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RESEARCH ARTICLE

Intraspecific variation in plant–soil feedback depends on plant dominance while interspecific variation is unrelated to plant community structure

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Abstract

1. Plants interact with a variety of soil biota; the accumulation of which can affect their growth and that of subsequent plants. This plant–soil feedback (PSF) can both positively and negatively affect plant populations. Diverse plant communities should dilute pathogens and increase beneficial soil biota, which can mitigate negative PSF. Plant dominance, conversely, should result in reduced microbial diversity and increased pathogens or mutualists of the dominant plant, enhancing negative or positive PSF. Genetic diversity within the dominant species may dilute PSF, yet it is unclear whether species and genetic diversity can have additive effects.
2. Using field-conditioned soils from *Medicago sativa* production systems varying in dominance and species diversity, we inoculated multiple plant species and *Medicago* cultivars to assess effects on PSF. In the field, we measured multiple aspects of the biotic and abiotic environment, including sequencing bacteria, fungi, arbuscular mycorrhizal fungi and oomycetes. Using structural equation modelling, we linked the dominance and diversity of the plant community to intraspecific and interspecific (community-wide) means and variances in PSF via changes in microbiome community composition and diversity.
3. Intraspecific PSF was more negative and variable as *Medicago* dominance increased, whereas the mean and variance in interspecific PSF were largely unlinked to plant composition. While the microbiome was strongly linked to both the mean and variance of intra- and interspecific PSF, only the oomycete community had similar effects within and among species, suggesting they are important generalist pathogens and drivers of plant population and community dynamics. Nonetheless, each microbiome component was linked to the mean PSF of either the community or *Medicago*. The diversity of the eukaryotic microbiome, however, was more important for determining variability in PSF within and among species.

4. *Synthesis*. Plant dominance had stronger effects on microbiome assembly and plant–soil feedback (PSF) than plant diversity. Although plant diversity did not reduce negative PSF, independent variation in PSF within and among species suggests additive benefits of genetic and species diversity for dilution of plant responses to pathogens. Understanding this variation, however, requires quantifying microbiome components beyond bacteria and fungi.

KEYWORDS
arbuscular mycorrhizal fungi, bacteria, forage production, grassland, interspecific variation, intraspecific variation, *Medicago sativa*, oomycetes, plant diversity, plant–soil feedback

1 | INTRODUCTION

Most plants interact with soil microbes, with interaction outcomes ranging from mutualism to antagonism (Bever et al., 2012). These interactions not only affect the growth of that plant but can also cause shifts in the soil microbiome that persist and affect the recruitment of new individuals into those soils (Bever et al., 2010). These plant–soil feedbacks (PSFs) can thus increase or decrease population growth rates and alter the structure of plant communities (Bennett et al., 2017; Teste et al., 2017). In low-diversity agricultural systems, accumulation of crop diseases can cause negative PSF and significant crop losses (Mariotte et al., 2018). Increasing plant diversity can dilute species-specific pathogens, and thus overall pathogen densities, and increase the diversity and abundance of beneficial microbes (Bennett et al., 2020). Consequently, diversification of agroecosystems should reduce negative PSF and increase positive PSF.

Plant–soil feedback is commonly measured as the effect that plants of a particular species have on conspecific recruitment via changes in the soil; however, in diverse systems, many plant species may condition the soil and respond to these changes (Baxendale et al., 2014; Kulmatiski, 2018). This concept is partially reflected in the measurement of PSF as a pairwise interaction between species (Crawford et al., 2019), yet pairwise PSF is often a poor indicator of plant-community dynamics (Reinhart et al., 2021). This lack of prediction may be because plant neighbourhoods influence microbiome assembly (Mommer et al., 2018) or because root systems are intermingled within soils (Frank et al., 2015) meaning that soils are simultaneously being conditioned by multiple species, even over small spatial scales. Consequently, soil conditioning is likely dependent on the community context. The variability in PSF is further increased by varying responses of plant species to changes in the microbiome (Baxendale et al., 2014), thus limiting our ability to understand the dynamics of diverse plant communities from traditional PSF approaches.

The role of PSF in plant communities may be better considered by integrating the effect and response of multiple species. Averaged across species, the mean effects of soil biota would thus be an estimate of the soil quality and its effect on plant growth. Variability among species may also be important and could have implications for the structure and functioning of ecosystems: An increase in PSF variability could either exacerbate or mitigate fitness inequalities among community members, whereas a reduction in variability suggests that PSF will have limited effects on community dynamics as all plants are affected equally. From a functional perspective, reduced variability coupled with positive or negative community mean PSF could indicate shifts in community productivity, whereas greater variability in PSF should reduce the likelihood that function is affected due to compensatory responses among less affected community members.

Understanding variability in PSF among species can help in designing sustainable cropping systems by mitigating negative and strengthening positive PSFs (Koyama et al., 2022), yet increasing genetic diversity may be more practical in low-diversity agroecosystems as plant genotypes differ greatly in their interactions with soil biota (Gundale & Kardol, 2021; Van Nuland et al., 2016). Whether increasing both species and genetic diversity would have additive benefits depends on whether variability among genotypes and species is correlated and whether genotypes and species respond to different components of the soil microbiome (Schöb et al., 2015). If species are affected by different pathogens or mutualists (i.e. there is a high degree of specificity), we should expect the presence of specific specialized soil biota to increase PSF variability, whereas the presence of shared pathogens or mutualists should reduce variability (Semchenko et al., 2022; Wang et al., 2023). Consequently, regardless of the type of microbiota, microbiome composition could be positively or negatively related to PSF variability. As many crop varieties are bred for resistance to specific pathogens and specialist soil microbes are unlikely to have similar effects on unrelated species (Gilbert & Parker, 2016; Semchenko et al., 2022), we hypothesize that intra- and interspecific variation in PSF responds to different aspects of soil microbiome composition. Microbiome diversity, however, should increase the likelihood that strong pathogens or mutualists are present via selection effects and thus increase intra- and interspecific variability.

