Corn-soybean rotation, tillage, and foliar fungicides:
Impacts on yield and soil fungi

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IN A BEAN POD

▶ Rotating crops is key to achieving high soybean and corn yield.

▶ Prophylactic foliar fungicides resulted in a small increase (2 bu ac^{-1}) in soybean seed yield.

▶ Continuous corn and soybean had different bulk soil fungal communities, while annually rotated corn and soybean plots had similar fungal communities.

▶ Foliar fungicide treatments did not have an impact on bulk soil fungal communities.

INTRODUCTION

Tillage, crop rotation, and foliar fungicides are three of the major tools conventional corn and soybean producers rely on to manage fungal disease. Tillage aids in reducing disease inoculum by burying crop residue that may harbor disease-causing organisms and by speeding up the decomposition of fungal survival structures (Roth et al., 2020). Crop rotation helps keep disease pressure low by reducing the frequency of a susceptible host plant's presence. Although used to protect yield, prophylactic foliar fungicide use does not consistently increase yield or profitability for corn and soybean, especially when disease pressures are low. For soybean, yield response to fungicides is generally positive, but small (3-5%), and may not warrant the cost of product and application (Mourtzinis et al., 2017). For corn, the yield benefits are often tied closely to the disease pressure present in the field (Paul et al., 2011). This study looks at the potential effect of two fungicides (prothioconazole and picoxystrobin) on yield under variable management (crop rotation and tillage).

Environmental impact of fungicides such as their possible effect on non-target species living in the soil is also something to consider. We know that foliar fungicides can impact plant-associated fungal communities, including rhizosphere or root-associated, fungi shortly after application (Santísima-Trinidad et al., 2018). However, many modern cropping practices also impact soil microbial communities. Crop rotation and crop species seem to be consistent predictors of community composition for soil bacteria and soil fungi. This study used two methods, ITS Sequencing and PLFA-FAME to characterize the soil fungal communities in 4 crop rotation treatments (CC, SS, SC, CS) under no-till and with or without foliar fungicides.

Overall, our objectives were to 1) identify the effect of crop rotation, tillage, and foliar fungicide use over three growing seasons on corn and soybean yields, and 2) explore the impact of both crop rotation and three continuous years of foliar fungicide applications on bulk soil fungal communities. The data for this study was collected from a long-term corn-soybean rotation study in Arlington, WI. See table 1 for the treatments, and the original publication in *Field Crops Research* for more details on the materials and methods.

Table 1. Treatments and treatment codes for part 1 (yield) and part 2 (soil fungi) of the study.

Treatment		Part 1 Yield	Part 2 Soil Fungi
Tillage	Conventional (CT)	x	
	No-Till (NT)	x	x
Crop Rotation	Continuous corn (CC)	x	x
	Continuous soybean (SS)	x	x
	Annually rotated corn (CS)	x	x
	Annually rotated soybean (SC)	x	x
	First year corn after 5 years soybean (1C)	x	
	Second year corn (2C)	x	
	Third year corn (3C)	x	
	Fourth year corn (4C)	x	
	Fifth year corn (5C)	x	
	First year soybean after 5 years corn (1S)	x	
	Second year soybean (2S)	x	
	Third year soybean (3S)	x	
	Fourth year soybean (4S)	x	
	Fifth year soybean (5S)	x	
Fungicide	Untreated control (UTC)	x	x
	Prothioconazole ("Proline")	x	x
	Picoxystrobin ("Approach")	x	x

RESULTS & DISCUSSION

Yield

For soybean seed yield, there was a significant impact of fungicide treatment, as well as a significant interaction between tillage and crop rotation treatments. For corn grain yield, there was a significant impact of tillage treatment. There was also an interaction between crop rotation and fungicide treatment for corn (Table 2). We only present means for these four groups of treatments, since main effects involved in interactions can be misleading.

Soybean treated with either picoxystrobin or prothioconazole yielded 3.6% higher than untreated soybean (Table 3). The interaction between tillage and crop rotation treatments resulted in 6 statistically similar groupings. The highest yielding group included SC, 1S, and 2S in both no-till and conventionally tilled treatments and 5S no-till. Annually rotated (SC) in conventional till was also included in the lowest-yielding group, as well as SS and 4S in both tillage treatments, and 3S and 5S in conventionally tilled plots. The highest yielding treatment combination (NT-1S) produced 11.9 bu ac⁻¹ greater seed yield than the lowest-yielding treatment combination (NT-SS), about a 19% increase (Table 3).

