, although we cannot rule out plant-induced abiotic effects.

Our study was intended as a proof of principle of how soil inoculations may alter plant-soil feedbacks for use in restoration. There are however two important limitations for direct translation of our results to restoration projects in the field. Our experiment was conducted on a sterilized common soil and it is well-known that establishment success of inoculated soil biota depends on the abundance and diversity of the resident soil community (Van Elsas et al., 2012; Mallon et al., 2015). The effects may therefore be smaller when the resident soil community is left intact by not sterilizing the common soil, as would be the case in restoration projects. However, in previous soil inoculation trials on undisturbed field soils, we did observe that novel soil biota could be successfully introduced and alter local soil and plant community composition (Wubs et al., 2016, 2019) and therefore the local resident community may not be a strong barrier to establishment of late-successional soil biota. Secondly, as is the case with most plant-soil feedback studies (Kulmatiski et al., 2008; Van der Putten et al., 2013), we only documented plant biomass responses over a period of 9 weeks. It is unclear how these short-term responses translate into longer-term fitness differences in the field (Trinder et al., 2013; Heinze et al., 2016; Kulmatiski et al., 2016) as plant-soil feedback strengths may change with plant ontogeny and may take longer to develop in later-successional species (Hawkes et al., 2013; Bezemer et al., 2018; Dudenhöffer et al., 2018). Field observations suggest that the reciprocal interactions among plants and soil biota strengthen over time (Meyer et al., 2016; Wubs et al., 2019) and extrapolation may thus be possible. Nevertheless, field based quantifications of plant-soil feedback strength, e.g., using phytometer plants, are needed to test our conclusions under conditions relevant for restoration.

An important aspect of our study is that both in the conditioning and in the response phases we used plant communities rather than individual plants or monocultures of plants. Most PSF studies have been carried out with soil conditioned by individual plants or monocultures (Kulmatiski et al., 2008). Plant competition can alter plant-soil feedbacks (Casper and Castelli, 2007; Kardol et al., 2007; Jing et al., 2015; Xue et al., 2018), for instance *via* altered root exudation (Bais et al., 2006), the interaction between resource depletion and defense (Lind et al., 2013), and because of altered food web interactions driven by different species mixtures (Bezemer et al., 2010; Bakker et al., 2013; Kulmatiski

et al., 2014). In nature, plants typically do not grow in isolation or in monocultures, and we urge that an important step forward in PSF research is understanding these mixed community feedbacks.

CONCLUSIONS

Our results show that soil inoculation alters the nature of individual species' plant-soil feedback (PSF) when these species are grown in competition with other species. The effects were species specific and differed from effects observed in other experiments. This suggests that net inoculation effects on natural vegetation depend importantly on the individual PSFs induced by the soil inoculation, which in turn depend on the soil biotic community and abiotic conditions the plants are growing in. Inoculation with late-successional soil led to suppression of the positive PSF of ruderal species found on arable-inoculated soils. Thus, we conclude that inoculation with late successional soil can reduce the colonization by non-target plant species from elsewhere if our results can be extrapolated to non-sterilized soils and long-term differences in plant fitness.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

EW and TB designed the experiment. TH and PM collected the data. EW analyzed the data and wrote the first draft. All authors contributed to the final version of the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00451/full#supplementary-material

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frontiers in Environmental Science

BRIEF RESEARCH REPORT
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Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated

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Plant-soil feedbacks (PSFs) have become a commonly invoked mechanism of plant coexistence and abundance. Yet, most PSF experiments have been performed in greenhouse conditions. To test whether or not greenhouse-measured PSF values are of similar magnitude and positively correlated with field-measured PSFs, we compared PSF values from five different studies that measured PSF values in both greenhouse and field conditions. For 36 plant species, greenhouse-measured PSF values were larger than and not positively correlated with field-measured PSF values. Similarly, these 36 species produced 269 soil-specific PSF values, and for each site there was no positive correlation between these greenhouse- and field-measured PSF values. While PSFs were observed in both greenhouse and field conditions, results provided no support at the soil, site or species level that a positive correlation exists between greenhouse- and field-measured PSF. Further, greenhouse-measured PSF appear to overestimate field-measured PSF. Although from five studies, results strongly suggest that field experiments are needed to understand the role of PSFs in plant communities in natural settings.

Keywords: plant-soil feedback, environmental factors, above-belowground interactions, experimental environment, field experiment, greenhouse experiment

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INTRODUCTION

Plant-soil feedbacks (PSFs) are increasingly used to explain plant community dynamics including succession, invasion, legacy effects, landscape abundance, coexistence, and biodiversity (Klironomos, 2002; Kardol et al., 2006; van der Putten et al., 2013). However, PSF research continues to rely mostly on greenhouse experiments (**Figure 1**). Greenhouse PSF studies are useful for developing conceptual models of plant community dynamics (Bever et al., 1997; Bonanomi et al., 2005; Aguilera, 2011), however, it remains largely untested whether or not PSFs measured in the greenhouse are correlated with PSFs measured in the field (Kulmatiski and Kardol, 2008; Schittko et al., 2016).

Plants can alter soil biota, and these changes in soil biota may subsequently affect their own growth and the growth of neighboring plants (Reynolds et al., 2003; Ehrenfeld et al., 2005). PSFs are typically investigated by testing a plant's growth response to soils cultivated by different plant species (Bever, 1994). Many approaches have been used to test PSF effects (Kulmatiski and Kardol, 2008) including unsterilized vs. sterilized soils,

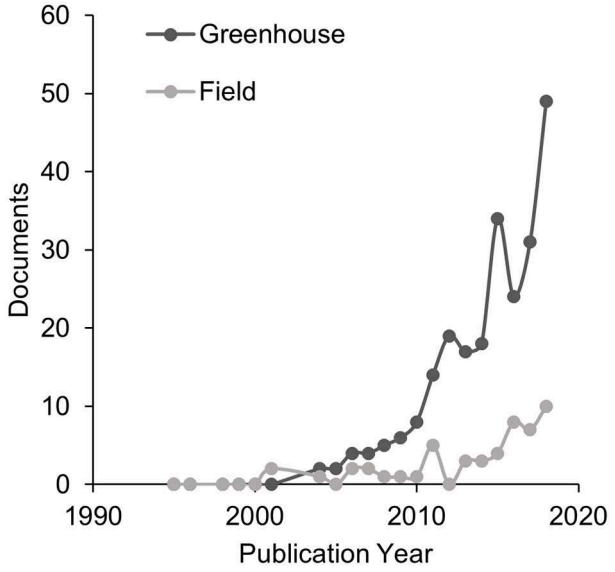

FIGURE 1 | A Scopus search performed on March 18, 2019 for "plant-soil feedback" OR "plant-soil feedbacks" in abstract, title, or keyword of published articles from 1995 to 2018 demonstrated an exponential increase in PSF research over the past 20 years. Dark gray indicates greenhouse studies and gray indicates field studies.

comparisons among different field soil inoculum into sterilized soils, microbial filtrate inoculations, and two-phase experiments in which soil types are cultivated during an experiment. The two-phase approach remains a standard approach (Bever et al., 1997; van der Putten et al., 2013). In a two-phase experiment, during the conditioning phase of the bioassay (Phase 1), plants are used to create a soil with biota specific to that species. In the response phase (Phase 2), phytometers are planted to test the growth response of a species to the altered soil biota. Growth of the Phase 2 species on soil previously conditioned by the same plant ("home") is compared to growth on soil previously conditioned by a different plant ("away"). By using soil from Phase 1 to inoculate sterilized soils, this approach can isolate microbial from soil chemical (Ehrenfeld et al., 2005; Morris et al., 2009; Ke et al., 2015) and physical (Kyle, 2005; Kulmatiski et al., 2017) effects.

PSF studies are typically executed in the greenhouse for several reasons. Greenhouse studies allow for many isolated replicates and can be performed throughout the year in rapid growth conditions. Because it is relatively easy to sterilize greenhouse soils, greenhouse studies more easily control legacy effects and separate soil nutrient effects from soil microbial effects, relative to field studies. However, completely isolating microbial from nutrient PSF may be unrealistic (Kulmatiski and Kardol, 2008; Ke et al., 2015). Greenhouse studies also lack microsite variability which can increase the likelihood of detecting PSFs in the greenhouse (Burns et al., 2015; Rinella and Reinhart, 2017).

Abiotic and biotic conditions can be very different between the greenhouse and the field (Heinze et al., 2016; Schittko et al., 2016). Greenhouse soils are typically sterilized and inoculated with small amounts of live soil; this likely creates soil conditions favoring fast-growing microbes and fast-growing plant species (Eno and Popenoe, 1964; De Deyn et al., 2004; Howard et al., 2017). Frequent fertilization and watering can cause arbuscular mycorrhizal fungi to become parasitic as conditions change from low to high fertilization regimes, and dry to wet water regimes (Johnson et al., 2003; Schmidt et al., 2011). This could cause PSF to appear neutral or positive in dry field conditions, and negative or neutral in a greenhouse with a consistent water regime (Mohan et al., 2014). Large soil organisms are typically absent in greenhouses which would affect plant-soil interactions (Kutáková et al., 2018), such as below-ground herbivory (Hol et al., 2010; Bezemer et al., 2013). More broadly, stressful conditions found in field studies may induce greater facilitation and a more positive PSF in the field (Maestre et al., 2009). These differences have led several authors to recommend greater field experimentation (Kulmatiski and Kardol, 2008; Heinze et al., 2016; Schittko et al., 2016).

Here, our goal was to test whether or not greenhouse-measured PSFs are of a similar magnitude and positively correlated with field-measured PSFs. We predicted that greenhouse- and field-measured PSF would be positively correlated because we expected that plants have a dominant effect on soil microbial community composition and subsequent PSF; these effects should be similar in both settings due to similar plant species and soil microbial communities. A negative correlation or a lack of correlation between greenhouse- and field-measured PSF suggests that greenhouse conditions change plant-soil interactions in ways that reverse or change PSF values. To test this prediction, we compared greenhouse- and field-measured PSF values from published studies and publicly available datasets. To assess whether PSF is overestimated in greenhouse or field conditions, we compared the magnitude of PSF values (regardless of sign) by taking the absolute values of greenhouse- and field-measured PSF.

METHODS

A Scopus search for PSF studies with the term "plant-soil feedback" or "plant-soil feedbacks" in the title, abstract, or keywords was performed on March 19, 2019. Of the resulting 515 studies, meta-analyses, modeling papers, reviews, and non-English studies were removed. The remaining studies were reviewed to identify studies containing (1) a home/away PSF method (Brinkman et al., 2010), (2) aboveground biomass or cover as the response variable, and (3) grasslands as the study ecosystem (Burns et al., 2017; Teste et al., 2017), which left 297 studies. Species from grassland ecosystems were selected as the focal organisms because most PSF research has been conducted in grassland ecosystems, so sufficient sample sizes from non-grassland ecosystems were unlikely (Kulmatiski et al., 2008; van der Putten et al., 2013). Of these 297 studies, 237 occurred in the greenhouse, 50 in the field, seven in mesocosms, and three included both greenhouse and field approaches. Of these three studies, data was collected from two, but one possible study did not respond to requests for data. An additional three datasets

produced by the authors, which are publicly available at the USU Digital Commons, were also included.

The resulting dataset contained paired greenhouse-measured and field-measured PSF values for 36 species derived from 2,975 field observations and 2,907 greenhouse observations at five different study sites. We used the paired dataset to (1) calculate PSF values using a single method for all data, (2) test for correlations between greenhouse- and field-measured PSF, and (3) compare PSF values and PSF magnitudes (absolute values) between greenhouse- and field-measured PSF.

Study Sites

Of the five study sites included, three were from Europe (Berlin, Potsdam, and Jena in Germany) and two were from North America (Winthrop, Washington and Cedar Creek, Minnesota in the United States). At all sites, the focal species selected were abundant in local plant communities. Four species were common among at least two study sites (**Supplementary Material**).

All five studies compared phytometer growth responses to "home" and "away" conditioned soil (Bever, 1994). When more than two species are used in a PSF experiment, this comparison can be undertaken by mixing all conditioned "away" soils together to create a single "away" treatment. This approach was used in the Berlin study; it eliminates site-by-site variation in soil microbes (Reinhart and Rinella, 2016; Rinella and Reinhart, 2017), and can be useful when the research question is not focused on spatial variability (Cahill et al., 2017; Gundale et al., 2017). Alternately, phytometer responses can be measured on each "away" soil creating a species*soil-level design. This approach was used in the studies at Cedar Creek, Jena, Potsdam, and Winthrop. Data from species*soil-level PSF experiments were converted to species-level PSF values by averaging a species' growth across "away" soil types.

Greenhouse Experiments

The experiments at Cedar Creek, Jena, and Winthrop implemented a cultivated two-phase approach (Rinella and Reinhart, 2018). The experiments at Berlin and Potsdam collected conditioning soils from underneath monotypic stands in the field (Kulmatiski and Kardol, 2008) (**Table 1**).

For Phase 1, the Cedar Creek greenhouse experiment steam-sterilized a six-to-one mixture of sand and sphagnum peat inoculated with ten percent field soil. The prepared 1-L pots were planted and grown for a 6-months Phase 1. The Jena greenhouse experiment inoculated a three-to-one mixture of compost and sand with ten percent field soil. The prepared 1-L pots were planted and grown for an 8-months Phase 1. The Winthrop greenhouse experiment steam-sterilized a six-to-one mixture of coarse sand and sphagnum peat and inoculated with five percent field soil. The prepared 1-L pots were planted and grown for a 3-months Phase 1. At the end of Phase 1, plants were removed by hand-clipping; 2,282 pots at Cedar Creek, 239 pots at Jena, and 216 pots at Winthrop had growth.

For Phase 1, the greenhouse experiment at Potsdam collected field soil from underneath three different species' monotypic stands and filled 90 0.41-L pots with 100% field soil (Heinze et al., 2016). In Berlin, Schittko et al. (2016) collected field soil from underneath eight different species' monotypic stands. The soil for the "away" treatment was mixed, where the soil for the "home" treatment was not mixed. A steam-sterilized sandy loam soil was inoculated with 23% "home" or "away" soils collected in the field and used to fill 240 pots, 80 of which were retained for the greenhouse experiment.

For the greenhouse experiment at Cedar Creek, the Phase 2 length was 6 months; at Jena 3 months; at Winthrop, 3 months; at Potsdam two and one-third months; and at Berlin 4 months. Pots were clipped and aboveground biomass weighed for all species at the end of Phase 2 (**Table 1**).

TABLE 1 | Methods for the paired greenhouse and field experiments.

	Berlin	Cedar Creek	Jena	Potsdam	Winthrop
Field plot size	1.4-L pots except for *Cichorium intybus* and *Medicago* × *varia*, which were in 3.1-L pots	0.75/0.35 m	0.75/0.35 m	0.4/0.4 m	1.5/1.5 m
Greenhouse pot size	1.4-L and 3.1-L (see above)	1-L	1-L	0.41-L	1-L
Phase 1 Type	Inoculum	Cultivated	Cultivated	Inoculum	Cultivated
Greenhouse live soil rate	23%	10%	10%	100%	5%
Greenhouse experiment length	4-months Phase 2	6-months Phase 1 and 6-months Phase 2	8-months Phase 1 and 3-months Phase 2	Two and one-third months Phase 2	3-months Phase 1 and 3-months Phase 2
Field experiment Length	0.5 months spent in the field out of a 4-months experiment	24-months Phase 1 and 24-months Phase 2	24-months Phase 1 and 24-months Phase 2	2.5-months Phase 2	48-months Phase 1, 32-months Phase 2
Greenhouse N	80	2,282	239	90	216
Field N	160	2,066	345	89	315

Field Experiments

At Cedar Creek and Jena, the field site area was sprayed with glyphosate and disked. Experimental plots (0.35/0.75 m) were established with 0.75 mm thick HDPE root barrier inserted to 35 cm deep between each plot. For Phase 1 at Cedar Creek, ten grams of pure live seed per m^2 was applied to each of the plots. At Jena, 2,000 total pure live seeds per m^2 were applied to each of the plots. After a 2-years Phase 1, the area was sprayed with glyphosate and hand-tilled using a garden claw. Non-target species were removed by hand-weeding. At Cedar Creek, plots containing C_3 grasses and forbs were hand-tilled using a garden claw, but vigorous root growth in the C_4 grasses necessitated tilling using a miniature tiller on plots containing that functional group. Seed was re-applied at the same respective rates. After a 2-years Phase 2, aboveground biomass was clipped, dried and weighed; 2,066 Cedar Creek plots and 345 Jena plots had growth.

At Winthrop, the top 10 cm of vegetation and soil was removed (Kulmatiski, 2019). A one-to-one mix of native soil inoculum and sand was applied to the prepared site, and disked to 15 cm to homogenize. A grid of 1.2 m wide geotextile cloth was laid down to create 315 1.5/1.5 m PSF plots in the area. Ten grams of pure live seed per m^2 was applied to each plot, and allowed to grow for a 4-years Phase 1. After 4 years, Phase 1 plants were sprayed with glyphosate. Seed was re-applied for Phase 2 and plots were allowed to grow for 3 years. Growth was estimated using percent cover in June 2013.

At Potsdam, 30 (0.4/0.4 m) plots were prepared by cutting the first 25 cm of roots under three different monotypic stands to create three Phase 1 treatments (Heinze et al., 2016). Three individuals of each species were planted in each plot. Individuals were spaced 10 cm apart and allowed to grow for 10 weeks. After the 10 weeks, aboveground biomass was harvested, and 89 individuals had growth.

At Berlin, at week 14 of the greenhouse experiment, 160 pots were transferred to the field and left to sit on top of the soil for a period of 2 weeks (Schittko et al., 2016). After 2 weeks, the aboveground biomass was harvested. Extended methods for Cedar Creek and Jena are in **Supplementary Material**; for Potsdam, Winthrop, and Berlin extended methods are in Heinze et al. (2016), Kulmatiski et al. (2011, 2017), and Schittko et al. (2016).

Statistical Analyses

To avoid bias from different calculation methods, original plant growth data on "home" and "away" soils was used to calculate PSF values using a single method for all data (Brinkman et al., 2010). PSFs were calculated as (H-A)/maximum (H,A), where H is the aboveground growth (ground cover or biomass) produced by a species in Phase 2 on "home" soils, and A is the aboveground growth produced by a species in Phase 2 on "away" soils. The denominator refers to the maximum aboveground growth produced by a species regardless of soil type. This calculation has similar mathematical properties to the commonly used ln(H/A) metric (i.e., values that are symmetric around zero and bounded between +1 and −1). In addition, it has the advantage of being easily interpretable as the proportion increase or decrease in growth due to soil type (Brinkman et al., 2010). Plots or pots where the Phase 1 or the Phase 2 realized no growth were removed from the dataset. To prepare the data from species*soil-level PSF studies for a species-level analysis, one PSF value was calculated for each "away" species by taking the mean PSF value for each species across soil types.

To determine if the mean PSF value for each experiment was different from zero, we took the standard error of the mean. For data from species-level PSF studies, one home vs. away PSF was calculated for each species. For species-level PSF values, we used linear models to test for a correlation between greenhouse- and field-measured PSF within each study site and overall. For species*soil-level PSF values, we used linear models to test for a correlation between greenhouse- and field-measured PSF within each study site only, to control for the outsized effect of Cedar Creek's data on the overall dataset. Linear models were performed using the polyfit and fitlm scripts in MATLAB (The MathWorks Inc., 2015). Residuals for the species-level data were checked for normality using the Shapiro-Wilk test.

Comparisons

To compare PSF values and PSF magnitudes (absolute values) among study sites and regions, we performed a one-way analysis of variance (ANOVA) using the script anova1 in MATLAB. Significance was evaluated at $\alpha = 0.05$. When significant, differences were explored with a Tukey's Honest Significant Difference test in MATLAB using the script multcompare.

RESULTS

From species-level data, 36 paired PSF values were compared. Of these 36 values, eight came from the mixed-soil PSF experiment at Berlin, and the remainder from species*soil-level studies where the mean PSF value across all soil types was calculated to create a single PSF value per plant species: 16 PSF values came from Cedar Creek, five from Jena, three from Potsdam, and four from Winthrop. Greenhouse PSF values were positive in Berlin and Potsdam, and neutral in Jena, Winthrop, and Cedar Creek (**Figure 2A**). Field PSFs were positive in Berlin and Winthrop, neutral in Jena and Potsdam, and negative in Cedar Creek (**Figure 2A**). For the species-level greenhouse-measured data the average PSF was 0.046 and the coefficient of variance was 5.14; for the field-measured data the average PSF was −0.008 and the coefficient of variance was 24.01.

A total of 269 PSF values from species*soil-level field/greenhouse paired experiments were compared. Of these values, 239 came from the Cedar Creek study, 20 from the Jena study, six from the Potsdam study, and four from the Winthrop study. PSF values for Berlin were excluded from the species*soil-level dataset because the study was not species*soil-level in design. Greenhouse PSF values were positive in Potsdam and Jena, and neutral in Winthrop and Cedar Creek (**Figure 2B**). Field PSF values were positive in Winthrop, neutral in Jena and Potsdam, and negative in Cedar Creek (**Figure 2B**). For the species*soil-level greenhouse-measured data the average PSF was −0.007 and the coefficient of variance was 50.59; for the field-measured data the average PSF was −0.064 and the coefficient of variance was 4.81.

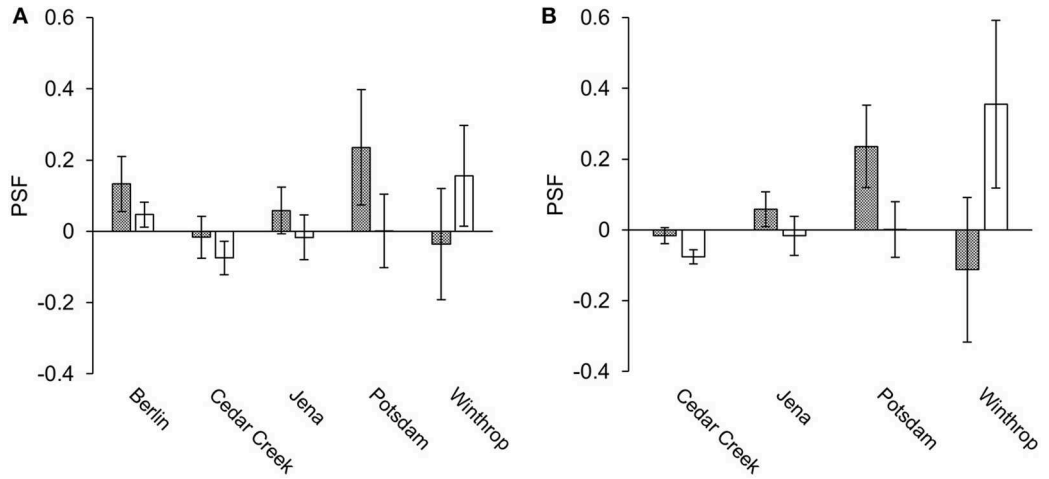

FIGURE 2 | Average greenhouse- and field-measured species-level **(A)** and species*soil-level **(B)** PSF values (mean ± SE). Gray indicates greenhouse studies and white indicates field studies.

We tested for correlations in species-level data both within and among sites. For species*soil-level data we tested within but not among sites because 86% of species-level data was from one site. For species-level data, there was no correlation between greenhouse- and field-measured PSF values across all study sites ($F_{1,34} = 0.179$, $P = 0.675$, **Figure 3A**). Similarly, there was no correlation between greenhouse- and field-measured PSF values within study sites ($P > 0.05$, **Figure 3A**). For the species*soil-level PSFs, there was no correlation between greenhouse- and field-measured PSF values at the Cedar Creek, Jena, and Winthrop sites ($F_{1,237} = 0.001$, $P = 0.972$; $F_{1,18} = 0.003$, $P = 0.959$; and $F_{1,2} = 0.039$, $P = 0.801$; respectively; **Figure 3B**). There was a negative correlation between greenhouse- and field-measured data from the Potsdam site ($F_{1,4} = 10.129$, $P = 0.034$, $R^2 = 0.717$; **Figure 3B**).

We tested for differences in magnitude (absolute value) for species-level data only because of the strong effects Cedar Creek had on species*soil-level data. While there were few correlations between greenhouse- and field-measured PSF values, there were differences between the magnitude of greenhouse- and field-measured PSF values, indicating that PSF (either positive or negative) were larger in greenhouse than field conditions ($F_{1,70} = 5.056$, $P = 0.028$).

DISCUSSION

Although PSFs are commonly invoked as a mechanism to explain complex plant community dynamics in the field, the majority of PSF experiments take place in controlled greenhouse conditions. We had predicted that greenhouse- and field-measured PSF would be positively correlated due to the dominant effects of plants on their soil microbial communities, but found no evidence to suggest that greenhouse-measured PSF data are positively correlated with field-measured PSF. We also found greenhouse-measured PSF values were exaggerated relative to field-measured PSF values. Together, results suggest that the greenhouse-measured PSFs that predominate in the literature both overestimate and provide little direct inference into PSF effects in the field. Although our dataset is derived from only five sites, our results strongly suggest that PSFs are sensitive to growth conditions (Casper et al., 2008). Consequently, field experiments are likely to be needed to fully understand the role of PSFs in natural systems.

There are several potential reasons that could explain why PSF values were smaller in the field than in the greenhouse. More stressful growing conditions (for example, competition, drought, or herbivory) may minimize PSF effects (van der Putten et al., 2016; Crawford and Knight, 2017; Fry et al., 2018). Although researchers in all five field experiments attempted to decrease competitive effects by hand-weeding, it is likely that competitive pressure was still greater in the field than greenhouse experiments due to the larger seed bank in unsterilized field soils (Lekberg et al., 2018). Similarly, greater aboveground herbivory in the field was likely to decrease PSF values directly by removing aboveground biomass and potentially indirectly by inducing increased belowground growth (Heinze and Joshi, 2018). Drought in the field may also decrease PSF values by decreasing plant growth, microbial growth, and nutrient cycling rates (van der Putten et al., 2016). With only five studies and many potential factors affecting differences between greenhouse and field results, it was not possible to test these hypotheses, but they are consistent with our observation of larger PSF values in the greenhouse.

Methodological differences were likely to explain why there was no positive correlation between field and greenhouse PSF values, though we were unable to isolate any specific methodological difference that would explain our results. Compared to the field, growing space is restricted, experiment length is shorter, and dominant soil microbes differ in the greenhouse. Excepting Berlin, greenhouse pots were smaller than field plots. Yet, we did not observe a qualitatively different

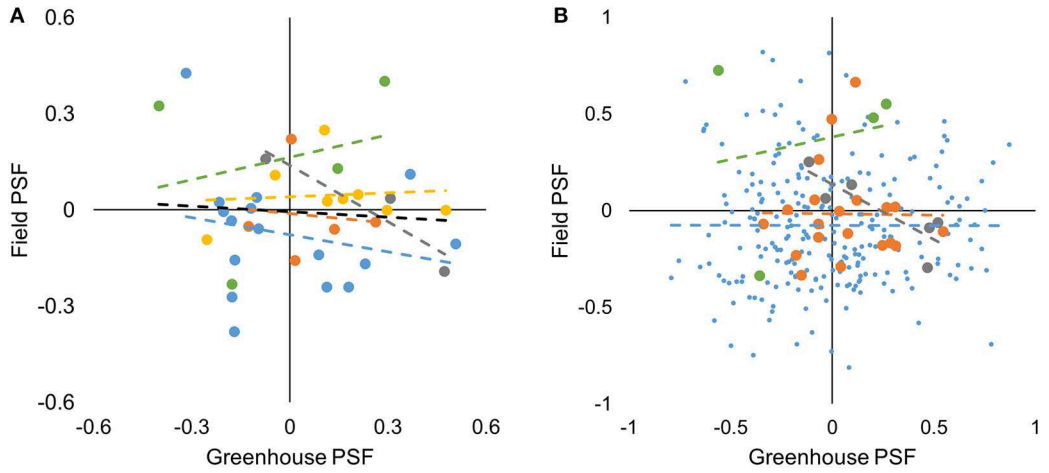

FIGURE 3 | Greenhouse- vs. field-measured species-level **(A)** and species*soil-level **(B)** PSF values from five study sites. Yellow, Berlin; blue, Cedar Creek; orange, Jena; gray, Potsdam; green, Winthrop; black is a best-fit line for all study sites. Though only significant for the Potsdam species*soil-level data, best-fit regression lines are shown for each site to demonstrate that slopes were close to zero.

relationship between greenhouse and field PSF values at Berlin. The Winthrop site had the largest difference between field plot and greenhouse pot size, yet PSF values were not notably different from other sites.

Differences in temporal scales among sites similarly did not appear to drive our results. PSFs have been suggested to accumulate over time (Kardol et al., 2006; Kulmatiski et al., 2008; Diez et al., 2010; Lepinay et al., 2018), but Potsdam, which had similar greenhouse and field experiment lengths, did not have a positive correlation between greenhouse- and field-measured PSF. Sterilized soils, which were used at three of the five reviewed experiments, often have higher nutrient availability and promote faster plant growth, changing PSF values and soil microbial communities that drive PSF (De Deyn et al., 2004). However, sites using sterilized soils and sites using unsterilized soils both had uncorrelated PSF values. Little can be inferred from the five studies reviewed, but results did not provide strong evidence to suggest that pot size, experiment length, or sterilization technique provided a strong explanation for the difference between greenhouse and field results.

The only correlation observed between greenhouse- and field-measured PSF, was a negative correlation at the Potsdam site. This site was the only site to use 100% field soil in the greenhouse experiment. It is possible that a negative correlation occurred because under decreasing light conditions PSF can be reversed (Smith and Reynolds, 2015), but it is not clear why this effect would only appear when 100% field soils were used. To the contrary, we would have expected that the use of 100% field soil would produce more similar results to the field.

Although our results and results from previous studies suggest that PSF values are very context-dependent (Casper and Castelli, 2007), the PSF concept remains relevant to plant community ecology. Greenhouse-measured PSFs have been found to improve predictions of plant growth in communities in the greenhouse (Kulmatiski et al., 2011, 2017) and field-measured PSFs have been found to improve predictions of plant growth in communities in the field (Klironomos, 2002; Kardol et al., 2006; Mangan et al., 2010; Mariotte et al., 2018; Kulmatiski, 2019). Thus, our results suggest that while greenhouse studies are useful for conceptual model development and predicting plant growth in greenhouse conditions, ecologists who wish to understand the role of PSFs for specific plant species in the field should rely on field studies.

While from five studies, our results suggest that the PSF literature, which is predominantly derived from greenhouse experiments, overestimates PSF effects and while it may provide insight into general patterns of interactions that occur in plant communities in the field, it provides little insight into the specific PSFs that determine the growth and abundance of specific plants in natural communities. Our findings are consistent with results from previous studies (Heinze et al., 2016; Schittko et al., 2016), and suggest that although greenhouse-measured PSFs are important for conceptual models, field experiments will likely be needed to understand the role of PSFs in complex plant community dynamics in the field.

DATA AVAILABILITY STATEMENT

The Potsdam dataset analyzed for this study is available on request from JH. The Cedar Creek, Jena, and Winthrop datasets analyzed for this study can be found at USU Digital Commons (https://doi.org/10.26078/52k0-jr94, https://doi.org/10.15142/T3XM19). The Berlin dataset analyzed for this study can be found in the Dryad Digital Repository (https://doi.org/10.5061/dryad.r7c23).

AUTHOR CONTRIBUTIONS

LF and AK coordinated the writing. LF coded the papers included in the literature search, and conducted the meta-analyses with input from AK, JG, JH, and CS. LF, JG, and AK performed the

studies at Cedar Creek and Jena. AK performed the study at Winthrop. JH performed the study at Potsdam. CS performed the study at Berlin. All co-authors participated in generating the ideas discussed here.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00184/full#supplementary-material

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Aboveground Competition and Herbivory Overpower Plant-Soil Feedback Contributions to Succession in a Remediated Grassland

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Plant-soil feedback (PSF) can provide a driving force during ecological succession by altering soil properties in ways that benefit or disadvantage other species in the successional sequence. Succession may be inevitable in disturbed sites remediated by planting early successional species, but information on PSF in such settings is lacking. We investigated whether gray birch (*Betula populifolia*), a native species but strong invader, alters succession from grassland to deciduous forest at a site contaminated with zinc, lead, and cadmium. We investigated PSF within the context of competition, herbivory, and soil contaminants, and evaluated whether gray birch, as a high metal accumulator, engages in elemental allelopathy, poisoning other species through its metal-contaminated leaf litter. We assessed the effects of gray birch on neighboring plant community structure, soil chemistry, fungal root symbionts, and the germination, growth, and herbivory of seedlings of black oak (*Quercus velutina*) and sugar maple (*Acer saccharum*), two tree species expected to follow gray birch in succession. Gray birch was associated with increased diversity in its neighborhood grassland community, increased herbivory on black oak seedlings, and influenced colonization by fungal root symbionts in both species. Seedling biomass was correlated with colonization by ectomycorrhizal fungi in black oak, but not with arbuscular mycorrhizal or dark septate fungal colonization in sugar maple. Gray birch had no effect on maple seedling performance or soil chemistry, and a small effect on black oak performance in the absence of aboveground competition. We found little evidence consistent with elemental allelopathy. Black oak and sugar maple seedlings responded more strongly to variation in soil nutrients than soil heavy metals, and they maintained leaf metal concentration profiles markedly different from those in their soils. We conclude that gray birch alters its environment in ways that could promote establishment and succession of woody species, potentially favoring those that are ectomycorrhizal, but most effects of PSF are overpowered by aboveground competition, herbivory, and/or existing abiotic soil factors. This study well illustrates why the potential for PSF to affect plant performance

and community structure must be examined within the context of other ecological processes. Such a broad understanding can inform decisions made in the remediation and management of disturbed sites, and our understanding of plant succession and coexistence in general.

Keywords: competition, heavy metals, herbivory, plant-soil feedback, succession, remediation, restoration

INTRODUCTION

Succession is the process by which the species composition of ecological communities changes over time, in ways that are often orderly and directional (Connell and Slatyer, 1977). In terrestrial systems, plant-soil feedback (PSF) is a driver of plant species replacements (Connell and Slatyer, 1977; Bauer et al., 2015; Wubs and Bezemer, 2017), and can either speed or slow succession, depending on whether plant-induced soil alterations improve or worsen conditions for contemporary species relative to later arriving species (Bever et al., 1997; Reynolds et al., 2003; Kardol et al., 2006; Castle et al., 2016; Lozano et al., 2018; Crawford et al., 2019). PSFs can act via soil organisms, including pathogens and symbionts such as mycorrhizal fungi, as well as any number of chemical or physical soil properties (Ehrenfeld et al., 2005; Png et al., 2018; Bennett and Klironomos, 2019). While PSFs have been shown broadly to be involved in ecological succession (Kardol et al., 2006; Kulmatiski et al., 2008; Bauer et al., 2015), connecting specific feedback mechanisms to succession remains rare in the literature, and calls are made for more such studies and for more field studies of PSFs in general (Van Der Putten et al., 2013, 2016; De Long et al., 2018).

