



Crop rotation but not cover crops influenced soil bacterial communities in a corn-soybean system

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IN A BEAN POD:

- ▶ Plots with continuously cropped corn and soybean had different communities of soil bacteria, while plots with annually rotated corn and soybean displayed similar communities.
- ▶ The first year of grass cover crops did not affect soil bacterial communities.
- ▶ Soil properties like pH and organic matter influence soil bacterial communities.
- ▶ Continuously cropped corn (no residue removal) had higher total organic matter than continuous soybean.

INTRODUCTION

Conservation practices like crop rotation and cover cropping are thought to have an impact on soil biology. Although it is a short rotation, an annual corn-soybean rotation has the potential to increase soybean yields 8 to 20% over continuous soybean, and corn yields 5 to 30% over continuous corn (Stanger and Lauer, 2008). Yield increases associated with crop rotation are generally attributed to improved pest (insect, weed, and pathogen) and nutrient management. These effects may include a contribution from microbial communities in the soil, especially regarding disease management and nutrient availability. Beneficial soil microbes are able to compete with disease-causing organisms: in extreme cases, soil microbial communities are considered disease-suppressive (Peralta et al., 2018). Microbial communities aid in nutrient management by making nutrients plant-available, through decomposition, solubilization, siderophore (iron-scavenger) production, or in some cases symbiotic relationships (Muller et al., 2019).

Cover cropping involves growing a crop to protect and enrich the soil but not to harvest. Grass cover crops, such as cereal rye or oat, are potential options for farmers who choose to plant a cover crop after corn or soybean. Cereal rye is winter hardy, while oat is not. The window to plant cover crops in the North Central United States after corn and soybean is often narrow due to harvest timing relative to remaining growing degree units (GDU). While drilling grass seeds after harvest is an option, it is also possible to aerially broadcast the seed into a senescing crop before harvest. Aerial seeding before harvest allows for an extended growth period while reducing the time and labor required for the grower to plant the cover crop (Clark, 2012). This strategy however does come at some risk (conductive weather) for success.

There is currently substantial interest in developing microbial treatments to enhance crop growth and decrease input needs, much like the development of probiotics in humans or animals. Probiotics are living microorganisms that confer a health benefit when introduced into a host: rhizobia inoculants are an example of a probiotic application. In soils, an organism or group of organisms must be able to survive in storage and successfully colonize the soil environment once applied in order for this method to be effective.



Figure 1. Main plot (crop rotation) and split plot (cover crops) in the long-term crop rotation study in Arlington, WI. Crop rotation treatments have been in place since the study was established in 2002, cover crops were established in 2017. Note that these cover crop photos are only for illustration of the treatments, and do not represent cover crop success in the first year of the study, these photos were taken in 2019.



An alternative approach to manipulate a host's microbiota is through the use of a prebiotic, which provides specific substrates to encourage growth of beneficial microorganisms (Foo et al., 2017). Supplementing agricultural soil with organic materials (plant residue, manure, or other waste products) that could feed and manipulate the existing microbial communities could be viewed as a prebiotic approach. Crop rotation may serve as a method for such manipulation: growing different plant species over time may promote a more functionally diverse community of soil microbiota that may ultimately benefit plant growth (Lakshmanan et al., 2014). Cover cropping is also an opportunity to add organic matter to the soil and influence the activity and abundance of microbial communities.

Both crop rotation and cover cropping can increase plant biodiversity over time. By increasing the variety of biomass and exudates entering the soil over time, the biodiversity of plant communities may influence microbial communities in the soil (Fierer, 2017). There is some evidence already that crop rotations and cover crops have an impact on microbial communities, which we sought to investigate further.