The conventionally tilled treatment yielded higher than no-till for corn grain yield (Tables 4). The interaction between crop rotation and fungicide treatment also significantly affected corn grain yield, resulting in 7 statistically similar groupings (Tables 2 and 4). The highest yielding group included all CS combinations and 1C combinations that were treated with a fungicide. The lowest yielding group included all combinations for rotations 3C, 4C, 5C, and CC, as well as 2C-UTC and 2C treated with picoxystrobin (Table 4).

Crop rotation treatments primarily drove crop performance

Foliar fungicide application in soybean can have a small yield benefit, a conclusion reflected in our data (Table 3) and in previous research (Mourtzinis et al, 2017). The yield increase we observed with either fungicide treatment over untreated soybean was about 2 bu ac^{-1}, which would likely not be sufficient to warrant the cost of fungicide application with recent soybean prices. Instead of using foliar fungicides on all soybean acres, we recommend scouting for disease pressure, and using disease prediction tools like the Sporecaster app to manage white mold (Willbur et al., 2018). Rotating crops is key for achieving high soybean yields. Similar yields can be achieved with conventionally tilled soybean and no-till when comparing the same rotations (Table 3). As with soybean, rotation is one management practice for maintaining good

Table 2. Analysis of variance for fixed main effects and interactions for soybean seed and corn grain yield for 2017, 2018, and 2019 growing seasons.

	Soybean p-value	Corn p-value
Tillage (T)	0.003	0.003
Crop Rotation (CR)	< 0.001	< 0.001
T × CR	0.013	0.297
Fungicide (F)	< 0.001	< 0.001
T × F	0.212	0.686
CR × F	0.346	0.041
T × CR × F	0.962	0.846

Table 3. Effects of foliar fungicide and the interaction between tillage (T) and crop rotation (CR) on soybean seed yield across 2017, 2018, and 2019. Yields followed by the same letter are not statistically different, determined by Tukey's HSD (α = 0.05).

Fungicide	Soybean Seed Yield (bu ac^{-1})	
Prothioconazole	66.2	a
Picoxystrobin	66.1	a
UTC	63.9	b

T × CR	Soybean Seed Yield (bu ac^{-1})	
NT - 1S	72.3	a
NT - 2S	69.8	ab
CT - 1S	68.5	ab
CT - 2S	67.9	abc
NT - SC	67.8	abcd
NT - 5S	67.5	abcd
NT - 3S	66.5	bcde
CT - SC	65.8	abcdef
CT - SS	62.2	def
CT - 5S	61.9	def
NT - 4S	61.8	cdef
CT - 4S	61.8	def
CT - 3S	61.7	def
NT - SS	60.4	f

Table 4. Effects of tillage (T) treatment and the interaction between crop rotation (CR) and foliar fungicide (F) on corn grain yield for 2017, 2018, and 2019. Letters indicate statistically similar treatment groups, determined by Tukey's HSD (α = 0.05).

Tillage	Corn Grain Yield (bu ac^{-1})	
CT	245.0	a
NT	233.1	b

CR × F	Corn Grain Yield (bu ac^{-1})	
CS - Picoxystrobin	267.7	a
1C - Prothioconazole	263.9	ab
1C - Picoxystrobin	258.8	abc
CS - Prothioconazole	258.2	abc
CS - UTC	253.4	abcd
1C - UTC	246.3	cde
2C - Prothioconazole	245.8	bcdef
2C - Picoxystrobin	236.1	defg
4C - Prothioconazole	235.4	defg
2C - UTC	235.1	defg
CC - Prothioconazole	235.0	defg
3C - Prothioconazole	234.6	efg
5C - Prothioconazole	231.8	efg
4C - Picoxystrobin	230.7	efg
5C - Picoxystrobin	230.6	efg
3C - Picoxystrobin	228.5	efg
3C - UTC	227.4	fg
5C - UTC	226.6	g
CC - UTC	226.5	g
4C - UTC	225.5	g
CC - Picoxystrobin	221.8	g

corn yields. The only corn yield response to fungicide treatment within a rotation treatment was 1C-Prothioconazole over 1C-UTC (Table 4). Both fungicide treatments within 1C were part of the highest yielding group, with all three CS treatment combinations. For both corn and soybean, results suggest using integrated pest management strategies and scouting for disease or using disease prediction tools to decide where foliar fungicide applications may be beneficial. Additionally, to manage fungal resistance to a particular fungicide, alternating between different fungicide groups or using multiple groups together is important, especially for prophylactic applications.

