

Patterns of microbiome-mediated plant-soil feedback intensity in organic versus conventional farm soils

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Abstract

Plants influence soil properties, impacting subsequent growth via plant-soil feedback (PSF). We collected rhizosphere soils from common ragweed (*Ambrosia artemisiifolia*) across 24 organic and conventional farms to create microbial inoculants. The inoculants were used to condition soils in greenhouse pots that were re-planted with new seedlings to simulate a PSF cycle. We observed negative PSF in 21 out of 24 farming system treatments. However, more intense PSF effects were observed in systems with microbiomes derived from conventional farm soils. Higher soil bacterial diversity was correlated with less negative PSF in systems with microbiomes from organic farm soils. Most of the plant growth-suppressive microbiomes were derived from conventional farms, whereas microbiomes that had weakly negative, neutral, or positive effects on plant growth originated largely from organic farm soils. Network analysis revealed distinctly different bacterial interactions between samples with high versus low PSF, as well as between organic and conventional farm soils. Our findings suggest that field management practices structure the rhizosphere microbiome of *A. artemisiifolia*, potentially allowing the bacterial microbiome to intensify plant-soil feedback. Microbiome properties, like microbial diversity, could play a role in influencing the trajectory of these feedback processes.

Introduction

Interactions in the rhizosphere between plants and soil microorganisms influence biogeochemical cycling and ecosystem functions of plant systems that help shape the growth of successive plant populations (Kardol et al., 2007). Plant-soil feedback (PSF), defined as plant-induced changes to soil physical, chemical, and biological properties that influence plant growth, can result in positive, neutral, or negative effects on successive generations of plants. Previous research has demonstrated that the strength and direction of PSF involving the soil microbiome often reflect plant-specific accumulation of beneficial or harmful microorganisms in the rhizosphere (Klironomos, 2002; Miki et al., 2010; van der Putten, 2017). In addition, processes mediated by microorganisms, such as decomposition and mineralization, can have indirect effects on plant growth and performance. Many knowledge gaps still exist regarding the biotic and abiotic drivers of PSF and how to assess and quantify such complex above- and belowground interactions.

There is growing evidence that negative PSF contributes to the maintenance of plant diversity and plant invasiveness (Bever, Westover & Antonovics, 1997; Klironomos, 2002; Teste et al., 2017; Thakur et al., 2021), including providing benefits for more diverse intercropping agricultural systems compared with monocultures (Marques et al., 2020; Wang et al., 2021; Kama et al., 2024). Empirical studies suggest that microbe-mediated negative PSFs result from accumulation of deleterious soil microbes such as host-specific pathogens (Kardol et al., 2007), and even generalist pathogens may induce host-specific effects (Semchenko et al., 2022). However, mechanisms underlying PSFs are difficult to study in isolation because the soil microbiome is a complex network of organisms. Observed PSFs are likely to reflect a combination of antagonistic and synergistic effects and therefore are treated as a “black box” in most PSF studies without knowing the composition of the soil biota associated with the effects.

Utilizing PSFs to control invasive or weedy plant species is particularly relevant in organic farming systems that have limited options for weed control. Soil microbiomes may differ between organic and conventional systems because management practices (e.g., tillage, application of fertilizers and pesticides, and crop rotations) can affect soil microbial activity, diversity, and community composition (Alguacil, 2008; Ge et al., 2013; Xue et al., 2013; Hartmann et al., 2015). The resulting differences in soil biota can result in contrasting PSF effects. Reduced-tillage methods in organically managed systems are often characterized as supporting greater microbial activity and diversity relative to conventionally managed soils (Alguacil, 2008; Ge et al., 2013; Xue et al., 2013; Hartmann et al., 2015). In general, organic cropping systems are expected to have more positive PSFs than conventional cropping systems (van der Putten et al., 2016; Johnson et al., 2017).

The effects of farm management system on PSFs could be system-specific. For example, plant debris from farms relying on reduced tillage can harbor pathogens and increase pathogen outbreaks, reducing plant health (van der Putten et al., 2016). Moreover, the effects of soil microbiota on plants can vary across plant species and functional groups, and between crops and weeds. More positive effects of soil biota on plant growth were reported in organic soils compared with conventional soils for wheat (*Triticum aestivum*) and redroot pigweed (*Amaranthus retroflexus*) but not for wild oat (*Avena fatua*) (Johnson et al., 2017). PSF strength has been shown to vary based on plant functional characteristics, typically having more negative effects in fast-growing plants (Xi et al., 2021), suggesting that weeds and fast-growing annual crops are good candidates for exploring negative PSFs. Further research is required to understand how weed species are affected by PSF, to identify the key soil microbial species driving these processes in conventional and organic agricultural systems, and to explore how PSF may be harnessed in integrated weed management strategies. While the role of soil fungi in driving PSF is well-documented, the extent to which soil bacteria influence feedback intensity remains less understood.

We investigated the taxonomic and functional diversity of soil bacteria from organic versus conventional farming systems to examine how differences in these microbiomes influence PSF for a model weed species, *Ambrosia artemisiifolia* L. (common ragweed) [Asteraceae]. This herbaceous annual species is an important agricultural weed that is native to North America and rapidly spreading across Europe and Asia (Sun & Roderick, 2019). *Ambrosia artemisiifolia* is also known for releasing large amounts of allergenic pollen that contributes to public health problems (Ziska et al., 2003). We hypothesized that (1) the composition and diversity of soil bacteria will influence PSF effects on *A. artemisiifolia*, and that (2) the strength or direction of PSF would differ between treatments with microbiomes from organically- versus conventionally-managed farms. We expect that soils derived from organic farming systems harbor greater bacterial diversity and with potentially more beneficial microbiota relative to conventional farming systems because organic farms typically rely on practices that promote soil health, such as cover cropping and adding organic matter inputs (Hartmann et al., 2015; Ling et al., 2016). To test the hypotheses, we conducted a two-stage (pre-feedback and feedback stages) greenhouse study in which *A. artemisiifolia* was grown in soil conditioned with microbiome inoculants derived from 13 organic versus 11 conventional farms.

Materials and Methods

Overview. Plant-soil feedbacks (PSFs) in this study are defined as changes to the biotic and abiotic properties of soil that subsequently have positive, neutral, or negative effects on plant performance. We grew *Ambrosia artemisiifolia* L. (common ragweed) for two planting cycles (generations), referred to as the pre-feedback and feedback stages. In the pre-feedback stage, *A. artemisiifolia* plants were grown in sterile potting mix inoculated with microbiomes derived from organic or conventional farm soils collected across New York State, United States. In the feedback stage, new *A. artemisiifolia* plants were grown in the same soil used for the pre-feedback stage. The net effect of the soil microbiome on *A. artemisiifolia* was assessed by measuring plant biomass differences between sterilized versus non-sterilized inoculants. The direction and magnitude of PSF were determined by comparing microbiome effects between the pre-feedback stage and the feedback stage. Microbial DNA sequencing, supervised learning, and network analysis were used to help explain the observed microbiome effects and PSF.

