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RESEARCH

Soil microbiome response to integrated perennial weed management practices in semi-arid organic cropping systems

Jed O. Eberly · Asa Hurd · Jenni W. Hammontree · Patrick M. Carr

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Abstract Creeping perennial weeds are difficult to manage on organic farms in semi-arid regions of the northern Great Plains. Integrated weed management practices that combine biological, cultural, and mechanical controls can improve management of these weeds, but little is known about the soil microbial response to these practices. Our work investigated the soil microbiome response to contrasting, 4-year crop sequences with standard and reduced tillage. The crop sequences included a range of crop competition phases from high (three years of alfalfa, *Medicago sativa* L.) to low (two years of continuous fallow), within the longer 4-year period, with intermediate levels of crop competition between those two extremes. Soil samples were collected, and bacterial 16S and fungal ITS amplicon sequencing was performed. Differences in alpha diversity were not significant ($p > 0.05$) between tillage methods. Across all six locations, bacterial alpha diversity was negatively correlated with soil organic matter ($R = -0.37$, $p < 0.001$) while fungal alpha diversity was positively correlated ($R = 0.17$, $p = 0.043$). Bacterial community composition was not affected by crop sequence or tillage treatment. Fungal community composition was affected by crop sequence ($p = 0.00163$) and tillage ($p = 0.02$). The fungal genera *Neosetophoma*, *Boeremia*, and *Paraphoma* were 10 – 35-fold more abundant in continuous alfalfa compared to the mean abundance in the other crop sequences. Reduced tillage led to a 40% reduction in the fungal genus *Fusarium*, which contains many plant pathogen species. These results suggest that diversified crop sequences and altered tillage methods have minimal impact on bacterial communities, but fungal communities are sensitive to these management changes.

Keywords Microbiome · Integrated weed management · Organic · Soil health

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J. O. Eberly (✉) · A. Hurd · J. W. Hammontree · P. M. Carr
Central Agricultural Research Center, Montana State University, Moccasin, MT, USA
e-mail: jed.eberly@montana.edu

Introduction

Management of perennial weeds is a major challenge to the long-term sustainability of organic grain production in the United States northern Great Plains (USNGP) and is one of the greatest problems growers are facing (Tautges et al. 2017). While conventional growers can use a variety of synthetic herbicides for perennial weed control, organic growers have few effective products that are approved for use in organic systems. Failure to control these weeds can result in substantial economic loss to organic farmers. Grain yield reduction resulting from infestations of perennial creeping weeds such as Canada thistle

can approach 70% (McLennan et al. 1991). Perennial weeds are difficult to manage in organic systems due to their physiological characteristics such as extensive root systems with carbohydrate reserves that enable them to resist a variety of management approaches (Orloff et al. 2018).

Integrated weed management (IWM) strategies that incorporate annual and perennial crops, tillage, and biological controls are promising approaches for dealing with challenging perennial weed species, but few studies have systematically evaluated the effect of these practices on the soil microbiome. Implementing IWM strategies for control of perennial weeds can have unintended consequences on soil health and microbial community structure and function. Organic systems rely on tillage as a method of weed control due to the lack of herbicides approved for organic use. Tillage has negative effects on soil, including loss of soil structure and moisture and increased risk of soil erosion (Carr et al. 2019). Tillage can also decrease soil C and N (Grandy et al. 2006; Crystal-Ornelas et al. 2021). In addition to effects on soil chemical and physical parameters, tillage impacts the structure and function of the soil microbiome which can lead to a reduction in microbial species richness compared to reduced tillage or no-till systems (Lupwayi et al. 1998; Legrand et al. 2018). Conventional tillage is particularly detrimental to fungi due to hyphal breakage and can decrease the abundance of arbuscular mycorrhizal fungi (AMF) relative to reduced tillage (Rosner et al. 2018; Somenahally et al. 2018). Soil microbial functions can also be altered by tillage. Research has shown minimum tillage systems have higher abundances of genes involved in N cycling compared to conventional tillage (Kaurin et al. 2018; Liu et al. 2022; Zhang et al. 2022).

In addition to tillage, specific crop sequences can also drive changes in microbial communities. Studies have shown higher microbial diversity and greater fungal biomass under wheat (*Triticum aestivum* L.) following a legume compared to continuous wheat or wheat fallow (Lupwayi et al. 1998; Somenahally et al. 2018). Previously we found a 50% reduction in fungal alpha diversity in wheat following safflower compared to wheat following wheat (Eberly et al. 2024) and work in the Pacific Northwest of the US found a reduction in bacterial and fungal abundance when camelina was introduced into a wheat-fallow rotation (Hansen et al. 2020). Other studies have shown specific cover crop species can affect microbial community composition and function (Finney et al. 2017; Schmidt et al. 2018) and increased crop diversity can enhance fungal community richness (Guzman et al. 2021). These findings indicate that specific crops included in a rotation can alter microbial community structure and function. More work is needed to elucidate these interactions, particularly in response to IWM in organic systems.

Nutrient management can also alter fungal community composition. High inputs of mineral fertilizer can decrease fungal alpha diversity (Ma et al. 2021). In contrast, organic manure application can increase fungal diversity (Ding et al. 2017). Research has also shown that soil organic matter is positively correlated with fungal alpha diversity (Li et al. 2021a, b) which suggests management practices that increase organic matter will be beneficial for soil fungi.

Microorganisms do not function in isolation but form complex associations. One approach to evaluating these complex ecosystem interactions is through network analysis which has been applied to wide variety of interactions including food webs and ecosystems (Krause et al. 2003; Barberan et al. 2012). Co-occurrence network analysis, which considers associations between taxa, can reveal insights into community dynamics beyond community composition metrics (Gao et al. 2022). This approach also enables identification of keystone taxa, which are taxa that are highly connected within the network and have a disproportional influence on the community (Banerjee et al. 2019).

There are a lack of integrated studies evaluating perennial weed control and soil health responses to management practices in organic systems even though this has been identified as a topic of interest to organic growers (Osterholz et al. 2020). The objectives of this work were to evaluate the effects of IWM strategies for creeping perennial weeds on microbial richness and community composition across diverse ecosystems in the USNGP. We hypothesized that IWM approaches that incorporate perennial crops or reduced tillage would have greater fungal diversity while bacterial diversity would not be affected. We further hypothesized that these practices would improve both bacterial and fungal network connectivity compared to conventional tillage practices with only annual crops.

Methods

Site description

Field trials were performed at certified organic sites in North Dakota, Montana, and Washington, USA. In North Dakota, trials were located at the Dale E. Herman Research Arboretum near Absaraka, ND (46.988319, −97.352284) and an on-farm location near Turtle Lake, ND (47.401058, −100.879758). These sites were characterized by differences in annual precipitation (177–765 mm) and soil properties (Table S1). Trials in Montana were located at the Montana State University (MSU) Central Agricultural Research Center (CARC), Moccasin, MT (47.062422, −109.947653), and on-farm locations in Chinook, MT (48.755550, −109.353601) and Conrad, MT (48.206262, −111.476927). In Washington the trial was located at the Washington State University, Palouse Field Station, Pullman, WA (46.76242, −117.19837). Soil properties for each study location can be found in Supplemental Tables S1 and S2.