RESULTS & DISCUSSION: SOIL FUNGI

Microbial biomass and fungal community changes are indirect effects of continuous cropping

As a preface to this discussion, note that we do not recommend DNA sequencing methods for farmers to assess soil microbial communities, because the results will likely not impact management decisions. Coarser methods like PLFA-FAME may provide similar information for farmers interested in tracking soil health and microbial biomass on their farm. We used both of these methods, but just present a fraction of the information that were available from these tests.

From the total lipids extracted with PLFA-FAME, we inferred microbial biomass, which includes bacteria and other microbiome members beyond fungi. Fungicide treatment did not have an impact on total lipid biomass extracted ($p = 0.399$), but rotations planted to corn in 2019 (CC and CS) had higher lipid biomass than continuous soybean (Figure 1).

With the ITS sequence data, we looked at the overall structure of the fungal communities; this is similar to a census that asks, "who lives here?" Crop rotation explained 26.6% of community variation ($p = 0.001$), while fungicide treatment did not explain variation within the community ($R^2 = 0.038$, $p = 0.484$). The two continuous rotations (SS and CC) differed the most (Figure 2).

The most notable results in terms of fungal community structure are the differences between continuous corn and continuous soybean treatments and lack of difference between annually rotated treatments. The same relationship has been noted for bacterial communities in a field experiment adjacent to this one at the research station (Chamberlain et al., 2020). The lack of difference in fungal community composition between annual rotations suggests that this is primarily a rotational effect and not a short-term, crop-specific effect. Changes in the fungal community are likely indirect effects of crop rotational treatments; continuously cropping corn and soybean has an impact on soil properties, which result in shifts in microbial community structure. A small amount of community variation was explained by differences in soil test values (Table 5), some of which were associated with crop rotation treatments (Table 6).

Generally, factors like nutrient status, pH, and organic matter content are more important than plant community for bulk soil microbial communities, which are not in direct contact with plant roots. This is not true for rhizosphere (area surrounding the root) communities, which are influenced by the plant to a much greater extent (Fierer, 2017). Rhizosphere fungi may have an impact on bulk soil fungi over time as well, which likely contributes to the differences between our crop rotation treatments. Another explanation for different fungal communities in continuous soybean and continuous corn focuses on crop residue. Heavy residues from continuously cropping corn without removing stalks may drive differences in community structure. Although there was not higher organic matter (as tested by loss on ignition) in continuous corn than continuous soybean, the quality of that residue (C:N ratio) determines how easily, and by which microbes, the residue can be decomposed (Table 6). Corn harvested for grain leaves behind more residue, with a higher C:N ratio than soybean. Consequently, this difference in organic matter additions puts selection pressure on microbial communities. The difference in residue amount and quality may also explain the increased microbial biomass in continuous corn (Figure 1). A higher amount of organic material to break down would support an overall increase in microbial

PLFA-FAME (phospholipid fatty acid – fatty acid methyl ester) analysis is a lab technique that uses unique signatures of the molecules that make up cell membranes to identify different groups of organisms. This method can be used to estimate total microbial biomass, and the fraction of that biomass associated with arbuscular mycorrhizal (AM) fungi, non-AM fungi, and bacteria.

ITS Sequencing is a DNA-based method that sequences a small portion of a gene shared across many different types of organisms, including fungi. By comparing the sequence of this barcode-like gene to the sequence for known organisms, we can identify members of the fungal community in our sample. This method is generally accurate to a genus level, but there are a lot of soil-dwelling fungi that have not been identified yet.