Field studies are essential for evaluating the importance of PSF in the presence of other prevalent ecological processes affecting a species' performance, such as plant-plant competition and herbivory (Casper and Castelli, 2007; Heinze and Joshi, 2017). Both competition and herbivory have the potential to augment or reduce effects of PSF, based on the strength of these other processes and whether their effect on plant performance is in the same or opposite direction as that of PSF (Casper and Castelli, 2007; Lekberg et al., 2018; Heinze et al., 2019). Conversely, PSF effects on plant performance could indirectly alter a plant's susceptibility to herbivory based on information that herbivory levels can vary with mycorrhizal status (Gehring and Bennett, 2009; Koricheva et al., 2009), leaf nutritional quality (Ayres et al., 1997), or plant vigor (Cornelissen et al., 2008), and evidence that soil biota can influence plant nutritional quality (Kos et al., 2015) and herbivory defenses (Kostenko et al., 2012; Zhu et al., 2018). PSF may contribute to plant-plant competition directly through altered soil characteristics or indirectly (Hortal et al., 2017; Bezemer et al., 2018) since competitive ability is usually related to plant size (Weiner and Thomas, 1986).

Understanding how plant communities change over time and which mechanisms underlie these changes is deeply important for managing ecosystems and particularly pertinent for previously disturbed sites undergoing restoration, where installed vegetation may represent an early successional stage. Grasses and other early successional plants are often planted on abandoned mines or metal-polluted sites in order to curtail erosion and leaching, achieve quick phytostabilization through ground cover, and create a surface soil enriched in organic matter (Turnau et al., 2008; Bolan et al., 2011), even in regions where woody plants are the expected successional climax (Turnau et al., 2010; Zhang et al., 2012). We need to know more about succession in these systems and the possible role of PSFs, particularly in heavy metal contaminated soils (Krumins et al., 2015).

Heavy metal soils hold the potential for a particular PSF mechanism called elemental allelopathy. The idea is that plants with high leaf metal uptake create allelopathy (Inderjit et al., 2011) when their contaminated leaf litter locally increases soil metal concentrations to the detriment of less metal tolerant plants and/or soil microbes growing nearby (Baker and Brooks, 1989; Wilson and Agnew, 1992; Boyd and Martens, 1998). Some field studies have found elevated levels of metal contaminants around hyperaccumulating plants, sometimes with negative effects on other species, consistent with elemental allelopathy (Boyd and Jaffré, 2001; Mehdawi et al., 2011; Jaffe et al., 2017), but most cannot eliminate alternative explanations (Morris et al., 2009; Mehdawi et al., 2011).

We looked for evidence of PSF and elemental allelopathy in particular during succession from grassland to deciduous forest at a revegetated site. The site was historically mostly forested but was then severely contaminated and devegetated by Zn, Pb, Cd, and SO_x emissions from two zinc smelters operating for approximately 80 years, with the combination of metal pollution and acidity from SO_x likely responsible for most plant deaths (Buchauer, 1973; Jordan, 1975; EPA, 2007a,b). Land managers chose a phytostabilization approach and planted primarily C_4 grasses with low metal uptake rates (EPA, 2007a,b). Among the most successful species that have since encroached from forest remnants or colonized *de novo* (Dietterich and Casper, 2017) is gray birch (*Betula populifolia*), a native pioneer tree that accumulates high foliar concentrations of the metal pollutants (Gallagher et al., 2011).

Our main goal was to determine whether gray birch affects succession through PSF at this revegetated, heavy metal polluted site. We examined how gray birch affects local plant community structure; soil organic matter, metal, and nutrient concentrations; and soil temperature and moisture. To address PSF, we crossed soils conditioned by either gray birch or grasses with the presence or absence of aboveground competition in the field to identify any interactions between these two factors in affecting succession. In particular, we examined seed germination and seedling size, root colonization by fungal symbionts, herbivory levels, and plant metal uptake of two later successional tree

species, black oak (*Quercus velutina*, Fagaceae) and sugar maple (*Acer saccharum*, Sapindaceae).

Because of gray birch's high metal uptake, we predicted (1) lower seedling growth under birches than grasses, but higher growth in plots where aboveground competition was removed. Because gray birch and black oak both associate with ectomycorrhizal fungi (ECM) while the grasses and sugar maple are only colonized by arbuscular mycorrhizal fungi (AMF) (Harley and Harley, 1987; Dickie et al., 2001; Wang and Qiu, 2006), we predicted (2) black oak would have greater ECM colonization in soils conditioned by gray birch, sugar maple would have more AMF colonization in soils influenced primarily by grasses, and increased root colonization would translate to increased plant growth. To examine the effects of PSF and competition in the context of standing variation in soil metal contamination, we further explored whether spatial variation in soil contaminant and nutrient concentrations explained variation in seedling performance or leaf chemistry, independent of our experimental treatments. We predicted (3) strong relationships between soil contaminant concentrations, plant growth, and leaf contaminant concentrations, on the premise that the presence and availability of metals in the soil would shape plant uptake of those metals. Finally, through the field experiment and a companion greenhouse experiment, we evaluated (4) whether gray birch engages in elemental allelopathy.

MATERIALS AND METHODS

Our study was based in the portion of the Palmerton Zinc Superfund Site (Palmerton, PA, USA) owned and managed by the Lehigh Gap Nature Center. The large area (>800 ha) and steep, rocky terrain made removing the metal contaminants unfeasible (EPA, 2007a,b). To remediate the devegetation and high levels of soil metal contaminants, land managers applied fertilizer and compost to inoculate the soil with microbes, including decomposers and mycorrhizal fungi. They planted C_4 grasses, known to have low metal uptake rates, with the goal of creating an organic enriched surface layer, sequestering the metals away from humans and the rest of the food web (EPA, 2007a,b). The planted grasses remain abundant but now represent a minority of the site's species, as many others have encroached from forest remnants or colonized since the revegetation efforts (Dietterich and Casper, 2017). Gray birch, the most striking colonizer, is spreading rapidly and forming dense stands.

Our field experiment to determine how seed germination and seedling performance of black oak and sugar maple respond to grass vs. birch, as two dominant vegetation types, was set up from May 2014 to September 2015. At each of 10 locations where grassland communities grew in close proximity to dense gray birch stands, we established four 1.5 × 3.0 m experimental plots; two dominated by gray birch and two dominated by the grasses. At each location, to examine possible interactive effects of aboveground competition and vegetation type, every ∼2 weeks during the growing season, we clipped and removed all aboveground vegetation from one plot of each vegetation type while leaving the other intact. We chose not to remove belowground vegetation in order to preserve soil structure and possible variation in soil chemistry with depth.

Gray Birch Effects on Community Structure and Soil Characteristics

Before setting up the experiment, we evaluated plant community structure and soil nutrients, metal contaminants, and organic matter. In a May 2014 census, we identified all plants, most to species, and visually estimated their percent cover within each plot. Because the plots' dominant grasses could not consistently be identified to species at that time of year, we grouped them into a single taxon for analysis. We examined total cover of the birch and grasses as well as cover, species richness, and Shannon diversity of newly colonizing species apart from birch and grasses.

To investigate soil chemistry, we collected ∼100 g of surface soil (primarily organic horizon material from the top ∼10 cm of soil) at the center of each plot using a clean trowel. Soils were air-dried in the lab and sieved to 2 mm. We used inductively coupled plasma optical emissions spectroscopy (ICP-OES; details below) to measure concentrations of the nutrients Ca, Mg, and K, the known contaminants Zn, Cd, and Pb, and the heavy metals Cu, Mn, and Ni, which were not expected to be enriched in the site. The procedure yields total extractible concentrations of these metals and nutrients, which we interpret as the amount of the metals and nutrients a long-lived plant may have access to in its lifetime, but more than would be bioavailable to a plant at any given time. We also measured soil organic matter content as percent loss on ignition (LOI).

To further characterize growing conditions, in the second growing season, we periodically measured soil moisture and temperature at three locations in each plot with a WET-2 sensor connected to an HH2 moisture meter (Delta-T Devices Ltd, Cambridge, UK). We measured leaf area index as a proxy for shading intensity with a CI-110 Plant Canopy Imager (CID Bio-Science, Inc., Camas, WA, USA).

Gray Birch and Abovegound Competition Impacts on Tree Species Establishment, Mycorrhizal Colonization, and Leaf Metal Content

We planted six bare-root 1–2 year old seedlings each of black oak and sugar maple (Musser Forests, Indiana, PA, USA) into each plot. For each seedling, we dug a hole with shovels and trowels, put the seedling into the hole, and backfilled the excavated soil up to the top of the root collar. Each seedling received 10–20 mL of water at planting and one or two days later, but otherwise were irrigated by rainfall. To protect seedlings from deer, we used plastic mesh tubes (Forestry Suppliers, Jackson, MS, USA, Stock No. 17048), supported by wooden garden stakes and cable ties. Every 2–3 weeks during both growing seasons, we re-cleared all aboveground vegetation from cleared plots and examined all planted tree seedlings, adjusting displaced protective tubes as necessary. In spring 2014, we also assessed germination by planting 20 cold-stratified seeds of each species (Sheffield's Seed Co., Inc., Locke, NY, USA) near the center of each plot and counting seedling emergence after nine weeks. Seeds were planted under a ∼40 × 40 cm plastic ∼0.5 cm mesh to reduce seed herbivory and aid in seedling recovery.

We measured seedling height at planting and again at harvest, where we conducted additional size and biomass measurements

at the end of the second growing season. We separated seedlings into aboveground and belowground parts by clipping at the top of the root collar. We recorded aboveground plant height, fresh and dry leaf biomass, dry stem biomass, leaf area, number of leaves, and the length of apical and lateral branches grown during the current and previous growing seasons. We estimated whether leaf herbivory was absent, mild (5–20% of leaf area), moderate (20–50% of leaf area), or severe (>50% of leaf area) after Johnson et al. (2016). We restricted our analysis to herbivory that appeared to be due to insects based on the pattern of leaf damage. We saw evidence of deer herbivory, including stem and leaf damage in the shape of a single large bite mark, as well as live deer and deer scat in the area. We did not analyze deer herbivory because it was impossible to know how many leaves were missing.

We also measured root biomass and fungal colonization. We washed roots thoroughly with tap water and recorded the fresh weight of the whole root system. For all 228 black oak seedlings harvested, we recorded percent colonization by ECM as the proportion of colonized root tips out of a representative sample of at least 50 root tips, unless fewer were available. For sugar maples, we removed and weighed sub-samples of fine roots to clear and stain for colonization by AMF and dark septate endophytes (DSE). We estimated dry biomass of roots used for endophyte colonization by obtaining wet and dry weights of additional fine root sub-samples. We dried root systems at 60°C for at least 48 h, then manually separated and recorded the dry biomass of fine (≤1 mm diameter) and coarse (>1 mm diameter) roots.

To quantify AMF and DSE colonization in sugar maple, subsamples of fine roots were cleared in 10% KOH for 12–24 h or as needed to remove abundant dark pigments, bleached in 9:1 household H_2O_2: household NH_3, acidified in 5% HCl for 15–30 min, and stained in hot 0.01% Trypan blue in 1:1:1 lactic acid: glycerol: water. For each of 44 seedlings sampled at random, at least 10 root segments at least 1 cm long were mounted in parallel on a microscope slide and fixed with polyvinyl lactic acid glycerol (INVAM, 2014). We recorded percent colonization of AMF and DSE by assessing the presence or absence of their structures on each 1-mm section of each root segment (modified from McGonigle et al., 1990).

We measured metal concentrations in leaves collected at the end of the first growing season, in September 2014. We collected one leaf from each planted seedling with three leaves and two leaves from each seedling with four leaves or more, and combined them by species and plot. We washed leaves thoroughly with tap water, dried them at 60°C for at least 48 h, and ground them with mortar and pestle using liquid nitrogen as necessary. We then weighed 0.25–0.50 g of the samples into ceramic crucibles, ashed them in a muffle furnace at 475°C for at least 4 h, and weighed them again to estimate organic matter content by LOI. We digested the residue in 2 mL concentrated HCl for 10 min at 90–100°C. Digest solutions were diluted to 25 mL with ultrapure (18 Ω) water and stored at 4°C.

Concentrations of Ca, Mg, K, Zn, Pb, Cd, Cu, Ni, and Mn in digest solutions were measured by ICP-OES using standard methods modified from Zarcinas et al. (1987). We included as standard reference materials peach leaves (NIST 1547) and either olive leaves (BCR 062) or citrus leaves (NIST 1572), as well as a reagent blank, to confirm the quality of each digest. We further verified ICP-OES measurements by including standard solutions of known concentrations in each run. We used a similar method to measure metal concentrations in soils collected at the beginning of the experiment. Soils were sieved to 2 mm, air-dried, then ashed, digested, and analyzed by ICP-OES as above, a protocol modified from EPA method 3050B.

Testing Elemental Allelopathy

We performed a series of experiments to test three fundamental components of the mechanism of elemental allelopathy. To determine the extent that gray birch trees accumulate heavy metals in their leaves relative to their neighbors, a criterion for elemental allelopathy, we compared foliar metal concentrations in birch and the planted grasses. To test whether birch trees were associated with local increases in soil metal concentrations, we measured topsoil metal concentrations as a function of distance from gray birch trees in the field. Finally, we conducted a greenhouse experiment to evaluate phytotoxicity of gray birch leaf litter.

We collected leaves from 12 gray birch trees and 4–8 individuals each of nine planted grass species in July 2014 and measured metal and nutrient concentrations for each individual plant by ICP-OES as described above. To determine potential effects of birch on soil chemistry, in August 2013, we sampled soil from the top ∼10 cm at points located 0, 50, 100, and 200 cm from the stems of 18 gray birch trees located throughout the Palmerton site. We selected from among larger trees (∼5–10 years old, 2–4 m tall, canopy radius <2 m), and established our soil collection points along a cardinal direction chosen randomly for each tree such that the focal tree was the closest gray birch tree to each collection point. We then measured soil metal concentrations and organic matter content as described above.

We tested the phytotoxicity of metal-contaminated gray birch leaves from the site using a greenhouse experiment. We grew seedlings of autumn bent grass (*Agrostis perennans*; Poaceae), the forb white snakeroot (*Ageratina altissima*; Asteraceae), black oak, and sugar maple individually in pots of contaminated soil collected from the site, into which we manually mixed dried, crushed gray birch leaves, with three levels of leaf treatments: collected from inside the contaminated site, collected at nearby uncontaminated sites, or no leaves. To confirm differences in elemental concentration between contaminated and uncontaminated leaves, we measured metal and nutrient concentrations in 40 samples of each using ICP-OES. We collected seeds of autumn bent grass and white snakeroot from the Palmerton site in Fall 2013, and germinated them in autoclave-sterilized sand in Spring 2014 before transplanting into experimental pots. These two understory species colonized the site naturally and are abundant there, so they evidently have some degree of heavy metal tolerance. Seedlings of black oak and sugar maple came from the same batch as those used for the field experiment, and were transplanted into experimental pots upon arrival. We replaced any seedlings that died within the first week of the experiment.

Soil for the greenhouse experiment came from 10 locations within the grassland-dominated area in the Palmerton site, with

each location supplying soil for one replicate of every species-treatment combination. Soils were mixed with sand in a 6:1 soil:sand ratio to improve drainage. For each species, we planted similarly sized seedlings in the different leaf litter treatments to avoid confounding experimental treatments with initial size. We planted autumn bent and white snakeroot in 150 mL pots filled with soil mixed with 3 g of crushed leaves, and black oak and sugar maple in 550 mL pots with soil mixed with 4 g of crushed leaves. Leaf amounts were chosen to strike a balance between similar leaf masses and similar leaf:soil ratios in each pot. Pots were randomized with respect to replicates and treatments upon planting and repeatedly throughout the experiment. Because of differences in life history, we grew autumn bent for 11 weeks, white snakeroot for 21 weeks, and black oak and sugar maple for 23 weeks. To assess performance at harvest, we measured for autumn bent grass: height, number of tillers, and aboveground and belowground biomass; for white snakeroot: height, number of leaves, and aboveground and belowground biomass; for black oak and sugar maple: height, the length of apical and lateral branches from the current growing season in the greenhouse and past growing season in the nursery, number of leaves, and biomass of leaves, woody aboveground tissue, and fine and coarse roots based on a 1 mm diameter cutoff. For five replicates of each species, we also measured leaf metal and nutrient concentrations by ICP-OES.

Statistical Analysis

For the field experiment, we used linear models to test for effects of vegetation type (birch vs. grass) and aboveground competition (cleared vs. intact) on soil metal and nutrient concentrations, soil moisture, soil temperature, and light availability, as well as the growth, metal and nutrient uptake, and fungal root colonization parameters, analyzing black oak and sugar maple seedlings separately. For most parameters, we had multiple measurements per plot and thus included plot as a random effect in the models. Models depended on data distributions: ANOVAs for continuous variables such as branch lengths and biomass, which were $\log_{10}$-transformed as necessary to improve normality, MANOVAs for multivariate metal concentration profiles, and generalized linear models (GLMs) for leaf number (poisson) and root mycorrhizal colonization (binomial).

We tested for effects of vegetation type, aboveground competition, and their interaction on several metrics of community composition: birch and grass cover, and the cover, Shannon diversity (ANOVA) and species richness (GLM) of other colonizing species. We used chi-squared tests to test for associations between herbivory levels and experimental treatments for black oak and sugar maple separately. We constructed three contingency tables, one to isolate vegetation type, another for clearing, and another combining the two treatments. We used three levels of herbivory for black oak but because insect herbivory on sugar maple was generally low, we re-coded sugar maple leaf herbivory as presence/absence to avoid violating assumptions of the chi-squared test.

To test for differences between birch and grass in metal and nutrient concentrations, we used two-tailed *t*-tests to account for the possibility that birches may have greater concentrations of some metals and grasses may have greater concentrations of others. We adjusted significance thresholds using the Dunn–Šidák correction for multiple comparisons. To determine whether soil concentrations of each metal or nutrient varied significantly with distance from a focal gray birch tree, we used linear regression, again correcting for multiple tests. Metal and nutrient concentration data was $\log_{10}$-transformed to improve normality. To account for potential differences in background metal and nutrient concentrations under the sampled trees, we repeated this analysis with each concentration expressed as the change in concentration from the base of the trunk.

We used MANOVA to investigate the effects of grass and birch on soil metal concentrations at the beginning of the experiment. To investigate the effects of soil metal concentration profiles measured at the beginning of the experiment on plant biomass at harvest, we used principal components analysis (PCA) to produce axes that captured the majority of the variation in our soil chemistry data and then regressed plant biomass against these axes. Finally, we used correlation matrices to investigate the relationships between leaf and soil metal and nutrient concentrations.

In the greenhouse experiment, we used ANCOVA to test for an effect of litter treatment (contaminated, uncontaminated, or none) on each measure of plant performance and metal and nutrient concentration. We included depth to soil surface as a covariate in all models because soil settled in the pots to different degrees during the experiment, creating differences in the available amount of soil, and potentially nutrient availability. We repeated analyses excluding the no litter treatment to be sure to detect differences contributed by contaminated leaves vs. uncontaminated leaves, if they were present.

RESULTS

Gray Birch Effects on Soil Characteristics and Plant Community Structure

Compared to the grasses, gray birch had approximately 10-fold greater leaf concentrations of the pollutants Zn and Cd ($P < 0.001$), but not Pb (**Figure 1**). Our spring 2014 measurements, before clearing and planting, showed that birch plots, excluding birch itself, had lower vegetative cover than grass plots ($P < 0.001$, **Figure 2A**). However, species that colonized the site independently, excluding birch and the planted grasses, had marginally greater species richness ($P < 0.1$, **Figure 2B**) and greater Shannon diversity ($P < 0.05$) in birch plots. Plots designated to be cleared did not significantly differ in cover, richness, or diversity from plots designated to be left intact.

Abiotic conditions in the plots differed with initial vegetation type and clearing of aboveground competition. Soil organic matter content, measured at the beginning of the field experiment, was marginally higher in grass plots than birch plots, but soil heavy metal and nutrient concentrations did not differ (**Table 1**). We observed marginal main treatment effects on soil moisture measured during the experiment; moisture was greater in grass plots ($P < 0.1$; **Table 2**) and in cleared plots ($P < 0.1$), with no significant interaction. Soil temperature was greater

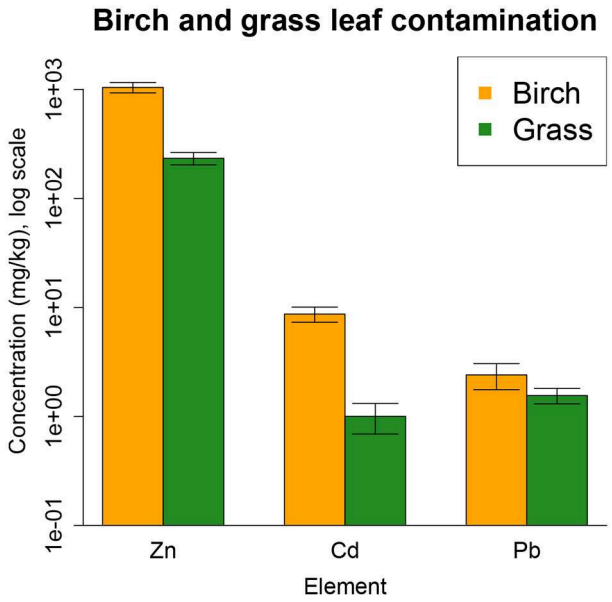

FIGURE 1 | Concentrations of the contaminants Zn, Cd, and Pb in leaves of gray birch (orange bars) and the planted grasses (green bars) in the Palmerton site (average ± SE). Grasses and birch differ in Zn and Cd ($P < 0.001$) but not in Pb. The y-axis is on a log scale for readability. Error bars are ± 1SE.

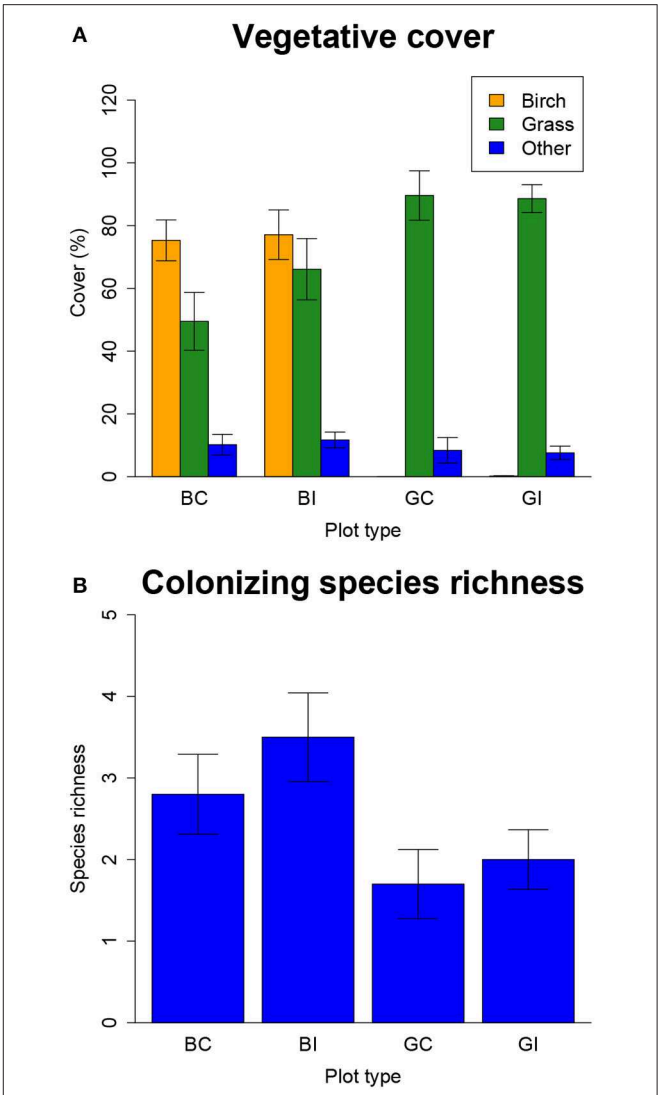

FIGURE 2 | Birch and grass effects on neighboring plant communities assessed in experimental field plots before beginning the experiment in May 2014. **(A)** Cover of gray birch (orange bars), grasses (green bars), and all other colonizing species (blue bars). **(B)** Species richness of colonizing species excluding birch and grasses. Plot treatments are abbreviated as follows: Birch, B; Grass, G; Cleared, C; Intact, I. Error bars are ± 1SE. Gray birch is excluded from all plant community data and analyses except gray birch cover. In the "grass" category, we include the planted C_4 grasses *Andropogon gerardii*, *Eragrostis trichodes*, *Panicum amarum*, *Panicum virgatum*, *Sorghastrum nutans*, *Schizachyrium scoparium*, and *Tripsacum dactyloides*, the planted C_3 grasses *Dichanthelium clandestinum* and *Elymus canadensis*, and the C_3 grass *Agrostis perennans*, which was not planted but occurred in the site before revegetation and grew abundantly in some plots. Grasses were treated as a single group because they were the dominant species in the grass plots at the beginning of the experiment and most species could not reliably be distinguished when this early-season census was conducted. All species other than gray birch or the above grasses were considered other colonizing species.

in cleared plots ($P < 0.05$), and leaf area index, a proxy for aboveground competition or shading, was greater in intact plots ($P < 0.001$; **Table 2**). Leaf area index was >0 in cleared plots because of the experimental tree seedlings and partial regrowth of vegetation between clippings. Neither soil temperature nor leaf area index differed between grass and birch plots.

Gray Birch and Aboveground Competition Impacts on Tree Species Establishment, Fungal Root Colonization, and Herbivory

Black oak germination approximately tripled with clearing in grass plots but decreased slightly with clearing in birch plots, producing a significant vegetation type x clearing interaction ($P < 0.05$; **Figure 3A**) but no main effect of either. There was no main effect of vegetation type or clearing. Sugar maple seed germination did not differ between experimental treatments (**Figure 3B**).

As seedlings, neither species performed differently in plots dominated by birch vs. grasses, as a main effect, based on any measure of performance. Growth of black oak responded to vegetation clearing, where seedlings in cleared plots had significantly more leaves ($P < 0.05$, **Figure 3C**) and lateral branches, greater lateral branch length (**Table A1**), and marginally greater total biomass ($P < 0.1$, **Figure 3E**). For black oak, fine root biomass responded to a significant vegetation type x clearing interaction where clearing had little effect in birch plots but more than doubled fine root biomass in grass plots ($P < 0.05$, **Figure 3G**). Sugar maple seedlings showed no significant effects of experimental treatments or their interactions on any of the growth parameters we measured (**Figures 3D,F,H**). Seedling height increments from the beginning to the end of the experiment were often negative for both species, but especially

TABLE 1 | Initial plant community structure **(A)** and soil chemistry **(B)** of birch and grass plots before clearing treatment.

		Birch	Grass	Difference
(A)				
	Birch cover (%)	76.2 ± 5.0	0.10 ± 0.07	B>G***
	Grass cover (%)	57.8 ± 6.8	89.1 ± 4.4	G>B***
Colonizing species	Cover (%)	11.0 ± 2.0	8.0 ± 2.2	NS
	Species richness	3.2 ± 0.36	1.9 ± 0.27	B>G (.)
	Shannon diversity	0.82 ± 0.12	0.49 ± 0.10	B>G*
(B)				
	Organic matter (% LOI)	25.9 ± 3.3	34.6 ± 3.4	NS
Base cations	Ca (mg/kg)	1441 ± 344	1884 ± 571	NS
	Mg (mg/kg)	10624 ± 3776	9516 ± 3311	NS
	K (mg/kg)	1840 ± 96	1876 ± 96	NS
Contaminants	Zn (mg/kg)	7007 ± 1197	8417 ± 1270	NS
	Pb (mg/kg)	683 ± 136	1006 ± 187	NS
	Cd (mg/kg)	79.8 ± 17.2	110.3 ± 21.1	NS
Other heavy metals	Ni (mg/kg)	13.0 ± 1.7	15.8 ± 2.0	NS
	Cu (mg/kg)	128 ± 25	143 ± 21	NS
	Mn (mg/kg)	4934 ± 878	6281 ± 1033	NS

*Statistical significance of effects is denoted in the Difference column as follows: B birch, G grass, C cleared, I intact, (·) $P < 0.1$, *$P < 0.05$, ***$P < 0.001$. Values are average ± 1 SE.*

TABLE 2 | Effects of birch, grasses, and clearing of aboveground vegetation on soil moisture, temperature, and leaf area index (a proxy for aboveground competition) in experimental field plots.

Plot type	Soil moisture (% v/v)	Soil temperature (°C)	Leaf area index
BC	7.10 ± 1.59	32.5 ± 0.60	0.313 ± 0.045
GC	10.68 ± 1.48	30.8 ± 0.56	0.383 ± 0.239
BI	3.79 ± 0.79	29.9 ± 0.47	1.546 ± 0.232
GI	7.11 ± 1.28	29.8 ± 0.49	0.954 ± 0.109
Difference	G>B (.) C>I (.)	C>I*	I>C***

*Plot treatments are abbreviated as follows: Birch, B; Grass, G; Cleared, C; Intact, I. Statistical significance of effects is denoted in the Difference row as follows: (.) $P < 0.1$, *$P < 0.05$, ***$P < 0.001$.*

so for sugar maple, presumably due to both low growth and herbivory. Height increments did not respond to experimental treatments in either species (**Figures 3I,J**).

Vegetation type did affect root colonization by fungi. For black oak, ECM colonization was marginally greater in birch plots than grass plots ($P < 0.1$, **Figure 4A**), and explained significant variation in aboveground, belowground, and total seedling biomass ($P < 0.001$, **Figure 4B**). For sugar maple, AMF colonization did not respond to vegetation or clearing as main

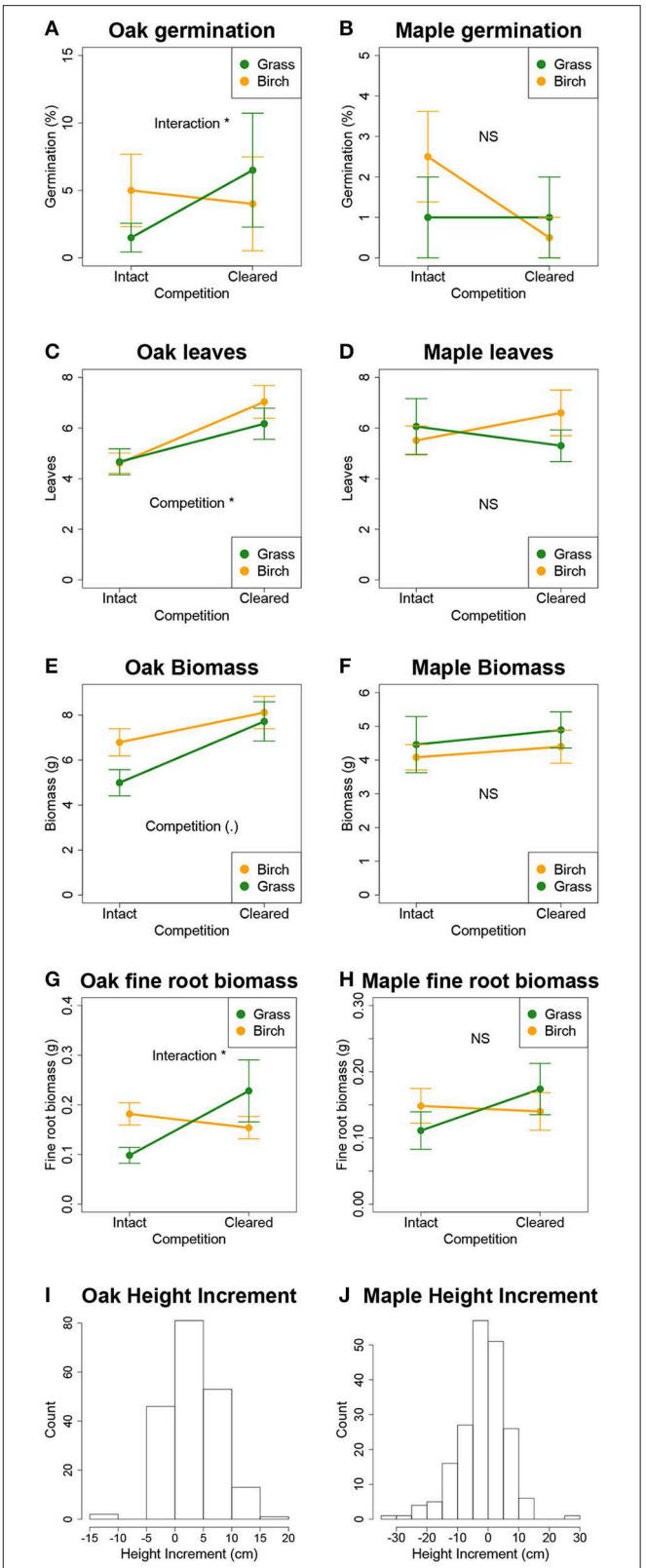

FIGURE 3 | Germination, leaf number, total biomass, and fine root biomass of black oak and sugar maple seedlings **(A–H)** in the field experiment, shown as *(Continued)*

FIGURE 3 | a function of the vegetation type into which they were planted and whether aboveground competition was eliminated by clearing. Distribution of changes in height from planting until harvest **(I,J)** combines seedlings in all four vegetation type*clearing treatments. Significance codes: (.) $P < 0.1$; *$P < 0.05$; NS not significant. Error bars are ±1 SE.

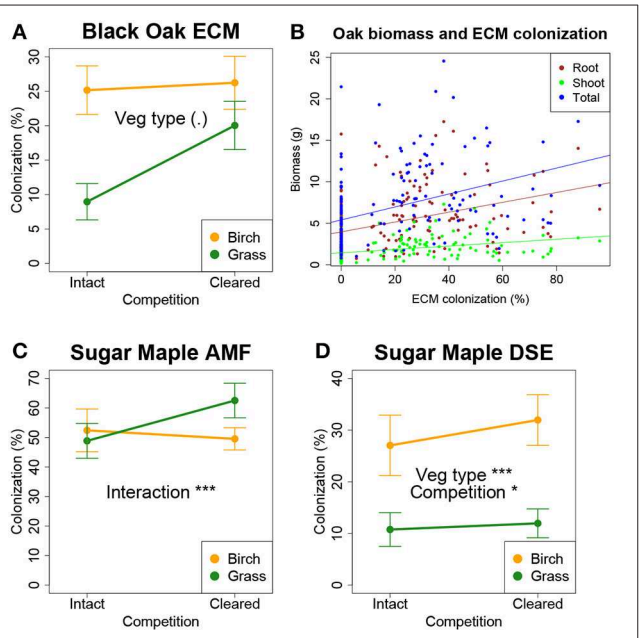

FIGURE 4 | Fungal root colonization for seedlings in the field experiment. **(A)** Percent colonization of black oak by ECM by vegetation type and clearing treatment. **(B)** Percent ECM colonization explains variation in aboveground, belowground, and total biomass ($P < 0.001$). Percent colonization of sugar maple roots by AMF **(C)** and DSE, dark staining fungal endophytes **(D)**. Significance: (.) $P < 0.1$; *$P < 0.05$; ***$P < 0.001$. Error bars are ±1 SE.

effects, but there was a vegetation type x clearing interaction ($P < 0.001$) reflecting greater colonization in cleared grass plots than in other treatment combinations (**Figure 4C**). Also for sugar maple, DSE colonization was greater in birch plots than in grass plots ($P < 0.001$, **Figure 4D**), and greater in cleared plots than intact plots ($P < 0.05$, **Figure 4D**), with no significant interaction. Neither AMF nor DSE root colonization explained variation in sugar maple biomass.

Insect herbivory on black oak leaves was greater in birch plots compared to grass plots ($P < 0.05$, **Figure 5A**). For sugar maple, insect leaf herbivory did not vary with vegetation type or clearing (**Figure 5B**).