Farms and field sampling. We collected *A. artemisiifolia* plants and rhizosphere soil samples from 24 different farms (within a 100 km radius of 43°13'55.2"N, 76°06'51.5"W), comprising 11 conventionally managed farms and 13 organically managed farms. Organically managed farms adhere to the United States Department of Agriculture (USDA) or the Northeast Organic Farming Association of New York (NOFA-NY) organic standards. Conventionally managed farms are permitted to use conventional pesticides and fertilizers, while organic farms are restricted to products approved by the Organic Materials Review Institute (OMRI). Other differences between conventional and organic farms may include management practices such as the use of cover crops, carbon amendments to enhance soil organic matter levels, tillage frequency and intensity, or weeding methods.

For each sample, surface litter around an *A. artemisiifolia* plant was removed. The plant and the top 15 cm of soil were collected with a shovel cleaned with 70% (v/v) ethanol to avoid any contamination. Bulk soils were removed and the rhizosphere soil adhering to the plant was shaken into a bag. This process was repeated for 15 to 20 randomly selected *A. artemisiifolia* plants at each site to gather 2 L of rhizosphere soil. Soils were stored overnight at 4°C and then frozen at – 15°C to preserve the soil microbiota (MacKay & Kotanen, 2008; Ram et al., 2017).

Soil inoculum preparation. To prepare the inoculum, 350 g of each collected soil sample was mixed into 1,400 mL autoclaved water in a Nalgene bottle that was shaken on a reciprocating shaker for one hour at 200 rpm. The slurry was then filtered through four layers of sterilized cheese cloth to allow bacteria and fungi to pass into the inoculum. The inoculum was separated into 100 mL aliquots.

Pre-feedback stage: the first cycle of planting. This experiment was conducted at the Cornell University Kenneth Post Laboratory, a controlled-environment greenhouse facility (Ithaca, New York, USA). An array of twenty PL2000 400 W HPS lamps (P.L. Light Systems Inc., Beamsville, Ontario, Canada) was used to deliver supplemental lighting daily for 16 hours. The bench surfaces were sterilized by spraying and wiping with commercial bleach (8% sodium hypochlorite). Customized mesh cages were built using

bamboo sticks and two layers of mesh on each bench to minimize cross contamination from air circulation.

A mixture of sterilized potting mix (Lambert LM-111 potting mix; Lambert Peat Moss, Inc., Riviere-Ouelle, Quebec, Canada) and sterile water were added to 300 15 cm-diameter pots. One hundred surface-sterilized *A. artemisiifolia* seeds were sown into each pot at 0.5 cm depth. The seeds were obtained from Roundstone Native Seed Company (Upton, Kentucky, USA). Each pot received 100 mL of either soil inoculum (treatment), autoclaved inoculum (control 1), or autoclaved water (control 2). Six replicate pots were included for each combination of sampled farm and sterilization (24 farms × 3 sterilization treatments × 6 replicates = 432 pots). Seedlings were thinned to three similar sized seedlings per pot upon emergence of the first set of true leaves. Pots were positioned in the greenhouse in a randomized block design. The pots were rearranged randomly in the greenhouse every week to avoid variation in microclimate effects. Plants were watered every two days with water filtered by a 0.1 µm filter (Model SP122; Sawyer Products, Inc., Safety Harbor, Florida, USA) for four weeks, then plants were watered every other day for three weeks with the same filtered water. Fertilizer was not used in this experiment. At the end of the experiment (eight weeks), aboveground plant biomass was harvested, oven-dried for three days at 60°C and weighed. Subsamples of the conditioned soil mixes were collected and stored following the same soil storage protocol described above.

Soil feedback stage: the second cycle of planting. Pots from the soil conditioning (pre-feedback) stage were prepared for reuse by removing any remaining plant material while maintaining 75% of the soil volume from the first planting. Double-autoclaved LM-111 potting mix was used to replenish the missing 25% soil volume. We adhered to other PSF studies in maintaining the conditions of the pre-feedback soils to capture the residual variation and minimize type I error (Reinhart & Rinella, 2016). One hundred surface-sterilized *A. artemisiifolia* seeds were added to each pot and seedlings were thinned to three plants of similar sizes and ages. The watering schedule was the same as in the soil conditioning stage and no fertilizer was applied. Aboveground plant biomass was harvested using the same protocol and weighed at the end of eight weeks. Subsamples of the feedback soil mixes were collected and stored following the same protocol described above.

Calculating PSFs. The net effect of soil microbiota on *A. artemisiifolia* was determined as the difference in plant biomass between the non-sterile treatment and the average of the two sterile controls. This net effect of soil microbiota is referred to as G1 for the pre-feedback stage and G2 for the feedback stage. For each replicate, G1 and G2 were calculated as (treatment biomass – average control biomass) / average control biomass.

Differences in the net effect of soil microbiota between the two planting cycles represent PSF effects. For each replicate, PSF was calculated as G2 – G1. This approach is conceptually similar to traditional "home-away" soil comparisons in other PSF studies. However, it utilizes a sterile control as the baseline rather than a home soil. By comparing plant performance to sterile counterparts both before and after conditioning, we can more precisely isolate the microbial contribution to plant growth at each stage.

Previous work has taken a similar approach in comparing sterile to treated soils after the conditioning phase (Idbella et al., 2024), yet our study includes an additional control before conditioning. This allows us to account for baseline microbial differences, ensuring that the observed effects are specifically driven by plant-induced soil legacy rather than the inherent properties of the initial microbial communities.

DNA extraction, PCR amplification, Illumina sequencing and sequence processing. The PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, California, USA) was used to extract soil DNA from 0.1 g of the stored soil samples from different experimental stages (initial inoculum, conditioned soil, and feedback soil). The 16S rRNA region V3–V4 was amplified by 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). The 16S amplicons were then prepared for and sequenced by the Illumina MiSeq platform at the Cornell Genomics Facility (Ithaca, New York, USA) following the protocol of Howard et al., (2017). The pipeline from the Brazilian Microbiome Project (<http://www.brmicrobiome.org/>) was modified to process the raw sequences. Briefly, Mothur v. 1.36 (Schloss et al., 2009) was used to merge paired-end sequences, trim off primers, remove singletons and classify sequences. The VSEARCH package in QIIME v. 1.9.1 (Caporaso et al., 2010) was used to cluster *de novo* operational taxonomic units (OTUs; 97% similarity) and remove chimeric reads. Taxonomy was assigned by matching representative sequences against the Greengenes v13_8 database.

Illumina sequencing of the feedback-stage rhizosphere soil 16S rRNA recovered a total of 8,627,505 sequences from the 144 inoculated samples with 349 to 128,613 sequences per sample (mean = 59,913). After denoising and removing chimeric and nonbacterial sequences, we obtained 2,240,225 high-quality sequences with 7 to 40,475 sequences per sample (mean = 15,557). *De novo* OTU picking generated 33,146 unique OTUs at the 97% similarity threshold.