Figure 1. Boxplot of total lipid biomass extracted using the PLFA-FAME hybrid method (linked closely to total microbial biomass). Boxes delimit first and third quartiles. The solid line inside the box indicates the median. Upper and lower whiskers represent the largest or smallest value, unless those values lay further than 1.5 times the range between first and third quartiles, indicated by black circles. In this case, whiskers represent the next largest or smallest point. Crop rotation treatments include continuous soybean (SS), continuous corn (CC), and annually rotated corn (CS) and soybean (SC). The rotations with the same letter are not different, as determined by Tukey's HSD ($\alpha = 0.05$).
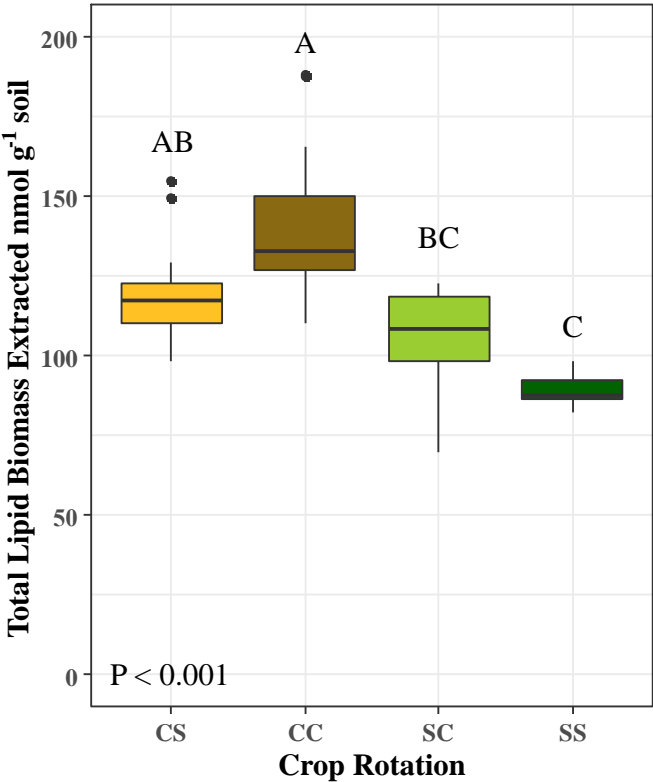
Figure 2. Non-metric multidimensional scaling (NMDS) plot of soil fungal Bray-Curtis dissimilarity (k=2, stress=0.16). The axes of this graph are arbitrary. The farther apart two points are, in any direction, the more different those fungal communities are. Each point represents one plot, from four crop rotations: continuous soybean (SS), continuous corn (CC), and annually rotated corn (CS) and soybean (SC), and three foliar fungicide treatments. Note that the foliar fungicide treatments were applied as split-plots within each crop rotational treatment.
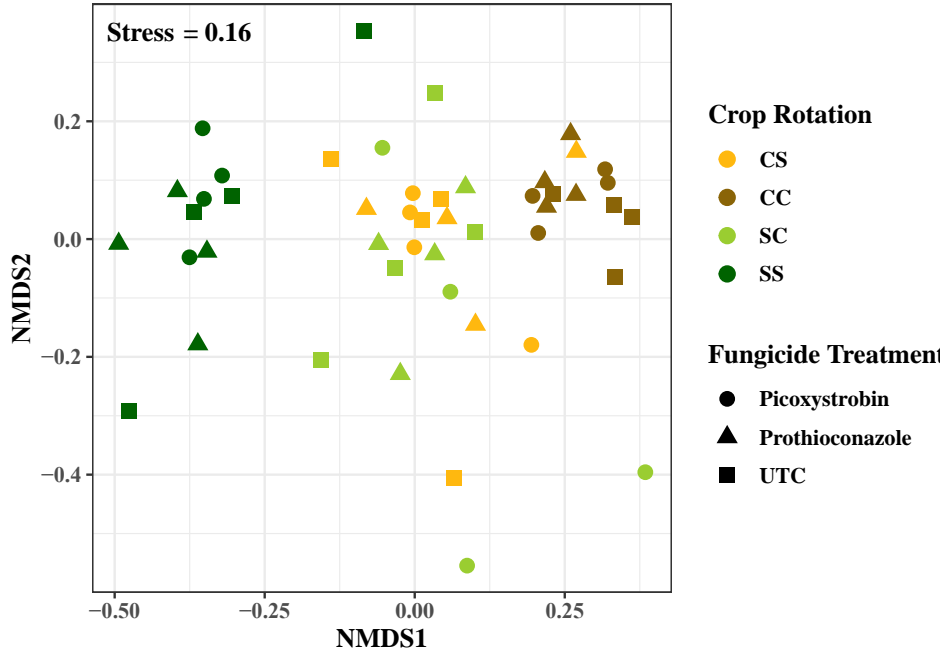

Table 5. Permutational analysis of variance to determine the percent of soil fungal community explained by routine soil tests, including pH, organic matter (OM % by loss on ignition), Bray-1 potassium (K), Bray-1 phosphorus (P), ammonium (NH_4^+), and nitrate (NO_3^-). For this type of analysis, R^2 can be interpreted as the proportion of variation in community structure (given $p < 0.05$), 1 being 100% of variation. Each factor was tested separately, so variation explained should not be summed.