Experimental design

At each location, 4-year crop sequences were established. Sequences were 1) three years of alfalfa (*Medicago sativa* L.) followed by hard red spring wheat (HRSW), 2) lentils (*Lens culinaris Medik*) followed by HRSW and field pea (*Pisum sativum* L.), and 3) a nine species cool season cover crop polyculture followed by HRSW. At the Moccasin, MT location a more extensive design was used which included barley (*Hordeum vulgare* L.), winter pea (*Pisum sativum* L.), winter triticale (*Triticosecale Wittm.*), foxtail millet (*Setaria italica* L.), hemp (*Cannabis sativa* L.), or fallow in rotation with HRSW. Crop sequences were designed to represent a spectrum of crop growth habits, competitive ability, and soil disturbance (Larson et al. 2024). Crop sequences are shown in Table 1 and additional study details are described in (Gramig et al. 2024; Larson et al. 2024). In 2021, field pea was seeded in sequences S3 and S4 at the Moccasin, MT location because of failure to establish yellow-flowered sweet clover in 2020.

Weed control consisted of a combination of cultural (crop sequence) and mechanical methods (tillage). Standard tillage consisted of inverting the soil using a tandem disk with four 46-cm diameter notched disk blades at a depth of 11 to 13-cm. At the Moccasin, MT location a reduced tillage treatment was also incorporated where sheep grazing was used to terminate nongrain crops and suppress weeds. Livestock grazing was a component of the reduced-tillage system in this study because previous studies have proven benefits in tillage reduction, improved soil quality, and nutrient cycling with livestock integration (Larson et al. 2024). Treatments were arranged in a randomized complete block design, with four blocks. At Moccasin, a 2 × 8 factorial design was used to account for both treatments (crop sequence x tillage). Plots within block

Table 1 Crop sequence grown from 2019 to 2022. Crop sequences 1, 4, and 7 were common to all locations while the remaining treatments were grown at Moccasin, MT

Crop Sequence	2019	2020	2021	2022
S1	Barley + Alfalfa	Alfalfa	Alfalfa	HRSW
S2*	HRSW[a]	Spring triticale	Foxtail millet	HRSW
S3*	HRSW	Lentil/yellow sweet clover	Field pea	HRSW
S4	Lentil	HRSW/yellow sweet clover	Field pea	HRSW
S5*	HRSW	Barley	Field pea	HRSW
S6*	Lentil/yellow sweet clover	Field pea	Hemp	HRSW
S7	CC[b]	HRSW	CC	HRSW
S8	HRSW	Fallow	Fallow	HRSW

[a]HRSW: Hard red spring wheat

[b]CC: 9-species cover crop polyculture consisting of barley, emmer, oats, field pea, lentil, faba bean, yellow mustard, radish, and turnip

*Indicates treatments planted at the Moccasin, MT location only

were separated by a 3-m buffer, with 15-m wide alleys between each block.

Soil sampling

Soil samples (0- to 15-cm depth) were collected in triplicate from each plot to establish base-line soil chemistry prior to planting, in 2019. Samples were transported on ice to the lab where cores from each plot were homogenized and a 10-g subsample was stored at −80 °C for microbial community analysis. The remaining soil was dried at 50 °C, passed through a 2 mm sieve, and submitted to Ward Laboratories, Inc. (Kearny, NE) for pH, nitrate–N, Mehlich-III P, cation exchange capacity, K, Ca, Mg, Na, and S. Soil properties are shown in Table S1. Additional samples were collected in the spring of 2022 as described above, to evaluate changes in the microbial community due to IWM practices that were implemented over the 4 years of the study.

Microbial biomass was determined by chloroform fumigation incubation (Voroney and Paul 1984). Soils were dried 3 days at 50 °C and passed through a 5 mm sieve. A 250 ml beaker with 50 g soil was placed in a desiccator along with a beaker containing 10 ml chloroform. Vacuum was applied until the chloroform began to boil and the soil was incubated 24 h at 25 °C. Unfumigated controls were prepared without the addition of chloroform. Following fumigation, DI water was added to bring soil to 55% (w/w) water holding capacity and soil was transferred to 1 L jars and a screw cap 50 ml vial containing 10 ml 1 M NaOH was placed in the jar to act as a CO_2 trap. A vial containing 10 ml water was added to maintain humidity. Jars were sealed and incubated 10 days at 25 °C. Vials with NaOH were removed and sealed immediately and CO_2 trapped was determined using a colorimetric method where 1 ml 1.5 M $BaCl_2$ was added as a color indicator and samples were titrated with 1 M HCl until color changed. Volume of HCl added was recorded and used to calculate the amount of CO_2 trapped.

Sequencing

DNA was extracted from a 250 mg soil subsample using a Qiagen DNeasy PowerSoil Pro DNA Isolation Kit (Qiagen Inc., Germantown, MD, USA). Purified DNA was submitted to Integrated Microbiome Resource (Dalhousie University, Halifax, NS) for amplicon sequencing of the bacterial 16S V6-V8 region using the 969F (5'-ACGCGHNRAACCT TACC-3') and 1406R (5'-ACGGGCRGTGWG TRCAA-3') primers and fungal ITS2 region using ITS86F (5'-GTGAATCATCGAATCTTTGAA-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') primers as previously described (Comeau et al. 2011). Sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) using a 2 × 250 PE kit.

Bioinformatics

Quality control and processing of sequence reads was performed in R 4.1.3 (Team 2023). Demultiplexed bacterial sequences were trimmed by 5 bases at the start position and truncated using a Q score threshold of 30 which resulted forward and reverse reads being truncated to 250 bp and 200 bp respectively. Fungal sequences were trimmed by 5 bases at the start position and sequences were not truncated but a minimum length threshold of 50 bp was used. Following quality filtering and chimera removal, reads were assembled into error-corrected amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). Taxonomic assignment of bacteria was performed using a naive Bayes classifier pre-trained on the weighted Silva 138.1 database with a 99% identity threshold while fungal taxonomic assignment was performed with the UNITE 9.0 database (Quast et al. 2013; Nilsson et al. 2019; Abarenkov et al. 2023). Statistical analyses and data visualization were performed using phyloseq, MicroViz, ggplot2, ggpubr, vegan, and ggstatsplot packages (McMurdie and Holmes 2013; Wickham 2016; Okansen et al. 2019; Barnett et al. 2021; Patil 2021). Alpha diversity analysis was performed using the Shannon diversity index (H), a measure of community diversity which accounts for richness and evenness (Shannon and Weaver 1998). A nonparametric Kruskal–Wallis test was used to compare changes in alpha diversity between treatments at each location. This test was chosen since it does not assume a normal distribution. Relationships between microbial biomass carbon, NH_3-N, organic matter, and alpha diversity were explored using Pearson's correlation.