Gray Birch and Aboveground Competition Effects on Seedling and Soil Metal Concentrations

Leaf metal and nutrient concentrations in seedlings responded to aboveground competition but not vegetation type. MANOVA showed a significant effect of aboveground competition on black oak leaf metal profiles ($P < 0.001$) and a marginal effect of aboveground competition on sugar maple leaf metal profiles ($P < 0.1$), but no effect of vegetation type in either case. Univariate analyses of black oak and sugar maple leaf metal concentrations revealed that black oak leaves had marginally higher Al, K, and Cu in cleared plots than intact plots ($P < 0.1$) and marginally higher leaf Cu in birch plots than in grass plots ($P < 0.1$). Sugar maple leaves had marginally greater Zn and Pb ($P < 0.1$) and significantly greater Cd ($P < 0.05$) in cleared plots than intact plots (**Table A2**). However, none of these univariate results was statistically significant after correcting for multiple comparisons.

Principal components analysis of soil metal and nutrient concentration profiles captured about 90% of the variation in the first three axes. Biplot analysis showed that the first axis was negatively related to the pollutants, other heavy metals, and organic matter. The second axis was positively related to Ca and Mg and negatively related to K, and the third axis was negatively related to K (**Figure 6**). Regression of plant biomass parameters showed that black oak root, shoot, and total biomass were all negatively related to axis 3 ($P < 0.001$), and shoot biomass was also positively related to axis 2 ($P < 0.05$). Because the negative relationship between oak biomass and axis 3 suggests a positive relationship between biomass and soil K, but the positive relationship between oak shoot biomass and axis 2 suggests the opposite, we performed univariate regressions to further investigate the relationships between oak biomass and soil K. We found that soil K was positively related to black oak aboveground, belowground, and total biomass ($P < 0.001$). For sugar maple, root and shoot biomass were each positively related to axes 1 and 2 ($P < 0.05$). Total sugar maple biomass was positively related to axis 1 ($P < 0.05$) but only marginally positively related to axis 2 ($P < 0.1$).

Correlations between soil and plant metal concentrations were weak but mostly positive. However, contrary to expectations, the foliar concentration of a given element was seldom as highly correlated with the soil concentration of that same element as it was with soil concentrations of other elements (**Figures A1A,B**). For black oak, the stronger relationships included the following: foliar Pb and Cd were negatively related to soil Ca and Mg, and foliar Zn was positively related with soil Zn, Cd, and Ni (**Figure A1A**). For sugar maple, soil Ca and Mg were strongly positively related to foliar Mg and negatively related to the rest of the elements measured. Foliar Pb and Cd were negatively related to soil Ca, Mg, and LOI. Foliar Zn, LOI, and to a lesser extent Cd, were negatively related to soil Ca and Mg and weakly positively related to soil Zn, Pb, Cd, Mn, and Ni (**Figure A1B**).

Elemental Allelopathy

Despite large differences in the Zn and Cd concentrations of birch and grass leaves (**Figure 1**), we observed no signature of heavy metal enrichment around birch trees. Levels of soil contaminants around birch trees were highly variable, and there were no significant trends in concentrations of soil organic matter, base cations, or heavy metals, as a function of distance from stem, whether we analyzed absolute concentrations or changes in concentration with distance from stem (**Figure A2**).

For our greenhouse experiment, we confirmed that gray birch leaves collected inside the Palmerton site had significantly higher

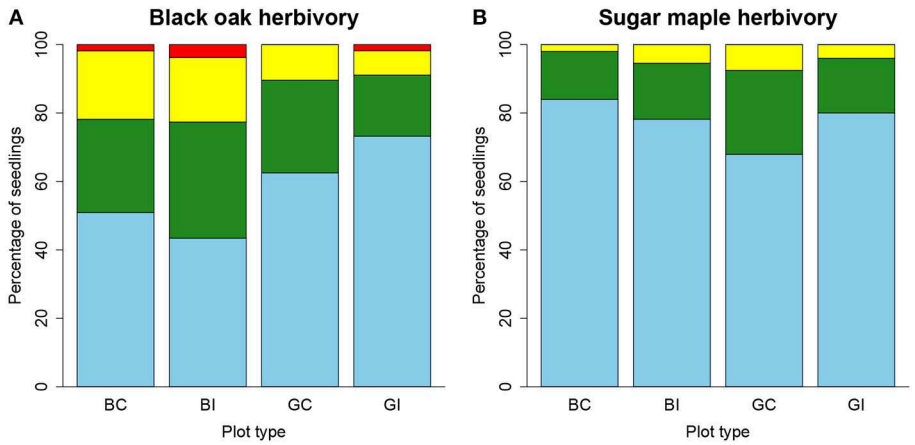

FIGURE 5 | Percentage of black oak **(A)** and sugar maple **(B)** seedlings experiencing different classes of insect leaf herbivory while grown in birch (B) or grass (G) dominated plots in the field with aboveground competition cleared (C) or left intact (I). Classes of leaf herbivory are defined and color-coded as follows by the amount of leaf area apparently missing: None, blue, <5%; Minimal, green, 5–20%; Moderate, yellow, 20–50%; Severe, red, >50%.

concentrations of the pollutants Zn, Pb, and Cd than gray birch leaves collected outside the site (all $P < 0.001$, **Figure 7**). After correcting for multiple comparisons, contaminated and uncontaminated gray birch leaves did not differ in any of the other chemical characteristics we measured, including organic matter, the base cations Ca, K, and Mg, and the heavy metals Cu and Ni.

Growth and elemental uptake responses by our four target species exposed to birch litter treatments in the greenhouse were species-specific and often minimal. Moreover, none showed predicted evidence of elemental allelopathy, which would be reduced growth and/or greater foliar Zn, Pb, or Cd concentrations when grown with contaminated litter. Black oak grew taller and had more fine root biomass with contaminated gray birch leaf litter than with uncontaminated litter or no litter (**Figures 8A,B**), but aboveground and total biomass did not respond to the litter treatments. Autumn bent grass grew more in the absence of birch leaves, having about twice as much aboveground biomass, belowground biomass, and tiller number in pots without litter added (**Figure 8C**) but showed no response to litter contamination. Growth of white snakeroot and sugar maple also did not respond to the litter treatments. No species showed an effect of litter treatment on their leaf contaminant concentrations.

DISCUSSION

As succession proceeds in this remediated grassland, gray birch is more likely to engage in PSF by influencing the types of fungal root symbionts than by performing elemental allelopathy, despite gray birch's elevated foliar levels of Zn and Cd. Black oak performance was more responsive to soils conditioned by gray birch vs. grasses than was sugar maple, demonstrating species-specific consequences, and some soil-mediated effects only occurred in the absence of the neighboring plant canopy, indicating an interaction between aboveground competition and soil factors. Herbivory by deer may also have obscured PSF effects on seedling performance. We suggest that gray birch could influence succession broadly by lowering soil moisture, soil organic matter, and cover of light-demanding species, increasing plant species diversity, and changing soil microbial communities, compared to grasses.

Based on our findings, gray birch should help steer succession to other woody species that likewise associate with ECM. Gray birch apparently fosters a shift in mycorrhizal communities, from an AMF-dominated community under grasses to a more mixed community of AMF, ECM, and DSE. Although DSE are not formally considered mycorrhizal fungi, they can have very similar functions (Jumpponen, 2001). Shifts between AMF and ECM have been associated with succession and other plant community shifts before (Treseder et al., 2004, AMF to ECM; Williams et al., 2013, ECM to AMF), and have been shown to facilitate recruitment or growth of ECM-reliant woody species (Pringle et al., 2009). It is possible that different soil microbes under birch and grasses, including the fungi we analyzed, also contributed to greater black oak seed germination in cleared grass plots (e.g., Maighal et al., 2016; Varga and Kytöviita, 2016). Similarly, AMF colonization in sugar maples was greater in plots conditioned by AMF-dependent grasses as we predicted, but only when aboveground competition was removed and with no linkage to seedling biomass. Perhaps sugar maple seedlings become more favorable fungal hosts because aboveground clearing reduces photosynthate production in surrounding grass-dominated vegetation, making the maples relatively more desirable fungal hosts.

Any potential effects of fungal root colonization or experimental treatments on sugar maple might have been hidden because of this species' overall poor growth. Sugar maple may be less tolerant than black oak of heavy metal soils, or may have experienced too much herbivory by deer, despite our efforts to shield seedlings from vertebrate herbivores. Herbivory can mask effects of other ecological processes, especially if herbivores

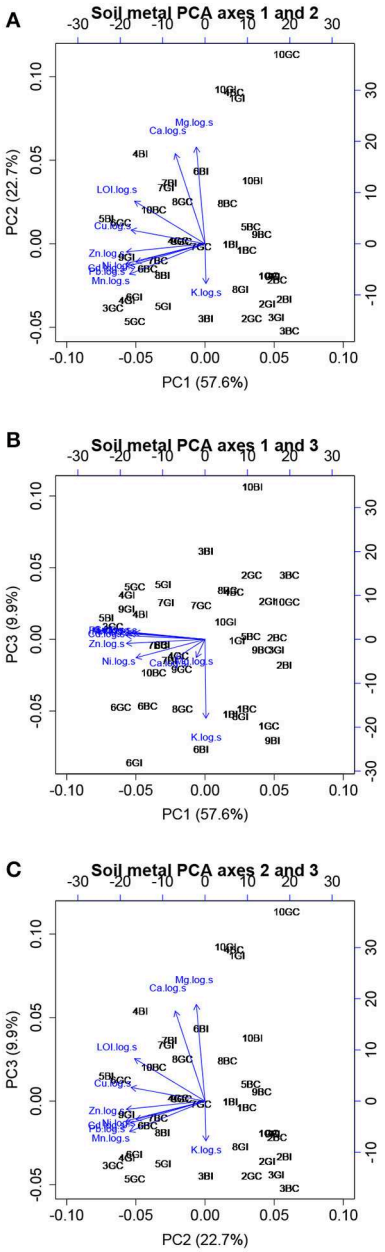

FIGURE 6 | Principal components analysis of log-transformed soil chemistry data, including concentrations of soil organic matter, the nutrients Ca, Mg, and K, the contaminants Zn, Pb, and Cd, and the heavy metals Cu, Ni, and Mn not thought to be enriched in the site. Axes 1 and 2 **(A)**, 1 and 3 **(B)**, and 2 and 3 **(C)**. Points show the location of each plot in the ordination space, and arrows are biplots showing contributions of each chemical concentration to the ordination space.

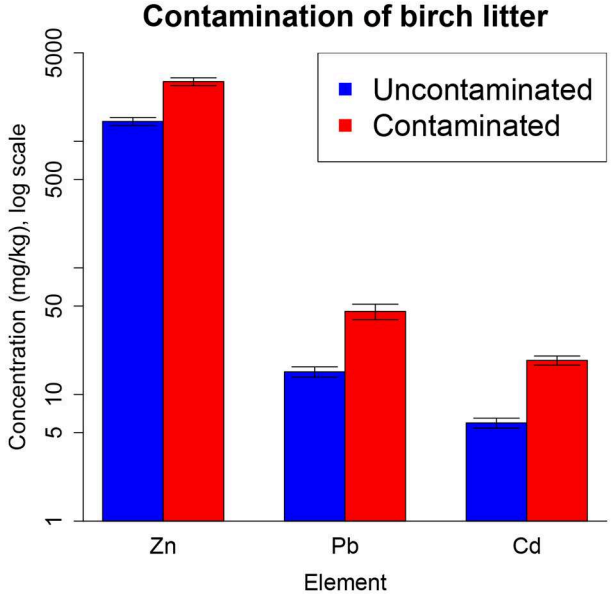

FIGURE 7 | Concentrations of the heavy metal contaminants Zn, Pb, and Cd in gray birch leaves collected inside (red) and outside (blue) of the contaminated site and used for the greenhouse experiment. Birch leaves from inside the contaminated site had significantly higher concentrations of all three contaminants ($P < 0.001$, $N = 40$). Error bars are ±1 SE.

preferentially attack larger, healthier seedlings (Price, 1991; Blaisdell et al., 2015), or otherwise interact with PSF in affecting plant performance (Bennett and Klironomos, 2019; Heinze et al., 2019).

Aboveground competition also proved consequential for black oak performance, both as a main effect on leaf number and seedling biomass and as an interaction with vegetation type affecting seed germination and fine root biomass. The expression of soil effects on black oak germination and fine root biomass only in the absence of aboveground competition is consistent with findings of several other studies, where competition masks or reduces effects of PSF (Casper and Castelli, 2007; Bezemer et al., 2018; Lekberg et al., 2018; Xue et al., 2018). Our measures of soil temperature, soil moisture, and LAI confirmed that shading by uncleared vegetation impacted the abiotic environment.

The greater insect herbivory on black oak seedlings growing in soils conditioned by gray birch, compared to soils conditioned by grasses, has multiple possible explanations. Soil characteristics under grasses and gray birch, including differences in mycorrhizae, might have differentially influenced herbivore activity (Koricheva et al., 2009) by altering leaf properties such as nutritional quality or defense chemistry (Kostenko et al., 2012; Kos et al., 2015; Wang et al., 2015), although in this case herbivory was greater in gray birch plots where ECM colonization was also greater. Plant neighbor identity has been shown to influence plant herbivory defense strategies as well (Broz et al., 2010). Neighboring gray birch might also have elevated herbivory on black oak through association susceptibility (Barbosa et al., 2009; Kim, 2017), in which gray birch served as an herbivore attractant or black oak was a preferred, alternate food choice.

We found no strong evidence in either the field experiment or the greenhouse experiment that gray birch engages in elemental allelopathy. Soil concentrations of Zn and Cd did not decrease with distance from gray birch stems as would be expected if gray birch litter locally enriches these soil contaminants (Jaffe

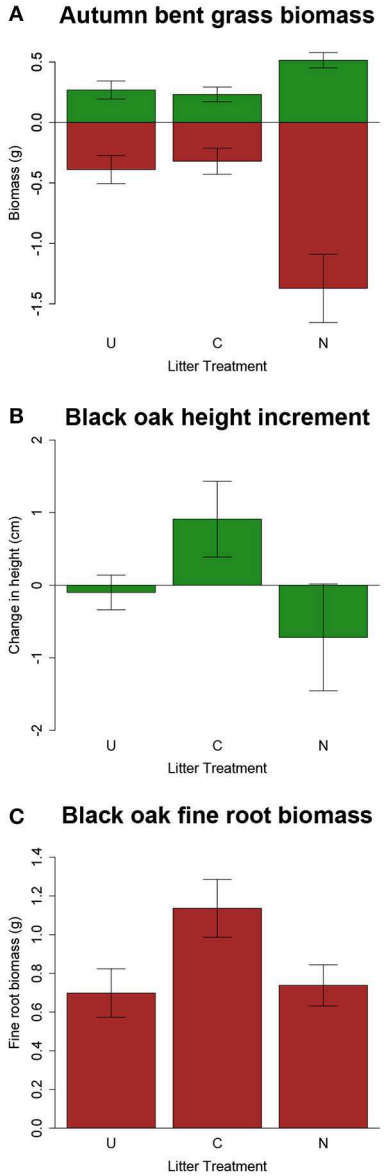

FIGURE 8 | Effects of birch litter treatments on plant growth in the greenhouse experiment, showing only results that differed between contaminated (C), uncontaminated (U), and no (N) litter treatments. **(A)** Biomass of autumn bent grass: bars above the x-axis represent aboveground biomass, and bars below the x-axis belowground biomass. **(B)** Change in height from planting until harvest and **(C)** fine root biomass in black oak seedlings. Green bars represent aboveground growth and brown bars represent belowground growth. Error bars are ±1 SE.

et al., 2017). It is possible that elemental allelopathy by gray birch impacted black oak seed germination and fine root production in the field, since both were greater in soils conditioned by grasses in the absence of aboveground competition, but it seems more likely that soil metal concentrations are sufficiently high and variable as to overpower any signal of birch-mediated metal movement. Furthermore, in the greenhouse experiment, contaminated birch litter actually increased oak height and fine root biomass, suggesting a positive effect on oak performance. Leaching of contaminants from leaf litter is likely (Maunoury-Danger et al., 2018). Zn has been shown to increase fine root growth in some concentrations (Feigl et al., 2019), but we cannot explain why contaminated litter increased black oak seedling height. The negative response of autumn bent grass to any birch litter, whether contaminated or not, could be due to the high phenolic content of birch leaves (Keinänen et al., 1999) and might indicate that gray birch, regardless of its metal uptake, could have negative consequences for some early successional species.

The ability to engage in elemental allelopathy has been offered as one explanation for the evolution of metal hyperaccumulation in plants (Rascio and Navari-Izzo, 2011), but examples in the literature are mostly inconclusive, unable to distinguish between a species contributing to locally elevated contamination and its preferential growth or establishment in pockets of high soil metal concentration (Morris et al., 2006; Jaffe et al., 2017). Even if plants do enrich soils in metal contaminants, there may be no consequences for co-occurring species that are likely to have some degree of metal tolerance (Morris et al., 2006; Mehdawi et al., 2011), and any impact may be greater with lower background levels of contamination (Jaffe et al., 2017).

We did detect signals that existing spatial heterogeneity in soil elemental composition influenced performance of both species independently of our experimental treatments, as indicated by plant biomass being correlated with PCA axes associated with soil heavy metals, nutrients, and organic matter. This analysis suggests that biomass in both species responds to spatial variation in nutrients, and that soil contaminants explain more variation in biomass of sugar maples than black oak. Strong spatial variation of metals in contaminated sites has been documented previously (Yang et al., 2013), including at Palmerton, where a survey of plant and soil metal concentrations in white snakeroot, autumn bent grass, and three other herbaceous species found that plant and soil metal concentration profiles were closely related, such that plant species identity explained more variation in soil metals than spatial proximity (Dietterich et al., 2017). Thus, in this study, we hypothesized stronger positive relationships between plant and soil elemental concentrations than we observed. Either black oak and sugar maple are strongly regulating elemental uptake (Baker, 1981), the total extractible pools we measured do not accurately reflect available pools (Remon et al., 2013), or close relationships between plant and soil chemistry take more than one growing season to develop (Waring et al., 2015).

Our study illustrates that the role and importance of PSF in natural systems must be evaluated in the context of other ecological processes and the background soil environment, and provides valuable information to land managers about how gray birch, as a strong colonizer, may influence succession. We found that gray birch contributes to greater species diversity initially, and we provide evidence that it favors ECM-reliant, later successional woody species like black oak. Some stakeholders express strong desire to eliminate gray birch mechanically or via controlled burns (Van Auken, 2009) because grassland habitat is rare in Pennsylvania and hosts several rare plant and insect species (Latham et al., 2007), yet controlled burns are expensive and risk releasing metals in smoke (Pereira and Úbeda, 2010;

Abraham et al., 2017, 2018). The deciduous forest that is likely to develop, if left alone, holds similar potential for soil stabilization as grasses, but lingering high topsoil metal concentrations and potential mobilization and bio-accumulation of metals from gray birch leaves into the local food chain will still need monitoring. Herbivory by deer may compromise the return of many desirable native forest and understory species (Horsley et al., 2003; White, 2012), but we are encouraged by findings of red oak recruitment in gray birch dominated areas nearby (Cullen et al., 2016), and by coniferous and broad-leaved species coexisting with gray birch in all but the steepest, rockiest slopes of a coal mine site near Palmerton (pers. obs.). We support recent recommendations that parts of the site be allowed to return to forest while others be maintained as grassland (LGNC, 2016). This approach will provide a field laboratory to study the ecology of plant communities in heavy metal polluted soils, including roles of PSF, under two very different management regimes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

LD and BC conceived and designed the study. LD, AL, and SG performed the experiments. LD performed the statistical analysis with contributions from all authors and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00459/full#supplementary-material

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Simulated Herbivory Weakens Plant-Soil Feedbacks in Competitive Mixtures of Native and Invasive Woodland Plants

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Plant-soil feedback (PSF), the process by which plants influence con- or heterospecifics via alteration of abiotic or biotic soil properties, is a known driver of plant coexistence and invasion. Yet there is limited understanding of how PSF interacts with other important drivers of plant community structure and dynamics, such as aboveground herbivory. Aboveground herbivory and PSFs are ubiquitous processes in plant communities, but traditional PSF experiments in the greenhouse eliminate herbivory as an experimental factor. Aboveground herbivory can affect plant-soil systems in multiple ways and therefore is likely to strongly interact with PSF. Herbivores can be selective, preferring certain species over others, which could influence PSF dynamics. Aboveground herbivory could also affect PSF dynamics by influencing photosynthate allocation, defense compound production, and soil nutrient levels. An existing conceptual framework predicts that aboveground herbivory should generally weaken pathogen-, mutualist- and soil nutrient-driven feedbacks, and a logical extension of these predictions is that aboveground herbivory will weaken PSF as a driver of plant species invasion. Using a Midwest urban woodland study system, we first measured aboveground mammalian herbivore pressure on native woodland perennials used in local restoration efforts. We then simulated these levels of herbivory in a greenhouse experiment to assess whether and how aboveground herbivory alters net pairwise PSF interactions between these native species and *Euonymus fortunei*, a common invasive plant of Midwest urban woodlands. Results support predictions that aboveground herbivory weakens PSF interactions. In our experiment, simulated herbivory eliminated PSF among *E. fortunei* and a co-occurring community of native species, although this effect depended on competitive context. When the native community and the invasive species were grown separately, net feedback was neutral regardless of herbivory, but when grown in a competitive mixture, feedback between the native community and the invader switched from negative to neutral when herbivory was imposed. To assess the generality of these findings, future studies are needed that examine herbivory-PSF interactions across multiple native-native and native-invasive species combinations, and for a range of plant community types.

Keywords: plant-soil feedback, herbivory, invasion, Midwest deciduous forest, native-invasive competition

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INTRODUCTION

While decades of research have established that plant-soil feedbacks (PSFs) can be a key force structuring plant communities and plant invasions (Bever et al., 1997; Klironomos, 2002; Kulmatiski et al., 2008), understanding of the context-dependency of PSFs, i.e., how PSFs change under different environmental conditions—is only beginning to be appreciated (Smith-Ramesh and Reynolds, 2017). The presence or absence of aboveground herbivory as a potentially strong influence on PSFs is our focus here. Plant-soil feedback refers to the process wherein plants affect biotic and/or abiotic conditions of the soil in which they are grown (a process commonly referred to as "soil conditioning"), which influences subsequent growth of con- or heterospecific plants (Bever et al., 1997). Plants affect biotic soil properties through various mechanisms, including root exudates, deposition patterns, and susceptibility to belowground enemies, ultimately generating species-specific microbial associations with roots and/or rhizospheres (Ehrenfeld et al., 2005; Kulmatiski et al., 2008). These associations consist of various symbionts like mutualists and pathogens (Bever et al., 1997; Packer and Clay, 2000; Reynolds et al., 2003; Ehrenfeld et al., 2005). Plants also directly and indirectly affect abiotic soil properties such as nutrient and water availability, pH and allelochemicals through uptake, exudates, and litter inputs (Bertin et al., 2003; Elgersma et al., 2012; Bardgett et al., 2014).

Plant-soil feedbacks can be positive, negative, or neutral. Individual feedbacks refer to plant performance in conspecific vs. heterospecific soil, and net pairwise feedbacks refer to plant performance in conspecific and heterospecific soil relative to that of another species (Smith-Ramesh and Reynolds, 2017). Net pairwise feedbacks are emphasized here because they predict the effects of PSFs on plant community dynamics (Smith-Ramesh and Reynolds, 2017). Net negative PSF, wherein plant species perform relatively better in soil conditioned by heterospecific vs. conspecifics, is a stabilizing mechanism that maintains species diversity (Bever et al., 1997). Net negative PSFs stem from host-specific enemy accumulation in many systems (Packer and Clay, 2000; Reynolds et al., 2003) but could also be driven by uptake of soil resources by niche-partitioned species (Smith-Ramesh and Reynolds, 2017). Net positive PSF, wherein plant species perform relatively better in soil conditioned by conspecifics vs. heterospecifics, is a destabilizing mechanism that can ultimately lead to increased abundance of a particular species or even competitive exclusion of other species (Bever et al., 1997), and may contribute to invasive species spread (Jordan et al., 2008; Zhang et al., 2010; Smith and Reynolds, 2012). Plants that form soil mutualisms could generate positive PSFs (Bever et al., 1997, 2010). Neutral feedbacks occur when plant performance is unaffected by soil conditioning and may indicate that PSFs do not structure plant community dynamics. Neutral PSFs could also promote invasion if native-invasive PSFs are neutral or less negative than native-native PSFs, due for example to complete or partial escape of invasive species from belowground enemies (Smith and Reynolds, 2015).

An existing conceptual framework predicts that aboveground herbivory should generally weaken PSF (Smith-Ramesh and Reynolds, 2017). Aboveground herbivory may weaken pathogen-driven negative PSF through a cross-induction of belowground defenses (Bezemer and van Dam, 2005; Kaplan et al., 2008). (Although if herbivory weakens plant condition and thus increases susceptibility to soil pathogens, it is possible that the strength of negative PSF could increase; Smith-Ramesh and Reynolds, 2017). Aboveground herbivory may weaken nutrient-driven negative PSF by reducing plant size and/or via nutrient inputs (e.g., excreta), either of which could reduce plant nutrient demands (Smith-Ramesh and Reynolds, 2017). Heavy or long-term aboveground herbivory may reduce the ability of plants to supply carbon subsidies to microbial mutualists (Bardgett et al., 1998), which could in turn weaken mutualist-driven PSF [In contrast, intermediate, or short-term herbivory could enhance below-ground resource allocation (Bardgett et al., 1998), strengthening mutualist-driven PSF]. A logical extension of these predictions is that aboveground herbivory will generally weaken PSF as a driver of plant species invasion.

Relatively few studies have examined herbivory in the context of PSFs. Several studies on herbivory and PSFs have investigated the effects of PSFs on herbivores, specifically herbivore growth and performance (Kos et al., 2015a,b; Heinen et al., 2017). However, research is needed to assess the effects of herbivory on PSFs. One study examined the effects of moth and beetle herbivory on PSF interactions with *Jacobia vulgaris*, finding that belowground (beetle) herbivory weakened negative PSFs (measured with respect to unconditioned soil) whereas aboveground (moth) herbivory strengthened negative PSFs (Bezemer et al., 2013). Another study examined the effects of aboveground insect herbivory on individual PSF in three native grasses and found that herbivory neutralized negative PSFs (Heinze and Joshi, 2017). The nature of herbivory-PSF interactions in other systems, and for native-invasive dynamics, is unknown.

Urban woodlands are highly disturbed systems that pose a unique opportunity to study herbivory-PSF interactions with native and invasive species. Urban woodlands are typically small, habitat fragments, with high edge to surface area ratios, close proximity to conventionally landscaped properties, and high populations of urban-adapted mammals such as rabbits and deer (Bauer and Reynolds, 2016). These conditions promote high levels of both plant invasions and herbivore pressure. Furthermore, in an instance of the general phenomena of enemy escape (Keane and Crawley, 2002; Shea and Chesson, 2002; Mitchell and Power, 2003), invasive woodland plant species may be consumed to a lesser extent by mammalian herbivores than are native woodland species (Knight et al., 2009; Relva et al., 2010; Averill et al., 2016). Concern for biodiversity loss and appreciation of ecosystem services has motivated efforts to restore urban ecosystems (DiCicco, 2014; Elmqvist et al., 2015). Besides advancing basic science understanding of context-dependency in PSF, study of how mammalian grazing pressure interacts with native-invasive PSF dynamics in urban woodlands may contribute to more successful restoration efforts.

We used field exclosure experiments to assess the intensity of mammalian herbivore pressure on seven native Midwest U.S. woodland perennials commonly used in urban woodland

restorations in Bloomington, IN, U.S. We then simulated these levels of mammalian herbivory in a full reciprocal PSF greenhouse study to determine their effect on net pairwise PSF dynamics amongst a community of three native woodland perennials and *Euonymus fortunei*, a common perennial invasive species in Midwest U.S. urban woodlands (Smith and Reynolds, 2015). Based on common findings of weakened negative or positive PSF for introduced species (Klironomos, 2002; Callaway et al., 2004) and our previous findings (Smith and Reynolds, 2012, 2015), we predicted that PSFs would be neutral to positive favoring the invader in the absence of herbivory. Furthermore, consistent with conceptual predictions (Smith-Ramesh and Reynolds, 2017), we also expected that PSF dynamics between native and invasive species would weaken in the presence of herbivory.

MATERIALS AND METHODS

Herbivore Pressure Experiment

Aboveground herbivore pressure from mammals such as deer and rabbits was assessed in two predominantly beech-maple urban woodlands in Bloomington, IN, U.S.: Dunn's Woods on Indiana University's campus and Latimer Woods, a municipal woodland preserve. Both of these woodlands are a focus of invasive species mitigation and native species restoration efforts by the Bloomington Urban Woodlands Project, a consortium of local non-profit, city government and Indiana University partners (https://sustain.iu.edu/buwp.html). Three separate exclosure experiments were conducted to assess herbivore pressure on transplanted native plants in these woodlands. In each of these experiments, native plants were transplanted into closed or open cages or uncaged control plots established in eight (Dunn's Woods) or 10 blocks (Latimer Woods) randomly located throughout the woodlands in areas without invasive plants. As an index of herbivore pressure, aboveground biomass of plugs after 12–16 months was measured.

Native plants for all experiments were propagated under natural light in a temperature-controlled greenhouse at Indiana University, from Indiana-genotype seed purchased from Spence Restoration Nursery (Muncie, IN, U.S.). Seeds were germinated in 10 cm × 10 cm flats filled with MetroMix 360 (Sun Gro Horticulture, Agawam, MA, U.S.). Once large enough to handle without breaking (∼4 weeks), seedlings were transplanted to stubby cone-tainers (Steuwe & Sons, Corvallis, OR, U.S.) filled with additional MetroMix and grown to the mature plug stage (∼2–3 months), wherein roots fully filled the cone-tainer. Seven perennials native to central Indiana, U.S. deciduous woodlands were used across the three experiments. Experiment one, conducted in Dunn's Woods from May 2013-September 2014, involved *Solidago flexicaulis* (zig-zag goldenrod), *Elymus hystrix* (bottle-brush grass) and *Aster cordifolius* (heart-leaved aster). Experiment two, conducted in Dunn's Woods from October 2013-September 2014, involved *Aster lateriflorus* (calico aster), *Carex normalis* (spreading oval sedge) and *Conoclinum coelestinum* (blue mistflower). Experiment three, conducted in Latimer Woods from October 2013-September 2014, involved *Lobelia siphilitica* (great blue lobelia) *Elymus hystrix* (bottlebrush grass) and *Conoclinum coelestinum* (blue mistflower).

Herbivore exclosures were 40-cm diameter, 1-m tall cylindrical cages constructed from hardware cloth (Midwest Air Tech Import, Grandview, MO, U.S.) fastened with cable ties (Gardner Bender, Milwaukee, WI, U.S.). Closed cages did not have openings and were not accessible to mammalian herbivores such as deer and rabbits. Open cages had two entrances (18 cm × 15 cm) opposite one another at the bottom of each cage and were accessible to small mammals such as rabbits. Open cages served as cage controls and also enabled the effects of small mammal vs. deer herbivory to be distinguished. Closed and open cages were secured to the forest floor with four ground staples (Easy Gardner Inc., Waco, TX). Uncaged plots (40 cm × 40 cm) were marked with four 30 cm bamboo stakes and were accessible to all herbivores. Within each block, caged and control areas were spaced approximately 1 m apart from one another. Mature plugs were removed from cone-tainers and transplanted into caged and uncaged plots using a dibble sized for the cone-tainer volume (Steuwe & Sons, Corvallis, OR, U.S.). Three plugs of each of three species were transplanted into each caged or uncaged plot, for a total of nine plugs per plot. Plugs were planted 5 cm apart in a 3 × 3 array, and plug locations were randomized.

Herbivory-PSF Experiment

Propagation of Study Species and Greenhouse Conditions

We focused on examining herbivory-PSF interactions amongst *Euonymus* and the three native perennial woodland species, *Solidago flexicaulis*, *Elymus hystrix,* and *Aster cordifolius,* shown to be most vulnerable to herbivory in our herbivore pressure experiment (see Results). Plants were germinated, propagated and grown in a temperature-controlled greenhouse at Indiana University. Supplemental light was provided by 1,000-watt high pressure sodium lights set to a summer photoperiod of 15 h light, 9 h dark. Native seeds were purchased from Spence Restoration Nursery (Muncie, IN) and *Euonymus* cuttings (one-node shoot tips) were collected from three urban woodland sites where soil was obtained (described below in Phase I—soil conditioning). Seeds and *Euonymus* cuttings were surface sterilized with 10% bleach. Likewise, all containers used to grow plants, as well as greenhouse bench surfaces, were surface-sterilized with 0.52% Physan 2.0 (Maril Products, Inc., Tustin, CA). Seeds were cold stratified for 4 weeks in moist fine sand that had been autoclaved twice at 120°C, 24 h apart, 2 h intervals then germinated in 10 × 10 cm flats filled with MetroMix 360 (Sun Gro Horticulture, Agawam, MA; autoclaved twice at 120°C, 24 h apart, 2 h intervals). Four-week old seedlings and *Euonymus* cuttings were rooted in 5 cm deep, 128-cell trays (Hummert International, St. Joseph, MO) filled with a 50/50 mix of river-washed sand (Rogers Group Inc., Martinsville, IN) and Indiana topsoil (sourced from Bloomington, IN) that had been autoclaved twice (120°C, 24 h apart, for 2 h intervals). Seedlings and cuttings grew in cell trays for a 4 week period.

Phase I—Soil Conditioning

For phase I pots, live soil was collected June 20th through June 22nd, 2017 from Latimer Woods and two other urban, predominantly beech-maple woodlands in Bloomington, Indiana: a woodland on Indiana University's campus near Ballantine Hall and Wapehani Woods, a municipal woodland preserve. At each site, a spade-tipped shovel was used to collect soil to a 21.5 cm depth in equal amounts from areas that were uninvaded and areas invaded by *Euonymus*. Soil from uninvaded and invaded areas was combined and thoroughly mixed by passing it through a 6 mm sieve. Mixed soil was stored in 5-gallon plastic tubs with lids. One plastic tub was collected from each site per day and used to establish a block of 12 experimental pots (four pots for each of the three sites), such that one block was established per day for 3 consecutive days. Soil was not mixed between sites to allow for site-specific effects to be observed. Shovels, sieves, tubs, gloves, and all other materials were washed with soap and sprayed with 70% ethanol in between sites to prevent cross contamination.

Phase I vegetation treatments were established in 16-cm diameter, 18-cm deep pots (Dillen Products Inc., Middlefield, OH) that had been surface-sterilized as described above and bottom-lined with 10-cm squares of clean newspaper. The native community treatment consisted of one individual of each species planted equidistant in each pot, and the *Euonymus* vegetation treatment consisted of three equidistant *Euonymus* cuttings per pot. To guard against cross contamination, pots were spaced at least 50 cm apart on greenhouse benches. After being planted, all pots were given an acclimation period of 2 months (June-August 2017) before herbivory treatments were assigned. Plants of each vegetation treatment were assigned to an herbivory treatment group, for which plants were clipped with scissors to simulate mammalian herbivory, and an unclipped control group (**Figure 1**). Scissors were washed with soap and rinsed with water between pots to prevent cross contamination. In the herbivore pressure experiment, we observed that plugs in open cages or uncaged controls were heavily browsed (see Results, **Figure 2A**). To simulate this strong degree of herbivory, plants were cut twice weekly to maintain a height of 5 cm for 3 months (September-November 2017) prior to collection of phase I soil. Each block consisted of one pot for each vegetation and herbivory group and soil site (12 pots per block). In total there were nine replicates for each vegetation and herbivory group for a total of thirty-six phase I pots. Pots were randomized within each of the three blocks.