Soil Test	R^2	p-value
pH	0.0385	0.001
OM	0.0298	0.004
K	0.0480	0.001
P	0.0237	0.078
NH_4^+	0.0356	0.001
NO_3^-	0.0238	0.148

Table 6. Values for the three soil tests that differed by crop rotation: organic matter (OM % by loss on ignition), Bray-1 potassium (K), and ammonium (NH_4^+). Values followed by the same letter were not different, as determined by Tukey's HSD ($\alpha = 0.05$).

Rotation	OM %	K (ppm)	NH_4^+ (ppm)
Continuous Corn	3.45 ab	206 a	5.95 a
Annual Corn	3.90 a	157 b	5.93 a
Annual Soybean	3.45 ab	147 b	4.94 ab
Continuous Soybean	3.13 b	159 b	4.58 b

biomass. On a global scale, fungal biomass has been significantly and positively correlated to soil C:N ratio; as the C:N ratio increases, fungal biomass increases as well (Bahram et al., 2018).

Arbuscular mycorrhizal fungi were enriched in continuous corn plots

Arbuscular mycorrhizal fungi are soil-dwelling fungi that associate with plants. In exchange for fixed carbon, the fungi help plants take up water and nutrients, and protect them against pathogens. These fungi make up the phylum, or taxonomic group, called *Glomeromycota*. We used both ITS Sequencing and PLFA-FAME to assess relative abundance of AMF in the soil. Remember that these methods differ – one looks at DNA sequences, while the other reflects cell membranes and (approximate) biomass. Both methods indicated a higher relative abundance of AMF in continuous corn, compared to rotations planted to soybean in 2019 (Figure 3).

Corn tends to be a better host for AMF than soybean; AMF associates more readily and has higher populations in fields with a history of corn (Gosling et al., 2013). Interestingly, continuous corn is associated with decreased yield, despite the high relative abundance of AMF. It is possible that in a phosphorus-limiting environment, AMF would increase yield potential, but with sufficient nutrient status, this high relative abundance of AMF may be detrimental to yield. Continuous cropping may also select for more parasitic strains of AMF than beneficial ones for the plant (Verbruggen and Toby Kiers, 2010). One reason AMF may be more abundant in continuous corn rotations is its sensitivity to disturbance; as an obligate biotroph, even rotating crops is a disturbance to AMF (Chagnon et al., 2013). Continuously cropping corn in a no-till system allows AMF to thrive but might be selecting for less beneficial strains.

Fusarium levels were higher in continuous soybean

Management of disease-causing organisms is a significant reason for rotating crops. By limiting the frequency of a susceptible host, the build-up of pathogenic inoculum can also be limited. Five genera that are home to some corn and soybean pathogens were selected for analysis, including *Fusarium* (causal organisms for *Fusarium* Ear Rot in corn and Fusarium Root Rot in soybean), *Septoria* (causal organism for Brown Spot in soybean), and *Macrophomina* (*M. phaeolina* causes Charcoal Rot in soybean). Other genera tested are home to the anamorph stage of plant pathogens, including *Exserohilum* (teleomorph *Setosphaeria turcica* causes Northern Corn Leaf Blight) and *Mycosphaerella* (teleomorph of several *Cercospora* species, some which can cause Frog Eye Leaf Spot in soybean and Grey Leaf Spot in corn) (Agrios, 2004; Roth et al.,