Differences in microbial communities between treatments were visualized using unconstrained principal coordinate analysis (PCoA) of the Bray–Curtis disimilarity matrix with ellipses drawn at the 95% confidence interval. Venn diagrams were used to compare the number of common and unique bacterial and fungal taxa among the three crop sequences common to all locations in the final year of the study.

Random forest models were used to identify taxa with the largest contribution to differences in microbial communities between year, crop, or tillage intensity. Differential abundance testing performed using Random Forest with Kruskal–Wallis rank sum tests as implemented in the microeco R package (Liu et al. 2021) with ntrees set at 10,000 and 100 bootstrap tests. The resulting ROC (Receiver Operator Characteristic) curve and area under the curve (AUC) values for the model are shown in Fig. S6.

Co-occurrence network analysis

To explore the impacts of IWM on microbial co-occurrence patterns, networks were constructed using the SPIEC-EASI (Sparse Inverse Covariance Estimation for Ecological Association Inference) method implemented in the SpiecEasi (v1.1.3) (Kurtz et al. 2015) and microeco (Liu et al. 2021) R packages was used to construct the microbial co-occurrence network associations for the 3 treatments common to all locations using the relative abundance of ASVs. SPIEC-EASI was chosen because it is more robust when dealing with compositional data and sparse networks (Kurtz et al. 2015). For network inference, only ASVs with a relative abundance greater than 0.025% were used and only associations with correlation coefficients greater than 0.7 and FDR corrected p-values less than 0.05 were included in networks. Networks were visualized in Gephi v.0.10.1 (Bastian et al. 2009). Graphical model inference was performed by the Meinshausen and Buehlmann neighborhood selection method (Kurtz et al. 2015). Keystone taxa were defined as those with a hub score greater than 0.1 within the co-occurrence network associations as previously described (He et al. 2024). Hubs are defined as network nodes that act as connectors between other nodes (Salavaty et al. 2020) and these connections are important for ecological network robustness (Dominguez-Garcia and Kefi 2024).

Results and discussion

Microbial biomass was significantly correlated ($R = 0.67$, $p < 0.001$) with soil organic matter (Fig. 1) and was higher ($p < 0.001$, FDR corrected) at Turtle Lake, ND compared to other locations with a mean concentration of 612 CO_2-C Kg^{-1} soil (Fig. S1). This was likely due in part to higher soil N compared to other locations with similar precipitation (Table S1). Microbial biomass was also higher at Moccasin, MT compared to the other locations in MT and Absaraka, ND. No difference in microbial biomass was observed between crop sequence or tillage treatments (data not shown). Crop sequences did not affect microbial biomass, and this contrasts with other reports that have shown changes in microbial biomass in response to crop rotations. These changes in microbial biomass have been associated with the quantity and diversity of crop residues (Moore et al. 2000). Given the relatively low crop biomass yields in dryland organic systems (Gramig et al. 2024; Larson et al. 2024), and drought conditions experienced in some years (Table S1), it is likely that the different crop sequences in our study did not produce sufficient biomass to affect microbial biomass concentrations.

Bacterial and fungal community profiling was performed to investigate the effects of crop sequence and tillage intensity on community composition in organic systems. A total of 6,895,063 16S and 10,113,566 ITS reads were generated. Subsequent ASV assignment and filtering resulted in a total of 16,779 bacterial and 1,379 Fungal ASVs. Kruskal–Wallis tests revealed similar bacterial and fungal alpha diversity as measured by Shannon's diversity index when crop sequences were compared (Fig. 2).

Bacterial (Fig. 2A) and fungal (Fig. 2B) alpha diversity were not different between crop sequences. While increased soil microbial diversity is frequently promoted as a benefit of increased crop diversity (Esmaeilzadeh-Salestani et al. 2021; Stefan et al. 2021), our results demonstrate that in semiarid environment this response is more nuanced and increased crop diversity does not always increase soil microbial diversity relative to a wheat fallow system. Similarly, studies that have found a positive effect on microbial diversity from increased crop diversity have shown these effect are relatively less important than abiotic factors (Stefan et al. 2021). Differences in alpha diversity were

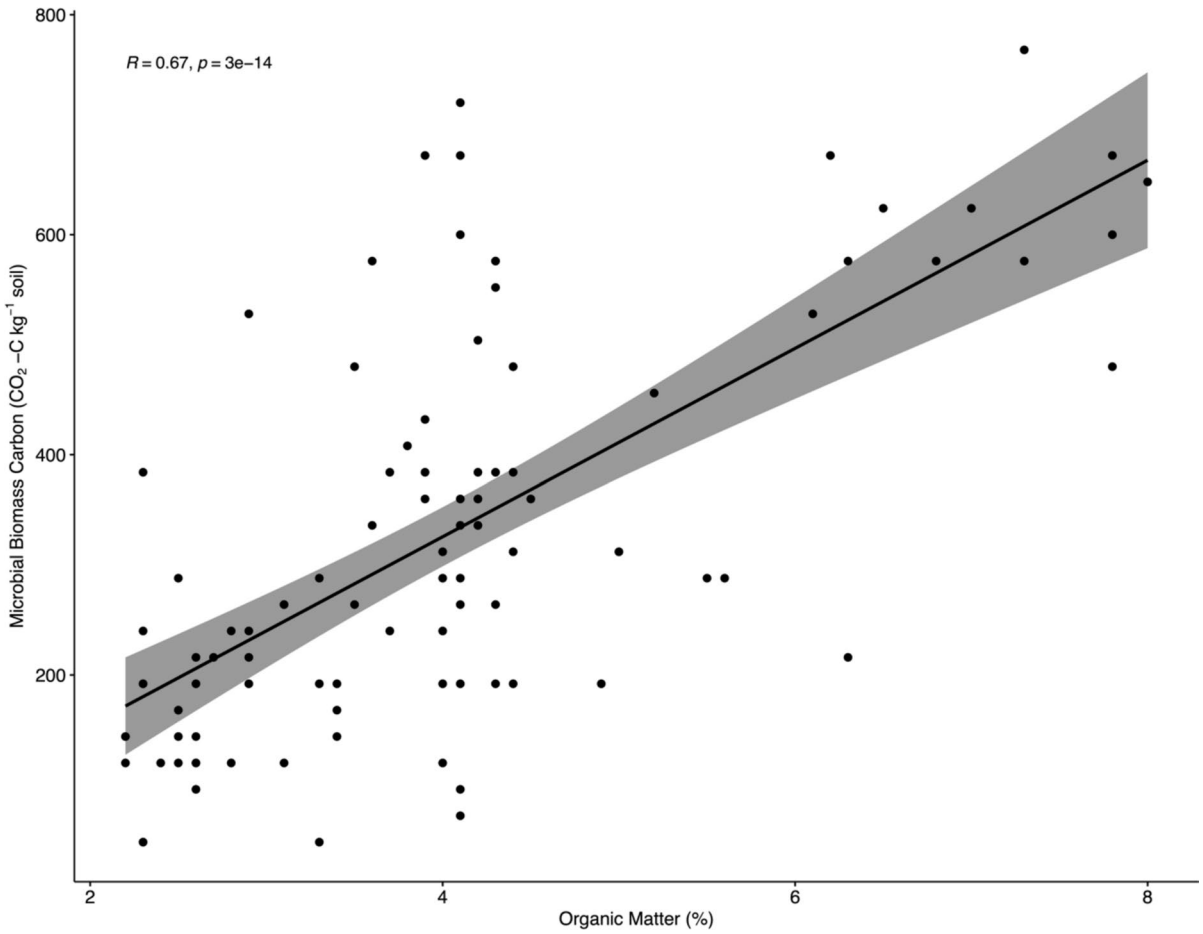


Fig. 1 Pearson's correlation showing the relationship between microbial biomass carbon and soil organic matter across all study locations and treatments (Pearsons R = 0.67)