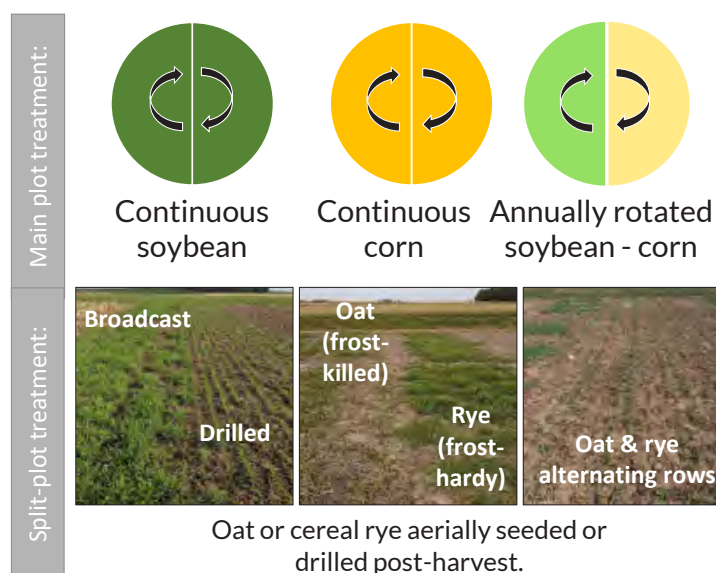
METHODS

Field Experiment

We took advantage of a long-term crop rotation study at the UW-Madison Arlington Agricultural Research Station to test the impacts of rotating corn and soybean, as well as the first season of grass cover crops, on bulk soil bacterial communities. This field experiment was established in 2002 and has been managed with no-till practices exclusively. The experimental design is a randomized complete block, replicated three times. The whole plot treatments are various crop rotation schemes of corn, soybean and wheat, a subset of which were sampled for this study. The crop rotation treatments sampled were continuous corn, continuous soybean, and annually rotated corn and soybean (both soybean and corn phase). A split-plot treatment of cover crops was established in the fall of 2017 (Fig 1). Two different grass cover crop species were planted: cereal rye and oat. Establishment was at two different time points: broadcast before harvest into the senescing crop (R6 soybean and R4-R6 corn) or drilled after harvest. Broadcast treatments were seeded in mid-September at a rate of 75 lbs ac⁻¹. Drilled treatments were planted in mid-October with a seeding rate of 50 lbs ac⁻¹ for oat and 64 lb ac⁻¹ for cereal rye. A fifth cover crop treatment involved both species of grass drilled in alternating rows. Cover crop vigor was measured as percent ground cover before frost in the fall of 2017 and the spring of 2018 before herbicide termination using the Canopeo app (Patrignani & Ochsner, 2015).

Bacterial Community Census

Microbial communities, or the microbiome, includes all microscopic inhabitants of an environment (bacteria, fungi, protists, and more). In this study, we looked at bacterial communities only, since bacteria tend to respond to change more rapidly than other





groups of soil microorganisms. Soil samples were collected at harvest time in 2017, and at planting in 2018. Five soil cores were taken from each split-plot, 0-6 inches depth and mixed together. A portion of each soil sample was sent to the University of Wisconsin Soil and Forage Lab in Marshfield for soil nutrient analysis (Ammonium-N, Nitrate-N, Bray-1 P, Bray-1 K, organic matter %, and pH).

The method used to characterize bacterial communities is called amplicon sequencing which utilizes the 16S gene that is shared across all bacteria species. A combination of factors makes the 16S gene great for identifying different bacteria without sequencing the entire genome (Goodrich et al., 2014). This gene can be thought of like a barcode for bacteria. For this project, DNA from 0.5 g of soil was extracted and a small region of 16S sequenced.

The two major ways to compare communities are 1) Diversity or richness measures that look at the number of species within one sample or community and 2) Measures that compare the composition of species between two samples or communities.

Diversity or richness measures count the total of different species but don't account for the abundance of each group. For example, in a forest that consists of 50 maples and 50 pines, the richness measure is 2. In another forest, with 3 pines and 97 maples, the richness measure is also 2. Other diversity measures, like Shannon's Diversity Index, take the distribution or evenness into account, so the forest with a 50 maples and 50 pines would have higher diversity than that with the 3 pines and 97 maples.

Comparing community structure allows us to see differences between communities that might have similar total numbers of species but differ in their composition. For example, a forest may have a similar number of different plant species to a prairie, but the types of species present are very different. To identify these types of differences, the Bray-Curtis Dissimilarity Index compares the number of species unique to two given communities to the total number of species in both communities (Bray and Curtis, 1957).