We determined the optimal sampling depth through examination of exploratory rarefaction curves of observed species plotted against sampling depth for both the selected 10% OTUs (see supervised ordination) and the entire dataset. Our selected 10% OTU table was rarefied to 1,800 sequences per sample while the whole OTU table was rarefied to 5,000 sequences per sample. Both rarefactions removed twelve samples with fewer reads. Alpha diversity metrics (Shannon diversity index, Chao 1 index, and observed OTUs) and the Bray-Curtis distance matrix were computed within QIIME by `alpha_rarefaction.py` and `beta_diversity.py`, respectively.

Supervised ordination. Supervised classification is a well-developed method for building prediction models in which information about predefined groups (labels) is used to identify discriminatory features. In the case of microbiome studies, the predefined groups are the studied microbiome feature or function and the discriminatory features are the identified taxa. Supervised learning of sequencing data has been widely used in human gut microbiome research to select features (OTUs) for classification and microbiome functions prediction on physiology or disease state, diet, genotype, etc. (Knights, Costello & Knight, 2011; Goodrich et al., 2014).

Microbial community profiling based on soil samples has demonstrated the large number of species in the soil microbiome (Mendes et al., 2011; Panke-Buisse et al., 2015). Although microbiome functions are often performed by a consortium of microorganisms, only a small proportion of the species captured by such surveys will be related to the studied function. Therefore, the true signal could be masked by the vast background noise, which makes it difficult to establish links between host traits and the associated microbiome. In the context of our study, the true signal is an association with PSF. Although PSF is likely to be driven by a complex network of soil organisms (Mariotte et al., 2018), the high number of OTUs identified from environmental samples makes it difficult to identify true PSF-responding signals. Reducing the number of OTUs included in an analysis will not only increase prediction accuracy but facilitate meaningful interpretation of the data (Knights, Costello & Knight, 2011).

We used filter-based feature selection (Knights, Costello & Knight, 2011) to create a subset of our data for analysis. Specifically, we selected the top 10% of microbiota (3,314 OTUs) that are most correlated with PSF in *A. artemisiifolia* systems, according to the absolute value of the Pearson correlation between OTU presence and PSF (Python code on GitHub). Random Forest and Support Vector Regression (SVR) models were built by QIIME2 sample-classifier plugin (Bokulich et al., 2018) on both the full and the reduced datasets to evaluate the correlation-based filter. The filtered OTU set is primarily used for ordination rather than for identifying mechanistic drivers of PSF. As a result, the machine learning component currently functions more as a dimensionality reduction step rather than as a tool for mechanistic insight.

A Bray-Curtis distance matrix was calculated based on relative abundances within the reduced (filtered) community and was used to generate a Principal Coordinates Analysis (PCoA) to visualize the relation between OTUs and suppressive feedbacks. For better visualization, we colored each sample by PSF strength. Dissimilarities in treatment group samples are indicated using PERMANOVA analysis.

Network analysis. Network analysis techniques have been widely used to explore interactions within large datasets through mathematical, statistical, and structural properties of the various entities being studied. The entities are referred to as nodes, and the connections between nodes are referred to as edges. Microbial community sequencing datasets are often complex and large, but network analysis is used less frequently in this field relative to some other fields. Network analysis is a good approach for microbial sequencing data because it takes full advantage of large datasets. Network analysis may provide in-depth insights not only into the direct effects of individual microbes on plants, but also into microbial community structure and interactions between microbes.

We performed co-occurrence and co-exclusion network analysis based on the method by Barberán et al., (2012). To remove poorly represented OTUs and reduce network complexity, OTUs that did not occur at least once in all the samples with > 1% relative abundance were removed. Next, relative abundance matrices were generated separately for the high and low PSF groups or the conventional and organic groups. Within each relative abundance matrix (OTU table), Spearman's rank correlation coefficient was calculated independently between all OTUs. This information was used to create a network in which

nodes represented OTUs at 97% identity and links (edges) between OTUs were assigned when the Spearman's correlation coefficient (ρ) was > 0.5 and statistically significant ($P < 0.01$). The edge tables were imported into Cytoscape (layout) and Gephi (fruchterman reingold) for visualization. Modularity analysis was run in Gephi using the "greedy modularity optimization mode". Different groups had different sample sizes, which is an issue in network analysis because more samples or more diversity will generally result in a weaker network. To make the network analysis results comparable between different groups, we used groups with fewer samples as the benchmark. Groups with higher sample numbers were randomly subsampled to produce the same number of samples.

Statistical analyses. We determined the significance of differences in response variables (plant biomass, PSF strength and diversity indices) across fixed factors (treatments and PSF strength groups) using one-way analysis of variance (ANOVA). We used the student's t-test to determine whether response variables differ significantly between microbiomes from conventional versus organic farms. All statistical analyses were performed in R. P values < 0.05 were considered significant, and P values < 0.01 were noted.

Results

Soil microbiome influence on *A. artemisiifolia* growth

Plant-soil feedback refers to the difference between plant biomass in the two planting cycles, represented in this study as pre-feedback and feedback stages. Figure 1a shows stronger suppression of *A. artemisiifolia* aboveground growth in the feedback stage relative to the pre-feedback stage ($t = -3.84566$, $P = 0.00009$). In the pre-feedback stage, plant growth suppression was similar between soils containing microbiomes from conventional farms and soils containing microbiomes from organic farms (Fig. 1a). However, in the feedback stage, there was greater plant growth suppression in soils containing microbiomes from conventional farms ($58.59\% \pm 1.49\%$) compared with organic farms ($43.15\% \pm 2.26\%$; Fig. 1a). This finding indicates stronger PSF ($G2 - G1$) responses in soils with microbiomes from conventional farms ($t = -5.45063$, $P < 0.00001$).

Figure 1b presents data for individual farms rather than as the mean of all farms combined into organic or conventional farming treatment. In the pre-feedback stage (i.e., first planting cycle), microbiomes from 15 of the 24 farm sites reduced plant growth. The relative growth inhibition ranged from -11.30% to 55.75% . There was strong negative PSF, with 21 out of 24 sets of microbiomes having a greater suppressive effect on plant growth in the feedback stage relative to the pre-feedback stage.

Microbiome diversity and composition

A reduced table of 3,314 OTUs was obtained after applying the Pearson correlation-based filter (data not shown). To evaluate the effects of this filtering step, Random Forest and Support Vector Regression (SVR) models were built with both the unfiltered and the reduced OTU tables. Both models showed

better performance (ability to predict PSF effects) when built with the reduced OTU table (Fig. S1). When predicted PSF values were graphed against observed PSF values, R^2 values were approximately 1.5 times higher and slopes much closer to 1 when the reduced OTU table was used to generate predictions (Table S1). Because analyses based on the reduced OTU table would be more likely to reveal microbial contributions to PSF, the reduced OTU table was used for the alpha and beta diversity analyses presented below.