Much effort has gone into understanding how PSFs change as a function of ecological conditions, highlighting the roles of climate, soil properties, resource availability and plant community structure in shaping plant–microbe interactions (Beals et al., 2020; De Long et al., 2023; Jiang et al., 2024; Lundell et al., 2022). Many of these studies, however, include relatively limited microbiome data and those

that do typically focus on bacteria or fungi (De Long et al., 2023). Few studies have focused on the role of oomycete pathogens in PSF (e.g. Burrill et al., 2023; Domínguez-Begines et al., 2021), despite their importance as plant pathogens (e.g. *Phytophthora* and *Pythium* spp. Kamoun et al., 2015). Furthermore, we know that interactions among microbiome components vary among environments and can have strong effects on microbial community assembly and plant–soil feedback (Bahram et al., 2018; Bennett et al., 2017). Nonetheless, interactions among microbiome components are rarely explicitly accounted for when testing the mechanisms of PSF.

To better understand inter- and intraspecific variation in plant responses to soil biota, we focused on how the plant community influences soil microbiomes and PSF in alfalfa (*Medicago sativa*) agroecosystems. Alfalfa is the most commonly grown forage species globally and is incredibly important to the livestock industry (Annicchiarico et al., 2015). It is a perennial legume that benefits from both rhizobia and mycorrhizal fungi (Püschel et al., 2017), while being susceptible to multiple soil pathogens, which can result in either positive or negative PSF (Awodele & Bennett, 2022). Alfalfa cultivars and other species, however, differ in response to inoculation with soil from alfalfa fields (Awodele & Bennett, 2022). Here, we focus on understanding the drivers of variation in PSF using structural equation models linking the plant community to intra- and interspecific variation in PSF via the soil microbiome (Figure 1). We used this model to test the following hypotheses: (1) Intra- and interspecific variation in PSF will be largely uncorrelated because they are affected by different aspects of the soil microbiome; (2) alfalfa abundance will cause negative intraspecific PSF and increase intraspecific variation in PSF due to increases in species-specific pathogens but will be unrelated to interspecific variation in PSF; and (3) plant species richness will increase positive PSF for both alfalfa and other species mediated by changes in both pathogens and beneficial microbes, resulting in reduced intra- and interspecific variation in PSF.

2 | METHODS

2.1 | Site selection and field sampling

To collect soils conditioned by alfalfa, 24 fields used for commercial alfalfa cultivation were selected in a previous study (Awodele & Bennett, 2022). All sites were within 300 km of Saskatoon, Canada, and at least 2 km apart, and located on private land so did not require permits. Of these, 12 were seeded to monocultures and 12 to mixtures between 1 and 6 years prior to sampling; however, stand composition was allowed to change naturally after seeding, so the stands varied greatly in their soils and plant composition (see Table S1). In summer 2019, we sampled three locations per site that were at least 50 m apart. We placed a 1-m^2 quadrat to estimate percent cover of vascular plants, then clipped the quadrat to 2 cm stubble height, separating alfalfa and other plants. Next, we collected 12 soil cores (2 cm wide and 15 cm deep) from the plot, pooling and mixing them before transporting them to the lab on ice. Soil for the PSF experiment was kept at 4°C for up to 1 week. Subsamples for sequencing were frozen at −20°C. The remaining soil was air-dried for chemical and physical analyses. Due to sampling and labelling errors, only 66 of the 72 samples were processed.

2.2 | Field plant and soil variables

For plants, we focused on five variables. Species richness and percent alfalfa were calculated from the cover data as estimates of community diversity and alfalfa dominance. Field plant biomass samples were dried at 60°C for 72 h, and then weighed, and we used total biomass to estimate productivity. We ground the alfalfa biomass and measured neutral detergent fibre using an ANKOM 2000 Fiber Analyzer™ (ANKOM Technology, New York, USA) and the percent

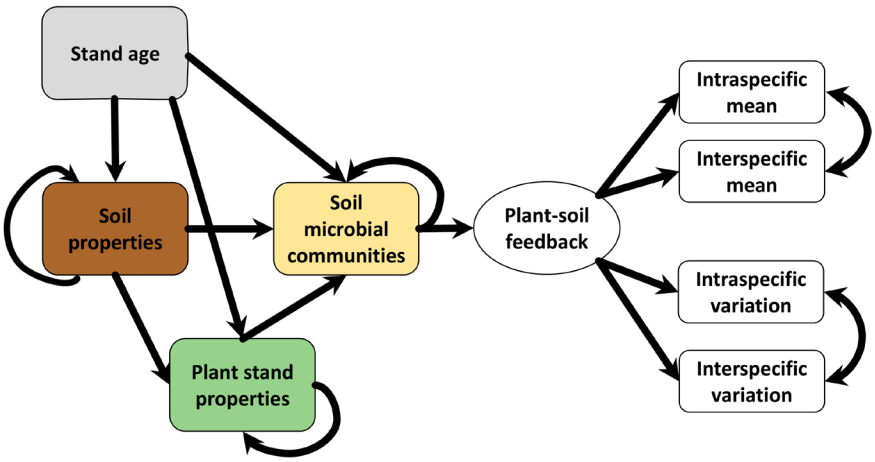

FIGURE 1 General structure of the initial structural equation model. We hypothesized that stand age would affect the properties of both soil (pH, texture, carbon, C:N ratio, phosphorus) and the plant stand (species richness, percent alfalfa, alfalfa N and fibre content), which would then alter the structure of soil microbial communities (richness, evenness and composition of bacteria, fungi, oomycetes and AMF). We also hypothesized that changes in the soil microbiome would affect the average and variation in plant–soil feedback among alfalfa cultivars and other plant species.

nitrogen using a Leco CN628 analyser (LECO, Michigan, USA). These variables are included as proxies for decomposability of plant litter, which can have strong effects on soil microbial communities and PSF (Ke et al., 2015).

Using the air-dried soils, we measured a series of physical and chemical properties associated with soil microbial communities and PSF (Awodele & Bennett, 2022; Ke et al., 2015; Leff et al., 2015; Tedersoo et al., 2014). We measured soil texture using the hydrometer method (Bouyoucos, 1962); however, as clay, silt and sand content were strongly correlated, we only use sand content in our analyses. We measured soil pH by shaking soil samples for 30 min in a 1:2.5 mixture of soil and deionized water, then measuring pH with a Fisher Accumet® AE150 pH meter (Fisher Scientific Canada, Ltd.). We measured total soil nitrogen and phosphorus by Kjeldahl digestion (Bremner & Mulvaney, 1982), followed by analysis on an AA2 Autoanalyser (SEAL Analytical, Inc. Wisconsin, USA) and percent soil carbon by combustion (Yeomans & Bremner, 1991) using a Leco TruMac™ elemental analyser (LECO, Michigan, USA). As soil carbon and nitrogen content were strongly correlated, we calculated the carbon to nitrogen ratio (C:N) to explore the effect of relative nitrogen availability.