Figure 3. Boxplots illustrating arbuscular mycorrhizae fungi relative abundance in the soil using (A) fungi in the phyla Glomeromycota (% of observed sequences), identified through ITS sequencing methods, and (B) AMF-associated phospholipid 16:1 ω5c extracted with the PLFA-FAME method (% of total lipids extracted by mol). Boxes delimit first and third quartiles. The solid line inside the box indicates the median. Upper and lower whiskers represent the largest or smallest value, unless those values lay further than 1.5 times the range between first and third quartiles, indicated by black circles. In this case, whiskers represent the next largest or smallest point. Within each graph, rotations with the same letter were not different, as determined by Tukey's HSD ($\alpha = 0.05$). Points are colored by crop rotation. Crop rotation treatments include continuous soybean (SS), continuous corn (CC), and annually rotated corn (CS) and soybean (SC).
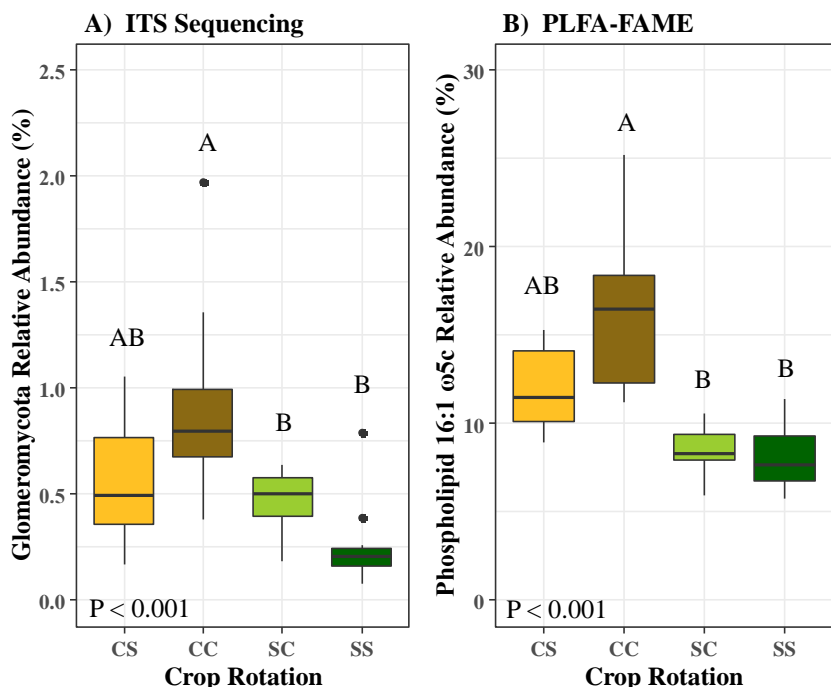
Figure 4. Relative abundance of the corn or soybean pathogen-associated genera *Fusarium*, *Septoria*, *Exserohilum*, *Mycosphaerella*, and *Macrophomina* (identified through ITS sequencing methods). Boxes delimit first and third quartiles. The solid line inside the box indicates the median. Upper and lower whiskers represent the largest or smallest value, unless those values lay further than 1.5 times the range between first and third quartiles, indicated by black circles. In this case, whiskers represent the next largest or smallest point. Crop rotation treatments include continuous soybean (SS), continuous corn (CC), and annually rotated corn (CS) and soybean (SC). Within each genus, rotations with the same letter were not different, as determined by Tukey's HSD (α = 0.05). NS: not significant at α = 0.05.
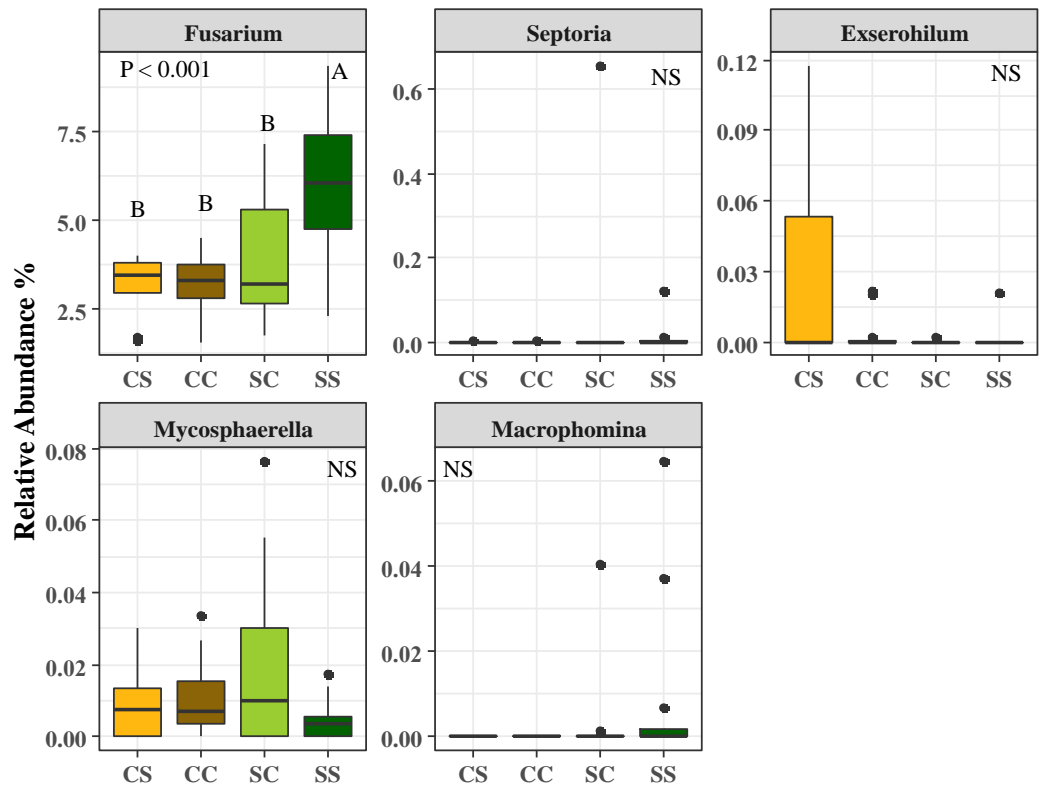
2020). The anamorph (asexual stage) of many fungi look different than the reproductive stages, which led to separate identification and naming as fungi were discovered in the past. Of the five fungal genera that are home to corn and soybean pathogens, only *Fusarium* showed a difference in relative abundance between crop rotations, with higher relative abundance in continuous soybean (Figure 4).