We used the Shannon diversity index to quantify alpha diversity of the microbial OTUs in the samples. More positive (less negative) PSF responses were positively correlated with Shannon diversity only when the inoculated microbiomes were derived from organic farm sites ($R^2 = 0.4702$, $p < 0.001$, Fig. 2a). We observed similar trends for other alpha diversity indices, including Chao1 and observed OTUs (Fig. S1). No significant correlation between PSF responses and the Shannon diversity index was observed for plant-soil systems that received microbiome inoculants derived from conventional farm soils ($R^2 = 0.008757$, $p = 0.48$, Fig. 2b). Similarly, PSF responses were not correlated with the Chao1 and observed OTU diversity indices in plant-soil systems with conventional farm-derived microbiomes (Fig. S2). Alpha diversity indices were generally similar between systems with organic farm-derived microbiomes and systems with conventional farm-derived microbiomes (Fig. S3).

Beta diversity is the difference or similarity between communities from different environments. We used the Bray-Curtis dissimilarity index to statistically quantify the compositional dissimilarity of OTUs across the samples. Bray-Curtis is based on the difference in taxonomic abundance profiles from different samples. Twelve samples were removed after rarefaction because of low sequence numbers. PCoA was based on the Bray-Curtis distance matrix of the top 10% of PSF-correlated OTUs.

Among microbiomes derived from organic farms, PCoA showed distinctly different microbiomes between samples with strongly negative PSF and samples with less negative, neutral, or positive PSF (Fig. 3a). However, this trend was not observed among microbiomes derived from conventional farms (Fig. 3b). Biplot analysis on the organic samples identified the top 10 OTUs that contribute most to the overall sample variances. Among these top bacteria taxa, *Opitutales* and *Myxococcales* were strong drivers of negative PSF. Other top taxa that contribute to the separation of samples along the direction of PSF include *Rhizobiales*, *Sphingomonadales*, *Rhodobacterales*, *Actinomycetales* and *Ellin329* (Fig. 3a).

Network analysis

We used a standard network analysis based on cooccurrences and mutual exclusions (Barberán et al., 2012). The 144 samples were grouped by PSF effects into three groups: 36 in “high”, 72 in “medium”, and 36 in “low”. The network analysis revealed markedly different topologies between the high and low PSF groups. For the high PSF group, the network had 99 nodes and 176 edges (79 negative correlations) and the modularity was 3.984 with 28 modules (Fig. 4a, Table 1). For the low PSF group, the network had 103 nodes and 295 edges (134 negative correlations) and the modularity was 3.895 with 18 modules (Fig. 4b, Table 1). Although the high PSF and low PSF networks had similar numbers of nodes, the low

PSF network had more positive and negative edges, representing more interactions. The two networks shared 12 edges. While both low PSF and high PSF groups had four major modules of more than five nodes, the low PSF modules had larger sizes (average of 21 nodes compared with 15.5 nodes).

Similarly, the organic and conventional samples showed different network topologies. The network of the conventional samples had 72 nodes and 141 edges, and the modularity was 20.687 with 19 modules (Fig. 4c, Table 1). The network of the organic samples had 101 nodes and 206 edges, and the modularity was 2.719 with 25 modules (Fig. 4d, Table 1). Thus, organic farm samples showed a more complex interaction network with more diverse OTUs compared with the conventional farm samples. Moreover, the modular size distribution analysis showed that with a smaller number of modules, the organic network had six major modules of more than five nodes, compared with two major modules in the conventional network (Table 1). On average, the two major modules in the conventional network were twice as large as the major modules in the organic network. In the organic network, there were more interactions across taxonomic groups (phyla) than within groups.

Table 1. Summary of network characteristics for high plant-soil feedback (PSF), low PSF, conventional farm, and organic farm groups.

Group	Nodes	Edges	Modularity	Modules	Major Modules*
High PSF	99	176	3.984	28	4
Low PSF	103	295	3.895	18	4
Conventional	72	141	20.687	19	2
Organic	101	206	2.719	25	6

* Modules with more than 5 nodes.

Discussion

In this study, we inoculated sterile potting mix with 24 sets of *A. artemisiifolia* rhizosphere microbiomes from 13 organic and 11 conventional farm sites. We found that most of the microbiomes reduced *A. artemisiifolia* growth in the first planting cycle, known as the pre-feedback stage. All 24 microbiome inoculation treatments led to significant plant growth inhibition compared to inoculation with sterile controls. In the second planting cycle, or feedback stage, a greater intensity of negative PSF (increased plant growth suppression) was associated with inoculants from conventionally managed farms compared with organically managed farms. This result likely indicates that initial differences in the microbiome from the organic versus conventional farm soils led to different changes to the microbiome during the first cycle of *A. artemisiifolia* growth (Pernilla Brinkman et al., 2010; Mariotte et al., 2018). The negative feedback response is consistent with the results of Semchenko et al., (2018), who found that the growth of 14 temperate grass species was on average 2.8 times greater in sterilized soil, compared

with the microbiome-mediated treatment. MacKay & Kotanen (2008) also showed negative PSF in *A. artemisiifolia* plants grown with soil microbiomes from their native habitats.

Negative effects of soil biota on plants have been demonstrated across many plant species with several potential mechanisms hypothesized (MacKay & Kotanen, 2008; Maron et al., 2016). These effects may involve the accumulation of pathogenic or saprotrophic species and complex plant-microbe and microbe-microbe interactions (Mills & Bever, 1998; Bever, Broadhurst & Thrall, 2013; Maron et al., 2016). For example, negative PSF was stronger in plant communities with more abundant plant species compared with rare species, which suggests a regulating role of the soil microbiome in modifying plant community structure. However, the identities and diversity of microbes responsible for PSF effects are not characterized in most studies. There are restrictive financial costs of including multiple soil biological taxonomic groups that could influence PSF, such as soil bacteria, fungi, viruses, arthropods, and other invertebrates. While amplicon sequencing was developed to provide an affordable method to analyze large sample sizes within a specific taxonomic group (e.g. fungi or bacteria), it is still cost prohibitive for many researchers to include multiple groups in a single study because of the cumulative costs of additional primer sets and independent sequencing runs.