2.3 | Soil microbial analyses

Soil bacteria, oomycetes and fungi, especially arbuscular mycorrhizal fungi, are all important in plant–soil feedback (De Long et al., 2023), so we focused our sequencing efforts on these groups. DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For sequencing fungi, we used the primers ITS1F/58A2R targeting the fungal intergenic transcribed spacer (ITS) (Gardes & Bruns, 1993; White et al., 1990). For bacteria, we used the primers 515F/806R targeting the V4 region of 16S rDNA (Bergmann et al., 2011). For AMF, we focused on SSU rRNA using the primers WANDA/AML2 (Hart et al., 2015), and for oomycetes, we amplified the ITS1 region using the ITS6/ITS7ae primers (Taheri et al., 2017). Library preparation and sequencing using the Illumina MiSeq platform were conducted by Genome Quebec (Montreal, Canada).

Sequencing data were analysed using AmpliconTagger (Tremblay & Yergeau, 2019). Briefly, raw reads were scanned for sequencing adapters and PhiX spike-in sequences. Primer sequences were removed using pTrimmer v1.3.4 (Zhang et al., 2019). The remaining sequences were processed to generate amplicon sequence variants (ASVs) in DADA2 v1.12.1 (Callahan et al., 2016). Chimeras were removed with DADA2 followed by UCHIME reference (Rognes et al., 2016). Bacterial ASVs were assigned a taxonomic lineage with the RDP classifier (Wang et al., 2007) using training sets containing the complete SILVA release 138 database (Quast et al., 2012) supplemented with a customized set of mitochondria and plastid sequences. The fungi ITS, AMF and oomycetes training sets were constructed from the UNITE database (Abarenkov et al., 2010). Taxonomic lineages were combined with the cluster abundance matrix obtained above to generate raw ASV tables. From these data, ASV richness and evenness of each group (Table S1) were calculated with RTK v0.93.2 (Saary et al., 2017). In addition, we summarized the ASV composition of each microbial group using principal coordinates analysis (PCoA) using Bray–Curtis distances in the r package 'vegan' (Oksanen et al., 2019). We then extracted the first two PCoA axes for each microbial group to represent community composition in subsequent analyses (see Figure S1).

2.4 | Plant–soil feedback experiment

Four alfalfa varieties and five additional species were originally selected for a PSF experiment (Awodele & Bennett, 2022). The four alfalfa varieties were selected by the seed producer (BrettYoung Seeds Ltd.) to vary in growth, disease resistance and root morphology. The additional species included both native (N) and non-native (A) species; however, the effects of species origin were inconsistent in the original study (Awodele & Bennett, 2022), so we group them together here. There were three legumes (*Trifolium pratense* (A), *Onobrychis viciifolia* (A) and *Vicia americana* (N)) and two grasses (*Agropyron cristatum* (A) and *Elymus lanceolatus* (N)). We chose to focus on legumes and grasses because only legumes and grasses are seeded into forage production systems in the region, and the choice of these species increased the applicability to stand management. More information on the varieties and species can be found in the original publication by Awodele and Bennett (2022).

The greenhouse PSF experiment was established in August 2019. The background soil was a 2:1 topsoil–sand mixture (both sourced commercially) that was sterilized in two 45-minute autoclave cycles at 121°C. Approximately 620 mL of background soil was added to 621 different D40L Deepots (volume: 656 mL; Stuewe and Sons Inc., Tangent, Oregon, USA), representing 69 pots per plant type: 66 different inocula and three uninoculated controls. To inoculate the pots, 30 mL of field soil was added to a 5-cm deep hole in the pot, with one pot inoculated for each of the nine plant types with each of the 66 field soil sources. For uninoculated pots, 30 mL of sterilized background soil was added. The upper 5 cm were then mixed with a sterilized spatula to ensure even distribution of the inoculum in the upper soil layer where contact with seedling roots was more likely. This inoculation method limited the amount of soil inoculum to <5% of total volume per pot to isolate the role of soil microbes from soil fertility (Brinkman et al., 2010). By comparing plant growth between plants grown in 100% autoclaved soil and >95% autoclaved soil, we also minimize any bias due to the effects of autoclaving on the soils. Each plant type was then seeded into the allocated 69 pots, which were split randomly between two Conviron™ growth chambers (model: GR48 and PGV36) located at the University of Saskatchewan. The chambers had a mean temperature of 24°C, humidity of 13% and light availability of 472 μM PAR, with 16 h of light per day. The pots were arranged in a completely randomized design. Plants were watered to capacity at 48-h intervals until harvest at approximately 4 months. At this time, the shoots and roots were

harvested; the roots were washed thoroughly; and then all biomass was dried at 60°C for 72 h and weighed.

In addition to PSF, mycorrhizal colonization of the fine roots (<0.5 mm diameter) was also estimated for all plants. Root fragments were cleared with 10% KOH at 96°C for 1.5–2 h, rinsed and acidified in 2% HCl at 96°C for 15–20 min, and then rinsed again before staining in 5% ink solution in lactoglycerol at 96°C for 15–20 min (Vierheilig et al., 2005). In all cases, processing times increased with root thickness. Root samples were then rinsed in a weak solution of lactic acid to de-stain. Colonization of the roots was assessed using the line intersect method at 40× magnification (McGonigle et al., 1990). Although colonization by all mycorrhizal structures was assessed, we only consider total colonization in our analyses.