Phase II—Feedback Response

A net pairwise design was implemented where vegetation treatments were grown in conspecific and heterospecific soil (**Figure 1**). Aboveground phase I plants were clipped, placed in paper bags, and dried at 60°C for 72 h. Belowground biomass was sieved from soil, placed in Ziplock bags, then washed, blotted dry on paper towels, placed in paper bags, and dried at 60°C for 72 h. Dried above- and belowground biomass was weighed for inclusion as covariates in phase II analyses. Phase I soil from each pot was passed through a 4 mm sieve into a clean plastic tub and stored in new Ziplock bag in a refrigerator for <12 h. Equipment was washed between pots and all pots and greenhouse benches were surface-sterilized as described in phase I. Once an entire block was sieved, soil from each phase I pot

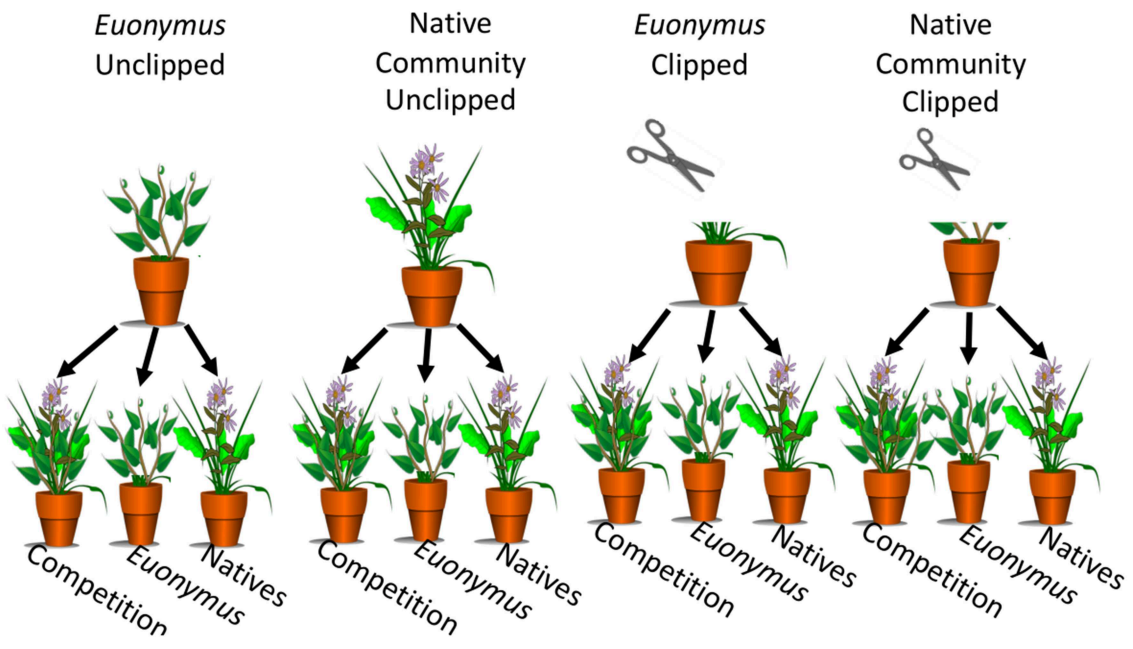

FIGURE 1 | Phase I vegetation groups included natives and *Euonymus*. Herbivory groups included unclipped controls (left) and simulated herbivory by clipping (right). In phase II, conditioned soil was used to grow the native community, monocultures of *Euonymus*, and a competition treatment with native community-*Euonymus* mixtures.

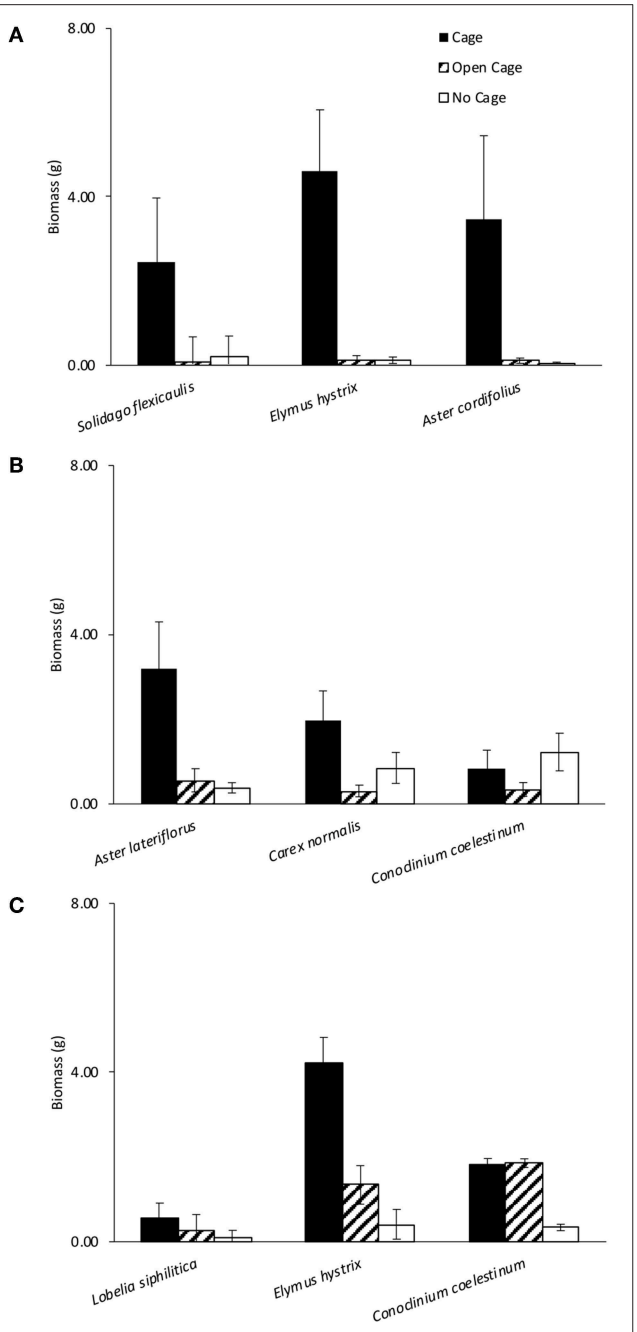

FIGURE 2 | Cage treatments had a significant effect on biomass at Dunn's **(A,B)** and Latimer **(C)** woods. Bars show +/– 1 SE.

was divided into three 950 mL pots lined with 10 cm squares of clean newspaper. Each set of three pots was then planted with one of three vegetation groups: a native community with one new seedling of each of the native species used in phase I, an invasive monoculture of three *Euonymus* cuttings, and a native-*Euonymus* additive design mixture of each of the three native seedlings and three *E. fortunei* cuttings (**Figure 1**). An additive design was used to best represent the process of plant invasion and native-invasive competition. Initial data including height, longest leaf length, and number of leaves was obtained from seedlings within 24 h after transplanting, for inclusion as covariates in Phase II analyses. Pots were randomized within each of nine blocks in the greenhouse. After a 3 month growth period (December 2017 to February 2018), above- and belowground biomass of phase II plants were harvested, dried and weighed following the same protocols as for phase I plants.

Statistical Analyses

To assess the degree of herbivore pressure that native woodland perennials experienced, we used nlme in R Core Development Team (2017, v3.4) to construct a mixed effects linear model for each experiment. with aboveground biomass as the response variable, exclosure treatment (cage, open cage, no cage), species identity, and their interaction as fixed effects, and block as a random effect. Based on graphical diagnostics of residuals, aboveground biomass was log-transformed [ln(x+min/2)] to meet normality and homogeneity of variance assumptions. Because there was no significant interaction between plant species identity and exclosure treatment, the interaction was dropped from the models. *A priori* contrasts were performed using R's contrast package to assess differences between exclosure treatments for each experiment.

In order to determine how herbivory influenced plant-soil feedback under different competitive conditions, we used PROC-MIXED in SAS Institute (2017, v9.4m5) to construct mixed-effects linear models. Given that our interest was mainly in examining herbivory × PSF interactions and that we had limited power to run a four-way model, we chose to run two separate three-way models, one for phase II plants grown in non-competitive mixtures and one for phase II plants grown in competitive mixtures. Phase II biomass was used as the response variable and herbivory, phase I vegetation group (i.e., conditioned soil type), and phase II vegetation group were included as fixed effects with all possible interactions. Phase II seedling size measurements (height, longest leaf length, and number of leaves) and phase I biomass (above- and belowground) were used as covariates. Soil collection sites (Ballantine, Latimer, Wapehani) and block were random effects. Graphical diagnostics of residuals were used to test for normality and heterogeneity of variance. No transformations were needed to meet model assumptions.

Plant-soil feedback is reflected in the interaction between phase I and phase II vegetation group, with non-neutral PSF indicated where the interaction is significant. A significant three-way interaction between herbivory, phase I vegetation group, and phase II vegetation group indicates that PSF was significantly different in the simulated herbivory treatment compared to the no-herbivory treatment. *A priori* contrasts were used to estimate net pairwise feedback (sensu Mangan et al., 2010; Smith and Reynolds, 2015), reflected as the interaction coefficient (I_s) developed by Bever et al. (1997):

$$I = (A_A - A_B) + (B_B - B_A) \quad (1)$$

where A_A represents species A growth in soil conditioned by species A and A_B refers to species A growth in soil conditioned by species B. Likewise, B_B refers to species B growth in conspecific soil and B_A refers to species B growth in heterospecific soil.

RESULTS

For each of the three herbivore pressure experiments, the mixed effects linear models revealed a significant effect of exclosure treatment and *a priori* contrasts indicated significant pairwise differences amongst exclosure treatments (**Table 1**). Although the exclosure × plant species identity interaction was dropped from all models due to non-significance, the effect of exclosure treatment was most consistent across species for Dunn's Woods experiment one, where on average, aboveground biomass of *Solidago flexicaulis*, *Elmus hystrix,* and *Aster cordifolius* was over 95% greater in closed cages compared to open cages or no-cage controls (**Figure 2A**, **Table 1**). Similar trends were evident for *Aster laterifolius* and *Carex normalis*, but not *Conoclinum coelestinum* in Dunn's Woods experiment two (**Figure 2B**) and for *Elymus hystrix*, but not *Lobelia siphilitica* or *Conoclinum coelestinum* in experiment three at Latimer Woods (**Figure 2C**), although the data were considerably more variable across species and *a priori* contrasts did not detect a significant difference between closed cages and no-cage controls in experiment two (**Table 1**).

In the herbivory × PSF experiment, when native communities and *Euonymus* were grown separately (non-competitive conditions), there was no significant two-way interaction between phase I and phase II vegetation type (i.e., PSF, aka "neutral PSF") and no significant three-way interaction between phase I vegetation group, phase II response group and herbivory group in the mixed effects GLM (i.e., no effect of herbivory on PSF, **Table 2**). Consistent with these non-significant interactions, mean interaction coefficients were not significantly different from zero regardless of herbivory treatment (**Figure 3**). There was a significant main effect of phase I vegetation group and a significant two-way interaction between herbivory group and phase I vegetation group (**Table 2**).

In contrast, when native communities and *Euonymus* were grown together (competitive conditions), the mixed effects GLM revealed a significant three-way interaction for herbivory group × phase I vegetation group × phase II vegetation group (**Figure 3, Table 3**). This indicates that PSF was significantly different in the simulated herbivory treatment compared to the no-herbivory treatment. Mean interaction coefficients reveal the nature of this shift; net pairwise PSF was neutral (0.081 ± 0.146, $p = 0.635$) under conditions of simulated herbivory and negative (-0.580 ± 0.130, $p = 0.047$) in the absence of herbivory. There were also significant main effects of phase I and phase II vegetation group and a significant two-way interaction for herbivory × phase I vegetation group (**Table 3**). In the absence of herbivory growth of both the native community and *Euonymus* was higher in soil conditioned by *Euonymus,* but this effect disappeared in the presence of herbivory.

TABLE 1 | Results of mixed effects linear models for herbivore pressure experiments.

Experiment	Factor	Contrast	d.f. (n,d)	*F* or *t*	Pr>*F* or Pr>|*t*|
DUNN'S EXPERIMENT 1					
	Exclosure treatment		2,60	25.52	<0.0001
	Plant ID		2,60	0.41	0.6638
		Cage vs. no cage	65	5.99	0
		Cage vs. open cage	65	−6.37	0
		Open cage vs. no cage	65	−0.37	0.7101
DUNN'S EXPERIMENT 2					
	Exclosure treatment		2,60	6.23	0.0035
	Plant ID		2,60	0.12	0.8902
		Cage vs. no cage	65	1.01	0.3177
		Cage vs. open cage	65	−3.43	0.001
		Open cage vs. no cage	65	−2.43	0.0181
LATIMER					
	Exclosure treatment		2,76	31.04	<0.0001
	Plant ID		2,76	9.54	2e-04
		Cage vs. no cage	83	7.83	0
		Cage vs. open cage	83	−3.19	0.002
		Open cage vs. no cage	65	−2.43	0.0181

For each experiment, exclosure treatment (cage, open cage, no cage) and plant species ID were included as fixed effects, and block was included as a random effect. A priori contrasts indicated pairwise differences amongst the three caging treatments. F-values are shown for fixed effects, t-values for contrasts.

DISCUSSION

Our results demonstrate that herbivore pressure on native understory plants can be intense in urban woodlands, and this can weaken net pairwise PSFs between native and invasive species, which is consistent with our original predictions. This is the first report of interactive herbivory × PSF effects in woodlands and for native and invasive species. Other findings support herbivory (from insects) as a factor that reduces the importance of PSF as a driver of plant community dynamics among native grassland species (Heinze and Joshi, 2017). There are several mechanisms that could explain aboveground herbivory weakening negative PSFs, including increased root growth (Bardgett et al., 1998) leading to an increase in soil mutualisms (Smith-Ramesh and Reynolds, 2017), a cross-induction of belowground defenses (Bezemer and van Dam, 2005; Kaplan et al., 2008), and a reduction in plant size and nutrient demands (Smith-Ramesh and Reynolds, 2017). Comparing microbial communities, soil defense compounds, and nutrient concentrations in phase I soils may yield insights into which mechanism operates. An important factor to consider is that our experiment did not pose an aboveground enemy

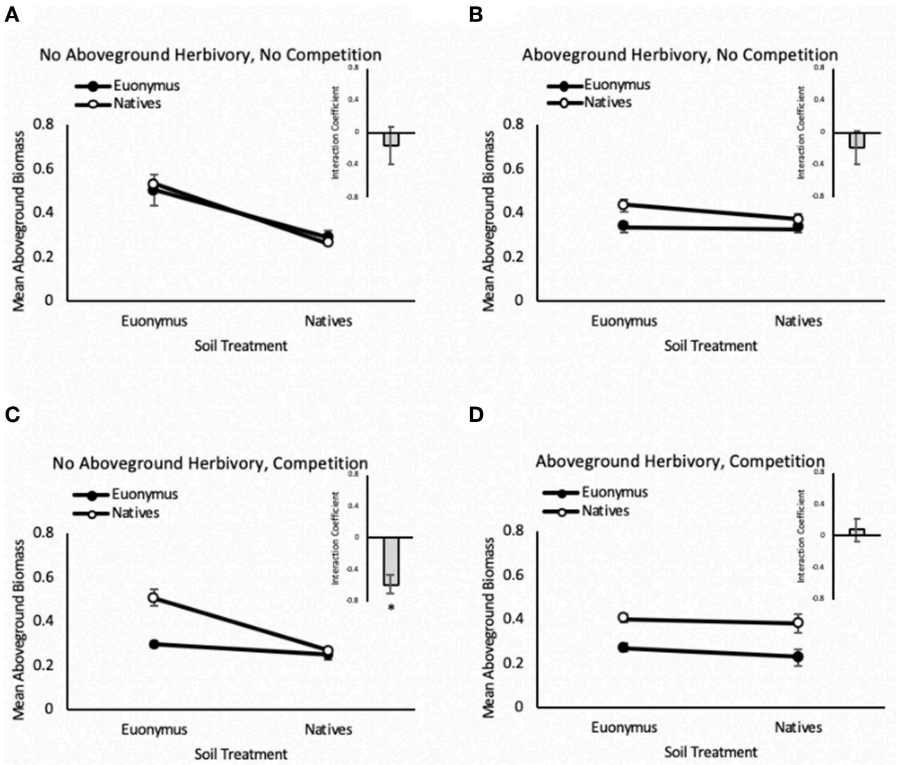

FIGURE 3 | Feedback between *Euonymus* (closed circles) and the native community (open circles) in the no aboveground herbivory, no competition **(A)**, no aboveground herbivory, no competition **(B)**, aboveground herbivory, competition **(C)**, and herbivory, competition **(D)** groups. Insets are mean interaction coefficients for each group. Bars show +/– 1 SE.

TABLE 2 | Results of mixed effects linear model for phase II plants grown in non-competitive conditions.

	d.f. (n,d)	*F*	Pr > *F*
Herbivory group	1,4	3.22	0.1474
Phase I vegetation group	1,4	26.36	**0.0068**
Phase II vegetation group	1,4	0.83	0.4139
Phase I vegetation group × phase II vegetation group	1,4	1.22	0.3315
Herbivory group × phase I vegetation group	1,4	16.96	**0.0146**
Herbivory group × phase I vegetation group × phase II vegetation group	1,4	0.00	0.9856

Herbivory, phase I herbivory group; Phase I vegetation group, identity of phase I plants (native community, Euonymus); Phase II vegetation group, identity of phase II plants (native community, Euonymus). Block and its interactions with all factors were included as random effects. Bold text indicates significance at the p = 0.05 level.

TABLE 3 | Results of mixed effects general linear model for phase II plants grown in competitive conditions.

	d.f. (n,d)	*F*	Pr > *F*
Herbivory group	1,4	1.86	0.2443
Phase I vegetation group	1,4	19.65	**0.0114**
Phase II vegetation group	1,4	9.3	**0.0380**
Phase I vegetation group × phase II vegetation group	1,4	6.87	**0.0588**
Herbivory × phase I vegetation group	1,4	12.05	**0.0256**
Herbivory × phase I vegetation group × phase II vegetation group	1,4	10.79	**0.0304**

Herbivory, phase I herbivory group; Phase I vegetation group, identity of phase I plants (native community, Euonymus); Phase II vegetation group, identity of phase II plants (native community, Euonymus). Block and its interactions with all factors were included as random effects. Bold text indicates significance at the p = 0.05 level.

escape scenario for *Euonymus*, which would be expected in nature even for more palatable invasive plant species (at least in the case of host-specific and/or specialist herbivores). In an aboveground enemy escape scenario, the native community would experience aboveground herbivory and any of its knock on effects to soil variables, while the invasive species would not. Depending on whether and how aboveground herbivory on the native community influenced soil microbial communities, defense compounds, and/or nutrient concentrations associated with native plants, net pairwise PSF among native and invasive plant species could range from negative to positive.

We observed net negative pairwise PSF dynamics for competitive mixtures of the native community grown with the invader *Euonymus* in the absence of herbivory. This result

is inconsistent with general expectations from the literature (Kulmatiski et al., 2008) and with previous studies examining PSF dynamics between native woodland species and *Euonymus*, which have found net neutral to positive feedback (Smith and Reynolds, 2012, 2015). Something overlooked, however, is that while neutral or positive PSF among native and invasive species is expected if either enhanced mutualisms or novel weapons are the driver of invasion (**Figures 4A,B**), negative PSF among native and invasive species is the expectation if belowground enemy escape is the main driver of invasion (**Figure 4C**). Our findings might therefore be explained if belowground enemy escape was operating for *Euonymus* under the conditions of our experiment.

Interestingly, our results also indicate that soil conditioned by *Euonymus* may have a general promoting effect on plant growth, although this effect disappeared in the presence of herbivory. While this finding that *Euonymus*-conditioned soil promoted greater phase II plant growth than native-conditioned soil may at first seem surprising, it could simply reflect greater soil resource drawdown by the three-species native community, perhaps due to niche complementarity of niche-partitioned species, ultimately reducing nutrient availability for phase II plants. The fact that this effect was lost in the herbivory treatment may be expected given that biomass—and presumably soil resource demand—of all species was kept consistently low by the simulated herbivory. Further studies are needed to confirm whether *Euonymus* has a promoting effect and the mechanisms by which this might occur.

Plant-soil feedbacks tend to be weaker in field studies compared to greenhouse experiments (Kulmatiski et al., 2008) and our results suggest that herbivore pressure in the field may contribute to such weakened PSF. Still, several limitations to our study may have prevented the magnitude of PSFs observed in our results from accurately reflecting field conditions. Cutting aboveground plant tissues with scissors may have a different effect on plant defense compounds or may damage plant tissues differently than an herbivore. In natural systems, herbivores may trample herbaceous vegetation and release waste products, both of which could potentially affect soil conditions (Bardgett and Wardle, 2003; Schrama et al., 2013) and consequently PSFs. Field PSF experiments that manipulate natural herbivores are therefore needed. While we used an additive design to best simulate the invasion process, results may have been different with a substitutive design (which holds total plant density constant).

Further research is also needed to confirm whether herbivory-PSF interactions affect invasion success. For example, future studies could examine *Euonymus*-native PSFs in the presence and absence of herbivory and quantify *Euonymus* invasion success in each treatment. Additionally, future studies should assess whether herbivore type (e.g., insect, mammalian) differentially affect PSFs. Herbivory-PSF interactions across a gradient of lower to higher herbivore pressure should also be examined. Exploring herbivory-PSF interactions among other native-native and native-invasive combinations, including palatable vs. unpalatable invasive species, and across different habitat types is also needed to assess the generalizability of our findings.

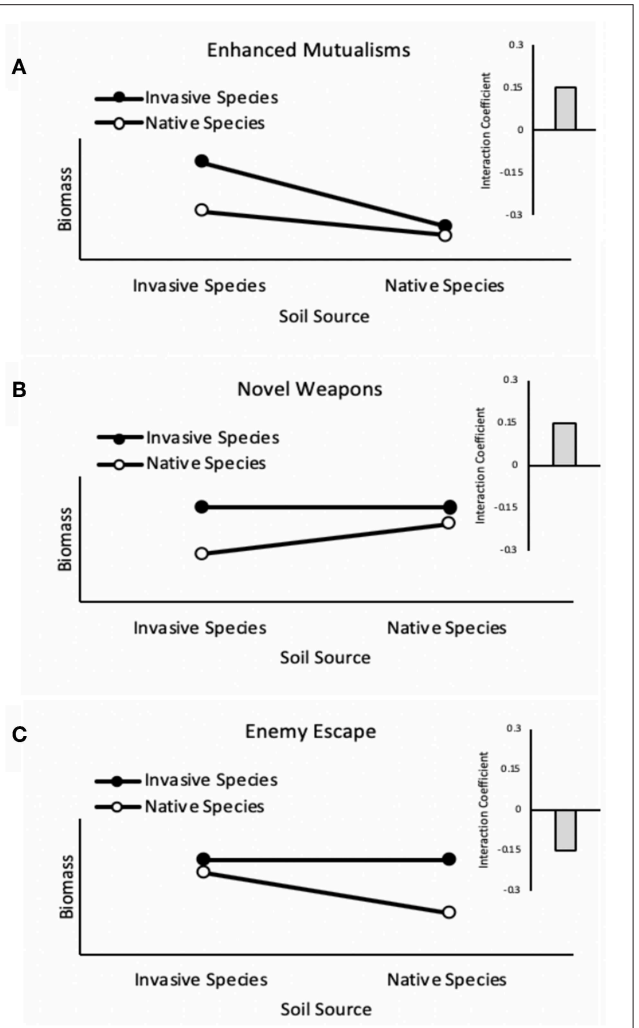

FIGURE 4 | Plant-soil feedback dynamics for enhanced mutualisms, novel weapons, and enemy escape hypotheses. If an invasive species exhibits enhanced mutualisms **(A)**, growth of the invasive species could be sufficiently greater in conspecific soil relative to heterospecific soil (positive individual feedback) to dominate the outcome of net pairwise feedback. An invader that exhibits novel weapons **(B)** would presumably be adapted to its own weapons and thus exhibit neutral individual feedback, whereas the invader's novel weapons would hinder native species growth in heterospecific soil, resulting in positive individual feedback for the native. Net pairwise feedback would therefore also be positive under the novel weapons hypothesis. In contrast, if an invasive species exhibits belowground enemy escape **(C)**, its growth would not be expected to be affected by soil conditioning (neutral individual feedback) whereas native species would exhibit negative individual feedback from host specific enemies, resulting in negative net pairwise feedback.

CONCLUSIONS

Our results suggest that herbivore pressure can be intense in urban woodlands and that such herbivore pressure can reduce the strength of PSFs, affecting species interactions with an invader and potentially influencing invasion success. Thus, plant-soil feedbacks may be less important in driving plant community dynamics in systems with high herbivore pressure. Our results also suggest that *Euonymus*' invasion success may in part be

attributed to belowground enemy escape, although research to confirm this mechanism of feedback is required. Further research is needed to determine whether herbivory-PSF interactions affect invasion success, identify the mechanisms of herbivory-PSF interactions, and to assess the generality of our results under more complex and varied field conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

HR, CH, and RA designed and executed the herbivore pressure experiments. HR and SB designed and SB executed the PSF × herbivory experiment. LS-R and SB did the statistical analyses. SB and HR wrote the manuscript, with input from LS-R. All authors contributed to the development of ideas.

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Individual Plant-Soil Feedback Effects Influence Tree Growth and Rhizosphere Fungal Communities in a Temperate Forest Restoration Experiment

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Plant-soil feedbacks have important implications for community composition and restoration. However, relatively few field trials test the influence of plant-soil feedbacks, especially on longer-lived species, such as trees. Here we present a field restoration experiment with 10 ectomycorrhizal fungal tree species native to eastern North America. Trees were inoculated with soils collected from conspecifics in the field or from a heterospecific: *Quercus rubra*. Following 16 months of growth in the field, *Carya ovata* diameter increase was significantly greater in trees receiving the heterospecific inoculant. This plant-soil feedback is consistent with *C. ovata's* natural co-occurrence with *Q. rubra*. Conversely, *Quercus macrocarpa* diameter increase and *Carya cordiformis* height increase were significantly greater when inoculated with conspecific soils, and this positive plant-soil feedback is consistent with their numerical dominance in natural communities. We found no evidence for phylogenetic Janzen-Connell effects or conservation of soil mutualists across tree species. We also quantified differences in soil fungal community structure with next generation sequencing methods (Illumina MiSeq) following 16 months in the field. Shannon's diversity of fungal taxa was greater in heterospecific soils of seven of our nine experimental species, consistent with a diversifying influence of *Quercus rubra* soil inocula. However, only one genus, *Ulmus,* exhibited differences in fungal community composition derived from conspecific and heterospecific sources, suggesting a stronger effect of focal tree species than of soil inocula source. The relatedness among focal tree species also influenced fungal community composition, with tree families and genera displaying different fungal communities. We suggest that future experiments should determine whether more diverse tree and fungal communities might have enhanced ecosystem functioning in tree restoration sites.

Keywords: plant-soil feedbacks, ectomycorrhizae, temperate trees, restoration, Illumina Mi-Seq

INTRODUCTION

Understanding the influence of plant-soil feedbacks on plant growth, community composition, and restoration ecology has become a growing area of focus in plant ecology (Kulmatiski et al., 2008; Brinkman et al., 2010; Bever et al., 2012), in part because plant-soil feedbacks have the potential to influence species coexistence (Mangan et al., 2010; Anacker et al., 2014; Bennett et al., 2017). For example, reciprocal negative feedbacks predict frequency-dependent coexistence and could be a diversity-enhancing mechanism in plant communities (Bever et al., 1997). Conversely, positive individual plant-soil feedback predict the numerical dominance of species that benefit from conspecific soils (Klironomos, 2002). Plant-soil feedbacks occur because plants influence the soils where they grow (Ehrenfeld et al., 2005) including soil microbial community composition and soil nutrient availability (reviewed in Ehrenfeld et al., 2005; e.g., Burns et al., 2017). A large body of greenhouse experiments suggest that individual plant-soil feedbacks can have a strong influence on plant growth (Kulmatiski et al., 2008). However, far fewer studies have determined whether such short-term greenhouse results translate into longer-term effects in the field (but see e.g., Schittko et al., 2016). Further, while plant-soil feedbacks are often quantified by comparing conspecific vs. heterospecific conditioned soils, the role of plant relatedness in plant-soil feedbacks is still unclear (Liu et al., 2012; Anacker et al., 2014; Mehrabi and Tuck, 2015; Crawford et al., 2019). Here, we ask whether individual plant-soil feedbacks influence soil microbial community composition and tree growth for nine temperate ectomycorrhizal fungal tree species in a field restoration experiment.

A common goal of ecological restoration is to facilitate the return of ecosystem function to disturbed environments, simultaneously providing substantial socio-economic and ecological benefits (BenDor et al., 2015). In eastern North America, the restoration of temperate deciduous forests is a common objective of many restoration projects, including those targeting abandoned agricultural land and anthropogenically disturbed urban habitats (Cernasky, 2018). While a multitude of factors influence the success or failure of these projects, soil microbial communities may have an important influence on restoration outcomes (Harris, 2009; Kardol and Wardle, 2010). Manipulation of soil microbial community structure may influence the result of temperate forest restoration and has become a commonly employed method intended to improve tree survival and/or growth.

One efficient method of manipulating soil microbial community structure is to inoculate trees with forest collected soils prior to outplanting in a restoration site (Maltz and Tresder, 2015; St-Denis et al., 2017). This method transfers potentially beneficial microbes, including mycorrhizal fungi, as well as potentially antagonistic microbes including pathogenic fungi and/or bacteria. Practitioners looking to collect soils for use as an inoculant could either avoid collecting from mature conspecific individuals if pathogens are found to have a primary influence on tree survivorship and growth (e.g., Packer and Clay, 2000), or conversely, target mature conspecific individuals if specific mutualisms elicit improved tree performance (e.g., den Bakker et al., 2004; Ishida et al., 2007). Plant-soil feedbacks that result from the conditioning of soil communities by different focal tree species have a profound influence on the microbial composition of forest soil transfers and the subsequent response of plants to inoculation during restoration (Wubs et al., 2016; Lance et al., 2019). Understanding the factors that influence the development of plant-soil feedbacks is essential to developing best practices for soil microbial community manipulation and is critical for ecological restoration of temperate forest communities. For example, the Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971), in which adult individuals inhibit the growth of conspecific or closely related recruits (Liu et al., 2012), may prove to be an important predictor of tree response to inoculation with forest soils.

Knowledge of the phylogenetic relationships between individuals that condition soil inocula and those receiving the inocula may be of value to both restoration practitioners and ecologists looking to further understand the connection between soil microbial communities, plant performance, and plant community composition (e.g., Reinhart et al., 2012a). Phylogenetic relationships amongst plants are known to influence the soil microbial communities with which they associate (Ishida et al., 2007; Burns et al., 2015). Phylogenetic conservatism in plant-soil feedbacks would result in closely related species responding in a similar fashion to inoculation with forest soil transfers. For example, pathogenic soil microbes are known to negatively influence closely related sub-tropical tree species (Liu et al., 2012), a pattern called "phylogenetic Janzen-Connell" effects. When pathogens effect close relatives similarly, practitioners should avoid collecting soils from close relatives. Alternatively, if closely related species response similarly to soil mutualists like mycorrhizal fungi (Reinhart et al., 2012b), then forest restoration might be enhanced by soils collected from close relatives. A lack of phylogenetic effect in plant-soil feedbacks might limit local soil collections to conspecifics for access to mutualists or heterospecifics for avoidance of pathogens (Fitzpatrick et al., 2017), at least where soil communities are species-specific (Ehrenfeld et al., 2005).

In this study we determine whether plant-soil feedbacks and plant relatedness influence tree growth and soil microbial community composition and diversity in a 16 month field experiment. We estimated a phylogeny for ten ectomycorrhizal tree species native to eastern North America, then performed a field experiment in which trees were inoculated with soil conditioned by a conspecific or heterospecific source. Our heterospecific source was *Quercus rubra*, a widely distributed ectomycorrhizal tree. We asked three primary questions: (1) Did individual plant-soil feedbacks (conspecific/heterospecific) influence tree growth and survival for ectomycorrhizal tree species? (2) Are plant-soil feedbacks consistent with a phylogenetic Janzen-Connell effect of conserved pathogens or do conserved mutualisms improve tree performance? (3) Were rhizosphere soil fungal diversity and community composition influenced by conditioning soil source (conspecific/heterospecific), focal tree species, and plant relatedness after 16 months in the field?

For question (1), we hypothesized that trees receiving the heterospecific inoculant would display greater growth and survival than those receiving the conspecific inoculant, if pathogens exhibit more host specificity than mutualists (but see e.g., Burgess et al., 2017). Host specificity is displayed in a small proportion of ectomycorrhizal fungal relationships and large numbers of ectomycorrhizal fungi can be found colonizing one tree (Bruns, 1995; Palmer et al., 2008). In addition, most cases of ectomycorrhizal fungal specificity occur in the genera *Pinus* and *Alnus*, neither of which was included in our study design (Bruns et al., 2002; Tedersoo et al., 2009). Thus we expect pathogen escape in heterospecific soils (i.e., negative plant-soil feedbacks). Alternatively, because our tree species are ectomycorrhizal, and because some prior work has observed positive plant-soil feedbacks in most ectomycorrhizal trees (Bennett et al., 2017), we might expect positive plant-soil feedbacks. To answer question (2), we explored how inoculation with distant relative's heterospecific conditioned soil might enable trees to form beneficial ectomycorrhizal fungal relationships while avoiding pathogens, consistent with a phylogenetic Janzen-Connell effect (Liu et al., 2012). Thus, we predicted more neutral plant-soil feedbacks in the close relatives of *Q. rubra* (e.g., *Q. palustris*) and more positive effects in distant relatives (e.g., pathogen escape). Alternatively, plant performance could be greater in conspecific and close relatives' soils, consistent with the positive plant-soil feedbacks observed in some ectomycorrhizal fungal trees (Bennett et al., 2017). In this case, we would predict more neutral plant-soil feedbacks in the close relatives of *Q. rubra* and more negative in distant relatives (e.g., loss of mutualist services). Conversely, if pathogens and mutualists are not influenced by plant relatedness, plant-soil feedbacks might not scale with phylogenetic distance (Mehrabi and Tuck, 2015). Finally, to answer question (3), we used next generation sequencing following 16 months in the field to explore the roles of soil treatment (conspecific, heterospecific), focal species, and plant relatedness in determining fungal community composition on roots and in rhizosphere soils. We predicted that fungal communities derived from heterospecific soils would be more diverse than those derived from conspecific soils, because heterospecific soils have both the fungal community in the inocula as well as any fungal species cultivated by the focal tree. We also predicted that rhizosphere fungal community structure would significantly differ between the heterospecific and conspecific treatments within each species. We predicted effects of focal tree species on fungal community composition, if focal trees influence soil microbial communities as they grow in our restoration site. Finally, we predicted that closely related focal tree species might have similar fungal community composition (e.g., Burns et al., 2015), if such effects are conserved (e.g., Liu et al., 2012; but see Mehrabi and Tuck, 2015; Sweet and Burns, 2017).