In our study, we focused on soil bacteria to assess patterns that potentially reveal a regulating role of the soil microbiome on PSF (Fig. 2a). As the Shannon diversity index increased in the plant-soil systems harboring microbiomes from organic farms, there was a correlative shift from negative to positive PSF. In contrast, a theoretical model proposed by Miki et al., (2010) predicted that increased diversity of microbial decomposers would lead to a shift from positive to negative PSF by buffering nutrient pool size, enabling increased plant coexistence and diversity. We did not observe any association between bacterial diversity and PSF strength in systems with conventional farm soil microbiomes. This contrast between the organic and conventional farm soils could reflect differences in the composition and function of microbiomes resulting from these contrasting management approaches. Greater microbial diversity was reported in organic farming systems, compared with conventional farming, in a 20-year experiment in Switzerland (Hartmann et al., 2015). Soil microbial diversity can be positively associated with plant productivity. For example, crop rotation increased total soil bacterial diversity and promoted cucumber plant growth through microbially-mediated PSF (Zhou, Liu & Wu, 2017). Another possibility for explaining different dynamics is increased tillage in organic systems compared with conventional, which was found to result in either increased or decreased strength of negative PSFs in different studies (Seipel et al., 2019; Menalled et al., 2020). Additionally, adding biochar was shown to increase both bacterial diversity and alleviate negative PSF (Wang et al., 2020), so it is possible that diversity in organic systems is tied to organic matter levels that modulate PSF. These possibilities highlight a number of key differences between organic and conventional management that may shape plant-microbiome interactions that ultimately determine weed persistence. Importantly, the observation that increased diversity may buffer negative PSFs in organic soils currently lacks a defined mechanistic basis, as 16S rRNA community profiling does not provide direct functional insights. Consequently, while the correlation between diversity and PSF intensity is a compelling trend, further research is required to identify the

underlying mechanisms and evaluate their potential for actionable applications in agricultural management.

Additionally, some studies indicate that microbial diversity and PSF are associated with specific microbial groups and not the whole microbiome of the rhizosphere. A study by Semchenko et al., (2018) showed that negative PSF in temperate grassland species was likely to be positively correlated with the species richness of putative fungal pathogens but negatively correlated with the relative abundance and richness of beneficial arbuscular mycorrhizal fungi (AMF). The negative PSF result was apparent only when the researchers focused on a subset of the microbiome, specifically the arbuscular mycorrhizal fungi (AMF). Like our study of PSF systems derived from organic farm microbiomes (Fig. 2a), the researchers found that greater microbial diversity shifted the PSF response from more negative to less negative or positive. In another study, Bever, Broadhurst & Thrall (2013) reported a correlation between PSF and microbial diversity of a nitrogen-fixing functional guild (rhizobial bacteria). They found that the presence of one acacia species increased rhizobia bacteria diversity, resulting in reduced growth of a second acacia species. The greater diversity in rhizome nodules of the second species likely led to more competitive interactions among rhizobia bacteria that reduced the symbiotic benefit to the host and thereby decreased plant productivity. These two studies highlight the need to examine subsets of the rhizosphere microbiome to observe associations between diversity and PSF. This represents both a future direction and a limitation for the current study, which does not incorporate fungi such as AMF. This is especially noteworthy given that fungal diversity can influence PSFs (Semchenko et al., 2018). Therefore, it is possible that part of the observed PSF is due to other soil biological interactions that were not examined in this study, such as fungal, viral or invertebrate associations. Nevertheless, the bacterial dynamics in this study highlight important links between agricultural management and PSFs that present an avenue for further research.

Subsets of the microbiome that potentially impact PSF could be detected through analyses of beta diversity. Variables that determine how microbiomes are similar or different across samples and treatments could provide insight into biotic regulation of PSF. In our study, we used the Bray-Curtis dissimilarity index to assess whether PSF strength was related to composition of the rhizosphere microbiome. The ordination indicated that the high PSF and low PSF groups harbored different microbiomes. A comprehensive study of PSF in 37 plant species showed that plant group (grasses, forbs, and legumes) significantly affected bacterial community composition, while the alpha diversity was not affected (Hannula et al., 2020). The differences in bacterial community composition involved bacterial phyla *Actinobacteria*, *Planctomycetes* and subphyla *Alphaproteobacteria* and *Deltaproteobacteria*. These subsets of the microbiome could play an important role in mediating PSF.

In our study, we used a Bray-Curtis biplot to identify the OTUs (microbial taxa) most associated with the separation between the low and high PSF microbiomes. Two of the OTUs driving the separation of the low PSF samples from other samples are N-fixing, free-living diazotrophic bacteria in the *Rhizobiales* order and another OTU influencing the separation is a *Sphingomonas* bacterium that could also possess N-fixing functions (Fig. 3a). The study conducted by Bever, Broadhurst & Thrall (2013) showed that

rhizobial species and their diversity could play an important role in PSF for two legume species. Nitrogen fixation has been reported in taxonomically different *Sphingomonas* bacteria (Videira et al., 2009). Also, inoculation of *Sphingomonas* sp. Cra20 promoted growth in *Arabidopsis thaliana*. In addition to highlighting the effects of N-fixing bacteria, our ordination showed that two species of the *Myxococcales* order contributed to the separation of strongly negative PSF samples (Fig. 3a). The *Myxococcales* are functionally important as producers of natural products, such as antibiotics and other secondary metabolites involved in plant-microbe and microbe-microbe interactions (Hoffmann et al., 2018). In fact, soil is an important reservoir for microbial natural products that include antibiotics or herbicides (Duke et al., 2000; Charlop-Powers et al., 2016). The natural products produced by *Myxococcales* species could have promoted negative PSF in this study. However, PCoA biplots are not a test of statistical significance, and their inclusion is primarily for visual representation and hypothesis generation rather than yielding conclusive results. The top 10% of OTUs associated with PSFs were used to visually highlight differences, and do not constitute direct evidence in the same way as statistical tests on alpha and beta diversity.

Alpha diversity and beta diversity measurements have become standard tools to analyze the increasing large datasets generated by high-throughput DNA sequencing. However, network analysis techniques may provide additional information about complex and diverse microbial communities. Network analysis techniques that investigate direct or indirect interactions between taxa may help decipher functional roles or identify environmental niches of uncultured microorganisms (Faust & Raes, 2012). The number and degree of species co-occurrences and mutual exclusions could reflect the level of cooperation or energy and material exchange events within the community (Garcia & Kao-Kniffin, 2019). Therefore, network analysis may provide deeper insights into ecosystem function than simple information about the presence or absence of specific taxa.

Our network analysis showed that the network created from organic samples had more nodes and edges than the network created from conventional samples, indicating a higher level of interactions in the organic network (Fig. 4). In addition, the organic network had more major modules and lower modularity than the conventional network, which suggests more intergroup interactions in the organic system soils. Together, these results suggest the organic farm microbiomes had more complicated microbial interactions, a finding consistent with other studies. For example, Ling et al., (2016) reported that microbiomes from organic fertilized soils had more complex interactions, including an almost doubled number of modules relative to soils that received synthetic fertilizer. A long-term trial showed that organic amendments increased bacterial network complexity, regardless of mineral fertilization (Schmid et al., 2018). A recent study on conventional, no-till, and organic wheat farms reported that organic farms had more complex fungal interaction networks (Banerjee et al., 2019). It has also been shown that PSFs shift network dynamics based on conditioning time (Huberty et al., 2022), raising the possibility that differences in PSF dynamics between conventional and organic farms may contribute to observed network differences.