2.5 | Data analysis

For our analyses, we calculated PSF as the natural log of shoot mass for each inoculated plant relative to the average of the plants of the same species grown in sterilized soil (Brinkman et al., 2010). To initially explore variability in PSF among cultivars and species, we calculated Pearson correlations among all cultivars and species in base R. The cultivar and species PSF values were then used to calculate four broader measures of PSF per field sample: (1) the intraspecific mean PSF as the average of all alfalfa cultivars; (2) the intraspecific variability in PSF as the variance among those cultivars; (3) the interspecific mean PSF as the average across all species, using intraspecific mean PSF as our estimate for alfalfa; and (4) the interspecific variability as the variance across species. We also calculated intra- and interspecific means and variances for AMF colonization in the same way. In both cases, we included alfalfa in the interspecific mean and variance as we intended these measures to be a community-level response to soil biota and alfalfa is an important part of the community; however, we also calculated the interspecific variance in PSF without alfalfa. As closely related species are likely affected by the same microbiome components (Gilbert et al., 2015), we also calculated the intrafamilial variance of PSF among legumes, including and excluding alfalfa. We then tested for correlations among intraspecific, intrafamilial and interspecific variation in PSF, both including and excluding alfalfa in the intrafamilial and intraspecific variation estimates to determine the effect of relatedness and including alfalfa on community variability and its relationship with intraspecific variation. We constructed the SEM using SPSS AMOS (IBM). Due to incomplete data, the total number of samples was reduced to 61 for SEM. Because of the number of variables, we constructed the model in stages and optimized the model at each stage. For the first stage, we hypothesized that stand age and the soil properties (carbon, C:N, sand, pH and phosphorus) would affect each plant community variable (alfalfa abundance, alfalfa nitrogen, alfalfa fibrousness, plant productivity and species richness) and included paths between these variables to represent these hypotheses. Soil carbon, soil C:N, productivity and species richness were log transformed and sand content and soil phosphorus were square root transformed to normalize the data and reduce the influence of outliers. We optimized the model first by examining modification indices and included additional covariances and paths among variables for suggested logical relationships. Whether we included covariances or direct paths depended on whether we judged the relationship likely to be directional (e.g. soil pH effects on phosphorus) or due to covariance with some other factor (e.g. soil carbon and phosphorus both driven by some variable affecting productivity). After including these new relationships, we removed non-significant paths when doing so reduced model AIC. After removing these paths, we repeated this procedure until no additional paths could be added or removed (Figure S2).

For the second stage of model construction, we hypothesized that stand age, soil properties and the plant community directly affected the microbial community (richness, evenness, PCoA axis 1 and PCoA axis 2 for bacteria, fungi, oomycetes and AMF). To reduce outliers and normalize the data, we squared AMF PCoA1 and square root transformed AMF richness. We optimized this stage using the same procedure as stage one, keeping all paths from stage one. All relationships among soil microbiome components were included as covariances as we could not ascribe causality (Table S2).

For the final stage of the model, we initially included direct paths between each component of the microbiome and each PSF measure. Both intra- and interspecific PSF variances were log transformed to normalize the response variables. Additionally, we hypothesized that the intra- and interspecific means and variances of AMF colonization would drive the mean and variation in PSF, so we included direct paths between those variables and the corresponding measure of PSF. We also included direct paths between the AMF components of the microbiome and each measure of AMF colonization. These final models were optimized as previously. As the model was multivariate non-normal, we tested the fit of the final model using Bollen–Stine bootstrapping (Grace, 2006). Relationship significance was determined using bootstrapped confidence intervals.

3 | RESULTS

3.1 | Pearson correlations in PSF

There were few correlations among the alfalfa cultivars and other plant species in how they responded to soil inoculation (i.e. PSF; Figure 2). For the alfalfa cultivars, PSF outcomes were not correlated among themselves or with other species (all $p > 0.1$). Among the other species, PSF was only correlated between *Vicia americana* and *Elymus lanceolatus* ($p = 0.018$) and between *Vicia americana* and *Trifolium pratense* ($p < 0.001$). Furthermore, we found no evidence that intraspecific variability in alfalfa PSF was correlated with variability among the other species, regardless of if alfalfa was included ($r = 0.16$, $p = 0.217$) or not ($r = 0.14$, $p = 0.273$). Similarly, there was no correlation between intraspecific and intrafamilial PSF variation with ($r = 0.15$, $p = 0.245$) or without alfalfa being included in the intrafamilial response ($r = 0.13$, $p = 0.329$).

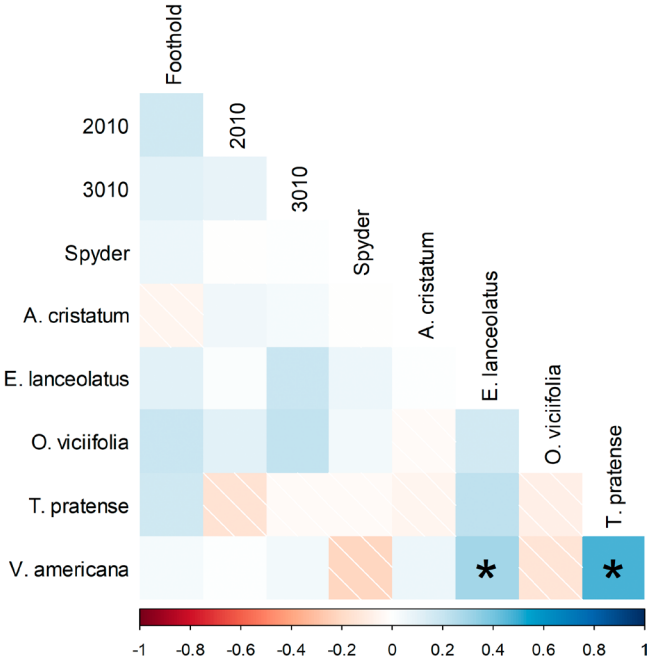

FIGURE 2 Pairwise correlations in plant–soil feedback effects among alfalfa varieties and other species. Colours correspond to Pearson correlation coefficients. Significant correlations are marked with an asterisk.

3.2 | SEM model fit and relationship among inter- and intraspecific means and variances

Although the chi-squared statistic indicated poor model fit for the SEM ($p=0.002$), Bollen–Stine bootstrapping showed good model fit ($p=0.976$). Intraspecific mean PSF was strongly explained by the SEM ($R^2=0.519$), whereas this was reduced for the intraspecific variation ($R^2=0.354$), interspecific mean ($R^2=0.346$) and interspecific variation ($R^2=0.350$) in PSF. Within the SEM, mean intra- and interspecific PSF were positively correlated ($r=0.412$, $p=0.005$), likely because alfalfa PSF was included in the interspecific mean, as, when we calculated the interspecific mean PSF without alfalfa, there was no pairwise correlation between mean intraspecific and interspecific PSF when using a Pearson correlation test ($r=0.13$, $p=0.299$). The SEM also indicated that the interspecific mean and variance in PSF were marginally negatively correlated ($r=-0.262$, $p=0.082$), suggesting that positive responses were less variable than negative responses. We found no similar relationship when considering intraspecific PSF within the SEM.