Fusarium is a large genus, with many non-pathogenic members, as well as both corn and soybean pathogens. In corn, a few *Fusarium* species cause Fusarium Ear Rot, a damaging disease for yield and quality due to mycotoxin production. Although the organism can overwinter on corn residue, it may not be present in soil (Agrios, 2004). In soybean, several *Fusarium* species can cause Fusarium root rot (Roth et al., 2020). The soil-dwelling nature of this pathogen and infection may explain the higher relative abundance of *Fusarium* in continuous soybean. The other four pathogen-associated genera we tested make up a tiny portion of the overall community. It should be noted that there are better methods for testing for disease-causing organisms, like qPCR, or even disease-ratings in the field.

Foliar fungicide treatments did not have a lasting impact on soil fungal communities

There was no effect of foliar fungicides on soil fungal community structure, microbial biomass, or any of the taxonomic groups assessed. This lack of response could be explained in many ways. First, that any impact on bulk soil communities was likely short-lived, and our sampling timepoint more than two months after the final year's fungicide application did not capture these changes. Applications were also made after canopy closure, so fungicide made limited direct soil contact. However, both picoxystrobin and prothioconazole are systemic fungicides, meaning the active ingredient can be translocated to the roots. Despite the fact that we did not observe effects of fungicides on community composition in the bulk soil, it is unlikely that there was no impact from these fungicide applications on plant-associated fungal communities. Off-target effects are likely when using broad-spectrum fungicides, but most research shows these impacts are primarily in communities closely associated with the plant, including endophytic, leaf surface, and rhizosphere communities (Santísima-Trinidad et al., 2018). As we targeted bulk soil communities, not the rhizosphere, it is not surprising that we did not see broad community-level effects in the soil.

CONCLUSIONS

Rotating crops is key to maximizing corn and soybean yield. The yield benefits of crop rotation may interact with other management factors like tillage practices and fungicide use. Foliar fungicides can result in small yield increases which may not be profitable. We recommend that farmers use integrated pest management strategies to manage fungal diseases, which may include fungicide applications if they are indicated by economic disease thresholds or prediction tools. Crop rotation did have an impact on bulk soil fungal community composition, but fungicide treatments did not. Soil microbial communities mediate many soil functions of interest. Understanding which cropping practices influence those communities, like crop rotation, may be useful as future cropping strategies are developed to leverage soil microbial functions.

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