Associations between microbial species within phyla often indicate functionally interrelated species or species with similar ecological niches, while inter-phyla co-occurrence suggests collaborations between functionally different species in the community (Barberán et al., 2012). In this study, the higher level of intergroup microbial association in organic farm samples indicates there might be more intergroup collaboration in the organic microbiomes, relative to the conventional microbiomes. Microbial interactions including collaboration are believed to play an important role in microbiome functions such as extracellular enzyme activity and nutrient cycling (Garcia & Kao-Kniffin, 2018). Consequently, changes in microbial interactions due to selection pressures or farming practices could alter microbiome function. For example, Garcia & Kao-Kniffin (2019) showed that 10 generations of selection for delayed plant flowering increased positive associations between microbial taxa, resulting in significantly stronger nitrogen-mineralization extracellular enzyme activities. A recent meta-analysis showed that intercropping increases potential microbial extracellular enzyme activity by 13% on average (Curtright & Tiemann, 2021). Microbial interactions could also be associated with other widely studied microbial functions related to plant performance, such as siderophore production and biofilm formation. Siderophore-producing bacteria can promote plant growth by suppressing plant pathogens and increasing iron availability (Pahari et al., 2018). Secreted siderophores could be shared between microbes and therefore their production may be a regulating mechanism for microbial collaboration, or for competition against other species that do not share the same siderophore system (Kramer, Özkaya & Kümmerli, 2020). Thus, different microbial interaction networks in organic and conventional farm soils could result in distinctly different microbial behaviors and functions.

In all the networks, the dominant bacterial phyla were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Acidobacteria*, which are widely distributed across a range of ecosystems. *Proteobacteria* contribute to organic matter decomposition by synthesizing many kinds of glycosyl hydrolases, such as cellulases, chitinases, xylanases and amylases. They produce oligosaccharides and aromatic alcohols that can be used as carbon resources by other bacteria, such as *Acidobacteria*. *Acidobacteria* are associated with low soil pH. The less abundant phyla in the network analysis tend to form their specific niches. For example, *Verrucomicrobia* as abundant and ubiquitous species in soils (Bergmann et al., 2011), was suggested to share a specific and undefined niche (Barberán et al., 2012).

Microbiome sequencing of agricultural soils can provide novel insights into PSF effects, which could potentially be managed to promote crop performance. In this study, we show that microbial effects on plant growth did not differ significantly between organic and conventional farm samples until the second generation of planting. In the second generation (feedback phase), conventional-farm microbiomes had more negative effects on plant growth, suggesting PSF differences between the farming systems. A correlation between soil microbial diversity and PSF occurred only in plant-soil systems that harbored microbiomes from organic farm soils. Adding to this trend, we discovered that organic farm systems had more intense and complex microbial interactions that signified stronger intergroup interactions. Altogether, our results suggest that organic farming systems cultivate soil microbiomes that have distinct functional and taxonomic traits that are likely responsible for buffering against the extreme effects of negative PSF.

Declarations

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Author Contributions:

LC and JKK conceived of and designed the study. LC set up and managed the experiment and also collected and processed samples. LC and JKK analyzed the data. LC and JKK wrote the initial manuscript draft. CG assisted with sequence data processing and submission to the NCBI repository. LC, JKK, CG, and AD contributed to editing and approved the final manuscript.

Data Availability

The raw sequencing data for the 16S rRNA gene of the soil microbial community were deposited into the National Center for Biotechnology Information Sequence Read Archive under BioProject ID: KJIL00000000. All other data generated during this study are available from the corresponding author upon request.

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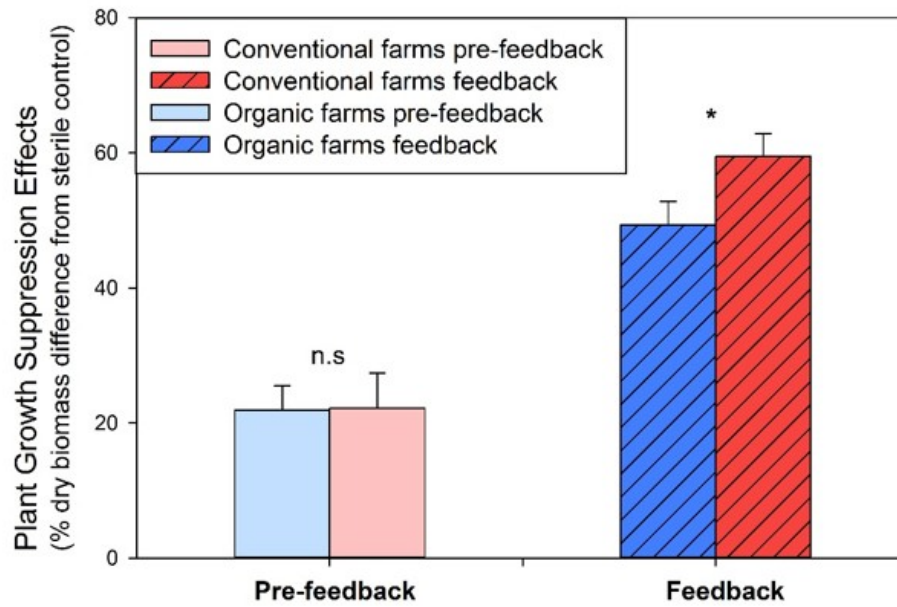
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Figures

a)



b)