also not significant ($p > 0.05$) between tillage methods (data not shown). A small but significant negative correlation was observed between bacterial Shannon diversity and microbial biomass carbon and soil organic matter ($p = 0.0081$ and $p < 0.001$, respectively) (Fig. 3A, E). In contrast, fungal Shannon diversity had a positive ($p = 0.043$) correlation with soil organic matter (Fig. 3F). Bacterial and fungal alpha diversity were not correlated to soil NH_3-N (Fig. 3C, D).

Other studies have found that higher organic carbon concentrations contribute to greater bacterial and fungal alpha diversity in single location trials (Mahanta et al. 2017; Esmaeilzadeh-Salestani et al. 2021). Increased soil organic matter can lead to an increase in saprotrophic fungi and this functional guild has been associated with increased litter decomposition under nutrient limiting conditions (Tunlid et al. 2022; Yin et al. 2022). The negative correlation we observed between bacterial community alpha diversity and soil organic matter suggests other location specific factors, such as differences in OM, N, P, and pH (Tables S1 and S2), also influence community diversity. Microbial diversity is correlated to organic carbon and pH is a primary driver of soil microbial diversity, while N can have a negative effect on microbial diversity (Bebber and Richards 2022; Chen et al. 2024). Given the variability of these soil factors among locations (Tables S1 and S2) it is likely that differences in N, P, and pH may have contributed to the lack of a positive correlation between bacterial diversity and organic matter in our study. These results highlight the importance of multilocation trials for evaluating microbial

community responses since soil variables can confound treatment response.

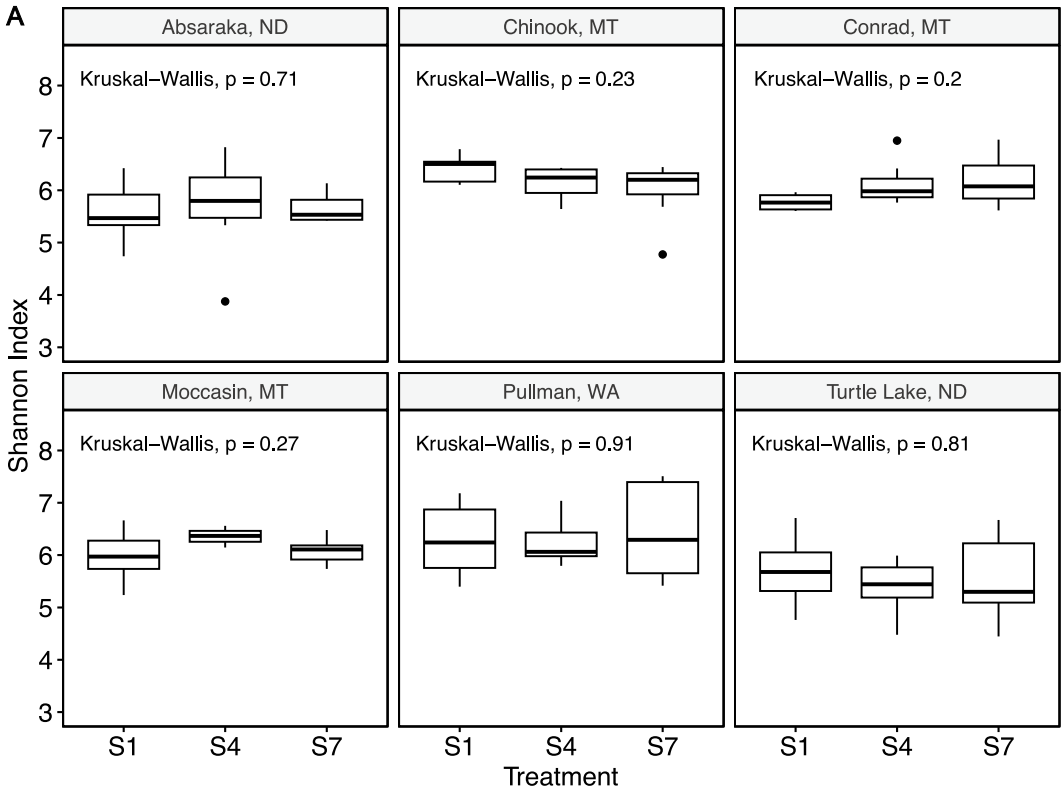
Across all locations, bacterial communities were dominated by Proteobacteria and Actinobacteriota (Fig. 4A) while fungal communities were predominantly Ascomycota and Basidiomycota (Fig. 4B). This is consistent with reports from other studies in similar dryland environments (Schlatter et al. 2020; Alleman et al. 2021; Eberly et al. 2024). Principal coordinate analysis (PCoA) of bacterial and fungal communities showed differences in community composition between years, and among locations in the final year of the study (Fig. 4C, D). Bacterial communities at Pullman, WA were dissimilar from the remaining locations in both years. Geographic differences explained a significant portion of the variance between microbial communities. This was expected in a study across a large region with diverse climates and soils and is consistent with other studies in dryland environments (Alleman et al. 2021).