3.3 | Direct effects on PSF

Mean alfalfa PSF was driven by the diversity and composition of each type of soil microbe (Figure 3). Mean alfalfa PSF was most strongly related to the bacterial (PCoA1) and AMF (PCoA2) communities (Figure 3), which were not correlated (Table S2). Alfalfa mean PSF was also positively related to bacterial evenness and fungal richness, but negatively related to fungal evenness and oomycete richness (Figure 3).

Intraspecific variation in alfalfa PSF was positively associated with oomycete richness and fungal evenness (Figure 3). As oomycete richness and fungal evenness were both negatively linked to intraspecific mean alfalfa PSF, this suggests that strong pathogen effects caused divergence in responses among cultivars. Intraspecific variation was negatively related to mean mycorrhizal colonization as well, indicating that high AMF abundance minimizes fitness differences among cultivars.

Multiple microbial community aspects affected mean interspecific PSF, with the composition of fungi (PCoA1), oomycetes (PCoA1) and bacteria (PCoA1) all having strong effects (Figure 3). AMF composition (PCoA2) was also associated with mean interspecific PSF to a lesser extent (Figure 3). Interestingly, mean mycorrhizal colonization of alfalfa was positively related to mean interspecific PSF, but not mean intraspecific PSF.

For interspecific PSF variation, bacterial richness and composition (PCoA2); oomycete richness, evenness and composition (PCoA2); and AMF composition (PCoA1) were all retained in the model (Figure 3). The only significant effects, however, were positive relationships with oomycete evenness, bacterial PCoA2 and soil carbon (Figure 3), indicating that increased abundances of non-dominant oomycetes, certain bacteria and more productive soils may enhance variability in PSF. Interestingly, the only factors associated with both intraspecific and interspecific PSF variation were oomycete richness and composition.

3.4 | Indirect effects on PSF

Alfalfa cover indirectly caused more negative and variable PSF in alfalfa (Figure 4). These effects were driven by increases in fungal evenness and oomycete richness (Figure 5), which were both strong drivers of the mean and variance of alfalfa PSF (Figure 3). Relative to alfalfa PSF variance, alfalfa cover had stronger indirect effects on alfalfa mean PSF (Figure 4) driven by additional positive effects of alfalfa cover on the bacteria (PCoA1) and AMF (PCoA2) communities (Figure 5) that affected mean alfalfa PSF (Figure 3). The indirect effects were weakened by increases in fungal richness in denser alfalfa patches (Figure 5), which generally promoted positive PSF for alfalfa (Figure 3). Alfalfa PSF was also more negative in older stands, suggesting pathogen accumulation over time (Figure 4). Precise pathways are shown in Figure S3.

The indirect effects of alfalfa cover on the mean and variance of interspecific PSF were non-significant (Figure 4), supporting our hypothesis. The lack of indirect effects on mean interspecific PSF was because of opposing effects on different components of the soil microbiome (see Figure S3). There were negative indirect effects mediated through bacteria PCoA1 and oomycete PCoA1, but positive indirect effects mediated through fungi PCoA1 and AMF PCoA2 (Figure 5). Conversely, alfalfa cover had no effect on oomycete evenness (Figure 5), which was the primary driver of the interspecific PSF variance (Figure 3) and weak effects on the other significant microbial driver of interspecific variation (bacteria PCoA2; Figure 5).

Plant richness had limited effects on PSF. There were no significant effects on the mean or variance of interspecific PSF, although