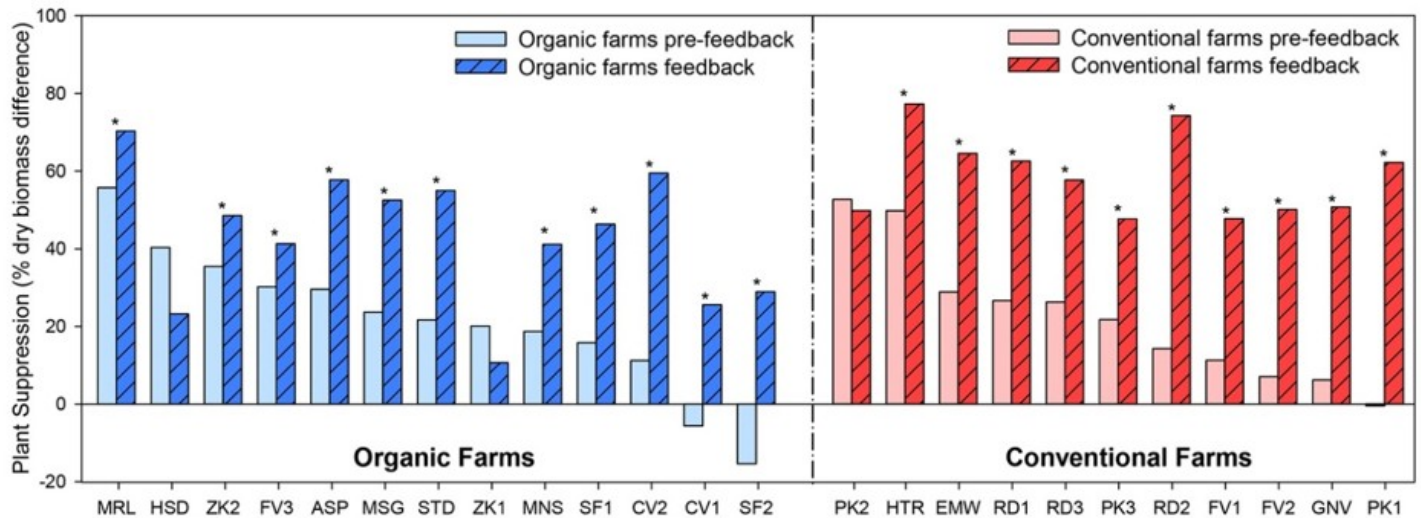
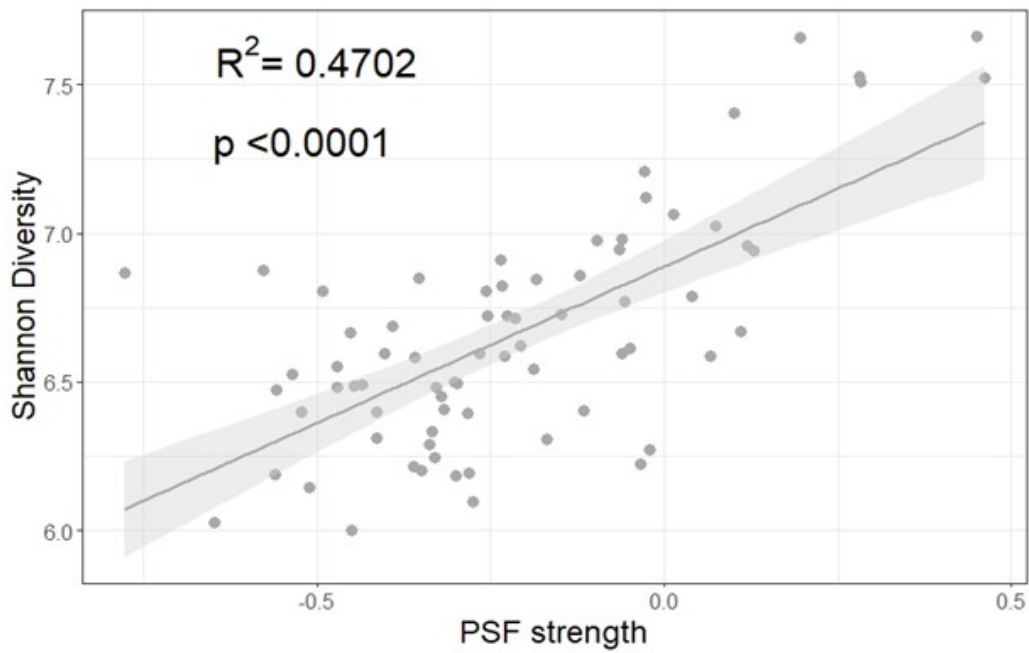


Figure 1

Growth suppression of *Ambrosia artemisiifolia* by soil microbes. Data represent percentage differences in plant dry biomass between the microbiome-inoculated treatment and autoclaved control. Plant growth-suppressive effects are shown for organic versus conventional farms during the pre-feedback and feedback stages. **a)** Columns represent mean $\pm$ SE across all organic or conventional farms. Significant differences between organic and conventional farms ($P < 0.01$) are labeled with an asterisk. n.s., not significant. **b)** Plant growth suppression is shown for individual organic ($n = 13$) and conventional farms ($n = 11$). Significant differences between pre-feedback and feedback stages ($P < 0.01$) are labeled with an asterisk.

a)

a) organic farm soil samples



b) conventional farm soil samples

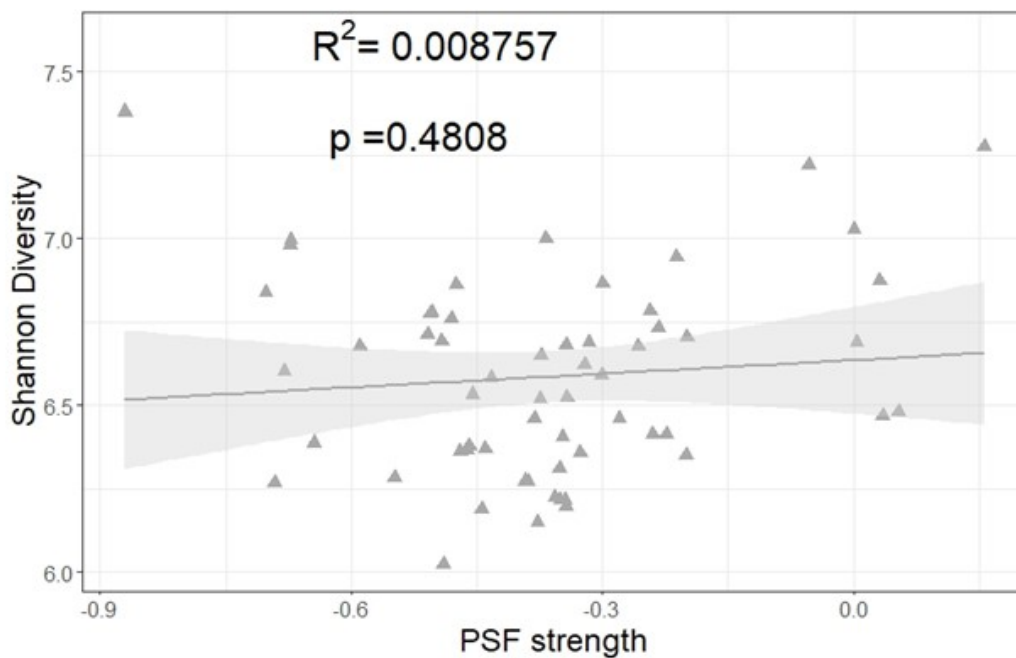
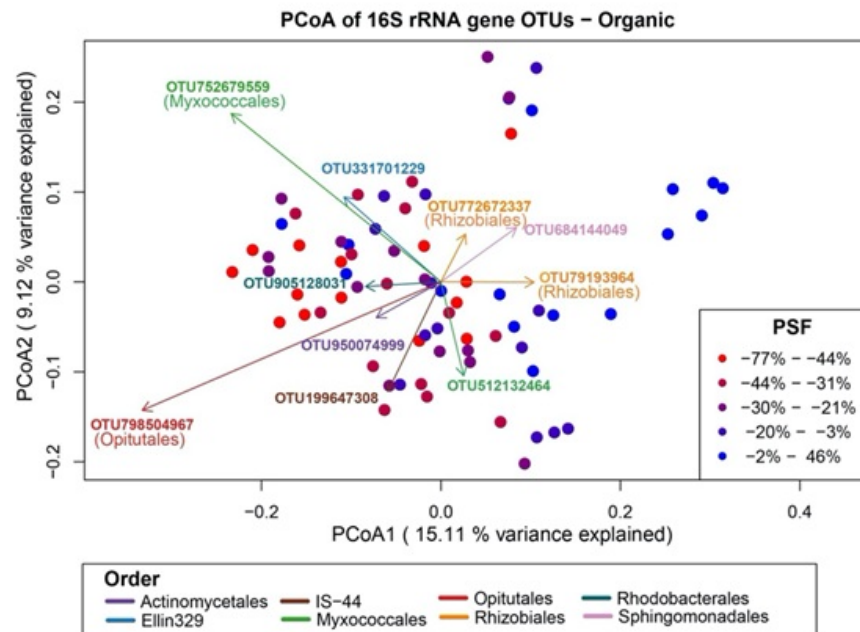