METHODS

Experimental Overview and Plant Material

Our experiment focused on the restoration of 10 ectomycorrhizal fungal tree species (see **Figure 1**), which are native to the northeastern United States. Our design included two experimental treatments: conspecific or heterospecific soil inocula, each with 10 replicates (except for *Q. rubra*, which received only conspecific soil). Thus, the total design was 9 species × 2 soils (conspecific/heterospecific) × 10 replicates (180 trees) + 20 *Q. rubra* in conspecific soil for a total of 200 trees. *Quercus rubra* served as the heterospecific soil source for all other tree species (see details below), because it grows in a number of forest types and can be found in association with all of our other experimental tree species (Lance, personal observation). We manipulated the soil microbial community associated with each tree by adding field collected soils to pots prior to planting in a former community garden at Squire Valleevue Farm (Hunting Valley, OH, USA). The region in which our site is located is characterized by a humid continental climate, with a mean annual precipitation of approximately 990 mm. Soils at our research site were classified as Ellsworth silt loams (USDA, 2019). We monitored tree growth and soil fungal community structure after two growing seasons to understand how plant-soil feedbacks may influence restoration efforts.

Trees for use as experimental phytometers were sourced from a native plant nursery in Indiana, USA in April 2017. We obtained 20 trees of each of our 10 focal species. Trees arrived in 4 to 12-liter pots, except for *Fagus grandifolia,* which arrived in 18-liter pots. Variation in initial size was accounted for by calculating relative growth rates (see *Statistical approach* below). Trees were watered during the period between arrival from the nursery and planting into the field site.

Inoculation and Planting

We collected soils to use as soil inoculum in April 2017. We collected soil from 3 separate mature (diameter at breast height > 20 cm) trees of each of the 10 species in our field restoration experiment [as suggested in (Reinhart and Rinella, 2016)]. Replicate soil collections were not mixed following collection. To obtain soils from all 10 species, soils were collected at two locations (Squire Vallevue Farm and Holden Arboretum) located approximately 15 km apart. The three dominant soil types at both locations are Mahoning silt loams, Ellsworth silt loams, and Haskins loams (USDA, 2019). Collection implements were sterilized with 80% ethanol and air dried between replicate soil collections. Trees targeted for soil collection were growing in natural temperate forests except for *Quercus macrocarpa* and *Quercus palustrus* where two of the three replicate collections were made from Holden Arboretum's planted collection. Approximately 0.5 kg of soil was collected within 1 m of the bole of each tree; collection locations were void of herbaceous vegetation and measured approximately 2 m × 2 m. Only the top 10 cm of soil was collected, and large root fragments were removed in the field. Following collection, soils were dried at ambient room temperature for 1 week then sieved with a 0.5 cm sieve. Each of the 3 replicate collections per species were kept separate throughout the inoculum preparation process; we avoid mixing soils to avoid potential problems with pseudoreplication, such as mixing a rare pathogen into all samples (Smith-Ramesh and Reynolds, 2017; Rinella and Reinhart, 2018).

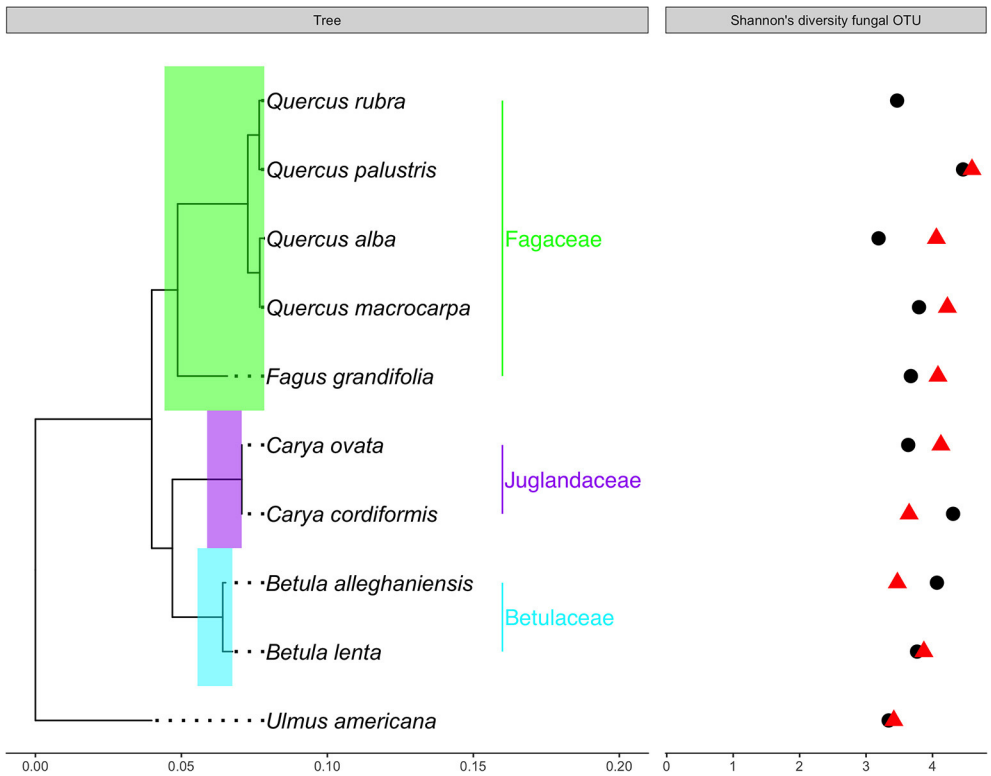

FIGURE 1 | A maximum likelihood phylogeny for 10 tree species, which associate with ectomycorrhizal fungi. Two DNA regions, *mat*K and *rbc*L were used to estimate this phylogeny (**Supplementary Material S2**). Branch lengths are in substitutions per site. Shannon's diversity of fungal communities following inoculation with conspecific soil (black dots) or heterospecific soils (red triangles) are represented to the right of the phylogeny.

Ten trees of each species were randomly selected for inoculation with either conspecific or heterospecific soil, resulting in a total of 20 trees per species being planted in the restoration site (except for 20 *Q.rubra,* which received only conspecific inoculation). We equally divided the three replicate collections per species during inoculation. Therefore, three trees received soil from one of two replicate collections while four trees received the randomly selected third replicate collection. We thus replicated both soil collections within species and phytometer/tree genotype within soil replicate. We abraded the surface of each pot with a sterile gloved hand, then applied 50 g of dried soil to the surface. Trees were immediately watered with 0.5 L water. Trees remained in their pots following inoculation for 2 weeks before being planted in May, 2017.

Our experimental site measured ∼50 m × 50 m; the site had previously been used as a community garden but was idle for 1 year prior to the start of our experiment. We tilled the site twice: once in the fall of 2016 and again in spring of 2017 to remove herbaceous vegetation and facilitate the planting process. Tilling has also been shown to increase ectomycorrhizal colonization in temperate tree restoration (Bauman et al., 2013). Trees were planted in 15 rows in completely randomized positions. Approximately 3 m separated each tree and row. We irrigated the site during planting and throughout the summer of 2017. Irrigation was not provided during 2018. Trees also received an ∼1 m diameter ring of fresh wood chips (not composted) immediately following planting. Trees were wrapped with plastic protective wraps (ArborGuard, Gempler's Supply, Janesville, WI, USA) to prevent small mammal herbivory. Areas surrounding trees and wood chips were seeded with a mixture of annual and perennial grasses.

Measurements and Soil Collection

We took a baseline measurement of tree size immediately following planting in May 2017. Subsequent measurements were collected in September 2018. Height was determined by measuring to the apical bud of each tree using a meter stick (height < 140 cm) or Sokkia telescoping height pole (Senshin Industry, Osaka, Japan). We measured diameter 10 cm above the root collar of each tree using a Mitutoyo digital caliper (Mitutoyo Corporation, Kanagawa, Japan).

Soils for molecular analysis were collected in September 2018. We sampled 6 trees per species: 3 inoculated with conspecific soils (one per replicate collection location) and three inoculated with heterospecific soils (one per replicate collection location) for a total of 57 samples [9 species × 2 soil origins (conspecific, heterospecific) × 3 collection locations + 3 conspecific inoculated *Q. rubra* (one per each of the 3 collection locations)]. Two separate cores of the top 15 cm of soil were taken for each sampled tree and immediately homogenized in the field.

Samples consisted of fine root fragments and soil that adhered to the roots (rhizosphere soil). Cores were sterilized with 80% ethanol between collections. Samples were immediately placed on ice in the field, then frozen at −70°C before processing.

Next Generation Sequencing With Illumina Mi-Seq

We utilized next generation sequencing methods to examine general fungal communities on roots and in rhizosphere soils of all 57 soil samples. DNA was extracted from samples following a phenol-chloroform protocol (Burke, 2008). We made amplicons of the fungal ITS-2 gene region using the primers 58A2F and ITS4 with Illumina overhang adapters. The primer sequences are included in the **Supplementary Material S1**. The "16S Metagenomic Sequence Library Preparation" (Illumina technology protocol) was utilized as a guide during primer selection. Each reaction included 2 units of FastStart Taq DNA polymerase (Sigma-Aldritch, Inc., St. Louis, MO, USA), 2 mM $MgCl_2$, 0.2 uM of both primers 58A2F and ITS4, 0.5 ug/ul bovine serum albumin, and 0.8 mM dNTP mix. Our thermocycling conditions were an initial denaturation at 95°C for 5 min, followed by 25 cycles of 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min, with a final extension of 72°C for 5 min. Amplicons were then purified, indexed, and sequenced as 2 × 250 bp reads on the Illumina MiSeq V3 sequencer at the Case Western Reserve University Genomics Core facility.

The Blaxter lab's metabarcoding processing pipeline (version 1.0.1) was used as a guide for our sample processing (Blaxter, 2016) with the UPARSE pipeline (Edgar, 2013). We merged forward and reverse reads with the fastq_mergepairs command in USEARCH, version 11.0 (Edgar, 2010). We removed control phiX prior to merging reads using the filter_phiX command. Primers were removed with Cut Adapt (v1.10) (Martin, 2011). We implemented the UCLUST algorithm (Edgar, 2010) for OTU clustering at 97% similarity and removal of chimeras using the UCHIME algorithm (Edgar et al., 2011). Singletons OTUs were removed. We made taxonomic assignments for each OTU by utilizing the SINTAX algorithm (Edgar, 2016) and comparing against the UNITE database (v 8.0, release date 2018-11-18) (Aberenkov et al., 2010; Koljalg et al., 2013).

Phylogeny Estimation

A molecular phylogeny based on *mat*K and *rbc*L gene sequences was estimated for 10 ectomycorrhizal fungal tree species (**Figure 1**). Sequences for both gene regions were available for all 10 species (see **Supplementary Material S2**). The program MUSCLE (Edgar, 2004a,b) in the MEGA platform (version 7.0.14) was used to align each gene sequence separately. Alignments were checked by eye and preliminary phylogenies for each gene region were used to diagnose outliers. Because both regions generated consistent preliminary phylogenies, they were concatenated to construct a "total evidence" or "super matrix" phylogeny. Garli (version.951) was utilized to conduct a maximum likelihood tree search with 100 bootstrap replicates. We rooted the tree using *Ulmus americana* (Hinchliff et al., 2015), which is in the Rosales (Rees and Cranston, 2017). All other taxa in our sample are in the Fagales (Rees and Cranston, 2017).

Statistical Analyses

We conducted all statistical analyses in R version 3.5.1 unless otherwise specified.

Did Plant-Soil Feedbacks Influence Tree Growth and Survival for Ectomycorrhizal Tree Species?

To compare growth rates across species, we first standardized by initial size by calculating relative growth rates (RGR), then compared responses to conspecific and heterospecific soil treatments using log response ratios (Brinkman et al., 2010). *Betula lenta* was not included in these analyses due to high mortality and *Quercus rubra* had only the conspecific soil treatment, leaving 8 tree species in these analyses. Relative growth rate (RGR) in each treatment was calculated as (ln X-ln Y)/16, where X was a size measurement (height or diameter) at the conclusion of the experiment and Y was the corresponding size measurement at the beginning of the experiment. Our experiment lasted for 16 months as indicated by the denominator in our relative growth rate equation. We examined differences in tree growth response (stem elongation and diameter increase) to conspecific and heterospecific inoculants by calculating pairwise natural-log response ratios (lnRR) (Brinkman et al., 2010; Larios and Suding, 2015). We averaged growth data by replicate soil collection location in order to avoid pseudoreplication. Ratios were calculated as ln(RGR in conspecific/RGR in heterospecific) (Brinkman et al., 2010). This procedure resulted in $n = 3$ lnRR per species. Ninety five percentage confidence intervals were calculated for each ratio in order to determine statistical significance. If plant-soil feedbacks are primarily positive (greater plant performance in conspecific soils), we would see positive log response ratios. If plant-soil feedbacks are primarily negative (greater performance in heterospecific soils), we would see negative log response ratios. Note that this metric is an individual plant-soil feedback metric, and measures the absolute difference in response between plant performance in conspecific and heterospecific soils. This is an important measure of plant-soil feedback effects, and corresponds to differences in abundance in the field in some cases (e.g., Klironomos, 2002). However, it does not make a coexistence prediction, as do net-pairwise plant-soil feedback metrics (Bever et al., 1997).

Tree survival was examined using a generalized linear model with a binomial error distribution in which survival was analyzed as a function of treatment (conspecific or heterospecific), soil replicate, species, row, and an interaction of treatment and species. We predicted significant differences in survival across species; furthermore, if tree species respond to conspecific and heterospecific soils in a species-specific manner, we predict a treatment × species interaction. We also analyzed the within species patterns to test for effects of treatment, again including row as a blocking effect. If trees had higher survival in the conspecific compared with heterospecific treatment, this is a positive plant-soil feedback. If tree had higher survival in the heterospecific treatment, this is a negative plant-soil feedback.

Were Plant-Soil Feedbacks Consistent With a Phylogenetic Janzen-Connell Effect or Conserved Mutualists?

To test for an effect of phylogenetic distance on the strength of plant-soil feedbacks, we conducted a phylogenetic "meta-analysis" on the plant-soil feedback log response ratio. Because log response ratios have an associated variance, we used this analysis method to take that variance into account. Plant soil feedbacks were again measured as the log response ratios for diameter and height, and we conducted two separate models, one for each log response ratio. As in standard meta-analysis, the log response ratios were weighted by the inverse of their variance (Koricheva and Gurevitch, 2013). We used the rma.mv function in the metafor (Viechtbauer, 2010) package with species treated as a random effect and phylogeny incorporated into the error structure as a variance-covariance matrix. This test takes non-independence of the branch lengths in the phylogeny into account and is thus preferable to a linear model. We asked whether the strength of plant-soil feedbacks were a function of phylogenetic distance to the heterospecific (*Quercus rubra*). If a phylogenetic Janzen-Connell effect is present, we predict that *Quercus* will have the most neutral plant-soil feedbacks, followed by *Fagus grandifolia*, then the *Caryas* and *Betula*, and *Ulmus americana* will have the most positive plant-soil feedback (i.e., a positive slope). In other words, we predict a positive slope for the phylogenetic distance effect in these models (Liu et al., 2012). Alternatively, if mutualist effects are phylogenetically conserved (Reinhart et al., 2012b), we predict the most neutral plant soil feedbacks for *Quercus*, followed *Fagus grandifolia*, then the *Caryas* and *Betula*, and finally *Ulmus americana* will have the most negative plant-soil feedback (i.e., loss of mutualist benefits). In other words, we predict a negative slope for the phylogenetic distance effect [as found in (Crawford et al., 2019)].

Were Soil Fungal Diversity and Community Composition Influenced by Plant-Soil Feedbacks, Focal Tree Species, and Plant Relatedness After 16 Months in the Field?

To characterize the soil fungal community, we utilized next generation sequencing on rhizosphere soils. We were able to generate OTU matrix tables for 52 of the 57 collected samples. Five samples did not produce useable reads (one *B. allegheniensis*, one *B. lenta, one C. cordiformis*, and two *Q. palustrus* samples). We normalized the matrix of sequence counts generated by our next generation sequencing effort with the RLE normalization using the edgeR package (version 3.22.5) in R prior to statistical analysis. Variance stabilizing normalizations such as the RLE normalization are superior to rarifying microbiome data during statistical analysis due to a higher retention of data (McMurdie and Holmes, 2014).

We compared general fungal community composition on roots and in rhizosphere soils with non-metric multidimensional scaling (NMDS) procedures in the "vegan" package of R (Oksanen et al., 2017). Our NMDS procedures utilized the Sorenson (Bray-Curtis) distance metric, a random starting configuration, and three dimensions. Three dimensions were selected in order to minimize ordination stress. All permutations consisted of 4,999 iterations and were stratified by row (position in the experimental design). Non-parametric permutation procedures (PERMANOVA) were utilized to test for significant differences in fungal communities between genera, families, and inoculant source (conspecific or heterospecific). We analyzed all species collectively.

To determine how focal tree species and soil treatment (conspecific/heterospecific) influenced ectomycorrhizal fungal abundance, we extracted normalized abundances for six focal ectomycorrhizal fungal taxa (*Entoloma*, *Laccaria*, *Russula, Scleroderma, Tomentella,* and *Tuber*) from the sequencing data set. Some of these ectomycorrhizal fungal genera contained several taxa. To summarize these data, we summed abundances for each of these six genera across the RLE transformed matrix counts for each species/fungal genus combination. These values represent normalized abundances of these fungal genera and were then plotted as a heatmap against our tree phylogeny.

To determine whether fungal community diversity differed across treatments (conspecific/heterospecific), we calculated Shannon's diversity on the total fungal OTU matrix. We used a paired *t*-test and a paired sign test to test the prediction that heterospecific soils will be more diverse than conspecific soils. The paired *t*-test tests the prediction that the diversity values for heterospecific soil are greater than conspecific soils within species. The paired sign test tests the prediction that the direction of the effect (*a priori* prediction: heterospecific > conspecific) is consistent across species, for the 9 trees with both heterospecific and conspecific treatments (note that *Quercus rubra* only had a conspecific treatment).

RESULTS

Plant-Soil Feedbacks Influenced Tree Growth or Survival for Three Ectomycorrhizal Tree Species After 16 Months in the Field

We found three significant trends in tree growth in response to inoculation with either heterospecific or conspecific soils. Following two growing seasons, *C. cordiformis* stem elongation was significantly greater when trees received the conspecific inoculant (**Figure 2**). Inoculation with conspecific soils also facilitated diameter increase in *Q. macrocarpa* (**Figure 2**). Conversely, *C. ovata* trees receiving the heterospecific inoculant displayed greater diameter increase following two growing seasons when compared to trees receiving the conspecific inoculant. We did not find additional significant growth trends in the remaining species (**Figure 2**).

We found significant differences in the survival of the 10 experimental species (**Table 1**). Only 33% of the *Betula lenta* trees survived through two growing seasons. Three other species, *C. ovata*, *C. cordiformis,* and *B. allegheniensis* exhibited more than more one fatality throughout the course of the experiment; 80% of the planted trees survived for each of the above three species.

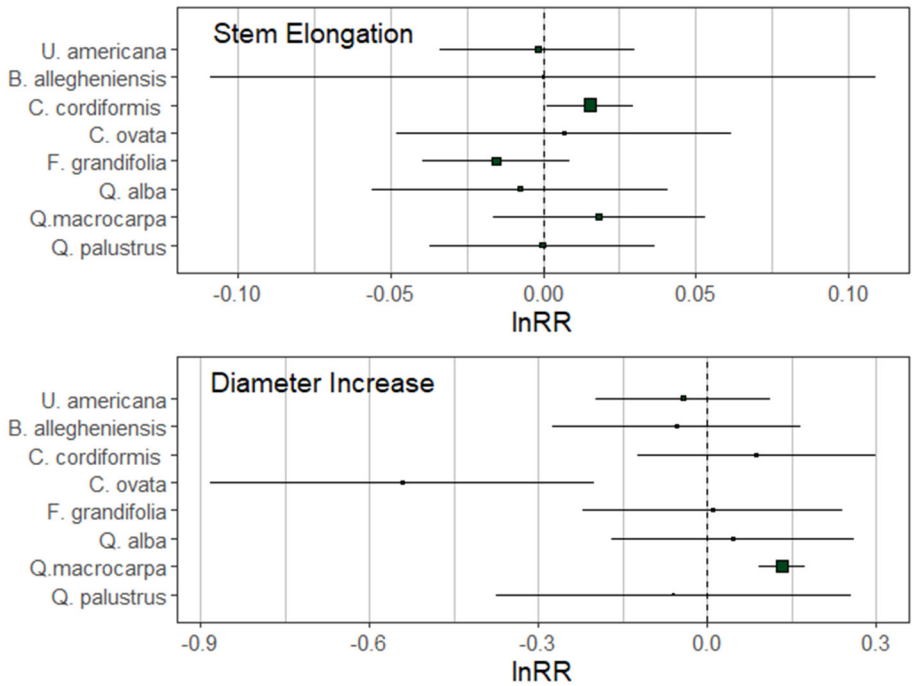

FIGURE 2 | Natural log-response ratios (lnRR) for stem elongation and diameter increase at the conclusion of the second growing season. Mean lnRR and boundaries of the 95% confidence intervals are represented on the figure. Positive plant-soil feedbacks have positive values and negative plant-soil feedbacks have negative values of lnRR.

TABLE 1 | Results of the survival analyses for tree survival across 9 species in a tree restoration experiment with two soil treatments (conspecific soil, heterospecific soil from *Quercus rubra*).

	DF	Residual DF	Deviance	Residual deviance	*P*
Species	9	187	60.27	89.63	0.0001
Treatment	1	186	0.385	89.63	0.54
Replicate	1	185	0.184	89.06	0.67
Row	14	172	16.93	72.32	0.26
Species × Treatment	9	163	4.95	67.36	0.84

TABLE 2 | Test for phylogenetic Janzen-Connell effects on plant-soil feedbacks (lnRR) (**Figure 3**).

	Estimate	SE	z-value	*p*
lnRR diameter ~ Phylogenetic distance	0.06	0.04	−1.60	0.10
lnRR height ~ Phylogenetic distance	0.0014	0.007	0.20	0.84

Phylogenetic distance is the distance in branch lengths between the focal tree and the heterospecific soil source (Quercus rubra). Plant-soil feedbacks were measured as the log-response ratio of relative growth rate in conspecific vs. heterospecific soils. A negative slope indicates that more distant relatives experienced more negative plant-soil feedbacks.

Carya cordiformis survival was significantly influenced by soil treatment ($p = 0.03$), with significantly greater survival following inoculation with heterospecific soil. The remaining species had 100% survival throughout the experiment, with the exception of *Q. rubra*, in which one tree (5%) died. We did not find a treatment × species interaction on tree survival over 16 months in the field (**Table 1**).

Plant-Soil Feedbacks Were Not Consistent With a Phylogenetic Janzen-Connell Effect or Conserved Mutualists

There was not an effect of phylogenetic distance on the strength of plant-soil feedbacks as measured by either growth in tree height or diameter (**Table 2**, **Figure 3**). In general, both diameter and height growth data suggested that the three closest relatives to heterospecific *Quercus rubra* (other *Quercus*) responded similarly to conspecific soil and heterospecific soil, performing weakly better in conspecific soil in most cases (**Figure 3**). Focal species at intermediate phylogenetic distances to *Q. rubra* were highly variable in their plant-soil feedbacks (**Figure 3**). The *Caryas* were especially diverse in their plant-soil feedbacks, with the largest and smallest effect sizes in diameter in our data. The most distant relative to heterospecific *Quercus rubra*, *Ulmus americana*, had a neutral feedback (responded similarly to conspecific and heterospecific soils (**Figures 2**, **3**).

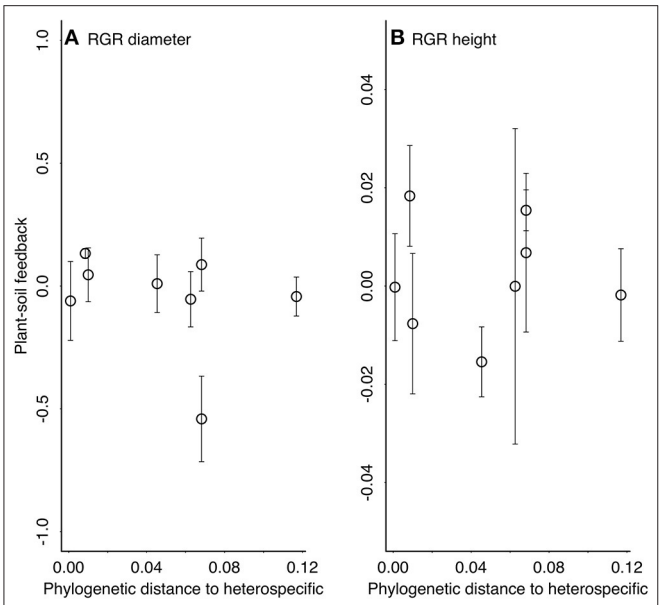

FIGURE 3 | The strength of plant-soil feedbacks [lnRR = ln(RGR conspecific/RGR heterospecific)] across the phylogenetic distance from the focal tree to the heterospecific soil inocula source (*Quercus rubra*) (**Table 2**). Relative growth rates were used to calculate lnRR for **(A)** plant diameter and **(B)** plant height across 16 months in the field. Positive plant-soil feedbacks have positive values and negative plant-soil feedbacks have negative values of lnRR. Means ± 1 SE.

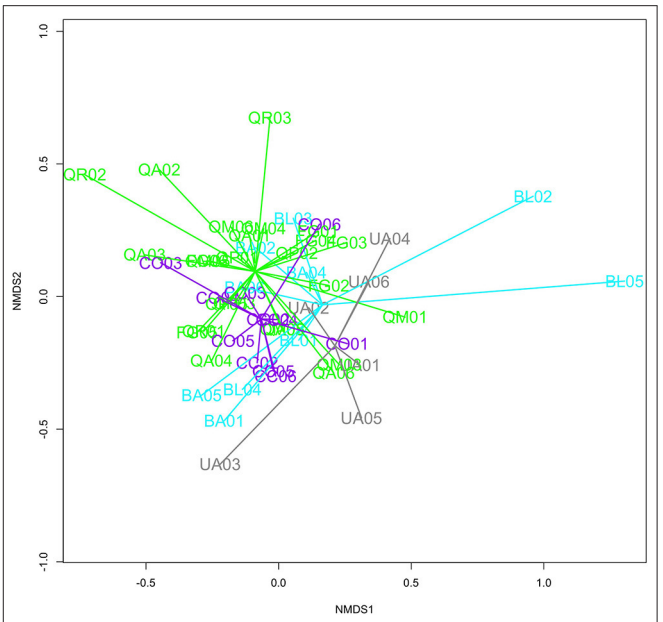

FIGURE 4 | Radar graph for general fungi communities in rhizosphere soils of each of the four experimental plant families at the end of the second growing season. Both conspecific and heterospecific treatments are represented. Families include Fagaceae (Green), Juglandaceae (Purple), Ulmaceae (Gray), and Betulaceae (Teal).

Were Soil Fungal Diversity and Community Composition Influenced by Plant-Soil Feedbacks, Focal Tree Species, and Plant Relatedness After 16 Months in the Field?

Our next generation sequencing methods generated over 14.1 million reads, which were mapped to 3,360 OTUs in the UPARSE pipeline. 780 OTUs (∼23%) could be assigned to either the genus or species level. 833 OTUs (∼25%) could not be assigned to a taxonomic level below "fungi." Richness ranged from 229 OTUs per sample to 1,186 OTUs per sample. A diversity of fungal functional groups was represented in our OTU database. OTUs included saprotrophic fungi (e.g., *Mortierella*, *Coprinellus,* and *Pleurotheciella*), ectomycorrhizal fungi (e.g., *Scleroderma, Tomentella,* and *Tuber*), and arbuscular mycorrhizal fungi including the genera *Glomus* and *Funneliformis*.

Plant-soil feedbacks influenced fungal diversity (**Figure 1**) but did not influence fungal community composition after 16 months in the field (**Figure 4**). We found marginally significantly greater ($p = 0.07$) Shannon's diversity of general fungal communities in heterospecific than conspecific soils (**Figure 1**). Trees receiving the heterospecific inoculant had greater diversity than those receiving the conspecific inoculant in seven of the nine species. Our paired *t*-tests, however, yielded insignificant results, suggesting that Shannon's diversity was not greater in heterospecific soils ($t = -0.64$, $p = 0.53$). Across all species, soil treatment (conspecific and heterospecific) did not have a significant influence on fungal community structure [$F_{(1,50)} = 0.87$, $p = 0.80$]. Our limited number of replicates precluded the use of PERMANOVA tests on single species; however, we implemented NMDS ordination plots to provide a visual representation of fungal communities derived from conspecific and heterospecific inoculants. We noted few visual differences in community structure between communities derived from conspecific and heterospecific inoculants, except in the genus *Ulmus* in which conspecific and heterospecific communities were visually distinct.

Focal tree species had different normalized abundances of ectomycorrhizal fungi and there was a great deal of variation in normalized abundances, with some ectomycorrhizal fungi being much more abundant than others (**Figure 5**). The most abundant ectomycorrhizal fungal genus in our experiment was *Tuber*. The genera *Entoloma* and *Russula* had low abundances in association with all of the tree species. *Tuber* associated strongly with distantly related tree species, suggesting limited host specificity, at least in this fungal genus. Additionally, the genus *Quercus* associated with more ectomycorrhizal fungi than other tree genera (**Figure 5**).

Our soil fungal community analysis indicated significant taxonomic conservatism amongst our experimental tree species. In other words, closely related focal tree species had similar fungal community composition after 16 months in the field. Our PERMANOVA analysis indicated that both tree genera [$F_{(4,46)} = 1.39$, $p = 0.0002$] and family [$F_{(4,47)} = 1.41$, $p = 0.001$] had significant influences on fungal community composition, indicating that closely related trees

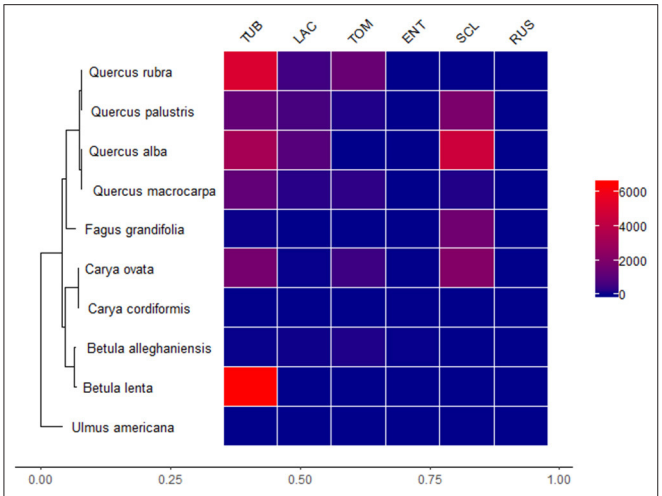

FIGURE 5 | Heatmap representing relationships between ectomycorrhizal fungi genera and the experimental tree species. Heatmap values indicate cumulative edge R normalized scores for each fungal genus in our OTU matrix. Ectomycorrhizal fungi taxa are TUB (*Tuber*), LAC (*Laccaria*), TOM (*Tomentella*), ENT (*Entoloma*), SCL (*Scleroderma*), and RUS (*Russula*). Red values are greater normalized abundances and blue colors are lower normalized abundances.

shared similar soil fungal community structure (**Figure 4**). Row also had a significant effect in our genera model [$F_{(1,47)} = 1.56, p = 0.03$].

DISCUSSION

Our experiment demonstrates that individual plant-soil feedbacks can persist for at least two growing seasons in temperate tree restoration. Tree responses to conspecific and heterospecific inoculation varied by species following two growing seasons. We found negative plant-soil feedbacks in growth for one species and positive for another, in addition to a negative plant-soil feedback in survival for a third species. These effects of soil inocula on tree growth were not consistent with a phylogenetic Janzen-Connell effect or conserved soil mutualists. Though greater fungal community diversity in heterospecific *Quercus rubra* soils persisted over 16 months in the field, our analysis of community composition suggests that trees planted into the restoration experiment might be "conditioning" the soils in their root zones. In other words, we likely observed convergence between soil general fungal communities derived from heterospecific and conspecific soil inoculants over time, though a baseline characterization of the fungal community would have been necessary to confirm this pattern. Further, we found a tree taxonomic influence on the structure of soil general fungal communities, with tree genera and families exhibiting different communities. Thus, plant-soil feedbacks are likely to interact with a species receiving an inoculant in a way that depends upon the plant species identity and evolutionary history.

Plant-Soil Feedbacks Influenced Tree Growth and Survival for Some Ectomycorrhizal Tree Species After 16 Months in the Field

Tree growth responses in our experiment varied by species, supporting the findings of a previous pot experiment, which utilized temperate trees (McCarthy-Neumann and Kobe, 2010). Prior studies have indicated that many ectomycorrhizal trees native to eastern North America facilitate the recruitment of their own seedlings/saplings (Bennett et al., 2017). Field experiments utilizing ectomycorrhizal fungal *Tsuga canadensis* provides support for these findings, as saplings of this species have shown improved performance in the soils of conspecifics compared to heterospecifics (O'Brien et al., 2011, but see Reinhart et al., 2012b). We identified conspecific facilitation in only one of our ten experimental species (*Q. macrocarpa*). The conspecific facilitation we observed in *Q. macrocarpa* is consistent with dominance patterns in natural plant communities, as this species commonly grows in open grassland communities where it alone can dominate the tree community.

Interestingly, *Q. rubra* and *C. ovata* are common associates in natural forests throughout the ecoregion in which our study took place (the glaciated Allegheny plateau). The facilitation of *C. ovata* growth by inoculation with *Q. rubra* conditioned soils may represent a diversity enhancing mechanism in these forests. *C. ovata* may have enhanced growth in tree fall gaps creating by *Q. rubra*, a pattern which would suggest that plant-soil feedbacks can persist for long periods of time and have important implications for structuring tree communities in natural forests (Bennett et al., 2017).

Plant-Soil Feedbacks Were Not Consistent With a Phylogenetic Janzen-Connell Effect or Conserved Mutualists

Our study differs from some previous work, which found evidence consistent with a phylogenetic Janzen-Connell effect (Liu et al., 2012; Sweet and Burns, 2017; Crawford et al., 2019). Some of these studies had comparable sample sizes to ours, including Liu et al. (2012) with 8 species of tree and Sweet and Burns (2017) with 7 species of herbaceous plants. There could be several, non-mutually exclusive, reasons for this apparent discrepancy. First, we have only a single species, *Ulmus americana*, that is highly phylogenetically distant from heterospecific *Quercus rubra,* and more distant relatives, might be needed to detect larger trends. Second, these trees have only grown for 16 months in the field, and increases in growth, especially height, were relatively modest. Longer time periods may be needed to detect significant effects of soil inocula on the growth of these tree species. Third, variance among species in tree sources could add variance to our data, potentially making detecting phylogenetic patterns more difficult. Fourth, meta-analyses using an alternative measure of plant-soil feedbacks, reciprocal pairwise plant-soil feedbacks (Bever et al., 1997), find for plants that share a mycorrhizal guild, more distantly related species have more negative plant-soil feedbacks (Crawford et al., 2019). Alternatively, we used the individual plant-soil feedback

metric, perhaps hinting that the type of metric used to measure plant-soil feedbacks influences the results (i.e., plants might perform relatively, but not absolutely, better in the soils of distant relatives, compared with close relatives). Finally, these analyses do not consider predictions about the variance in plant responses over evolutionary time, again because of the single sampled species at greater phylogenetic distances. However, Brownian motion evolution suggests that the variance in plant responses should be greater in the soils of more distant relatives (Cadotte et al., 2017). Thus we need more experimental tests with replication both across and, critically, within, phylogenetic distances (Burns et al., 2019). The greater variance among the *Betula* and *Carya* species than among *Quercus* (**Figure 3**) hints that this prediction might hold true with increased sampling at greater phylogenetic distances.