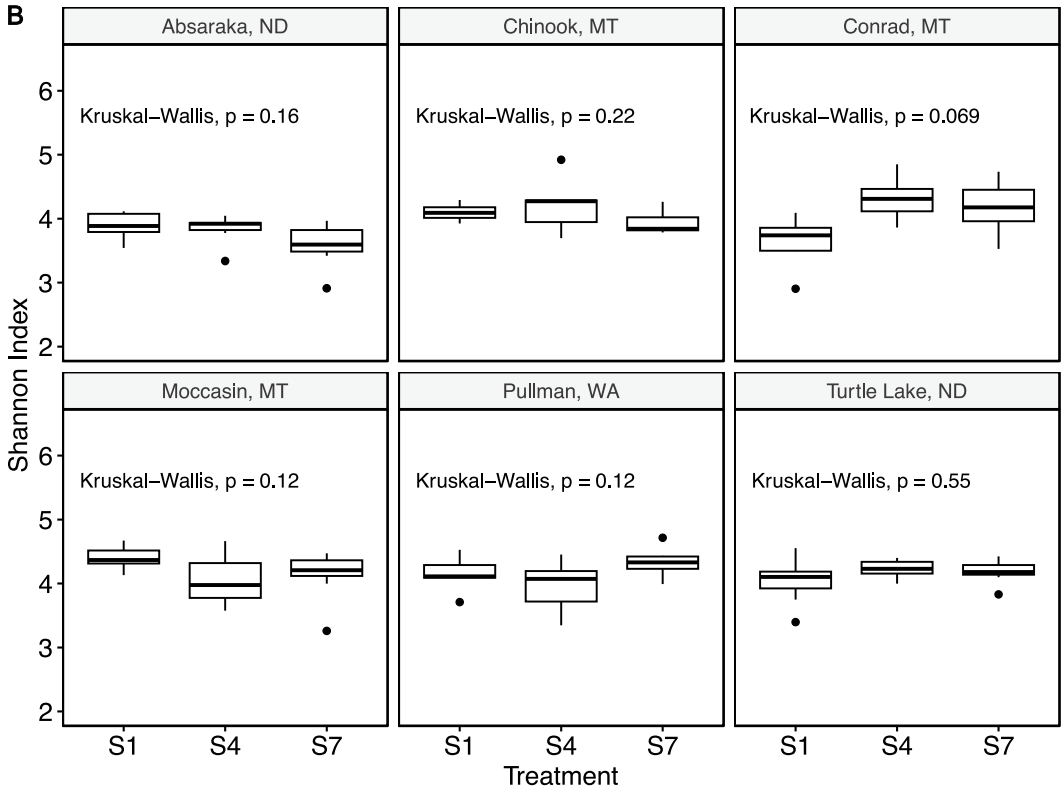
Most bacterial and fungal taxa were common among all crop sequences. A total of 907 bacteria taxa were shared among all treatments while cover crop, lentil, and alfalfa had 3, 2, and 4 unique taxa, respectively (Fig. 4E). In contrast, 657 fungal taxa were shared among treatments, 4 were common to cover crop and alfalfa while 14 taxa were common to alfalfa and lentil and cover crop and lentil crop sequences and no taxa were unique to any treatment (Fig. 4F). These findings suggest that specific crop sequences had minimal effect on the overall microbial community structure.

Random forest models were used to explore taxa that responded to treatments. No significant bacterial taxa were identified (data not shown). This is consistent with other research which compared continuous maize to a maize-soybean rotation and found bacterial communities differed by site but not crop rotation (Sible et al. 2024). Differences between treatments were observed in the fungal community. A total of 10 fungal genera were significantly different between crop sequences at Moccasin, MT (Fig. 5A). Ten of these genera belonged to the phylum Ascomycota, while two belonged to Basidiomycota. The genera *Neosetophoma*, *Boeremia*, and *Paraphoma* were 10 – 35-fold more abundant in Sequence S1 (alfalfa) compared to the mean abundance in the other crop sequences. Enrichment of specific taxa in the perennial alfalfa crop sequence relative to the annual crop sequences demonstrates how minimizing disturbances in the soil can alter the soil fungal community compared to more disturbed annual cropping systems.

The fact that these treatment effects were only observed at Moccasin, MT and not at the other locations demonstrates the environmental context dependence of practices that are intended to enhance soil health and sustainability. While IWM practices are often promoted for soil health benefits (McPheeters et al. 2022), soil type, climate, moisture regimes, and other abiotic factors vary significantly between locations and can strongly influence how agricultural management practices affect microbial communities (Kong et al. 2023; Gupta and Tiedje 2024). Historical land use and other legacy effects can also shape community response to management (Jangid et al. 2011; Turley et al. 2020).

Random forest models also identified indicator taxa for tillage intensity at the Moccasin, MT location. All genera that were differentially abundant between tillage treatments belonged to the phylum Ascomycota except *Minimedusa,* which belonged to the phylum Basidiomycota. *Fusarium*, a genus containing many plant pathogen species, was 1.4-fold more abundant in conventional tillage compared to reduced tillage (Fig. 5B). In general, tillage is considered beneficial for reducing fungal diseases (Sumner et al. 1981; Friberg et al. 2019) but work in dryland wheat-based cropping systems has shown that reduced tillage does not contribute to increased pathogen pressure from specific causal agents including *Fusarium*, *Pythium*, and *Rhizoctonia* that are known to be pathogenic on wheat (Yin et al. 2020). Our work is consistent with these findings and suggest reduced tillage will not lead to increased pathogen pressure in semi-arid regions. Our results also showed that the fungal genera *Chaetomium*, *Minimedusa*, and *Niesslia* were over twice as abundant in conventional tillage compared to reduced tillage. *Chaetomium* has been implicated in the degradation cellulose, cellulose disaccharides, and lignin while both *Chaetomium* and *Minimedusa* are known to have biocontrol effects on fungal diseases and other plant pathogens (Zhou et al. 2023). *Niesslia* are ubiquitous saprotrophs that are also associated with decay of plant residues (Gams et al. 2019). The observed enrichment of genera involved in organic matter degradation suggests that tillage may be beneficial for incorporating plant


A
Absaraka, ND
Kruskal–Wallis, p = 0.71
Chinook, MT
Kruskal–Wallis, p = 0.23
Conrad, MT
Kruskal–Wallis, p = 0.2
Moccasin, MT
Kruskal–Wallis, p = 0.27
Pullman, WA
Kruskal–Wallis, p = 0.91
Turtle Lake, ND
Kruskal–Wallis, p = 0.81
Shannon Index
Treatment
S1
S4
S7


B
Absaraka, ND
Kruskal–Wallis, p = 0.16
Chinook, MT
Kruskal–Wallis, p = 0.22
Conrad, MT
Kruskal–Wallis, p = 0.069
Moccasin, MT
Kruskal–Wallis, p = 0.12
Pullman, WA
Kruskal–Wallis, p = 0.12
Turtle Lake, ND
Kruskal–Wallis, p = 0.55
Shannon Index
Treatment
S1
S4
S7

◂**Fig. 2** Alpha diversity in different crop sequences as measured by Shannon's diversity index at each location. **A**) bacterial alpha diversity, **B**) fungal alpha diversity. A non-parametric Kruskal–Wallis test was used to identify significant differences between treatments. Crop sequences were S1: Alfalfa, S4: lentil – spring wheat – field pea – spring wheat, S7: cover crop – spring wheat

residues which can enhance organic matter mineralization (Sainju et al. 2010).

While reductions in the abundance of pathogen containing genera such as *Fusarium* are a benefit of some management practices, there is little evidence that changes in fungal abundance or diversity can directly benefit crop productivity. A review of multiple studies from diverse crops such as field peas, faba beans, oats, and wheat found no yield benefit to increased fungal abundance or diversity (Ryan and Graham 2018).