Figure 2

A strong positive correlation exists between Shannon diversity (top 10% of OTUs correlated with plant-soil feedback) and the spectrum of negative to positive plant-soil feedback responses in *A. artemisiifolia* plants in **a)** organic farm soil samples but not in **b)** conventional farm soil samples.

3A)



3B)

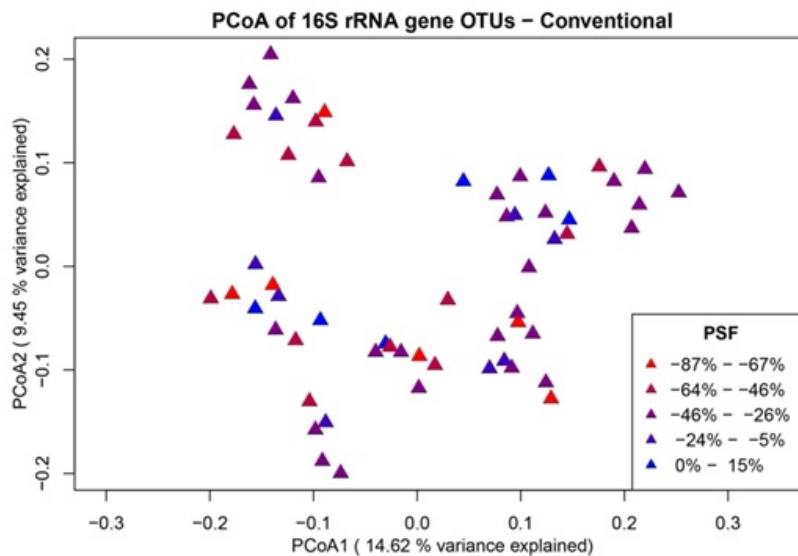


Figure 3

Principal Coordinates Analysis (PCoA) of Bray Curtis distance matrix generated from 16S rRNA sequence data from soils of the plant-soil feedback stage. The top 10% of OTUs correlated with PSF (3,314 OTUs) were used to calculate the distance matrix. The plant-soil systems with microbiomes from organic farm soils (n = 72) are shown in **(a)** with biplots indicating the top 10 bacterial OTUs contributing to the spread of the variance. The plant-soil systems with microbiomes from conventional farm soils (n =

59) are shown in **(b)**. Samples are colored by PSF strength (red indicating strongly negative PSF, purple indicating weakly negative PSF, and blue indicating neutral or positive PSF). The highest and lowest PSF groups in the organic plant-soil systems showed significance ($P = 0.003$) with 999 permutations using PERMANOVA.

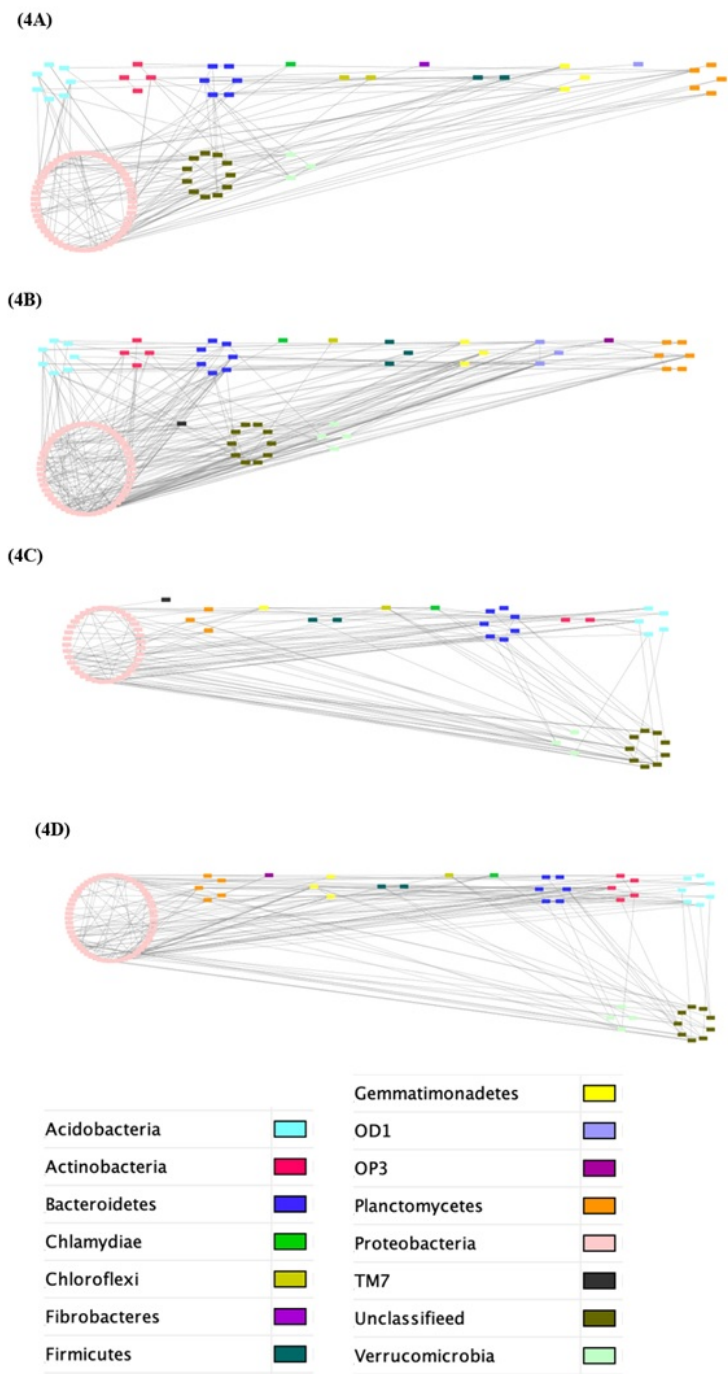


Figure 4

OTU networks based on correlation analysis in **(A)** high plant-soil feedback (PSF), **(B)** low PSF, **(C)** conventional, and **(D)** organic samples. Edges represent strong (Spearman's correlation coefficient, $\rho > 0.5$) and significant ($P < 0.01$) correlations. Nodes are colored and grouped by taxonomic affiliation.

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