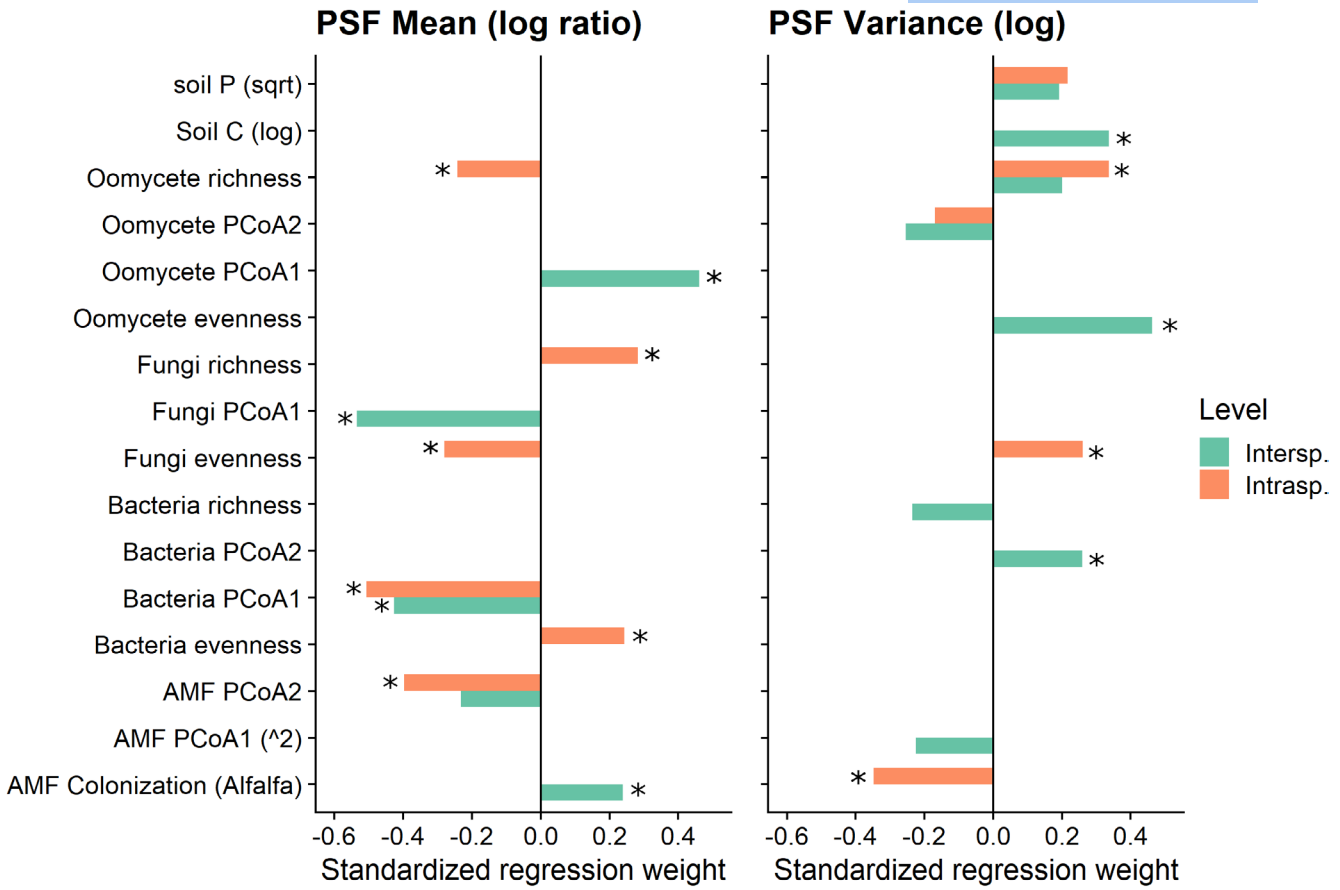

FIGURE 3 Direct effects of soil properties and microbiome components on plant–soil feedback. Shown are standardized regression weights for each path connecting the soil and microbiome to the intraspecific and interspecific means and variances in plant–soil feedback (PSF) that were retained in the model. Asterisks denote significant effects at $p < 0.05$.

PSF did become more negative in more diverse stands, contrary to our hypothesis. Plant richness only significantly affected the variance in intraspecific PSF, with more diverse stands causing greater variation in PSF among cultivars (Figure 4). This appears to be driven through increases in fungal evenness (Figure 5), which was positively associated with PSF variability (Figure 3). A full list of all relationships and their significance can be found in Table S3.

Beyond the hypothesized effects of alfalfa cover and plant species richness, soil pH was the strongest driver of intraspecific PSF and was positively associated with the mean and negatively associated with the variance (Figure 4), driven by strong effects on multiple aspects of the soil microbiome (Figure 5). Intraspecific PSF was also less variable in stands where alfalfa tissues had more nitrogen and were more fibrous, and interspecific mean PSF was more positive in soils that had more carbon and higher carbon to nitrogen ratios (Figure 4), but these effects were relatively weak.

4 | DISCUSSION

Consistent with our hypotheses, intraspecific and interspecific variation in PSF were uncorrelated and related to the diversity of different components of the microbiome. Microbiome composition was less important as a driver of PSF variability but had stronger effects on mean intra- and interspecific PSF. Also consistent with our hypotheses, alfalfa abundance was the main driver of the intraspecific mean and variance of PSF but had only weak effects on interspecific PSF. Contrary to our predictions, plant diversity increased intraspecific variation in PSF and had a negative effect on interspecific mean PSF but otherwise had limited effects, suggesting that plant diversity may not lead to pathogen dilution under field conditions.

As hypothesized, variation in intra- and interspecific PSF was not correlated. This lack of correlation was maintained regardless of whether alfalfa was included in these community-level estimates or if we included only closely related species (i.e. legumes), suggesting strong differentiation in microbiome responses within and among species irrespective of how closely related they are. Indeed, only one legume species, *Vicia americana*, exhibited PSF that was significantly correlated with any other species or cultivar, and those correlations were with one grass and one legume species. Consequently, we suggest that although specialized pathogens may be important for alfalfa, breeding efforts have led to microbiome differentiation (Annicchiarico et al., 2015) and that these specialized pathogens are not shared with other legume species (Semchenko et al., 2022).

The only shared response between intraspecific and interspecific PSF was an increase in variability with more diverse oomycete

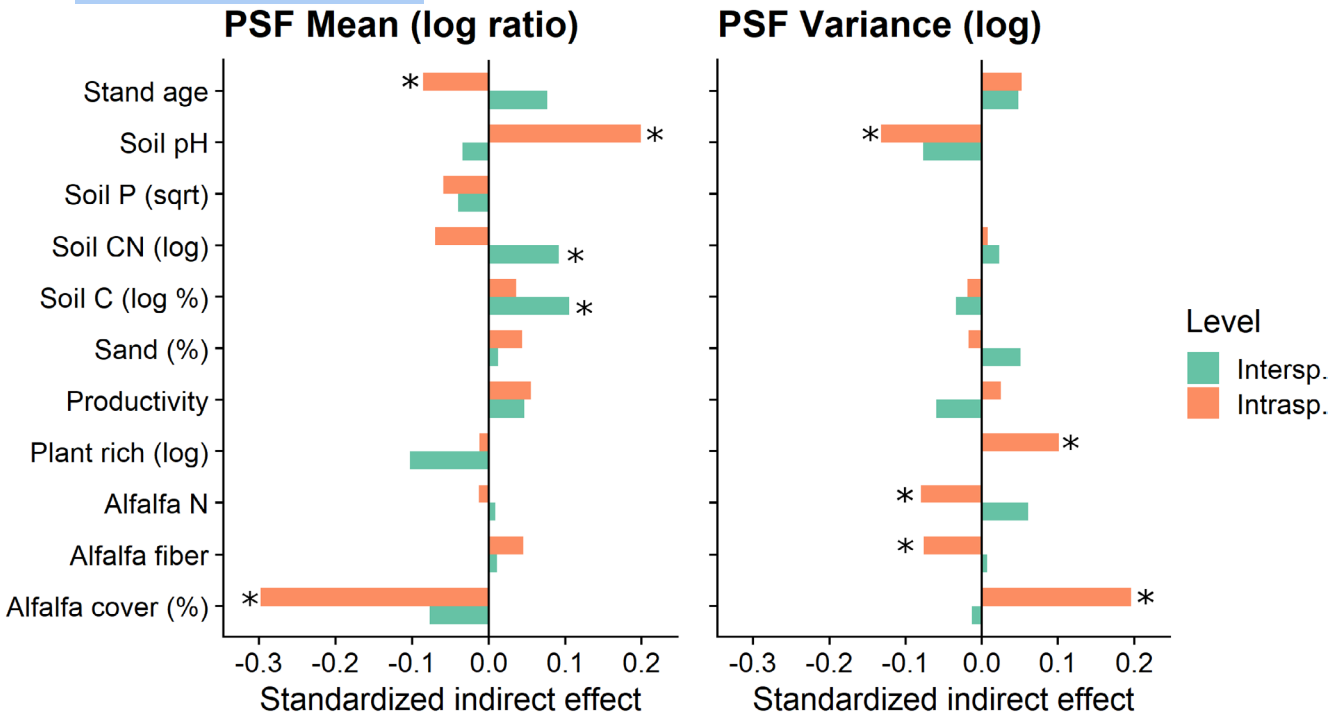

FIGURE 4 Indirect effects of the plant community and soil properties on plant–soil feedback. Shown are standardized indirect effects of each field-collected plant and soil variable on the intraspecific and interspecific means and variances in plant–soil feedback (PSF). Asterisks denote significant effects at $p < 0.05$.