Root and Rhizosphere Fungal Diversity or Community Composition Were Influenced by Plant-Soil Feedbacks, Focal Tree Species, and Plant Relatedness After 16 Months in the Field

Our next generation sequencing methods returned an incredible diversity of fungi in our early successional restoration site. Comparable hyperdiversity has previously been reported in general fungi communities of boreal forest communities (Taylor et al., 2014); however, we know of no similar estimations of fungal diversity from temperate forested systems or temperate restoration sites. While experimental treatments may have driven the diversity observed in rhizosphere soils, other sources of fungi such as nursery soils or the addition of wood chips could also have contributed to overall fungal diversity. The maintenance of such diversity through the establishment of a diverse array of tree species may result in increased fine-scale niche partitioning (Taylor et al., 2014) and increased microbial function (Carnovale et al., 2019). The marginally greater Shannon's diversity observed in trees receiving the heterospecific *Quercus rubra* inoculant suggests that heterospecific inoculation may introduce fungal taxa not typically associated with certain tree genera, and that these fungal taxa can persist for more than two seasons in field conditions.

Prior work in agricultural areas has also shown tree genus to be an important determinant of soil microbial community structure (Carnovale et al., 2019), although the mechanisms driving this pattern were unidentified in the cited study. Most studies connecting tree species or genera with soil microbial community structure investigate relationships between leaf litter traits and microbial community composition (e.g., Bardgett and Shine, 1999; Thoms and Gleixner, 2013). Our finding, however, could not be explained by interspecies variability in litter traits, as our soil samples were taken prior to leaf fall and excluded any leaf material which may have been present. Therefore, our finding is more likely the result of species having specific differences in belowground traits such as root carbon exudation (Broeckling et al., 2008). Above ground traits, however, can also influence belowground mechanisms. Growth rate, a trait in which our experimental species differed, has been shown to interact with certain microbial functional groups (Pei et al., 2016). The complexity of individual species responses to inoculation and the influence of tree relatedness on subsequent fungal community formation makes the development of broadly applicable methods for inoculation challenging.

We observed similar fungal community composition in the soils under congeneric and confamilial trees, consistent with some other studies (e.g., Burns et al., 2015). Such phylogenetic effects on soil fungal communities has the potential to lead to "phylogenetic" Janzen-Connell effects (Liu et al., 2012), where plants perform better in soils influenced by distant relatives and less well in soils from close relatives (see also Sweet and Burns, 2017). Escape from pathogens in the soil could potentially help explain such patterns. However, in this tree restoration experiment, tree growth over the first 16 months in the field did not suggest such phylogenetic Janzen-Connell effects. Rather, plant-soil feedback effects were generally species specific (McCarthy-Neumann and Kobe, 2010; St-Denis et al., 2017). We also noted that inoculation with heterospecific soils had the greatest influence on fungal community assembly within *Ulmus*, the genus most distantly related to *Quercus*. This suggests that increased phylogenetic distance between the soil conditioning species and the species receiving the soil transfer can result in more profound changes to fungal community structure than soil transfers between close relatives, though greater sampling of distant relatives would be needed to confirm this hypothesis.

In conclusion, we found plant-soil feedbacks after 16 months in the field for three tree species, with 2 out of 8 species showing effects on growth and 1 out of 9 for survival. Thus our results reinforce the hypothesis that the influence of plant-soil feedbacks may be exaggerated in glasshouse studies when compared to studies under natural conditions (Schittko et al., 2016; Heinze and Joshi, 2018). Our next generation sequencing approach found an influence of inocula treatment, tree species, and tree relatedness on rhizosphere fungal diversity or community structure. Therefore, planting a phylogenetically diverse tree restoration site could result in a more diverse fungal community. Future studies should measure ecosystem function (Lance et al., 2019), especially across a phylogenetic diversity gradient in tree restoration.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the https://osf.io/cv9kf/.

AUTHOR CONTRIBUTIONS

AL and JB designed the study. AL, SC-K, and DB conducted the lab work and analyzed the fungal sequencing data. JB conducted the phylogenetic analyses. AL and JB conducted the statistical analyses and wrote the first version of the manuscript. SC-K and DB contributed to manuscript revisions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00500/full#supplementary-material

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Abiotic and Biotic Soil Legacy Effects of Plant Diversity on Plant Performance

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Soil legacies are typically examined for individual plant species, and we poorly understand how soil legacy effects created by entire plant communities influence plant growth. We used soils collected from a biodiversity field experiment to examine how the soil legacy effects of plant diversity influence the growth of a focal plant species. In the field, we experimentally assembled and maintained grassland communities (0, 1, 2, 4, or 9 species) for two years. We collected soil from all plots and examined the growth of *Jacobaea vulgaris* in these soils under controlled conditions, and compared this to the performance of individuals that were planted directly in the plots. *J. vulgaris* was not part of the species pool used in the biodiversity experiment, but commonly occurs in the area. To disentangle different components of the legacy effects (soil nutrients vs. soil biota), in the pot experiment we tested the effects of plant growth in pure field soil and in sterilized background soil inoculated with live or with sterilized field soil. We found a weak positive legacy effect of plant diversity on *J. vulgaris* root biomass, but only in pure field soil and not in the inoculated treatments. Interestingly, for individuals planted in the field plots, plant biomass was negatively related to the diversity of the surrounding plant community but this was mainly due to high biomass in bare plots. In the pot experiment, plant biomass also varied among soils collected from different monocultures. Soil fungal community composition was not affected by the diversity of the plant community, but the biomass of the plants grown in pots with pure field soil correlated with fungal composition. The biomass of plants grown in pure field soil was also positively correlated with nitrogen availability in the soil, and negatively with the cover of three plants species in the communities. In conclusion, our study does not provide strong evidence for an important role of plant diversity on soil legacy effects on *J. vulgaris*, and shows that for this plant species, performance is related to both the biotic and abiotic characteristics of the soil in which it grows.

Keywords: biodiversity, *Jacobaea vulgaris*, plant-soil feedback, soil biota, plant species richness, soil fungi, T-RFLP

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INTRODUCTION

Plants can alter the biotic and abiotic conditions in the soil, and this can affect other plants that grow later in this soil. Most studies that examine these plant-soil interactions have examined the impacts of an individual species, via its effect on the soil, on the performance of another plant in pot experiments (Kulmatiski et al., 2008). In natural communities such as grasslands, plants do not

grow in isolation but interact with other plants. An important caveat in our understanding of the role of plant-soil interactions in natural plant communities is that we poorly understand how entire plant communities influence the soil, and, in turn, how these soil legacies influence plants that grow later in this soil (Bever, 2003; Petermann et al., 2008; Bever et al., 2010; van der Putten et al., 2013; Bennett et al., 2019).

Plant species vary greatly in how they influence the soil. In mixed plant communities the impact of a plant species on the soil will depend on how strongly this species influences the soil, but also on the abundance of this plant species in the community, on the identity of the other species that also influence the soil, as well as on the characteristics of the soil. Hence, it is very difficult to predict how soil legacy effects created by different plant communities will influence the growth of other plants in the soil. It is well known though, that different plant communities harbor unique soil communities (e.g., Bezemer et al., 2010; Heinen et al., 2018), and that these soil communities can differentially affect plants that grow later in the soil (e.g., Kardol et al., 2006). Whether this also depends on the number of species that make up the plant community is less well-understood. Most studies that examine the relationship between plant diversity and soil, so far, focus on the role of soil biota in driving diversity-ecosystem function patterns (e.g., Kulmatiski et al., 2012; but see Wurst et al., 2015). Various plant biodiversity experiments have shown that the number of species in a plant community is related to the diversity, composition and functioning of organisms in the soil (e.g., Wardle et al., 2003; Eisenhauer et al., 2010; Lange et al., 2015). Several studies have also shown that soil pathogens build up in soil of monospecific plant communities resulting in conspecific legacy effects (e.g., Maron et al., 2011; Schnitzer et al., 2011). Likewise, accumulation of pathogens that are less species-specific could give rise to heterospecific soil legacy effects. These changes in soil biota can result in positive relationships between the richness of the plant community and the performance of a plant when it grows in the soil of that community (Wurst et al., 2015; Luo et al., 2016). The opposite may be true for beneficial soil organisms such as mycorrhizal fungi.

While soil biota are a common mechanism of soil mediated effects between plants (e.g., Wardle et al., 2004; van der Putten et al., 2013), changes in nutrient availabilities, allelochemicals or other soil abiotic properties can also be a mechanism (Reynolds et al., 2003; Ehrenfeld et al., 2005; Perkins and Nowak, 2013; Bennett and Klironomos, 2018). Such negative effects could be strongest in monocultures and be diluted in mixed plant communities and hence also changes in abiotic soil conditions could result in a positive relationship between the plant diversity of a plot and the performance of plants that grow in the soil collected from these plant diversity plots.

Jacobaea vulgaris is a native plant species in the Asteracaea family. It occurs in varying densities in natural grasslands in Western Europe and is an important nectar source for numerous insects (Harper and Wood, 1957). For plants grown in pots we have previously shown that this species is highly sensitive to soil legacies created by other plant species (van de Voorde et al., 2011). Typically growth of *J. vulgaris* is reduced in soils conditioned by other species, compared to growth in sterile soil (Jing et al., 2015). Interestingly, these negative species-specific legacy effects are reduced when the soil is mixed with soil from other plant species, probably due to diluting the effects of e.g., soil pathogens, or allelopathy caused by specific plant species (van de Voorde et al., 2011). Hence, we may expect a positive effect of plant diversity on the overall negative soil legacy effects that influence this species (sensu Luo et al., 2016).

In this study we examine legacy effects of soils collected from grassland plots in which the diversity of the plant community was manipulated and maintained at levels of 0, 1, 2, 4, or 9 species per plot. The legacy effects were assessed using the phytometer plant *J. vulgaris*. This species was not present in any of the plots in which we maintained plant diversity and hence we tested how soil legacy effects of plant diversity influence the performance of a new species that colonizes the community. To evaluate to what extent the potential legacy effects were caused by soil biota we examined *J. vulgaris* performance in a pot experiment with pots filled with soil collected from the field plots and in pots filled with a standard sterilized background soil and inoculated with 20% live or sterilized soil from the field plots. After establishment of the plant communities in the field, in each plot we also planted *J. vulgaris* seedlings, and measured their performance in the field. This enabled us to compare the soil-mediated diversity effect on *J. vulgaris* observed in pots and the performance of this species in the field inside the diversity plots. Finally, to gain further insight on soil biota in comparison with soil abiotic properties as drivers of legacy effects, we measured the fungal community and we determined abiotic characteristics such as nutrient availability, organic matter and pH in the soil from each plot.

Specifically we examine:

(i) the soil legacy effect of plant diversity on the growth of *J. vulgaris*
(ii) the relationship between the abundance of plant species in the community and the soil legacy effect on *J. vulgaris*
(iii) whether the diversity-soil legacy relationship depends on the sterilization or inoculation treatments and how this is related to soil biotic and abiotic characteristics
(iv) how plant growth of *J. vulgaris* in pots filled with field soil or in pots inoculated with field soil is related to plant growth in the soils in the field.

MATERIALS AND METHODS

Plant Species

Jacobaea vulgaris Gaertn. is a biannual or short-lived perennial plant that commonly occurs in natural and semi-natural areas throughout Europe and Asia. As is characteristic of *Jacobaea* (*Senecio*) species *J. vulgaris* contains pyrrolizidine alkaloids known to deter generalist insect herbivores (Cheng et al., 2011). Pot experiments carried out in our lab have shown that *J. vulgaris* exhibits a strong negative conspecific plant–soil feedback but that plant growth also responds to soil legacy effects created by other plant species and that these effects range from positive to strongly negative (van de Voorde et al., 2011). Experimental *J. vulgaris* plants for this study were grown from seeds collected

from a single natural plant population from the area surrounding the grassland site in which the biodiversity experiment was carried out (Mossel, Ede, The Netherlands). *J. vulgaris* naturally occurs in this area.

Biodiversity Experiment

To study how the diversity of the plant community influences soil legacy effects we used an established biodiversity field experiment. For a detailed description of the experiment see Kostenko et al. (2012). In brief, in the summer 2008, seventy plots (3 × 3 m) separated by 1-m-wide paths were established in a fenced area within a former arable field that was restored to natural grassland (Mossel, Ede, The Netherlands). In September 2008, the plots were sown with a single plant species (monocultures) or with mixtures of 2, 4, or 9 species randomly chosen from a pool of 12 local grassland species that naturally co-occur in the studied area (**Supplementary Table S1**). *Jacobaea vulgaris* was not sown. Plots with the same species composition were replicated twice using a complete randomized design. There were 12 monocultures (one for each plant species), nine combinations of two species, 11 combinations of four species, and three combinations of nine species. One legume (*Trifolium arvense*), one forb (*Tripleurospermum maritimum*), and two grass species (*Agrostis capillaris* and *Anthoxanthum odoratum*) established poorly in monocultures, although these species were present in the mixed communities. The monocultures of these four species were therefore excluded from the analyses. Four plots were kept free of all plants. In the sown plots, the sowing density was 4,000 seeds per m^2. The sown species composition was maintained by hand weeding from the beginning of the growing season (late April) until the end of the growing season (late August) and paths between plots were regularly mown during each growing season. The experimental site was fenced to exclude large vertebrate herbivores.

At the end of August 2009, one year after sowing and after the different plant diversity treatments had established for one entire season, 25, 8-week-old *J. vulgaris* rosettes (5 cm diameter) were planted in a regular grid of 0.3 × 0.3 m in the central 1.2 × 1.2 m square of each plot. The rosettes were grown from surface-sterilized seeds (1 min in 2% sodium hypochlorite solution and rinsed with water) germinated on glass beads and transplanted into seedling trays filled with sterilized potting compost in a greenhouse (21/16°C day/night, 16 h photoperiod). The resident plant community around the rosettes of the *J. vulgaris* plants was not removed in order to test the effects of the surrounding community on the establishment of *J. vulgaris* seedlings. In bare plots, no other plants than the 25 *J. vulgaris* plants were present.

Soil Collection

In each plot, 25 soil cores (15 cm deep, 5 cm diameter) were collected in a regular rectangular grid 2 years after the establishment of the experiment. The soil cores were not taken

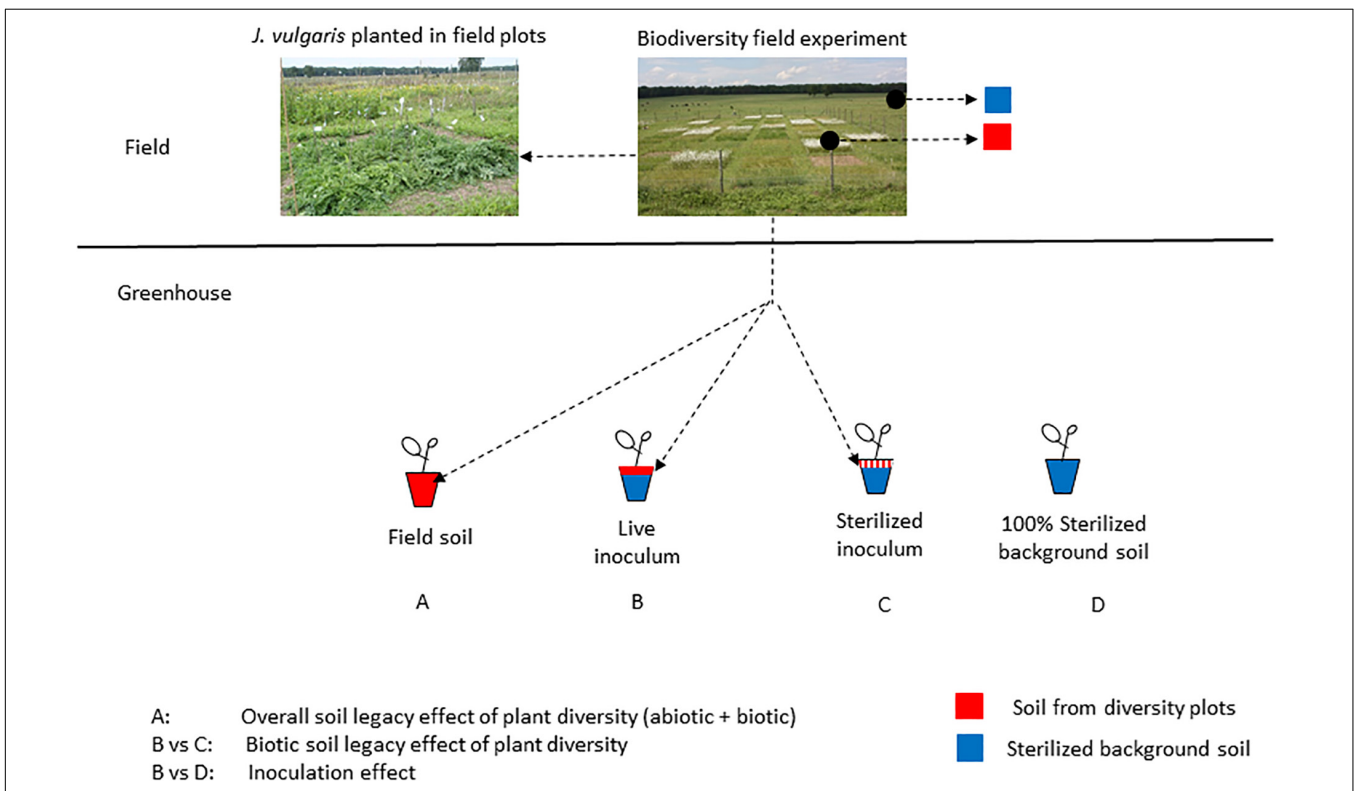

FIGURE 1 | Schematic overview of the experimental setup. Soil was collected from field plots that differed in plant diversity (0, 1, 2, 4, 9 species) and the legacy effect of these soils on *J. vulgaris* was determined in a pot experiment (pure field-conditioned soil; sterilized background soil mixed with live inoculum; sterilized background soil mixed with sterilized inoculum). The effects were compared to the performance of *J. vulgaris* planted in the plots (left photo shows *J. vulgaris* in a bare plot).

underneath or in direct vicinity of the *J. vulgaris* plants. The soil collected from each plot was stored individually in a plastic bag and transported to the laboratory 1 h after collection. In the laboratory, the soil samples were pooled per plot and sieved through a 0.5 cm mesh. Then, each soil sample was split in three subsamples: (1) A subsample of 1.0 g homogenized soil. This subsample was stored at −20°C for molecular analysis. (2) A subsample of 100 g soil. This subsample was oven-dried at 40°C and sieved (4 mm mesh size) to be used for chemical analysis. (3) The remaining soil was kept in a 4°C climate chamber for several days before using it for the soil legacy bioassay.

Soil Legacy Bioassay

Seeds of *J. vulgaris* plants were surface sterilized (1 min in 2% sodium hypochlorite solution and rinsed with water) and germinated on glass beads. Pots (800 ml) were filled with: (1) 800 mg of field-conditioned soil ("Field soil" treatment); (2) 160 g of field-conditioned soil mixed with 640 g (1:4 inoculation ratio) sterilized background field soil ("Live inoculum" treatment); (3) 160 g of sterilized field-conditioned soil mixed with 640 g sterilized background field soil ("Sterilized inoculum" treatment). Additionally, 10 pots were filled with 800 mg of sterilized background field soil ("Sterilized background soil" treatment; **Figure 1**). Background soil was collected from the grassland area surrounding the experiment (Mossel, Ede, The Netherlands). Sterilization of soils was achieved using gamma irradiation (>25 KGray, Isotron, Ede, The Netherlands). The difference between the live inoculum treatment and the sterilized inoculum treatment is an indication for the biotic soil legacy effect. The field soil treatment shows the overall legacy effects of plant diversity, while the comparison of the live and sterilized inoculum treatment with the 100% background soil treatment shows the effects of inoculation (**Figure 1**). The soil collected from each plot was kept separate and was used to fill two pots for each of the three treatments, resulting in 420 pots (70 plots × 3 treatments × 2 replicates per plot). One *J. vulgaris* seedling was transplanted into each pot. Seedlings that died during the first week of the experiment were replaced. Pots were randomly located within a greenhouse (21/16°C day/night, relative humidity 50–60%, 16 h photoperiod). Natural daylight was supplemented by 400 W metal halide lamps (225 μmol m^{-2} s^{-1} PAR). Plants were watered three times per week and randomly rearranged within the greenhouse once a week. After 8 weeks of growth, shoots were clipped; roots were carefully removed from the soil and rinsed. Shoot and root biomass of each pot was then oven-dried (70°C for 3 days) and weighed. Two root samples from the "live inoculum" treatment were lost during this process. These samples were not included in the statistical analyses.

Plant Measurements in the Field

In August 2010, all *J. vulgaris* plants were at the rosette stage. To estimate the size of the *J. vulgaris* plants in the field, we collected two random plants per plot and measured their shoot and root biomass and the number of rosette leaves and the length of the longest leaf. For all remaining *J. vulgaris* plants we recorded the number of rosette leaves and the length of the longest leaf. Then, based on the number of rosette leaves, the length of the longest leaf and the biomass of the collected plants, we constructed a model to predict the aboveground plant biomass (g) of all field-grown plants (for details see Kostenko et al., 2012). Furthermore, in August 2010 we determined the cover of all sown species in three squares (1 × 1 m) along a diagonal transect in each experimental plot.

Molecular Analysis of the Soil Fungal Community

The composition of the fungal community in the soil collected from each experimental plot was determined by T-RFLP (Terminal restriction fragment length polymorphism) analysis. Total DNA was extracted from 0.5 g frozen soil (−20°C) with a Power Soil DNA isolation kit (MOBIO laboratories, Inc.) using a bead beating system. DNA quantity was checked using 1.5% agarose gel electrophoresis. The ITS region of the fungal rDNA was amplified by PCR using the primers ITS1F (White et al., 1990) and ITS4 (Gardes and Bruns, 1993), which were labeled with FAM and NED respectively. The PCR reaction contained 13.8 μl Milli-Q, 2.5 μl 10× Fast Start High Fidelity Reaction Buffer (Roche Diagnostics), 2.5 μl DNTP Mix (2mM each), 2.5 μl ITS1F-6FAM primer (10 μM), 2.5 μl ITS4-NED primer (0.2 μM), 0.2 μl Fast Start High Fidelity Enzym Blend (5 U/μl) (Roche Diagnostics) and 1 μl template DNA. PCR program conditions were 5 min at 95°C, 34 cycles of 30 s at 95°C, 40 s at 55°C and 1 min at 72°C, followed by 10 min at 72°C before cooling. PCR product presence and quality were verified on 1.5% agarose gels prior to restriction digestion. Two restriction enzymes, *Hha*I and TaqαI (New England Biolabs, Ipswich, MA, United States), were used to digest dual end-labeled DNA amplicons. A mixture

TABLE 1 | Effects of plant species richness, soil conditioning treatment, and their interaction on root and shoot biomass of *J. vulgaris* plants in the pot experiment.

	DF	*F*	*P*
Bare plots included (0–9 species)			
Root biomass			
Plant species richness	1, 64	2.34	0.13
Soil treatment	2, 128	73.10	<0.0001
Interaction	2, 128	2.67	0.073
Shoot biomass			
Plant species richness	1, 64	0.53	0.47
Soil treatment	2, 128	148.08	<0.0001
Interaction	2, 128	0.84	0.44
Bare plots excluded (1–9 species)			
Root biomass			
Plant species richness	1, 60	0.53	0.47
Soil treatment	2, 120	67.83	<0.0001
Interaction	2, 120	3.06	0.050
Shoot biomass			
Plant species richness	1, 60	3.23	0.077
Soil treatment	2, 120	154.74	<0.0001
Interaction	2, 120	0.35	0.70

Analyses were carried out with and without bare plots.

containing 3.5 µl ddH_2O, 1 µl buffer, 0.1 µl Bovine Serume Albumin, 5 µl PCR product and 0.4 µl restriction enzyme was incubated at 37°C (*Hha*I) or at 65°C (TaqαI) for 3 h, and inactivated at 80°C for 20 min. Restriction products were purified using ethanol precipitation. Fragment length polymorphism analysis was performed on an automated 3130 Genetic Analyzer sequencer (Applied Biosystems) with GeneScan-500 LIZ, Applied Biosystems as a size standard. Samples which were over- (highest peak > 80,000 rfu) or under-loaded (highest peak < 1,000 rfu) were re-run with an adjusted concentration. Peaks were aligned to TRFs among the samples by applying a clustering threshold of 0.5 bp. Only peaks higher than 0.2% of the sum of all peaks in a sample were included.

Abiotic Soil Characteristics

Concentrations of plant available mineral nitrogen (NH_4^+ and NO_3^-), potassium (K) and magnesium (Mg) in the soil samples were determined colorimetrically in 1:10 (w/v) 0.01 M $CaCl_2$ using a Traacs 800 autoanalyser (TechniCon Systems Inc., Oakland, CA, United States). The C:N ratio in soil samples was measured on a FlashEA 1112 Series NC soil analyzer (Thermo Fisher Scientific, Waltham, MA, United States). pH was measured in 2:5 dry soil:water suspensions. The percentage organic carbon (C) was determined according to Nelson and Sommers (1982). Soil organic matter was determined by the loss-on-ignition method. Approximately 5 g of soil was oven dried at 105°C for 16 h and weighed. The sample was then burned at 550°C for 5 h and weighed again. Soil organic matter was calculated as the percentage weight loss between the oven-dried and burned samples. Total plant available phosphorous (P) was determined according to Olsen et al. (1954) and absorbance was measured at 720 nm (**Supplementary Table S2**).

Data Analyses

To examine the soil legacy effects of plant diversity on the growth of *J. vulgaris* plants we used mixed-effects models with plant community diversity (0–9 species) and soil treatment (field soil, live inoculum, sterilized inoculum) as fixed effects. Plant diversity

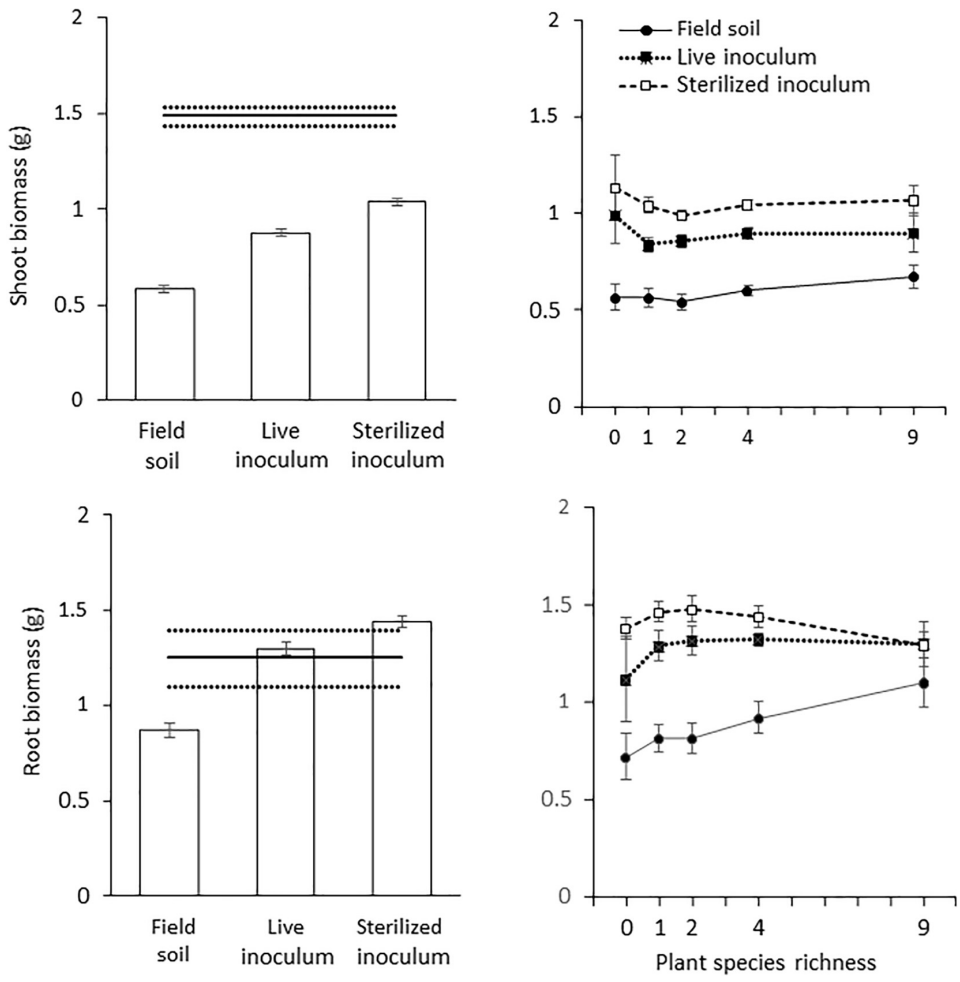

FIGURE 2 | Response of *J. vulgaris* to plant diversity. Shown are means (± SE) of all diversity levels (left panels) of shoot biomass (top panels) and root biomass (bottom panels) of *J. vulgaris* plants growing in pure field soil, in sterilized background soil mixed with live or sterilized inoculum; and for each diversity level (right panels). The solid black line with dashed lines represents the biomass (mean ± SE) of plants grown in sterilized background soil.

was transformed as log2(species richness + 1). We included plot identity as a random effect to incorporate that multiple pots were filled with soil collected from the same plot. Individual comparisons within each soil treatment were based on a Tukey HSD test. We repeated this analysis by excluding the bare plots. The effect of plant diversity on soil nutrients was analyzed using a general linear model with plant diversity as a log-linear factor [log2(species richness + 1)] and bare plots included or excluded from the model for all plots. Monoculture plots were compared using a general linear model with plant identity as a fixed variable. Univariate analyses were performed in R statistical language, ver. 3.4.3 (R Core Team, 2017).

The presence/absence matrix of T-RFLP fingerprints was analyzed using correspondence analysis and canonical correspondence analysis (CA and CCA) and the combined abiotic soil characteristics with principle component and redundancy analysis (PCA and RDA, centered and standardized data) in CANOCO version 5.03 (Šmilauer and Lepš, 2014). Significances in multivariate analyses were inferred by Monte Carlo permutation tests (999 permutations).

The association between the performance of the plants grown in the field-conditioned soil and plants grown in the field plots, as well as the relationship between plant growth and the cover of different species in the plant communities was analyzed using a Spearman rank-based correlation test. The association between the performance of the plants grown in the field-conditioned soil and fungal community composition of the soil was analyzed using CCA with forward model selection procedure. Finally, the association between the performance of the plants grown in the field-conditioned soil and soil nutrient composition was analyzed using multiple linear regression analysis with a stepwise model selection algorithm by AIC. In the latter two tests plant species richness was included as continuous covariate.

Structural Equation Modeling

Structural equation modeling (SEM, Shipley, 2002) was used to examine the strength of pathways linking soil legacies of plant diversity with the performance of *J. vulgaris* plants in the greenhouse bioassay. The conceptual model, presented in **Supplementary Figure S1**, considered direct effects of plant species richness on the performance of *J. vulgaris* and indirect effects via changes in soil nutrients or changes in fungal community composition. Plant species richness was included as a fixed continuous factor to incorporate the continuity of plant species richness in the analysis. We examined in separate models the "field soil" treatment, the "live inoculums" treatment, and the "sterilized inoculums" treatment and did this separately for root and shoot biomass. For multivariate variables (soil nutrient composition, soil fungal TRF composition) the first axis of a Principle Component Analysis (PCA) or Correspondence Analysis (CA) respectively was used in the SEM analysis. Plant biomass was log transformed prior to the SEM analysis. All variables used in the SEM were observed variables. Structural equation modeling was carried out using the lavaan package in R. All final models provided good fit to the data (**Supplementary Table S4**). Additional information about the SEM procedure is presented in the Supporting information (**Supplementary Table S4**).

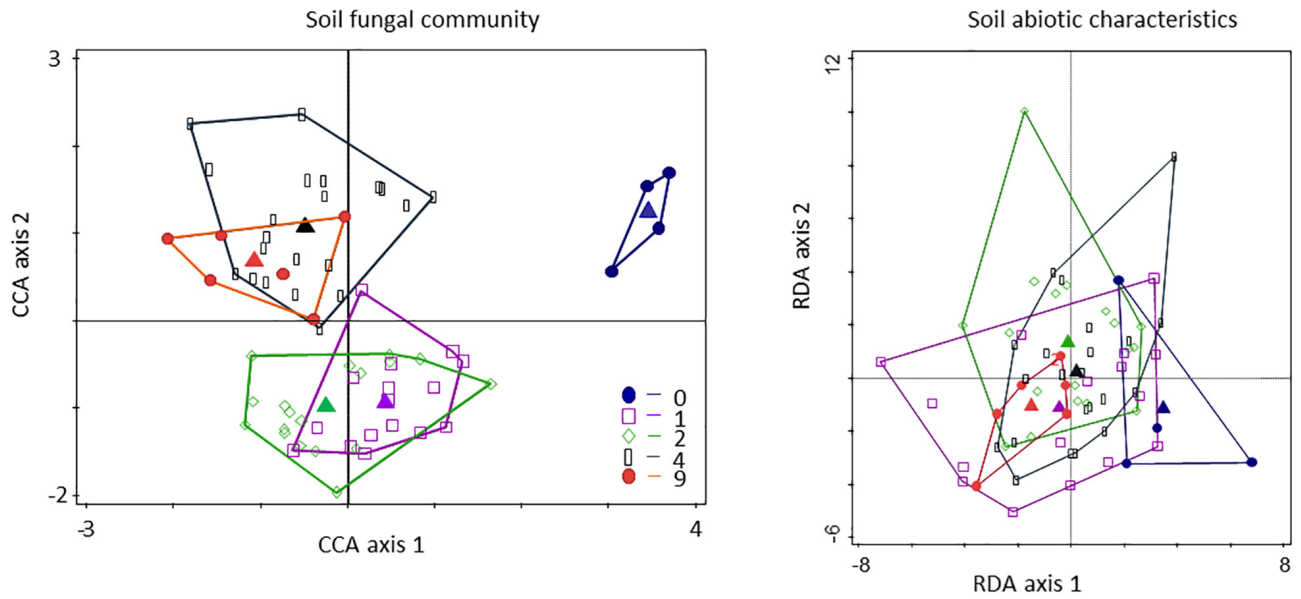

FIGURE 3 | Classified plot diagram (CCA) of soil fungi and soil abiotic characteristics (RDA) depicturing the separation of plots defined by the plant diversity treatment. For fungi score scaling is focused on T-RFLP scores. Symbols represent the individual plots (blue circles – plots without sown vegetation; purple squares – one species plots; green diamonds – two species plots; black rectangles – four species plots; red circle – nine species plots). Triangles represent the average relative composition of plots belonging to individual levels of a plant diversity (0, 1, 2, 4, 9 plant species). The distance between the triangles approximates the average dissimilarity of the plots with a certain diversity level as measured by their chi-square distance.

RESULTS

Soil Legacy Bioassay

There was a significant effect of the three soil treatments on shoot (**Table 1** and **Figure 2**) and root biomass of *J. vulgaris* (**Table 1** and **Figure 2**). Plants produced least shoot biomass when grown in field soil and the highest shoot biomass in sterilized background soil inoculated with sterilized inoculum independent of the plant diversity treatment (**Table 1** and **Figure 2**). Root biomass was also lowest in pure field soil (**Table 1** and **Figure 2**). When bare plots were excluded from the analyses, root biomass was higher in the soil of high diverse plant communities but this was only true for the pure field soil treatment (significant interaction; **Table 1** and **Figure 2**). Shoot biomass also tended to increase in soils originating from plots with higher plant diversity but this effect was not significant (p = 0.077; **Table 1** and **Figure 2**). Biomass of *J. vulgaris* did not differ significantly between soils from different monocultures (**Supplementary Figure S2** and **Supplementary Table S2**). Root and shoot biomass of the potted plants was larger in legume soils than in grass or forb soils but only in pots with pure field soil (**Supplementary Figure S3**).