Co-occurrence network analysis revealed differences in topological properties between crop sequences and tillage treatments (Table 2). Overall, the number of associations and cumulative weights of ASVs were largely positive within the bacterial and fungal networks (Fig. S2 – S5). This is consistent with previous reports and highlights the level of cooperation among distinct taxa within soil communities (Agler et al. 2016; Yu et al. 2021). Previous work has suggested that positive correlations among nodes represent cooperative relationships, such as commensalism and mutualistic interactions (Berry and Widder 2014). Fungal average degree and closeness centrality, which are measures of how well a node is connected to the rest of the network (Agler et al. 2016), were greater ($p = 0.030$ and $p < 0.001$, respectively) in the lentil treatment (Tables 2 and S3). Average degree was also higher in the reduced tillage treatment for both bacteria ($p < 0.001$) and fungi ($p = 0.0248$) (Tables 2 and S4). A significant ($p < 0.001$) difference in modularity was also observed for both bacterial and fungal networks and was associated with the cover crop treatment (Tables 2 and S3). In contrast, no difference in modularity was observed between tillage treatments (Table S4). Modularity can enhance species persistence in an ecosystem and improve network stability (Grilli et al. 2016). Modules are groups of highly connected nodes and higher network modularity is associated with greater network stability (Grilli et al. 2016; Shi et al. 2016). Greater network modularity in bacterial and fungal communities suggest a more stable microbial community with the diverse cover crop treatment.

Greater network connectivity occurred in the cover crop treatment even though bacterial and fungal richness did not vary significantly between crop sequences. Our results are consistent with other studies (Eberly et al. 2022; Gao et al. 2022), and suggested that microbial diversity is less important in determining community stability than the number of associations among taxa. More associations among taxa may lead to greater resilience in microbial communities under more diverse crop sequences.

Putative keystone microbes were identified among highly abundant ASVs using co-occurrence network analysis of the relative abundance data. A total of 18 bacterial and 22 fungal genera were identified as keystone taxa and 34.5% of bacterial and 52.8% of fungal keystone taxa were shared among the three treatments (Fig. S2 and S3). The majority of these fungal keystone taxa belonged to the phylum Ascomycota and 19 genera were shared among all crop treatments (Table S6).

A total of 18 bacterial and 20 fungal keystone taxa were identified between tillage treatments at the Moccasin, MT location. Reduced tillage systems had more bacterial and fungal keystone compared to CT and no keystone taxa were shared between the CT and RT treatments (Fig. S4 and S5). All taxa belonged to the phyla Ascomycota and Basidiomycota. In the CT treatment, 44% of the keystone taxa belonged to the phylum Ascomycota while 91% of the RT keystone taxa belonged to this phylum (Tables S7 and S8). Studies have shown that despite low relative abundance, keystone taxa can have high connectivity in the community and can thus indicate community shifts in response to changes in management practices (Herren and McMahon 2018; Banerjee et al. 2019). The greater abundance of keystone species under reduced tillage suggests these communities may have greater stability.

Keystone microbial taxa are highly connected taxa that play an important role as drivers of microbiome structure and function (Banerjee et al. 2019). Thus, functions associated with these taxa might play an important role in soil health and productivity. Within the phylum Actinobacteriota, four genera, *Actinosynnema*, *Oryzihumus*, *Crossiella*, and *Catenulispora*, were identified as keystone taxa in all crop treatments (Table S5). Members of the phylum Actinobacteriota are metabolically diverse and synthesize a variety

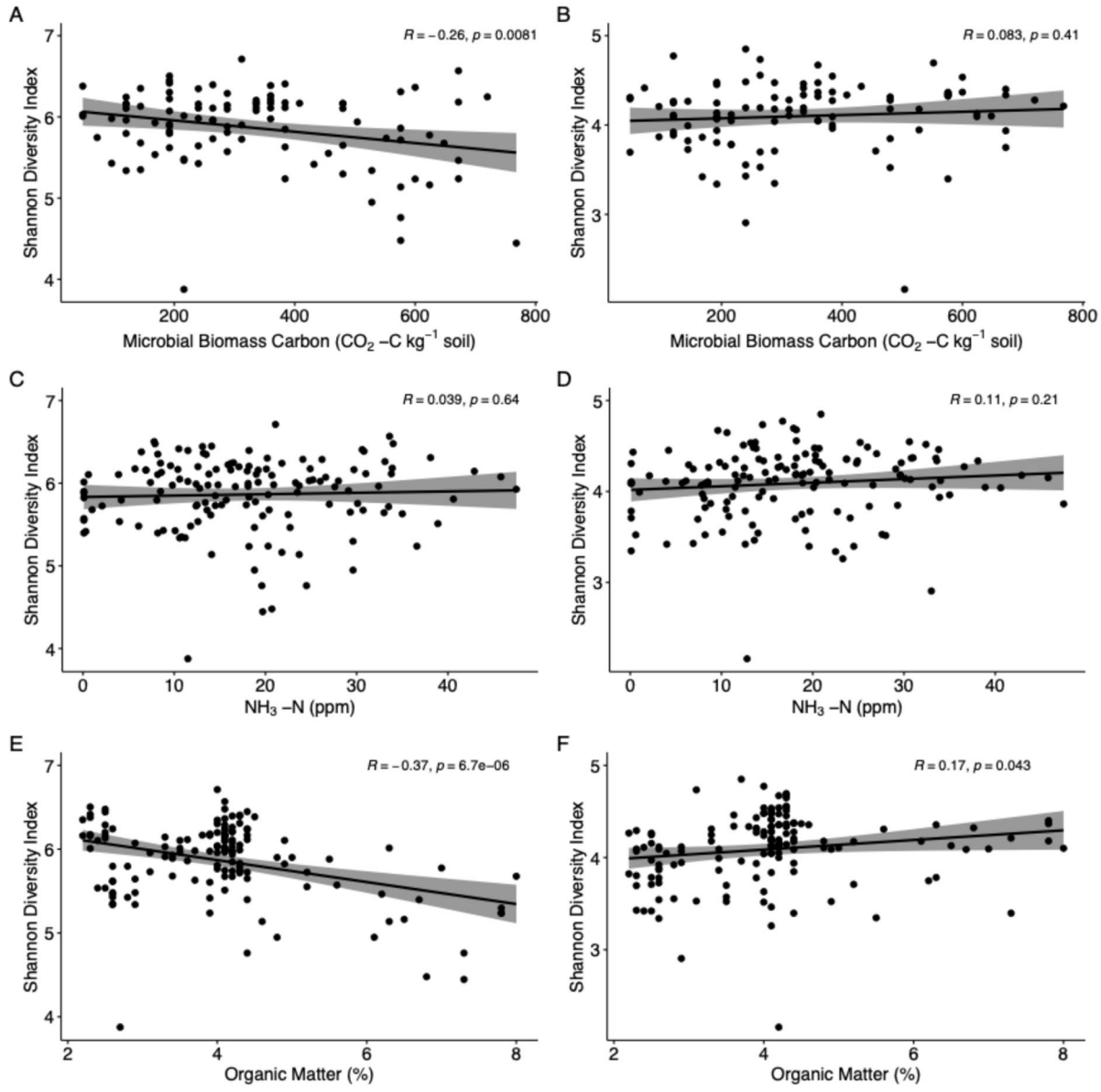


Fig. 3 Pearson's correlation showing the relationship between alpha diversity and soil parameters as measured by Shannon's diversity index. **A**) Bacterial diversity and **B**) fungal diversity correlation with microbial biomass carbon. **C**) Bacterial diversity and **D**) fungal diversity correlation with soil NH_3-N. **E**) Bacterial diversity and **F**) fungal diversity correlation with soil organic matter (%)