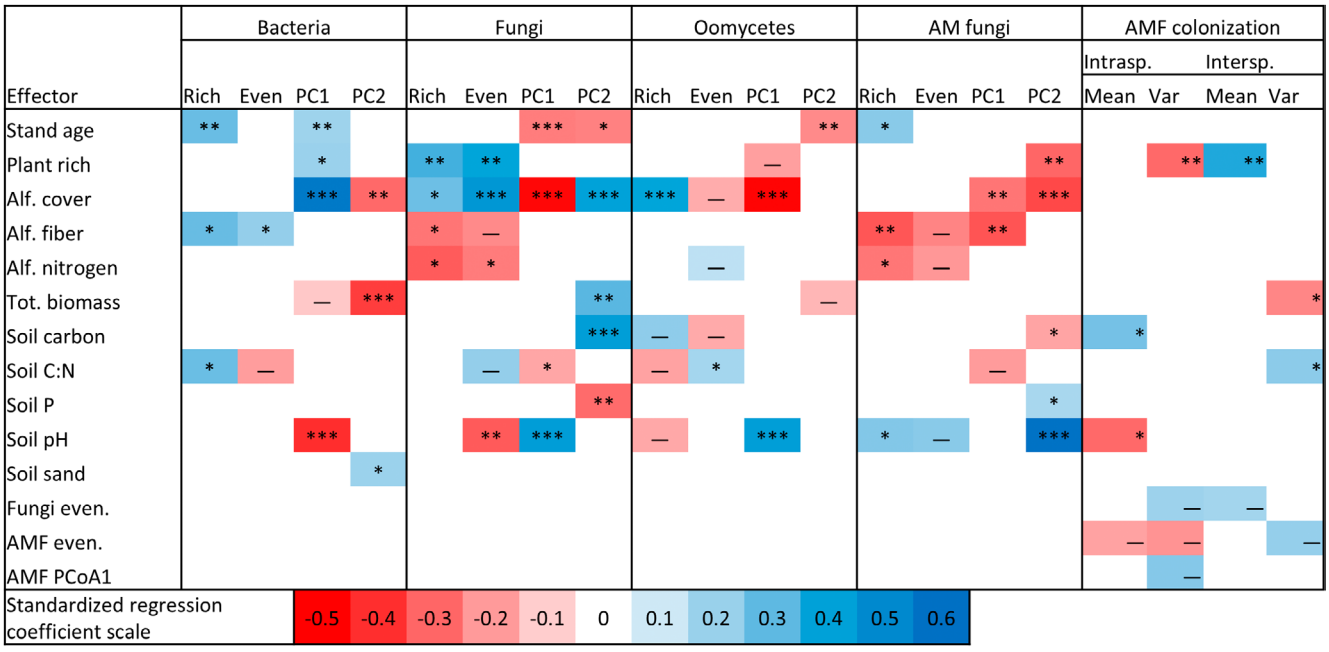

FIGURE 5 The effects of stand age, soil characteristics and the plant community on soil bacteria, fungi, oomycetes and AMF as indicated in the SEM. Predictors are shown in the first column with subsequent columns representing different aspects of the microbiome (ASV richness, ASV evenness and the scores for the first two axes from principal coordinates analysis) or AMF colonization from the growth chamber experiment (intraspecific and interspecific means and variance in AMF colonization). Colours correlate with the strength of the standardized regression coefficient, with negative relationships in red and positive relationships in blue. Symbols within cells denote the significance level of the relationship: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$, — = $p > 0.05$. SEM fit was modified to remove relationships that increased AIC scores and to add parameters suggested through modification indices. Only relationships retained in the final model are shown. Final model fit was assessed using Bollen–Stine bootstrapping ($p = 0.976$).

communities, consistent with the importance of oomycete pathogens in alfalfa agroecosystems (Abbas et al., 2022) and recent studies showing the importance of oomycetes for PSF (Burrill et al., 2023; Domínguez-Begines et al., 2021). Microbiome studies tend to be biased towards bacteria and fungi, and only recently have plant–soil feedback studies begun to include other microbial taxa when sequencing (De Long et al., 2023). By affecting variation within and among species similarly, oomycetes may have strong effects on diversity-productivity relationships. Further biotic effects may also have been missed due to our use of a small amount of inoculum, which could represent an incomplete sampling of the soil biome. Consequently, understanding of PSF will only come once we can enumerate all aspects of the microbiome.

Other than oomycetes, fungal evenness and specific bacterial communities enhanced intra- and interspecific variability, respectively. As fungal evenness also caused negative intraspecific PSF, increases in rarer fungal pathogens likely caused negative PSF, but only for some cultivars, potentially by including more virulent pathogens or increasing co-infection by multiple pathogens (Fang et al., 2021). The role of bacteria community composition in interspecific PSF variation is more difficult to explain as there was no main effect on interspecific mean PSF, and ecological roles are not well known for most taxa. The presence of certain rhizobacteria affecting legume species could be important (Andrews & Andrews, 2017) as could host-specific bacterial pathogens (Barrett et al., 2009) or some combination of these two mechanisms or others.