Soil Characteristics

The composition of the fungal community was not affected by the diversity of the plant community growing in the soil (pseudo-F = 1.1, P = 0.141; **Figure 3**). However, there was a clear discrimination of the fungal community composition of the bare plots, the fungal community composition of the low diversity plots (with one and two plant species), and the fungal communities of the highest diversity plots (with four and nine plant species; **Figure 3**). The fungal composition varied between monocultures (pseudo-F = 1.1; P = 0.004; **Supplementary Figure S4**). Individual soil abiotic characteristics did not vary significantly between the diversity treatments or among monocultures (**Figure 3** and **Supplementary Table S3**). Multivariate analyses (RDA), however, showed that the composition of the soil abiotic characteristics varied significantly

TABLE 2 | Relationship between the performance of plants (root and shoot biomass) from the three soil treatments (field soil; live inoculum; sterilized inoculum), and performance of plants grown in the field experiment, fungal community composition, and soil nutrient composition.

	Field soil		Live inoculum		Sterilized inoculum	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
Bare plots included						
Biotic variables						
Field-grown plants	0.45***	–	0.22	–	0.12	–
Fungal community	2.6***	1.9	–	–	–	–
Abiotic variables						
pH	–	–	↑5.13*	–	–	–
P (mg·kg^{-1})	3.17	2.68	↑3.23	↓4.73*	2.45	–
K (mg·kg^{-1})	–	–	–	–	–	–
Mg (mg·kg^{-1})	–	–	2.26	–	2.73	–
Mineral N (mg·kg^{-1})	↑15.94***	↑46.85***	–	–	–	–
Nitrogen (%)	–	–	2.56	–	–	–
Carbon (%)	–	2.70	–	–	–	–
Organic matter (%)	–	3.19	–	2.10	–	–
Bare plots excluded						
Biotic variables						
Field–grown plants	0.50***	–	0.22	–	0.13	–
Fungal community	2.8***	2.1	–	–	–	–
Abiotic variables						
pH	–	–	↑5.93*	–	–	–
P (mg·kg^{-1})	3.21	2.42	–	↓4.40*	–	–
K (mg·kg^{-1})	–	–	–	–	2.05	–
Mg (mg·kg^{-1})	–	–	2.46	–	↓6.07*	–
Mineral N (mg·kg^{-1})	↑17.64***	↑51.32***	–	–	–	–
Nitrogen (%)	–	–	–	–	–	–
Carbon (%)	–	3.53	–	2.00	–	–
Organic matter (%)	–	↑4.07*	–	–	–	–

*F-values are shown of the final models based on CCA with the forward selection procedure for fungal community and multiple linear regression with a stepwise model selection algorithm by AIC for soil characteristics; and Spearman's rank correlation rho's for shoot biomass of the field grown J. vulgaris plants. Analyses are carried out with and without bare plots. Asterisks indicate significant relationship at ***P < 0.001; *P < 0.05; the absence of asterisks indicates no significant relationship; – indicates that the variable was not included in the final model. ↑ indicates positive relationship and ↓ negative relationship.*

between monocultures (F = 1.80; P = 0.019), but not between diversity levels (**Supplementary Figures S5**, **S6**).

Relationship Between *J. vulgaris* Performance in Field Plots and in Pots

Shoot and root biomass of field-grown plants generally declined with increasing plant diversity but this was mainly due to the large size of plants in the bare plots where there was no competition with other plants (**Supplementary Figure S7**). The shoot biomass of *J. vulgaris* plants grown in field soil in the greenhouse positively correlated with the shoot biomass of the plants grown in the field (**Table 2** and **Figure 4**), but this correlation was not significant for plots with the highest level of diversity (**Figure 4**). There was no relationship between the biomass of field-grown plants and plant growth in pots with inoculated field soil (**Table 2**).

Direct and Indirect Effects of Biotic and Abiotic Soil Characteristics on Plant Growth

The shoot biomass of potted plants grown in pure field-conditioned soils significantly correlated with the fungal community composition and with soil nutrients (**Table 2**). The SEM analysis revealed that soil abiotic and biotic characteristics were related. In the field soil treatment, shoot biomass of potted plants was related to plant diversity and fungal community composition (**Figure 5**). Root biomass of potted plants grown in field soil, instead, was only influenced by soil abiotic characteristics (**Figure 5**). Plant biomass in the inoculated soils was not influenced by abiotic or biotic soil characteristics nor by plant diversity, except for an unexpected relationship between soil fungal composition and shoot biomass in the sterilized inoculum treatment (**Supplementary Figure S8**).

Relationship Between *J. vulgaris* Performance in Pots and Cover of Sown Species in the Field

The biomass of potted *J. vulgaris* plants grown in field soil negatively correlated with the cover of several plant species in the plots from which the soil was collected (particularly, *Achillea millefolium, Hypochaeris radicata*, and *Leucanthemum vulgare*), but this relationship was not significant for plants grown in inoculated soils (**Supplementary Figures S9**, **S10**).

DISCUSSION

In this study, we examined the soil legacy effects of plant diversity on the focal plant *J. vulgaris*. With our design, we tested the biotic and the overall soil-mediated effects of plant diversity on plant

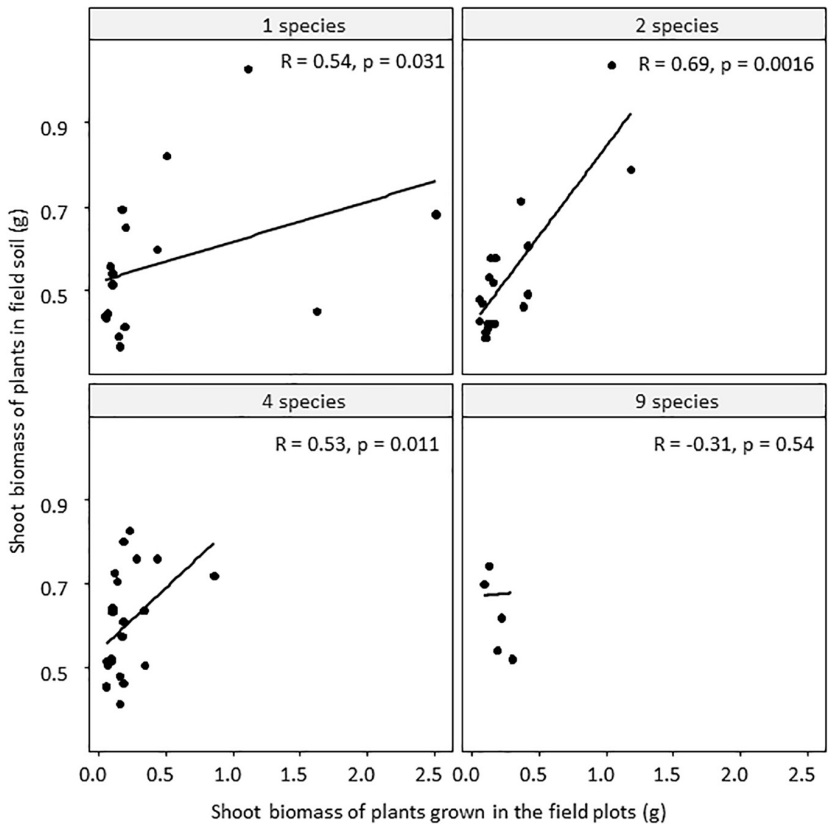

FIGURE 4 | Relationships between shoot biomass of plants grown in the field soil treatment and plants grown in the field in the biodiversity plots, for the different levels of diversity (1, 2, 4, or 9 plant species). R is the Spearman rank correlation coefficient, *p*-values are based on a Spearman rank correlation test.

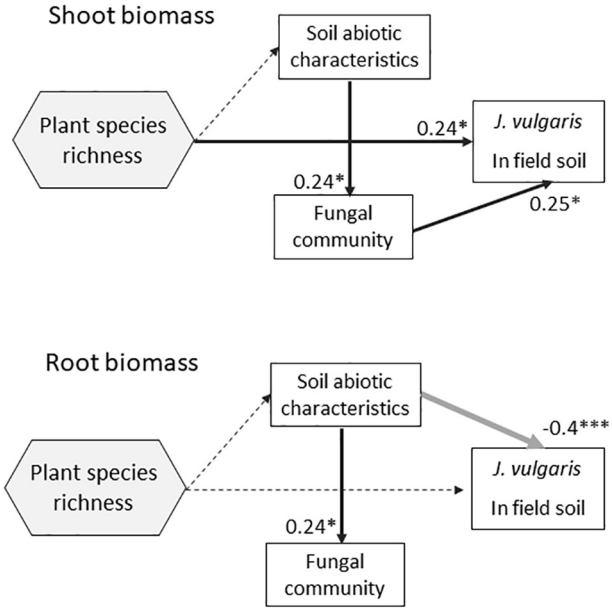

FIGURE 5 | Structural equation model testing the direct and indirect effects (mediated by the changes in soil abiotic characteristics or soil fungal community composition) of plant diversity on *J. vulgaris* root and shoot biomass in pots with pure field soil. Soil was collected from the field experiment with plots with plant communities that differed in diversity (0, 1, 2, 4, 9 species). Plant species richness was included as fixed continuous factor. For soil abiotic and fungal communities we included the first axis of a multivariate analysis of all soil abiotic characteristics or fungal TRF peaks respectively. The arrows show significant relationships (black = positive, gray = negative), dashed arrows indicate non-significant relationships. The explained variance is also presented and the significance (*$P < 0.05$; ***$P < 0.001$).

growth of *J. vulgaris*, and compared this to the performance of *J. vulgaris* planted in communities with different diversity levels. We observed that in the pot experiment there was a trend for a positive legacy effect of the diversity of the plant community on *J. vulgaris* root biomass, but this effect was only present in the field soil treatment. Similar results were observed in a study where genetic diversity of a single species was manipulated (Luo et al., 2016). That study focused on effects of inoculated soil and the authors concluded that dilution of pathogens was the likely mechanism (Luo et al., 2016). Other studies have shown that high species diversity reduces the inhibitory effects of soil biota in plant communities (Hendriks et al., 2013; Yang et al., 2015; Guerrero-Ramírez et al., 2019).

One of the questions that we set out to examine is how the diversity-soil legacy relationship depends on the sterilization or inoculation treatments. The results show that the diversity effect was only significant in the pure field soil treatment, and not in the inoculated treatments. Hence, our study provides no support for the role of soil biota in the legacy effects of plant diversity. It is interesting to note that sterilization of soil from high diverse plant communities (nine plant species) did not affect the root biomass of *J. vulgaris* while sterilization of soil from the communities with lower plant diversity (1, 2, or 4 plant species) enhanced *J. vulgaris* root biomass (**Supplementary Table S5**), similar to the results reported by Luo et al. (2016). This indicates that suppressive (biotic) effects may have been present in the low diversity soils but further research is needed to confirm this.

In our study, plant biomass was higher in pots with soil from plots in which only legumes had grown but this was only true in pots filled with pure field soil and not in the inoculated soil. Legumes live in symbiosis with nitrogen fixing rhizobacteria and we hypothesize that the observed effects were due to nitrogen availability, even though we did not detect differences in nitrogen availability in the soil chemical analysis among monocultures.

Soil legacies can be due to a myriad of changes in the soil that are caused by the first plant or plant community and then influence a second plant (Kulmatiski et al., 2012; Wurst et al., 2015). In this study, we examined the plant-diversity mediated effects on soil fungal composition and soil chemical properties. We expected that the build-up of species-specific fungal pathogens would be high in monocultures and be diluted with increasing plant diversity and that these pathogenic fungi would also negatively affect *J. vulgaris* at high densities in the soil. Hence, we expected a positive relationship between biomass of *J. vulgaris* and plant diversity. Earlier studies have shown that soil legacy effects on *J. vulgaris* can be explained by soil fungal composition (Bezemer et al., 2006; Wang et al., 2019). In our study, soil fungal community composition was not strongly affected by the diversity of the plant community but it was significantly related to the biomass of *J. vulgaris* when the plants were grown in the pure field soil. However, plant biomass was also positively related to nitrogen availability in the soil, highlighting that it is unlikely that soil legacy effects on plant growth can be explained by a single factor, and that most likely it is a combination of many components that change in the soil (e.g., changes in soil fungi and changes in nitrogen availability). It is important to note that the abundance of specific fungal species or groups of fungi in the soil will be much more important for plant growth than the composition of the entire fungal community. Future studies, therefore, should examine the effects of different fungi on plant growth and the absolute abundance of these fungi in the soil.

The diversity of the plant community can greatly affect plant performance (Tilman, 1997; Scherber et al., 2003; Agrawal, 2004) and these effects can be driven by changes in the soil (Maron et al., 2011; Kulmatiski et al., 2012). Interestingly, and in contrast to the potted plants, in the field, the growth of *J. vulgaris* plants tended to be negatively related to the diversity of the surrounding plant community, however, this was mainly due to high biomass in the bare plots. Previous work on *J. vulgaris* has shown that this ruderal species is strongly negatively affected by interspecific plant competition (McEvoy et al., 1993). Clearly, the *J. vulgaris* plants in the field were influenced by the effects of neighboring plants both above- and belowground on light and space availability, as well as by the changes in soil characteristics such as nutrient availability or e.g., soil pathogens caused by these neighbors. Our results indicate that plant diversity may negatively affect the performance of new colonizing plant species, i.e., invaders, that establish in these communities, as shown

by other studies (e.g., Hooper et al., 2005; Maron and Marler, 2008). However, for *J. vulgaris* this is mainly due to increased competition with other plants in more diverse communities, probably due to an increase in overall plant density in more diverse communities as a result of more complete use of resources due to niche differences between species (Scherber et al., 2010; Sun et al., 2014). Evidence from our study suggests that this diversity effect is not due to soil pathogens. It is important to note that we measured the performance of *J. vulgaris* plants at the rosette stage both in the field and in the greenhouse experiment and it is possible that the soil legacy effects of plant diversity will be greater when plants reach the flowering stage (Dudenhöffer et al., 2018).

An important question is whether soil mediated effects measured in potted plants can be extrapolated to plant growth in the same soil under field conditions (Heinze et al., 2016). In this study, we detected several relationships between growth of potted and plants that grew in the field plots. While this suggests that soil factors can explain the growth of *J. vulgaris* and that results from potted experiments can be extrapolated to plant performance in the field, we note that there are many differences between plants grown in pots and plants grown in field plots and that comparisons should be made with great caution.

We did not detect differences between the effects of the monoculture soils on plant performance. Several other studies carried out with soil collected from the same area have shown that the performance of *J. vulgaris* depends greatly on the identity of the species that grew previously in the soil. Some of these studies tested this with soil collected from potted plants (e.g., van de Voorde et al., 2011), but others have shown such effects with field collected soil from monocultures (e.g., Kos et al., 2015). It is possible that the period of 2 years of conditioning prior to collecting the soil for the current experiment was too short, so that the plants had not altered the soil sufficiently. The experimental plots were established in an area with a relatively homogeneous plant community consisting of roughly 12 species per m^2. The legacy effects of this previous plant community may still have been present in the soil and this may explain why we did not observe strong soil legacy effects. However, the analyses of soil abiotic characteristics and the soil fungi in the monocultural plots shows that there are clear differences among the soils from the different plots. Although we did not find strong differences in performance of *J. vulgaris* in the different monoculture soils, we did observe a negative relationship between the abundance of three of the sown forb species in the field and the performance of *J. vulgaris* in the soil legacy bioassay. However, as this effect was not detected in the monoculture soils, these results should be interpreted with caution.

In conclusion, our study provides only weak evidence that plant diversity-mediated soil legacies influence the performance of *J. vulgaris*. Hence, the importance of soil legacies in influencing plants in the field is still debatable.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

OK and MB conceived the original idea, conducted experiments, analyzed the data, and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

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Predicting Plant-Soil Feedback in the Field: Meta-Analysis Reveals That Competition and Environmental Stress Differentially Influence PSF

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Past research on plant-soil feedbacks (PSF), largely undertaken in highly controlled greenhouse conditions, has established that plant species differentially alter abiotic and biotic soil conditions that in turn affect growth of other conspecific and heterospecific individuals in that soil. Yet, whether feedbacks under controlled greenhouse conditions reflect feedbacks in natural environments where plants are exposed to a range of abiotic and biotic pressures is still unresolved. To address how environmental context affects PSF, we conducted a meta-analysis of previously published studies that examined plant growth responses to multiple forms of competition, stress, and disturbance across various PSF methodology. We asked the following questions: (1) Can competition, stress, and disturbance alter the direction and/or strength of PSF? (2) Do particular types of competition, stress, or disturbance affect the direction and/or strength of PSF more than others? and (3) Do methods of conducting PSF research (i.e., greenhouse vs. field experiments and whether the source of soil inoculum conditioning is from the field vs. greenhouse) affect plant growth responses to PSF or competition, stress, and disturbance, or their interactions? We discovered four patterns that may be predictive of what future PSF studies conducted under more realistic conditions might reveal. First, relatively little is known about how PSF responds to environmental stress and disturbance compared to plant-plant competition. Second, specific types of competition enhanced negative effects of soil microbes on plant growth, and specific environmental stressors enhanced positive effects of soil microbes on plant growth. Third, whether PSF experiments are conducted in the field or greenhouse can change plant growth responses. And, fourth, how the soil conditioning phase is conducted can change plant growth responses. With more detail than previously shown, these results confirm that environmental context writ large can change plant growth responses in PSF

experiments. These data should aid theory and predictions for conservation and restoration applications by showing the relative importance of competition, stress, and disturbance in PSF studies over time. Lastly, these data demonstrate how variation in experimental methods can alter interpretation and conclusions of PSF studies.

Keywords: plant-soil feedback, competition, stress, disturbance, environmental variation

INTRODUCTION

The past 25 years of research on plant-soil feedback (PSF), largely under highly controlled conditions, has established that plant species differentially alter abiotic and biotic soil conditions that in turn affect growth of other conspecific and heterospecific individuals in that soil (Bever, 1994; Bever et al., 1997; Wardle et al., 2004; Ehrenfeld et al., 2005; Kulmatiski and Kardol, 2008; Mangan et al., 2010; Putten et al., 2013). The broad conclusion from this research is that positive and negative PSF can shape community composition and ecosystem functioning by driving patterns of plant diversity, succession, and invasion (Bever et al., 1997; Mills and Bever, 1998; Klironomos, 2002; Reynolds et al., 2003; Mangan et al., 2010; Johnson et al., 2012; Van Der Heijden et al., 2018). Yet most PSF experiments are rarely conducted under the environmental conditions where large-scale ecological and evolutionary mechanisms occur, that is, in the field. Various findings indicate differences in PSF between greenhouse and field experiments (Putten et al., 2016; Schittko et al., 2016; Florianová and Münzbergová, 2018; Heinze and Joshi, 2018; Kivlin et al., 2018; Forero et al., 2019) which suggest that inferences from greenhouse studies may not accurately represent how PSF functions on the landscape (Kulmatiski and Kardol, 2008; Putten et al., 2013, 2016; Smith-Ramesh and Reynolds, 2017; Crawford et al., 2019; De Long et al., 2019). While greenhouse experiments have the advantage of maintaining control over non-focal variables and are crucial for confirming and falsifying mechanisms, recent field based PSF research has demonstrated that thoughtful field study designs can generate tractable and interesting results (Long et al., 2019). By not accounting for the surrounding environmental matrix in which plant-soil interactions occur, it is difficult to accurately predict community composition and productivity based on the plant species that are either inhibited by negative PSF or those that persist by positive PSF.

Competition, stress, and disturbance are common environmental pressures that occur across the landscape. To cope with these pressures, plants can differentially allocate resources to growth, reproduction, or maintenance. Selection for each of these strategies depends on tradeoffs for traits that allow tolerance to competition, stress, or disturbance events. This phenomenon, known as the competition-stress-disturbance (C-S-D) hypothesis (Grime, 1977), predicts that competitive plants thrive in ecosystems with high competition because of evolved traits such as large size and extensive fine roots. Likewise, traits such as high stem and root storage of resources allow stress-tolerant plants to thrive under highly stressful conditions, while disturbance-resilient plants thrive in frequently or intensely disturbed ecosystems due to evolved traits such as fast growth rates and fast reproduction. Changes in plant traits in response to these environmental pressures may subsequently impact plant-soil interactions by altering soil biota and chemistry. Although the C-S-D hypothesis is well-studied (Herms and Mattson, 1992; Reich, 2014; Rosado and de Mattos, 2017), it does not directly describe the role of plant-soil interactions in plant response to the biotic and abiotic environment (which C-S-D represents) that may enhance or impede a plant's ability to cope with competition, stress, or disturbance. For example, when accounting for soil microbial dynamics under well-watered vs. drought-stress conditions, a greenhouse study (Lau and Lennon, 2012) found that *Brassica rapa* fitness increased under drought when focal plants were grown with "dry-adapted" soil microorganisms. As such, examining PSF across environmental gradients of competition, stress, or disturbance will provide insight into plant-soil dynamics that influence plant species' persistence or decline under more realistic field conditions.

Competition is a prominent driver of plant fitness, community composition, and coexistence (Tilman, 1982; Callaway and Walker, 1997; Chesson, 2000; Aschehoug et al., 2016) and may influence the strength and direction of PSF in several ways. While competition reduces resource availability, a plant may benefit from its "home" microbiome and thus exhibit positive PSF if soil mutualists increase the availability of limiting resources (Bessler et al., 2012). Conversely, the phenomenon of reduced survival and density of conspecific seedlings near mature or parent conspecifics (Burkey, 1994; Bell et al., 2006; Mangan et al., 2010; Rolhauser et al., 2011; Reinhart et al., 2012; Comita et al., 2014) suggests that intraspecific competition may promote negative PSF due to accumulation of host-specific soil pathogens. The relative importance of competition and PSF on plant performance was examined in a recent meta-analysis (Lekberg et al., 2018). Distinguishing between both inter- vs. intraspecific and low vs. high-density competition and across multiple PSF treatments Lekberg et al. (2018) found antagonistic interactive effects of competition and PSF. In other words, the combined effect of competition and PSF was greater than the individual effects of PSF or competition alone, often leading to highly reduced plant growth and demonstrating how biotic interactions can alter PSF outcomes. Plant growth responses to PSF are likely also modified by plant responses to changes in the abiotic environment, although this was not tested by Lekberg et al. (2018).

As human-induced global change accelerates, stress (defined here as prolonged or continuous environmental pressures) and disturbance events defined here as sudden, temporally constrained changes in the environment) (Hillebrand and Kunze,

2020) are predicted to rapidly increase (Allen et al., 2010; Dai, 2013; Barbero et al., 2015). These types of environmental pressures will likely have additionally profound effects on plant distributions and plant-soil interactions. Increasing evidence suggests that plant response to environmental stressors including drought (Lau and Lennon, 2012; Vílchez et al., 2016; Kannenberg and Phillips, 2017), herbivory (Badri et al., 2013), and salt tolerance (Qin et al., 2016) can be mediated in part by interactions with soil biota.

As with competition, environmental stress, or disturbance can alter resource availability (e.g., soil nutrients, light, water). Fertilization is often considered a benefit to plant growth, however there is evidence that it can cause an imbalance in PSF. A surplus of nutrients from nutrient deposition could promote positive PSF if plant defense against pathogens is enhanced with high resource availability (Smith-Ramesh and Reynolds, 2017). Positive PSF may also occur under low-resource stress if plants rely on host-specific soil microbes for limiting nutrients (Reynolds et al., 2003; Revillini et al., 2016). Alternatively, nutrient inputs in excess of plant growth demands can shift the balance of plant-microbe interactions and prompt dissociations between plants and their home soil biota (Wallenda and Kottke, 1998; Treseder and Allen, 2002; Revillini et al., 2016). Nutrient inputs from fertilizer applications can lead to limitation of other micronutrients (Whalen et al., 2018) and changes in soil chemistry (Erisman et al., 2013) that modify a plant's association with the soil microbiome. Negative PSF could also occur if soil pathogens thrive under resource-rich conditions (Hersh et al., 2012; Spear et al., 2015).

Other types of environmental stressors like aboveground herbivory could promote negative PSF if grazed plants are less able to defend from soil pathogens that are more abundant in conspecific-conditioned soil (Smith-Ramesh and Reynolds, 2017). Negative PSF under herbivory may also occur if grazed plants reduce carbon allocation to roots and thus reduce the ability to support host-specific microbial mutualists (Smith-Ramesh and Reynolds, 2017). Alternatively, positive PSF could occur if grazed plants allocate growth to roots and thus support host-specific microbial mutualists (Smith-Ramesh and Reynolds, 2017). Disturbance events such as fire could promote either negative or positive PSF depending on how soil microbes respond to fire disturbance. Fire has generally been found to decrease microbial biomass and diversity (Dooley and Treseder, 2012; Pressler et al., 2019; Whitman et al., 2019) due to heat-sterilization of microbes and loss of soil carbon valuable for soil microbes (Dooley and Treseder, 2012). Negative PSF could occur if abundance of host-specific mutualists declines from fire, whereas positive PSF could occur if fire reduces soil pathogen abundance. However, it is possible that increases in ash deposition following fire could stimulate microbial growth by increasing availability of soil inorganic nitrogen and alleviating nutrient limitation (Rau et al., 2008; Schafer and Mack, 2010; Dooley and Treseder, 2012). In this scenario, negative or positive PSF could occur depending on whether host-specific mutualists or pathogens thrive from nutrient availability.

Predicting PSF accurately on the landscape also requires examining the methods under which plant growth response is measured and the methods that are used to condition the soil microbiome. PSFs have been found to vary between greenhouse and field experiments. An experiment by Schittko et al. (2016) for example, found that the majority of plant species in an experiment exhibited positive PSF under controlled greenhouse conditions, but did not exhibit any significant PSF under natural field conditions. A meta-analysis of the PSF literature seems to corroborate this observation—experiments conducted in the greenhouse often produce larger effect sizes in PSF than those conducted in the field (Kulmatiski et al., 2008; Forero et al., 2019). This broad pattern likely suggests that the true importance of PSF on the landscape is different than what is measured in greenhouse studies. Importantly, experiment location informs interpretation of the specific mechanisms of PSF. Field experiments testing PSF allow for understanding how a natural environment influences soil effects on plants, whereas PSF experiments conducted in the greenhouse do not provide this level of understanding (Smith-Ramesh and Reynolds, 2017).

Similarly, how the soil microbiome is conditioned can greatly influence plant growth. An important distinction between greenhouse-conditioned soil inoculum and field-conditioned soil inoculum is that greenhouse-conditioned soil contains primarily only microbes associated with the focal conditioning plant(s), whereas field-conditioned soil contains microbes associated with site-specific edaphic characteristics in addition to microbes associated with the focal conditioning plant(s). Subsequent differences in microbial composition can alter plant growth. For example, the perennial forb *Centaurea maculosa* exhibited negative PSF with field-conditioned soil inoculum and positive PSF with greenhouse-conditioned soil inoculum (Callaway et al., 2004). In this instance, greenhouse-conditioned soil could result in the accumulation of microbial symbionts or the loss of pathogens compared to field-conditioned soil (Kulmatiski et al., 2008). Similar to the role of experiment location, soil conditioning source can also inform interpretation of the specific mechanisms of PSF. Using soil conditioned by focal plants in the greenhouse limits understanding how the environmental context of a natural field environment influences plant effects on soil (Smith-Ramesh and Reynolds, 2017).

Several recent papers have specifically called for empirical studies that measure PSF under manipulations of abiotic and biotic variation (Putten et al., 2013, 2016; Smith-Ramesh and Reynolds, 2017; Lekberg et al., 2018; Crawford et al., 2019). However, no concerted effort has been made to assess the current extent and relative importance of PSF across environmental gradients of competition, stress, or disturbance. We expanded upon a recent effort (Lekberg et al., 2018) that analyzed the interaction between PSF and plant competition. Lekberg et al. found that PSF varied when plants were exposed to competitors or not. We build on that study by examining PSF when plants are exposed to environmental stress or disturbance, to leverage our understanding of plant C-S-D strategies in making predictions of PSF. We also investigated how PSF responses to C-S-D may vary in greenhouse and field conditions to further develop PSF predictive frameworks. Using data from 300 independent manipulations from 76 publications, we examined plant growth responses to multiple forms of competition,

stress, and disturbance across a range of PSF methods to identify trends in plant-soil interactions across biotic and abiotic environments and experimental conditions. To understand the relative importance of environmental context as well as common methods for PSF, we addressed the following questions to better understand and predict what PSF outcomes might be expected under realistic field conditions: (1) Can competition, stress, and disturbance alter the direction and/or strength of PSF? (2) Do particular types of competition, stress, and disturbance affect the direction and/or strength of PSF more than others? (3) Do methods of conducting PSF research (i.e., greenhouse vs. field experiments and whether the source of soil conditioning inoculum is from the field vs. greenhouse) affect plant growth responses to PSF or competition, stress, and disturbance, or the interaction of both?

METHODS

Paper Selection

We collected previously published, peer-reviewed data of PSF in response to experimental manipulations of competition from studies used in a recent meta-analysis (Lekberg et al., 2018) supplemented with a search conducted in Web of Science for more recent competition studies as well as stress or disturbance. We searched through April 2019 for publications that crossed PSF experiments with one of the environmental manipulations of competition, stress, or disturbance using the following search terms "plant-soil feedback*" OR "PSF" AND "competi*"; "plant-soil feedback*" OR "PSF" AND "stress" OR "drought" OR "herbiv*" OR "precipitation" OR "temperature" OR "salinity" OR "light" OR "fertiliz*" OR "enrichment"; "plant-soil feedback*" OR "PSF" AND "disturbance" OR "mining" OR "mine tailings" OR "wind" OR "hurricane" OR "tornado" OR "fire" OR "grazing" OR "agriculture." We identified additional publications not found in our initial Web of Science search by searching for studies that had cited publications included in our initial data set. Several publications were included twice in our data set because they measured PSF under multiple manipulations of competition, stress, or disturbance (Larios and Suding, 2015; Shivega and Aldrich-Wolfe, 2017; Yu et al., 2017; Hawkins and Crawford, 2018; Zhao et al., 2018), or under more than one stress or disturbance level (Heinze et al., 2016; Valliere and Allen, 2016).

We screened publications for studies that included (1) soil treatment methods indicative of a manipulative PSF experimental design (as detailed below), (2) plant growth responses to soil treatments, specifically, either aboveground biomass or plant height, (3) factorial design of PSF treatments crossed with some manipulation of either competition, stress, or disturbance, where the experiments were undertaken in the field or in the greenhouse and (4) measures of mean, error, and sample size for plant growth in all treatments. We did not have any criteria for the length of the study. We excluded one publication (Brandt et al., 2015) and experiments from several publications (Coykendall and Houseman, 2014; Maron et al., 2016; Zuppinger-Dingley et al., 2016) from the Lekberg et al. (2018) data set because of the absence of a full factorial design. We found nine additional PSF x competition publications that were not included in the Lekberg et al. (2018) meta-analysis (de la Peña et al., 2010; Chen et al., 2012; Zhang et al., 2014; Chung and Rudgers, 2016; Bezemer et al., 2018; Hawkins and Crawford, 2018; Xue et al., 2018; Zhao et al., 2018; Lozano et al., 2019). Soil feedback manipulations for PSF experimental design were conducted in three ways in the included publications: (1) soil conditioned from the focal species ("home" soil) or from a heterospecific species ("away" soil) (hereafter, home-conditioned vs. away-conditioned), (2) live soil (majority of soil biota active; hereafter referred to as "active soil") or sterilized soil (majority of soil biota absent inactive; hereafter referred to as "inactive soil"), or (3) soil untreated or treated with fungicide (non-fungicide treated soil hereafter referred to as "diverse soil"). All studies that included treatments of untreated vs. fungicide soil or treatments of live vs. sterilized soil used soil that had been conditioned by plants. Publications that measured competition included one or two types of competition treatments, either the number of plants differed between treatments and the focal plant grew alone or with other plants (here referred to as "alone-together"). In other instances, competition was quantified as equal number of plants between treatments and the focal plant was exposed to either intra- or interspecific competition (here referred to as "interspecific-intraspecific"). The soil treatments were then added factorially, either directly as field-conditioned inocula (at various quantities and according to treatments) or as a second phase conditioned soil from a two-part design in which plants were initially grown in pots in a greenhouse conditioning phase using a field soil inoculum. Soil conditioned from the first phase was then used as the inoculum in the PSF phase of the experiment (see Kulmatiski et al., 2008 for details of these designs).

Data Collection

Mean values and measures of plant growth were collected from text and tables in the main publication and/or **Supplemental Information**. We used GraphClick (Arizona Software) to extract mean and standard error values from figures when raw data was not provided. If not provided, standard deviations were back calculated from standard errors and sample sizes ($SD = SE \times \sqrt{n}$). In cases where data was not clearly available in the publication, we contacted the authors. We excluded two publications (Medina-Roldán et al., 2012; Kaisermann et al., 2017) for which we received no response from authors, and therefore could not include these studies. Some studies measured the performance of multiple focal species, and thus included multiple experiments. Some studies contained multiple trials within an experiment in which a focal species was examined under multiple treatments (i.e., multiple home/away soils, multiple competitors, or multiple levels of stress or disturbance).

For each record in our dataset, we recorded the type of soil feedback manipulation (as described above) and environmental manipulation (competition manipulation described above). Stress manipulations consisted of drought, fertilization (as a representation of nutrient deposition), grazing/herbivory, shade (light availability), mining, and temperature. Disturbance manipulations consisted of fire and tornado. We differentiated stress manipulations as those that represented prolonged

or continuous environmental pressures experienced by focal plant(s) and disturbance manipulations as those that represent sudden, temporally constrained changes in the environment (Hillebrand and Kunze, 2020).

We recorded duration of the feedback experiment and multiple descriptors of the focal plant. We also recorded the location of where the soil was collected, as well as the source of inoculum conditioning phase (i.e., collected directly from the field = conditioned in the field, or collected from a training phase in pots under controlled conditions = conditioned in the greenhouse). Field-conditioned soil represents microbiota associated with specific plant species in the field as well as microbiota associated with the edaphic conditions of that site (i.e., pH, nutrient levels, soil moisture), whereas greenhouse-conditioned soil represents mostly microbiota associated with the plants that were used to condition the inoculum. For each record we also recorded experiment location. Experiment location was defined as greenhouse if the plant growth response to soil phase was conducted in the greenhouse. Location was defined as field if the response phase was conducted in a natural field environment. Extracted data of all publications included in the dataset is available in the (**Table S1**).