of secondary metabolites (Zhang et al. 2020). Some genera, such as *Crossiella*, produce a variety of bioactive compounds that are active against bacterial and fungal pathogens while *Catenulispora* is known to produce diterpenes, a class of molecules with antimicrobial activity (Li et al. 2021a, b; Saha et al. 2021; Gonzalez-Pimentel et al. 2022). Keystone taxa are defined as taxa that have a disproportionately large effect on the community relative to their abundance (Wang et al. 2024). We found multiple members of the orders Rhizobiales and Burkholderiales were keystone taxa (Table S5). These orders have been consistently identified as keystone taxa in multiple studies across different ecosystems (Banerjee et al. 2018).

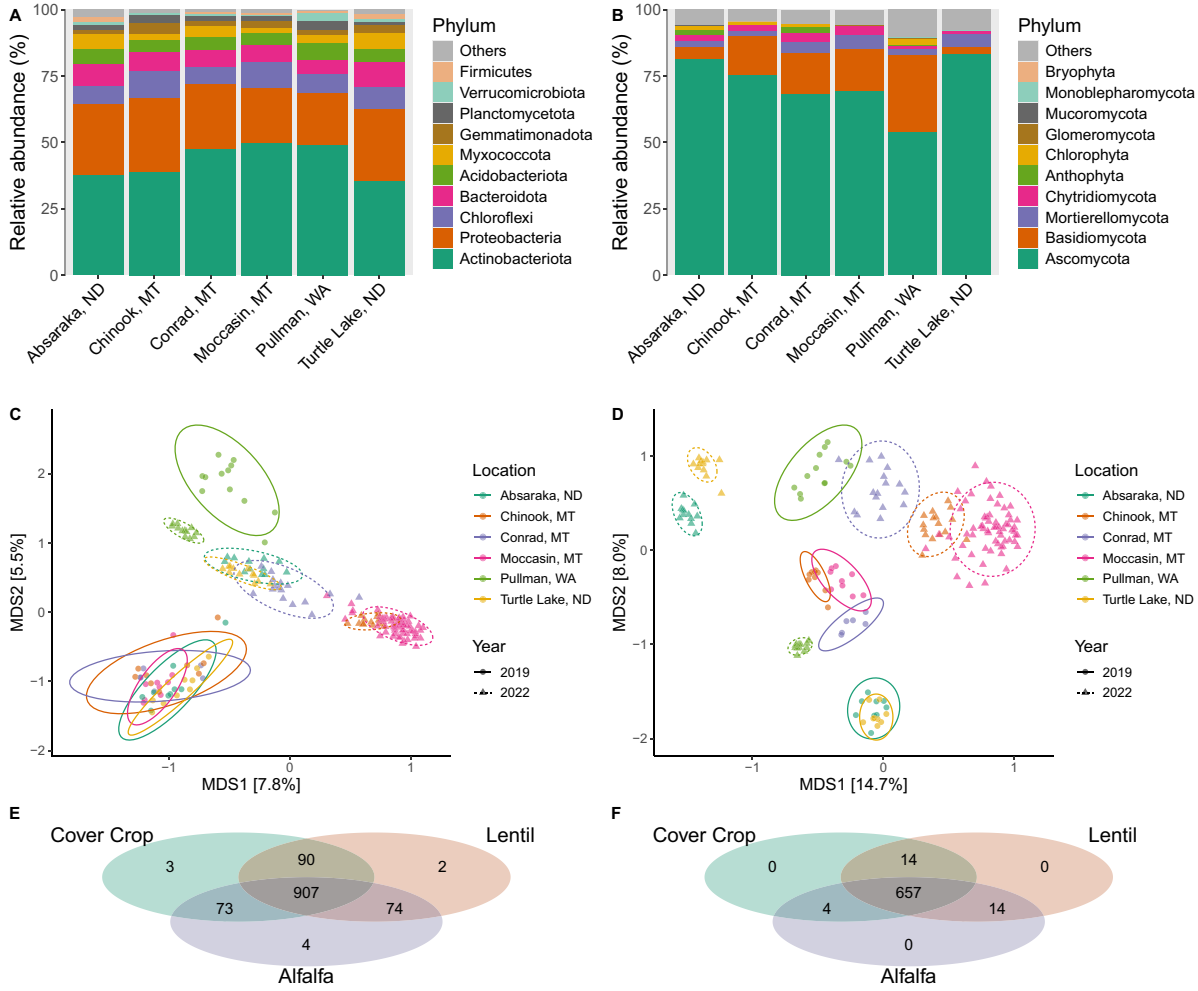


Fig. 4 Bar plot of relative abundance of the top 10 most abundant bacterial (**A**) and fungal (**B**) phyla at each location. Principal coordinate analysis (PCoA) of the Bray–Curtis distances for bacteria (**C**) and fungi (**D**) colored by location with shape indicating year. Ellipses show 95% confidence intervals. Venn diagram of the number of common and unique bacterial (**E**) and fungal (**F**) taxa among the three crop sequences common to all locations in the final year on the study

The order Rhizobiales contains multiple N-fixing genera and other endosymbionts while Burkholderiales contains multiple known pathogens (Banerjee et al. 2018). Keystone bacterial and fungal taxa have also been linked to organic matter decomposition (Banerjee et al. 2016). This suggests keystone taxa may play an important role in nutrient availability within the community, but more work is needed to identify functional roles.

Integrated weed management encompasses a wide range of practices, including herbicide application, tillage, grazing, crop rotation, and biological control. While numerous studies have evaluated the individual impact of these practices on the soil microbiome, few studies have considered the collective impact of multiple IWM practices. One recent study found IWM practices that incorporated conservation tillage, increased microbial biomass carbon and microbial diversity relative to conventional tillage or herbicide treatment (Nthebere et al. 2024). This study highlights the importance of considering the cumulative impact of multiple weed management practice on soil microbial communities and demonstrates the need for additional studies in other environments and cropping systems.