Neither the composition nor diversity of AMF affected PSF variability within or among species, although AMF abundances may still be important for PSF. This suggests that specificity in arbuscular mycorrhizas may be limited in this system, although AM community structure may be more important elsewhere (d'Entremont & Kivlin, 2023). Nonetheless, mean alfalfa AMF colonization reduced intraspecific variation in PSF. As AMF colonization is related to propagule densities (Jansa et al., 2009), this suggests that increases in alfalfa-associated AMF minimize soil-driven fitness differences among genotypes. AMF have been shown to increase the competitive abilities of rare species in grasslands (Bennett & Cahill, 2016) and to reduce competitive imbalances in other systems (Wagg et al., 2011) and are likely to do the same in genetically diverse alfalfa populations. Interestingly, increased AMF colonization of alfalfa was also associated with more positive interspecific PSF, suggesting that AMF abundances can broadly increase productivity in alfalfa agroecosystems, consistent with field trials (Pellegrino et al., 2022) and other grassland systems (Bennett et al., 2020).

Alfalfa abundance was the strongest indirect driver of PSF in alfalfa systems, strengthening negative PSF and increasing variability among cultivars. This is in line with our hypothesis and previous work in this system showing PSF to be more negative in monoculture than mixed stands (Awodele & Bennett, 2022), where monocultures had more alfalfa (Table S1). Most of these effects were driven by increases in oomycete and fungal richness in denser alfalfa stands. Increases in intraspecific PSF variability with fungal and oomycete diversity are not surprising as alfalfa suffers from multiple oomycete and fungal pathogens (Samac et al., 2015) and more diverse microbiomes should be more likely to host one of these disease agents (Rohr et al., 2020). The positive effect of alfalfa abundance on microbiome diversity suggests that increased host densities allow for more specific oomycete and fungal species to persist (Mommer et al., 2018). Alfalfa densities did not have strong effects on interspecific PSF or variability among species, indicating that host density has less consistent effects on more generalist microbes. Indeed, the multitude of pathways by which alfalfa cover was linked to the interspecific mean and variance (Figure S3) shows both promotion and inhibition of multiple important microbial groups.

Contrary to our hypotheses, plant diversity had weak effects on PSF, likely because plant diversity had relatively limited effects on the diversity of the soil microbiome. Many of the soil-mediated positive effects of plant diversity on productivity are expected via pathogen dilution (Wang et al., 2023), yet the links between plant and microbial diversity are typically weak (Liu et al., 2020) and were only found for fungi in this study. Significant dilution effects may be unlikely, but the change in fungal diversity was sufficient to increase variability among alfalfa cultivars, suggesting that increasing cultivar diversity in diverse stands may stabilize alfalfa productivity. Plant diversity effects may also operate through other mechanisms to affect PSF such as declines in root-feeding nematodes (Bennett et al., 2020) and increases in nutrient availability (Furey & Tilman, 2021); however, neither of these mechanisms were measured here.

Diverse plant communities have been shown to culture more beneficial microbiomes in experimental settings (Bennett et al., 2020), yet we found that diverse alfalfa stands promoted negative PSF across the community. Other studies have found negative diversity effects on community-wide PSF in natural grasslands (Lundell et al., 2022), and recent models have shown that increasing species richness of natural systems is unlikely to promote ecosystem function as many species are rare or weedy (Dee et al., 2023). Previous work in this system found less negative PSF in mixed than in monoculture stands (Awodele & Bennett, 2022). This appears contradictory to the current findings; however, maximum species richness was similar between monoculture and mixture stands (Table S1) and increasing weedy species in seeded monocultures may not contribute to a positive biodiversity effect (Dee et al., 2023).

Beyond our hypotheses, two other main results stand out. First, mean intraspecific PSF was more associated with the diversity of different microbial groups, but interspecific mean PSF was more associated with specific microbiome compositions. Increasing microbiome diversity may increase the odds that a strong alfalfa pathogen or mutualist would be present or abundant, whereas there may be certain generalist microbes that promote plant growth (Semchenko et al., 2022). Second, soil pH was the second strongest driver of alfalfa PSF. More basic soils had less negative and less variable PSF, indicating a less antagonistic microbiome for all cultivars. Acidic soils are known to inhibit mutualism functioning (Varga & Kytöviita, 2010), yet the minimum pH in this study (6.2) should not limit rhizobia or mycorrhiza functioning in alfalfa (El-Kherbawy et al., 1989) and overall AMF colonization declined when inoculated with more basic soils.

Rather, the effects appear to be driven by changes in both bacteria and fungi; however, more testing would be required to identify a specific mechanism.

5 | CONCLUSIONS

Plant dominance, rather than plant diversity, was the primary driver of both the mean and variability in PSF within the studied agroecosystem and is likely key in natural systems as well, although more explicit tests of this hypothesis are required. Importantly, variability within and among species was most tightly associated with soil microbiome diversity, whereas mean PSF was largely driven by multiple components of the microbiome. Consequently, any factor affecting either microbial diversity or composition is likely to affect PSF and its role in community assembly. As multiple aspects of the microbiome, especially oomycetes, caused changes in PSF within and among species, future work should explore other important soil biota (e.g. nematodes or protists), or potentially all soil eukaryotes, to complete our understanding of PSF and its role within plant communities. Nonetheless, the observed independence of intraspecific and intraspecific variability in PSF suggests that increasing genetic and species diversity should increase primary productivity via dilution of responses to pathogens, if not dilution of pathogen themselves. This may be especially important in agroecosystems where monocropping is common and disease prevalence can cause severe declines in productivity, but where plant species and genetic diversity can be easily manipulated.

AUTHOR CONTRIBUTIONS

Jonathan A. Bennett conceived of the study, obtained funding, conducted the fieldwork, conducted the data analysis, wrote and revised the manuscript. Stephen O. Awodele conducted the study and commented on the manuscript; Luke Bainard devised the molecular methods and edited the manuscript; Julien Tremblay conducted the bioinformatics and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data and code can be found on figshare https://doi.org/10.6084/m9.figshare.26305315. The raw sequencing data have been deposited under BioProject accession PRJNA1125521 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1125521).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Descriptive summary of field variables collected from alfalfa monoculture and mixture stands.

Table S2: Correlations among components of the soil microbiome.

Table S3: Direct pathways among variables in the structural equation model.

Figure S1: Biplots showing variation in bacterial, fungal, AMF, and oomycete composition as a function of whether the site was seeded to mixture or monoculture.

Figure S2: First stage structural equation model.

Figure S3: Path diagrams showing the indirect effects of (A) alfalfa cover (focal species abundance) and (B) plant species richness on the mean and variance of interspecific and intraspecific PSF mediated by the soil microbiome.

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