Effect Size Calculations

We conducted an interaction meta-analysis to assess effects of PSF, competition, stress, and disturbance across PSF methods on plant growth using the relative interaction intensity (RII) as the effect size metric (Armas et al., 2004). We preferred this metric over the log response ratio — a widely used metric in ecological meta-analyses—because unlike the log response ratio, RII is bounded between −1 and 1, and therefore symmetrical around zero, and it can be calculated if plant growth is zero in control groups. Following Armas et al. (2004), RII is calculated as:

$$\frac{treatment - control}{treatment + control}$$

We therefore calculated the effect of PSF manipulation as:

$$\frac{\overline{Y}_{c,f} - \overline{Y}_{c,n}}{\overline{Y}_{c,f} + \overline{Y}_{c,n}},$$

And the effect of competition, stress, or disturbance (i.e., C-S-D) manipulation as:

$$\frac{\overline{Y}_{t,n} - \overline{Y}_{c,n}}{\overline{Y}_{t,n} + \overline{Y}_{c,n}}.$$

To quantify the combined effect of PSF with C-S-D, we followed the calculation in Kivlin et al. (2013) modified from Armas et al. (2004) for a two-factor RII. The interactive effect of soil feedback and competition, stress, or disturbance can be described as the effect of PSF when competition, stress, or disturbance is present compared to the effect of PSF when competition, stress, or disturbance is absent. This was calculated as:

$$\frac{\overline{Y}_{t,f} - \overline{Y}_{t,n}}{\overline{Y}_{t,f} + \overline{Y}_{t,n}} - \frac{\overline{Y}_{c,f} - \overline{Y}_{c,n}}{\overline{Y}_{c,f} + \overline{Y}_{c,n}}$$

In these equations, $\overline{Y}$ is the mean plant growth for t = treatment or c = control for the competition, or stress, or disturbance treatment, and f = soil feedback imposed (away-conditioned soil, live soil, or non-fungicide treated soil) or n = no soil feedback imposed (home-conditioned soil, sterilized soil, or fungicide-treated soil). To calculate the 95% confidence intervals around the means for each record, variance was weighted by the sample size (n) and calculated using the standard deviation (s). For each record, we followed the calculations used in Kivlin et al. (2013) to calculate variance. Variance for the main effect of PSF was calculated as:

$$PSF\ Vi = \frac{s^2_{c,f}}{n_{c,f}\overline{Y}^2_{c,f}} + \frac{s^2_{c,n}}{n_{c,n}\overline{Y}^2_{c,n}},$$

and the variance of the main effect of competition, stress or disturbance as:

$$C - S - D\ Vi = \frac{s^2_{t,n}}{n_{t,n}\overline{Y}^2_{t,n}} + \frac{s^2_{c,n}}{n_{c,n}\overline{Y}^2_{c,n}},$$

Variance for the interaction of PSF and competition, stress or disturbance was calculated as:

$$Int\ Vi = \frac{s^2_{c,n}}{n_{c,n}\overline{Y}^2_{c,n}} + \frac{s^2_{t,n}}{n_{t,n}\overline{Y}^2_{t,n}} + \frac{s^2_{c,f}}{n_{c,f}\overline{Y}^2_{c,f}} + \frac{s^2_{t,f}}{n_{t,f}\overline{Y}^2_{t,f}}.$$

An RII_{PSF} significantly greater than zero indicates that away-conditioned, active, or diverse soil enhances plant growth. An RII_{PSF} significantly less than zero indicates that away-conditioned, active, or diverse soil inhibits plant growth. A RII_{PSF} not significantly different from zero indicates that away-conditioned, active, or diverse soil does not alter plant growth. An RII_{CSD} significantly greater than zero indicates plant growth is enhanced by competition, stress, or disturbance, whereas an RII_{CSD} significantly less than zero indicates plant growth is inhibited by competition, stress, or disturbance. An RII_{CSD} not significantly different from zero indicates that competition, stress, or disturbance does not alter plant growth. An RII_{Int} significantly greater than zero indicates a synergistic effect such that away-conditioned, active, or diverse soil enhances plant growth under competition, stress, or disturbance. An RII_{Int} significantly less than zero indicates an antagonistic effect such that away-conditioned, active, or diverse soil inhibits plant growth under competition, stress, or disturbance. An RII_{Int} not significantly different from zero indicates that the interactive effects of PSF and competition, stress, or disturbance are neutral. Specifically, a neutral

RII_{Int} indicates that away-conditioned, active, or diverse soil does not influence plant growth under competition, stress, or disturbance.

Data Analysis

We used the *rma.mv* function in the *metafor* package (Viechtbauer, 2010) in R 3.5.2 for all analyses. In all analyses, we separated the dataset by competition studies, stress studies, and disturbance studies and thus ran competition, stress, and disturbance models separately. Prior to analyses to assess our questions, we first identified the individual effects of PSF and C-S-D by building separate multivariate mixed effects models using RII_{PSF} and RII_{CSD} as response variables and *PSF Vi* and *CSD Vi* as the variance. These models tested for the main effects of PSF and C-S-D averaged across soil feedback manipulations and across competition, stress, disturbance manipulations. Thus, we tested for differences in plant growth in response to types of soil feedback and types of competition, stress, disturbance by including soil manipulation and C-S-D manipulation as moderators in separate models. A moderator in the *rma.mv* function is analogous to a fixed effect in an ANOVA model (Viechtbauer, 2010) and allows the model to calculate the effect size of specific levels of a factor.

To address the first question of whether competition, stress, and/or disturbance alters the direction and/or strength of PSF, we built multivariate mixed effects models using RII_{Int} as the response variable and as *Int Vi* as the variance to identify the main interactive effect of PSF x C-S-D (averaged across soil feedback manipulations and across competition, stress, disturbance manipulations). To address the second question of whether particular types of competition, stress, and disturbance affect the direction and/or strength of PSF more than others, we built separate multivariate mixed effects RII_{Int} models that included C-S-D manipulation as a moderator. To address the third question of whether methods of conducting PSF research alter the individual effects of PSF, or C-S-D, or the interactive effect of PSF x C-S-D, we built additional RII_{Int} mixed effects models using experiment location (field vs. greenhouse) and soil inoculum conditioning source (field-conditioned vs. greenhouse-conditioned) as moderators.

We included plant species as a random effect in all models because species are not independent and past evolutionary history may affect plant response regardless of treatment (Wooliver et al., 2017). Including plant species as a random effect also allows us to make comparisons across studies. To further account for phylogenetic variation of plant growth responses, we replicated the analysis using plant family as a random effect. Results did not vary between the species and family analyses. While it is likely that the strength of PSF varies with plant ontogeny (Kardol et al., 2013), the included studies lacked sufficient replication in experiment duration to use duration as another random effect. For all models we performed *post-hoc* tests using the *linearHypothesis* function in the *car* package (Fox et al., 2013) to test whether effect sizes differed from one another. We report QM as the test statistic for moderator coefficients of the *rma.mv* models. We considered results to be significantly different from zero if $\alpha \leq 0.05$.

RESULTS

Distribution of Studies

Using the selection criteria, we identified 300 studies from 76 publications that measured plant growth in response to different soil PSF methods in a specific environmental context (competition, stress, or disturbance). Of these, 199 studies (43 publications) measured PSF with competition, 95 studies measured PSF with stress (34 publications), and 5 studies measured PSF with disturbance (2 publications). The majority of studies were conducted in the greenhouse and 86% of all studies used grasses or forbs as the focal plant species. Field experiments comprised only 9% of all studies (29 studies from 7 publications); 26 field studies focused on competition (6 publications), and 3 field studies focused on stress (1 publication).

Main Effects of PSF, Competition, Stress, and Disturbance on Plant Growth

In general, plant growth responses to the main effects of PSF, stress, and disturbance were neutral and the response to competition was negative (**Figure 1A**). PSF had a generally neutral effect on plant growth (**Figure 1A**; $p = 0.28$; **Table S2**, row 3). However, the direction of PSF varied by type of soil feedback manipulation (**Figure 2A**; QM = 28.43, $p < 0.0001$; **Table S2**, rows 5–7). Active (i.e., live) soil reduced plant growth compared to sterile soil ($p < 0.0001$; **Table S2**, row 6), and the effect of PSF was neutral in studies that tested home vs. away-conditioned soil ($p = 0.28$; **Table S2**, row 7) or in those that tested diverse vs. non-diverse soil (i.e., untreated vs. fungicide treated soil) ($p = 0.97$; **Table S2**, row 5). *Post-hoc* analysis revealed that plant growth was reduced more in live vs. sterile soil manipulations than in home vs. away soil manipulations ($\chi^2 = 26.84$, $p < 0.0001$; **Table S2**, row 92) or untreated vs. fungicide manipulations ($\chi^2 = 3.97$, $p = 0.05$; **Table S2**, row 90). PSF was similarly neutral in studies that tested home vs. away soil manipulations and untreated vs. fungicide manipulations ($\chi^2 = 0.20$, $p = 0.66$; **Table S2**, row 91).

Plant growth responses to competition, stress, and disturbance were variable and depended on the type of competition, stress, or disturbance imposed. Competition in general significantly reduced plant growth (**Figure 1A**; $p < 0.0001$; **Table S2**, row 32), and the effect of plants grown together with a competitor was nearly 150% greater than the effect of inter- vs. intraspecific competition on plant growth (**Figure 2A**; $\chi^2 = 51.78$, $p < 0.0001$; **Table S2**, row 113). Generally, stress had marginally negative effects on plant growth (**Figure 1A**; $p = 0.22$; **Table S2**, row 56), and plant growth varied among different types of stress manipulations (QM = 94.94, $p < 0.0001$; **Table S2**, rows 58-63). As expected, drought (**Figure 2A**; $p = 0.0001$; **Table S2** row 58) and shade (**Figure 2A**; $p < 0.0001$; **Table S2**, row 61) greatly reduced plant growth, and plant growth was reduced similarly under drought and shade ($\chi^2 = 2.66$, $p = 0.20$; **Table S2**, row 132). Fertilization, on the other hand, increased plant growth (**Figure 2A**; $p = 0.0003$; **Table S2**, row 59). Increases in plant growth in response to fertilization were significantly different than reductions in plant growth in response to drought ($\chi^2 = 29.65$, $p < 0.0001$; **Table S2**, row 130) and shade ($\chi^2 = 70.51$, $p < 0.0001$; **Table S2**, row 137). Disturbance in general had no

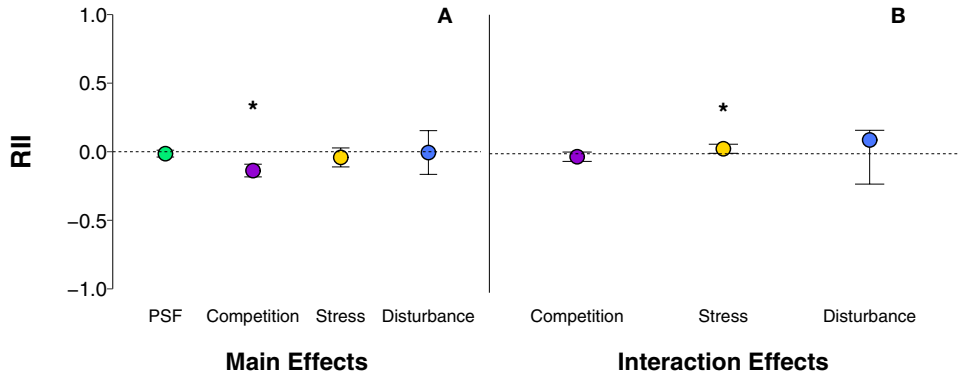

FIGURE 1 | (A) Main effects of PSF, competition, stress, and disturbance and **(B)** interaction effects of PSF x competition, PSF x stress, and PSF x disturbance on plant growth. Competition main effects are averaged across alone/together and interspecific/intraspecific competition manipulations. Stress main effects are averaged across all stress manipulations (drought, fertilization, grazing/herbivory, mining, shade, temperature). Disturbance main effects are averaged across fire and tornado disturbance manipulations. PSF main effects are averaged across all soil manipulations (i.e., live/sterile, away/home, untreated/fungicide). Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

effect on plant growth (**Figure 1A**; $p = 0.94$; **Table S2**, row 85). Disturbance caused by fire or tornado did not significantly affect plant growth ($p > 0.05$ for both; **Table S2**, rows 87-88) and plants responded similarly to these two disturbances ($\chi^2 = 1.86$, $p = 0.17$; **Table S2**, row 164).

Do Competition, Stress, and/or Disturbance Alter the Direction of PSF?

The strength of PSF was modified by plant-plant competition and environmental stress, but not disturbance. There was no general trend of competition affecting the outcome of PSF (**Figure 1B**; $p = 0.21$; **Table S2**, row 166), but specific types of competition interacted with specific soil feedback manipulations differently (QM = 8.22, $p = 0.22$; **Table S2**, rows 174-179). Interspecific competition reduced plant growth compared to intraspecific competition to a greater degree when plants were grown in away-conditioned soil ($p = 0.04$; **Table S2**, row 179). Interspecific competition affected plant growth similarly relative to intraspecific competition when plants were grown in active soils ($p = 0.42$; **Table S2**, row 178). Interspecific competition also affected plant growth similarly relative to intraspecific competition when plants were grown in diverse soils ($p = 0.96$; **Table S2**, row 177). *Post-hoc* analysis showed that interspecific competition effects in away-conditioned soil were marginally different than active soil ($\chi^2 = 3.62$, $p = 0.06$; **Table 2**, row 234) and similar to diverse soils ($\chi^2 = 0.43$, $p = 0.51$; **Table S2**, row 233). The effect of away-conditioned ($p = 0.82$; **Table S2**, row 176), active ($p = 0.25$; **Table S2**, row 175), and diverse soils ($p = 0.36$; **Table S2**, row 174) was similar when plants were grown together with a competitor compared to growing alone.

There was a general synergistic effect of PSF and environmental stress (**Figure 1B**; $p = 0.03$; **Table S2**, row 193), yet this trend was driven by the effect of drought stress which enhanced the effect of PSF (**Figure 2B**; QM = 19.06, $p = 0.0002$; **Table S2**, row 195). All other stressors had no effect on plant growth when in combination with PSF: fertilization (**Figure 2B**; $p = 0.12$; **Table S2**, row 196), grazing/herbivory (**Figure 2B**; $p = 0.18$; **Table S2**, row 197), shade (**Figure 2B**; $p = 0.63$; **Table S2**, row 198), mining (**Figure 2B**; $p = 0.34$; **Table S2**, row 199), and temperature (**Figure 2B**; $p = 0.98$; **Table S2**, row 200). *Post-hoc* analysis showed that the effect of drought on PSF was significantly greater than fertilization ($\chi^2 = 8.50$, $p = 0.004$; **Table S2**, row 252), shade ($\chi^2 = 10.01$, $p = 0.001$; **Table S2**, row 254), and temperature stress ($\chi^2 = 4.60$, $p = 0.03$; **Table S2**, row 256). Drought effects on PSF were similar to grazing/herbivory ($\chi^2 = 2.62$, $p = 0.11$; **Table S2**, row 253) and mining $\chi^2 = 0.07$, $p = 0.79$; **Table S2**, row 255), which were also positive but not statistically significant. All other environmental stressors had similar neutral effects on PSF ($p > 0.05$ for all other *post-hoc* comparisons). We were unable to analyze effects of different environmental stressors on different PSF soil manipulations due to lack of sufficient studies. There was no general effect of PSF with environmental disturbance (**Figure 1B**; $p = 0.80$; **Table S2**, row 217), and there were no disturbance-specific differences (QM = 0.45, $p = 0.80$; **Table S2**, rows 219-220). Plant-soils feedbacks were not modified by fire (**Figure 2B**; $p = 0.61$; **Table S2**, row 219) or tornados (**Figure 2B**; $p = 0.66$; **Table S2**, row 220). These disturbances had similar neutral effects on PSFs ($\chi^2 = 0.40$, $p = 0.53$; **Table S2**, row 278). Disturbance effects on different PSF soil manipulations could not be further differentiated due to the low number of studies.

Do the Effects of PSF and C-S-D Vary Between Methods Used to Conduct PSF Research?

Whether the experiment was conducted in the field or greenhouse altered the effects of competition and stress on plant growth, but not the effect of PSF (**Figure 3A**). There was no difference in the effects of away-conditioned vs. home-conditioned soils ($\chi^2 = 0.24$, $p = 0.62$; **Table S2**, row 103) or diverse vs. non-diverse soils ($\chi^2 = 2.61$, $p = 0.11$; **Table S2**, row 105) on plant growth between studies where the feedback

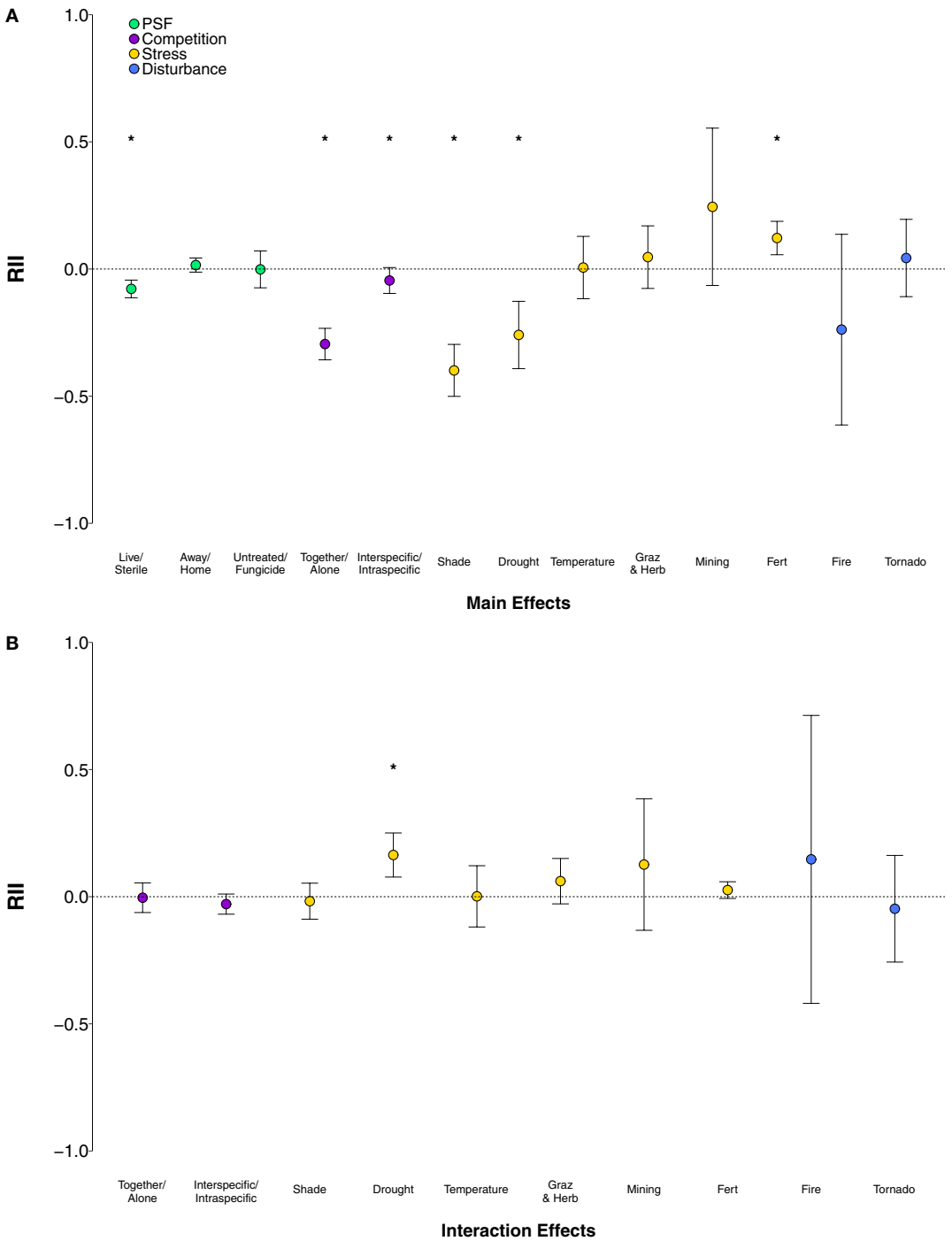

FIGURE 2 | (A) Main effects of PSF, competition, stress, and disturbance manipulations and **(B)** interaction effects of PSF x competition, PSF x stress, and PSF x disturbance manipulations on plant growth, averaged across soil manipulations (live/sterile, away/home, untreated/fungicide). Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

phase occurred in the field or greenhouse. Studies that used live and sterile soils as the soil feedback manipulation were only conducted in the greenhouse. Conversely, competition reduced plant growth when plants were grown in the field (**Figure 3A**; $p < 0.001$; **Table S2**, row 42) and the greenhouse (**Figure 3A**; $p < 0.001$; **Table S2**, row 43), but the effect was 138% stronger in field experiments than greenhouse experiments ($\chi^2 = 48.21$, $p < 0.001$; **Table S2**, row 122). This trend was driven by

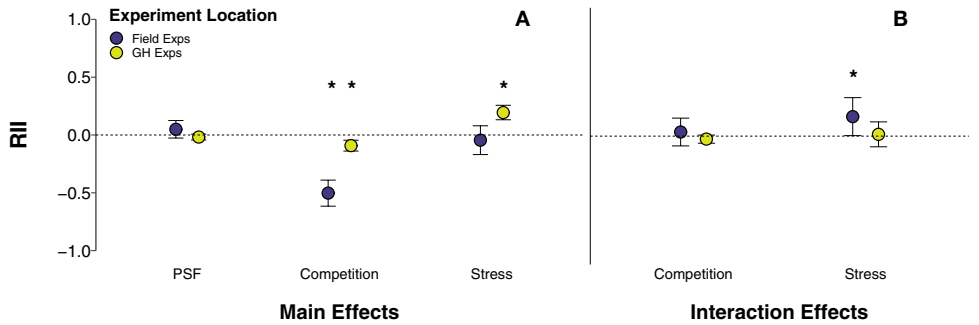

FIGURE 3 | (A) Main effects of PSF, competition, stress, and disturbance and **(B)** interaction effects of PSF x competition and PSF x stress between field and greenhouse experiments. Interaction effects of PSF x competition and PSF x stress are averaged across soil manipulations (live/sterile, away/home, untreated/fungicide) and across competition and stress manipulations. Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

alone/together competition studies because inter/intra-specific competition studies were only conducted in the greenhouse. Only grazing/herbivory stress was conducted in both greenhouse and field settings, and thus it was the only stressor we could analyze between experiment location. Grazing/herbivory stress enhanced plant growth in the greenhouse (**Figure 3A**; $p < 0.001$; **Table S2**, row 71), whereas its effects on plant growth were neutral in field experiments (**Figure 3A**; $p = 0.48$; **Table S2**, row 70). Grazing/herbivory stress enhanced plant growth over 300% more in greenhouse experiments than in field experiments (**Figure 3A**; $\chi^2 = 11.31$, $p < 0.001$; **Table S2**, row 157). Disturbance studies were only tested in greenhouse conditions.

The interactions between PSF and competition were neutral in both field and greenhouse experiments (**Figure 3B**; QM = 2.52, $p = 0.28$; **Table S2**, rows 181-182) and *post-hoc* analysis showed that these effects were similar between field and greenhouse experiments ($\chi^2 = 0.95$, $p = 0.33$; **Table S2**, row 245). Contrary to the finding that the main effect of grazing/herbivory was only positive in greenhouse experiments, there was a synergistic effect of PSF and grazing/herbivory only in field experiments (**Figure 3B**; $p = 0.04$; **Table S2**, row 202). In other words, away-conditioned, active or diverse soil enhanced plant growth under grazing/herbivory in field experiments. However, *post-hoc* analysis showed that the interaction of PSF and grazing/herbivory in field experiments was not significantly different than the neutral effect of PSF and grazing/herbivory in greenhouse experiments ($\chi^2 = 2.34$, $p = 0.13$; **Table S2**, row 268). Disturbance interactions with PSFs were only tested in greenhouse conditions.

In general, soil inoculum conditioning source modified the effect of PSF on plant growth (**Figure 4A**; QM = 34.93, $p < 0.0001$; **Table S2**, rows 23-24). Away-conditioned, active, or diverse soil reduced plant growth when soil inoculum was conditioned in the field (**Figure 4A**; $p < 0.0001$; **Table S2**, row 23) and enhanced plant growth when soil inoculum was conditioned in the greenhouse (**Figure 4A**; $p = 0.007$; **Table S2**, row 24). The various soil feedback manipulations also responded differently depending on the source of the conditioning phase. Active soil reduced plant growth when conditioned in the field ($p = 0.03$; **Table S2**, row 26) but had no effect on plant growth when conditioned in the greenhouse ($p = 0.40$; **Table S2**, row 27). The effects of away-conditioned soil on plant growth did not vary between field-conditioned ($p = 0.20$; **Table S2**, row 29) or greenhouse-conditioned soil ($p = 0.50$; **Table S2**, row 30).

The interaction of PSF and competition was similarly neutral between experiments that used field-conditioned and greenhouse-conditioned inoculum (**Figure 4B**; $\chi^2 = 0.04$, $p = 0.84$; **Table S2**, row 247). In general, the interaction of PSF and environmental stress was slightly synergistic when soil inoculum was conditioned in the field (**Figure 4B**; QM = 4.92, $p = 0.08$; **Table S2**, row 205). This trend was likely driven by drought, fertilization, and grazing/herbivory studies in which the average effects of PSF and each of these stressors was positive, though not statistically significant. However, *post-hoc* analysis showed that the interaction of PSF and stress with field-conditioned soil inoculum was not significantly different than the neutral effect of PSF and stress with greenhouse-conditioned soil inoculum ($\chi^2 = 0.52$, $p = 0.47$; **Table S2**, row 270). Conversely, the interaction of PSF and mining stress was slightly synergistic when soil was conditioned in the greenhouse (QM = 4.73, $p = 0.09$; **Table S2**, row 215). Away-conditioned, active, or diverse soil enhanced plant growth 300% more under mining stress when soil inoculum was conditioned in the greenhouse rather than conditioned in the field ($\chi^2 = 3.81$, $p = 0.05$; **Table S2**, row 276). Studies that manipulated temperature were not included in this analysis as these studies only included greenhouse-conditioned soil.

DISCUSSION

In general, our meta-analysis revealed important patterns that may aid predictions of PSF under natural or field conditions where plant-plant competition and environmental stressors are common. The results of this meta-analysis suggest that soil microbes may generally reduce plant growth where plant-plant competition for resources occurs and may enhance plant growth under drought stress conditions. These results also highlight an important research gap in examining PSF under environmental disturbance. Additionally, this meta-analysis suggests that where

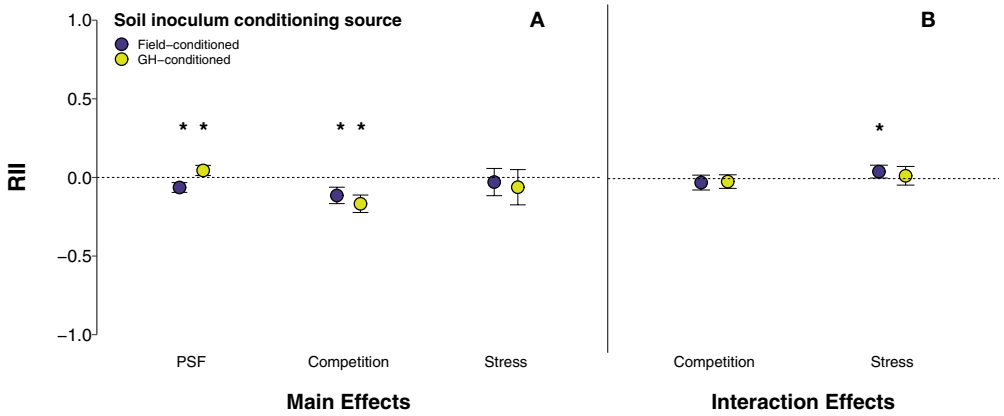

FIGURE 4 | (A) Main effects of PSF, competition, stress, and disturbance and **(B)** interaction effects of PSF x competition and PSF x stress between field-conditioned and greenhouse-conditioned soil inoculum. Interaction effects of PSF x competition and PSF x stress are averaged across soil manipulations (live/sterile, away/home, untreated/fungicide) and across competition and stress manipulations. Error bars represent lower and upper bounds of the confidence intervals. Asterisks indicate effect sizes significantly different from zero.

PSF experiments are conducted (field or greenhouse) affects the outcome of the study—especially when plants are competing. The nature of the soil conditioning manipulation (live vs. sterile, home vs. away, or fungicide application) can change plant growth responses, and again this is most evident when plants are competing for limiting resources. Our meta-analysis corroborates and expands upon Lekberg et al. (2018) to confirm that environmental context writ large can alter plant growth responses in PSF experiments, at least in grass and forb species with which the majority of PSF studies have been conducted.

Plant Competition, and Specific Environmental Stressors and Alter Direction and Magnitude of PSF

Competition shapes resource availability (Tilman, 1982, 1990) which can subsequently affect PSF, and the data to date show that PSF is often more examined in response to plant-plant competition than to environmental stress and disturbance. While growing next to a plant compared to growing alone more greatly reduced plant growth than when plants were growing with conspecifics or heterospecifics, types of competition reduced plant growth differently depending on the type of soil feedback imposed. When focal plants were grown in away-conditioned soil, interspecific competition reduced plant growth more than intraspecific competition. This likely indicates that interspecific competition either enhanced negative effects of non-host associated soil biota on plant growth or reduced beneficial effects of non-host associated soil biota on plant growth. When focal plants were grown in active or diverse soil, interspecific competition reduced plant growth to a similar degree to that of intraspecific competition. This could suggest that taxonomic composition of the soil microbiome is more important than microbial diversity for reducing plant growth under interspecific competition. Conversely, in alone vs. together competition studies, the effect of away-conditioned, active, and diverse soil was similar. This may suggest that just the presence of a soil microbiome is influential for inhibiting plant growth when a plant is in close proximity to a competitor.

While the frequency of environmental stress and disturbance are increasingly occurring in terrestrial landscapes, these data from 76 publications suggest that stress and disturbance can have both negative and positive effects on plant growth individually (e.g., drought and shade reduce growth but fertilization increases growth) but when combined with PSF, lead to few synergistic or antagonistic effects. While the combined effect of PSF and environmental stress was generally synergistic, the trend was driven by drought stress studies. Away-conditioned, active, or diverse soil enhanced plant growth under drought conditions. Though the exact mechanisms to explain this trend remain unclear, soil microbes may facilitate plant growth under low-resource stress if plants rely on microbes for acquiring limiting nutrients (Reynolds et al., 2003; Revillini et al., 2016). Although this meta-analysis found that the combined effect of PSF and grazing/herbivory was on average synergistic but not statistically significant, grazing/herbivory could potentially induce benefits of the soil microbiome to plant growth if plants allocated resources to roots and subsequently supported microbial mutualists (Smith-Ramesh and Reynolds, 2017).

This meta-analysis found that the combined effects of PSF and environmental disturbance were neutral for both fire and tornado disturbance treatments. It is unclear, however, whether this is a true pattern across fire and tornado disturbance or across many types of environmental disturbance because this dataset only includes five studies from two publications (Nagendra and Peterson, 2016; Senior et al., 2018). Soil microbiomes and their interactions with plants are likely to be affected by drastic landscape changes brought about by environmental stress and disturbance, yet this meta-analysis shows that there is not yet enough data to predict how PSF responds to environmentally disruptive events. This gap of knowledge highlights the necessity of PSF experiments to incorporate manipulations that are

representative of environmental variation under global climate change (Putten et al., 2016; Long et al., 2019).

Experiment Location and Soil Conditioning Source Affect the Outcomes of PSF Research

Comparisons of field vs. greenhouse studies showed that PSF did not vary by experiment location. Competition had a more negative effect when experiments were conducted in the field rather than in the greenhouse. However, the combined effects of PSF and competition were similarly neutral between field and greenhouse experiments. Grazing/herbivory only increased plant growth in greenhouse conditions. The combined effect of PSF and grazing/herbivory was synergistic in field conditions and neutral in greenhouse conditions, though further analyses indicated that the interactive effects of PSF and grazing/herbivory were not significantly different between field and greenhouse experiments.

It is important to note that only 9% of these studies were conducted in the field, suggesting that we have little inference for the strength or direction that environmental variation will demonstrate when interacting with PSF to affect plant growth. The few interactive effects shown here both support and contradict studies showing that PSF can differ between greenhouse and field experiments in different environments (Putten et al., 2016; Schittko et al., 2016; Florianová and Münzbergová, 2018; Heinze and Joshi, 2018; Kivlin et al., 2018) demonstrating how little is understood about the function of PSF in nature (Forero et al., 2019).

PSF varied by soil inoculum conditioning source. Away-conditioned, active, or diverse soil reduced plant growth if soil was conditioned in the field and enhanced plant growth if soil was conditioned in the greenhouse. The combined effect of PSF and competition was similarly neutral between studies that used field-conditioned and greenhouse-conditioned soil inoculum. The combined effect of PSF and stress was slightly synergistic when soil inoculum was conditioned in the field, though this was not significantly different than the neutral effect of PSF and stress with greenhouse-conditioned soil inoculum. The combined effect of PSF and mining stress was slightly synergistic when soil was conditioned in the greenhouse. Away-conditioned, active, or diverse soil enhanced plant growth 300% more under mining stress when soil inoculum was conditioned in the greenhouse rather than in the field. While it is difficult to identify detailed mechanisms, the trend from this meta-analysis of increased plant growth in greenhouse-conditioned soil relative to field-conditioned soil may be due to lower microbial diversity in greenhouse-conditioned soil. Specifically, greenhouse-conditioned soil may contain lower abundance of pathogens than field-conditioned soil (Callaway et al., 2004; Kulmatiski et al., 2008).

These results demonstrate that there are multiple methodological approaches that both allow us to infer how field studies may respond and to show how variation in methods can change interpretation of results. The finding that effects of PSF can differ between greenhouse and field experiments provides justification for pairing PSF experiments in the greenhouse with those conducted in the field, when possible (Smith-Ramesh and Reynolds, 2017). Additionally, that the source of inoculum for PSF experiments can also mediate interactive effects show that careful interpretation is required of studies that use each method (Smith-Ramesh and Reynolds, 2017). Studies that use field-conditioned inoculum for example, should be mindful to infer that focal plants are responding to microbes associated with the focal conditioning plant(s) in addition to microbes associated with site-specific soil characteristics. Focal plants in studies that use greenhouse-conditioned inoculum, on the other hand, are responding primarily to microbes associated with the focal conditioning plant(s). Moreover, it should be noted that 86% of the focal plants examined in this meta-analysis are grasses and forbs. While, overall, we did not see mean differences in effect sizes among plant functional groups (data not shown—see results in **Table S2**), this result is related to by the reduced power to detect an effect due to low sample sizes of trees and shrubs that have been studied to date.

An important distinction between greenhouse-conditioned soil inoculum and field-conditioned soil inoculum is that greenhouse-conditioned soil contains primarily only microbes associated with the focal conditioning plant(s), whereas field-conditioned soil contains microbes associated with site-specific edaphic characteristics in addition to microbes associated with the focal conditioning plant(s).

CONCLUSIONS

Environmental context writ large can change plant growth responses in PSF experiments, a conclusion supported by our analyses comparing the interactive effects of PSF with a range of competition, stress and disturbance types relative to their individual effects. Our results have direct application in ecological conservation and restoration because we show that positive PSF is synergistically enhanced by drought and stress. Additionally, our study suggests that field experiments may yield different responses than greenhouse experiments. Data from these 76 studies show the need for more research on PSF across environmental stresses and disturbances (i.e., two-thirds of these studies were conducted on competition) and the need for increased representation of a wide diversity of focal plant species, because the majority of PSF studies were conducted with grasses and forbs. The lack of studies investigating true gradients of stress and disturbance (i.e., multiple experimental levels, rather than presence-absence of different types of stress or disturbance) indicate that we know very little about how PSF effects will respond to stress and disturbance on the landscape. Our meta-analysis enables future research into plant community dynamics in a changing world. There is, therefore, much empirical work to look forward to.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR'S NOTE

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AUTHOR CONTRIBUTIONS

KB, JM, SB, CL, LM, MP, JLS, and IW screened all potential papers and coded data for papers that were included in the meta-analysis, curated by KB and JM. KB and JM ran all initial analyses and made figures. SK guided data analysis. KB ran all final analyses and made all final figures. KB, JM, JAS, and JB coordinated writing, to which all authors contributed. Ideas were conceived of by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00191/full#supplementary-material

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