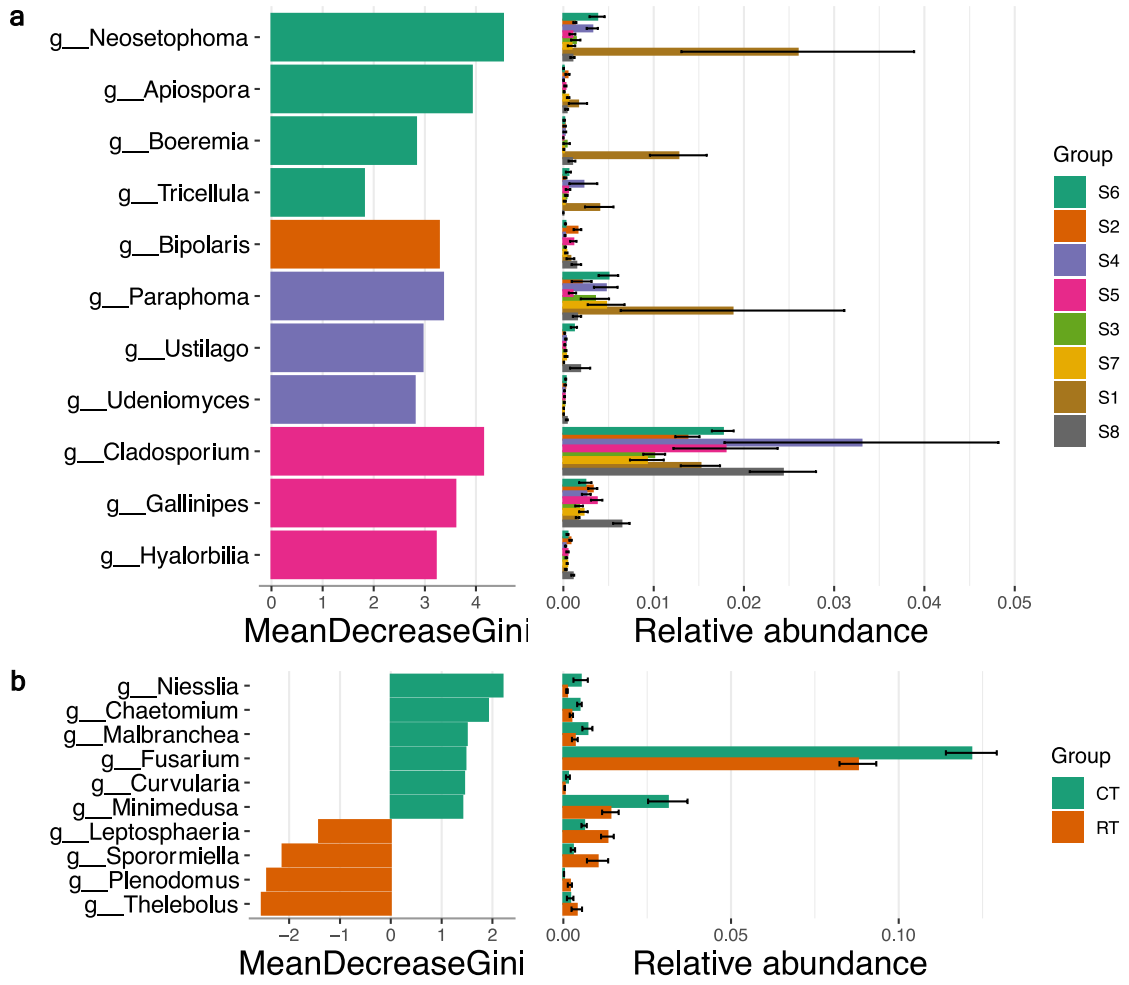


Fig. 5 Random Forest analysis of fungal communities at Moccasin by crop sequence (**A**) and tillage (**B**). Only taxa that were significant at $p < 0.05$ (FDR corrected) are shown. No bacterial taxa were different between crops or tillage treatments. CT; conventional tillage, RT; reduced tillage. Treatment groups correspond to crop sequences shown in Table 1

While this study provided new insight into the effect of management practices on soil microbial communities, there were limitations. Amplicon sequencing is subject to primer bias which can over or underrepresent certain taxa. Overly stringent quality filtering can lead to loss of data potentially biasing downstream analyses like differential abundance analysis and insufficient filtering of ambiguous bases can reduce the accuracy of taxonomic assignment (Guo et al. 2014). The study also had a relatively short timeframe, and some soil effects might not be observable unless crop sequences have been in place longer. Potential site-specific effects require more replication and the absence of functional microbial data such as metagenomic or transcriptomic data limit assessment of interspecies interactions.

Our results indicate that diversifying crop rotations as part of an IWM plan in organic systems will likely have minimal impact on the soil microbial community abundance and structure. Integrating alfalfa, along with reduced tillage, was also the most effective management strategy for controlling perennial weeds in organic systems (Larson et al. 2024). Our findings suggest planting a perennial forage, such as alfalfa, and reducing tillage intensity may provide an additional benefit by increasing the resiliency of the fungal community.

Table 2 Topological properties of microbial co-occurrence networks associated with crop sequences that were common across all study locations and tillage treatments at Moccasin, MT

	Treatment				
Network Property	Alfalfa	Lentil	Cover crop	CT	RT
Bacteria					
Nodes	549	565	538	94	86
Edges	2163	1872	1673	121	169
Average Degree	7.880	6.627	6.219	2.574	3.930
Modularity	0.763	0.765	0.789	0.914	0.803
Clustering coefficient	0.486	0.423	0.414	0.846	0.881
Closeness centrality	0.356	0.288	0.349	0.816	0.919
Fungi					
Nodes	197	222	208	152	174
Edges	850	1180	920	229	314
Average Degree	8.630	10.631	8.846	3.013	3.609
Modularity	0.684	0.658	0.691	0.921	0.913
Clustering coefficient	0.585	0.644	0.611	0.815	0.817
Closeness centrality	0.295	0.361	0.272	0.792	0.811

CT Conventional Tillage, *RT* Reduced Tillage

Conclusions

The structure and function of soil microbial communities play an important role agroecosystems but few studies have evaluated the impact of IWM practices on these communities in semi-arid organic farming systems. The effect of integrating various cultural (i.e., crop sequences) and mechanical (i.e., tillage) practices for perennial weed management on soil microbial communities in semi-arid organic cropping were compared in this study. Our results showed that soil bacterial communities were not affected by the IWM practices that were evaluated in this work. These results indicate that diversifying cropping systems and altering tillage approaches to improve IWM in dryland organic systems are likely to have minimal impact on soil bacterial communities. In contrast, fungal communities were affected by both crop sequence and tillage treatment which highlights the greater sensitivity of fungi to management practices. Crop sequence effects were not consistently observed across all locations which highlights the environmental context dependence. Management practices must be considered in the context of local soil, climate, and crop history and there is a need for more localized research to address these interactions. More work is also needed to assess functional changes in fungal communities to determine likely impacts on nutrient cycling and plant productivity. Additionally, growth rates and maturity of different crops occurs at varying times throughout the growing season, thus crop effects on fungal community structure may differ depending on plant growth stage. More studies are needed to evaluate the temporal changes in soil microbial communities throughout the growing season in response to management practices to better assess community response.

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Data availability The raw sequence data supporting the finding of this study are deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1176680).

Declarations

Competing interests The authors declare no competing interests.

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