RESULTS AND DISCUSSION

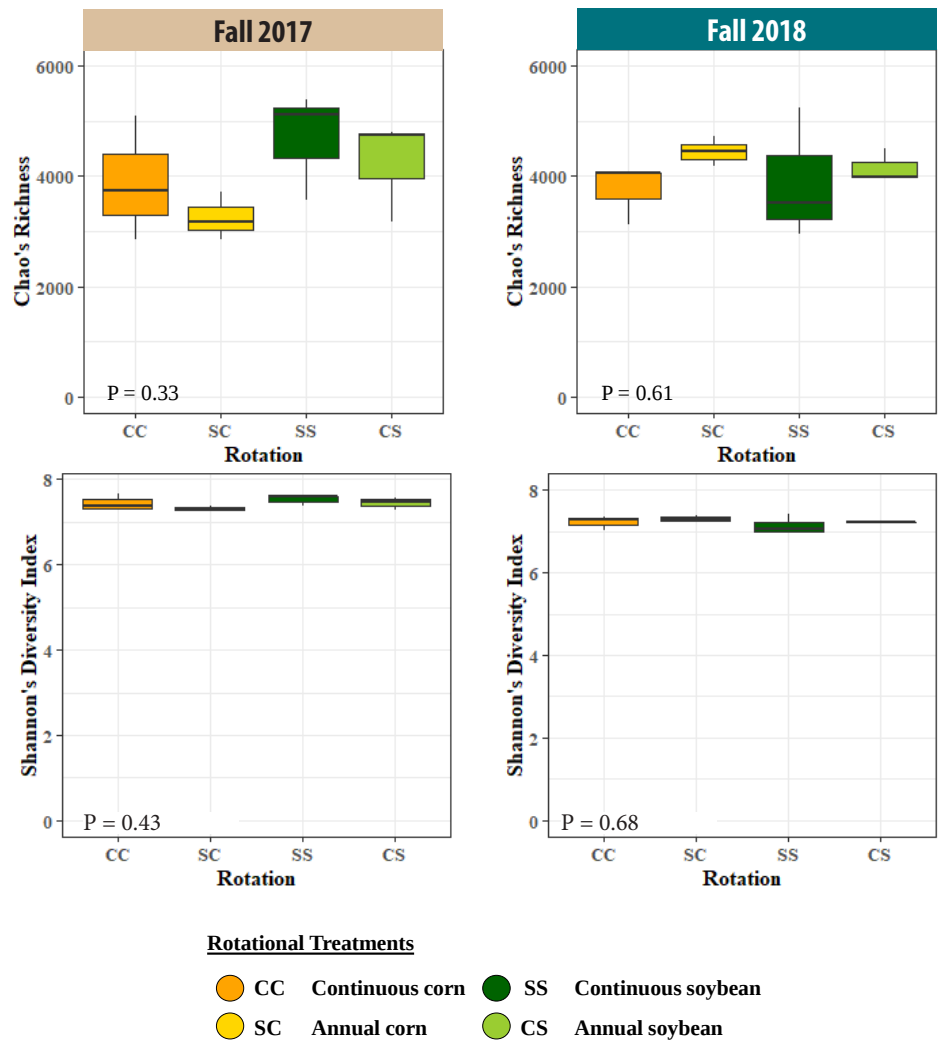
Crop rotation had impacts on community structure but not on diversity or richness.

There was no difference in bacterial communities between crop rotational treatments in terms of diversity or richness measures (Fig. 2). Although cultivated to two different crops, this finding was not unexpected as all the plots in this study are managed similarly. Generally, shifts in diversity or richness are expected with more profound changes in soil properties or management than a change of from one to two crop species. Our study only included a short rotation of two crops, so it is not surprising that the total number of different bacterial species did not change.

Research from other universities shows mixed results on the relationship between crop rotation and microbial diversity. A meta-analysis (research summarizing the findings of many studies) showed that often crop rotation did not have an impact on total diversity of bacteria in soil, unless rotations were very long (Venter et al., 2016). Depth within the soil profile had an impact on richness or diversity measurements, due to generally higher availability of organic carbon and oxygen in the topmost portion of the soil profile (Zhang et al., 2017). There are simply more microbes near the soil surface. Therefore, depth of soil sampling is important to keep in mind when interpreting results from other studies — shallow sampling may identify shifts in bacterial communities that deeper sampling does not show. Overall, soils tend to be a very rich microbial habitat that is dominated by many different taxa at low abundances. Therefore, richness and diversity measures may not be as useful for making ecologically relevant conclusions regarding soil bacterial communities as other measures, like community structure (Shade, 2017).

The major finding of this study is that there are distinct differences in bulk soil bacterial communities between continuously cropped corn and continuously cropped

Figure 2. Chao's Richness (top) and Shannon's Diversity (bottom) measures for four crop rotational treatments in fall 2017 (left panel) and spring 2018 (right panel). Reported p-values indicate overall ANOVA results, which indicated no differences in diversity or richness between rotational groups.



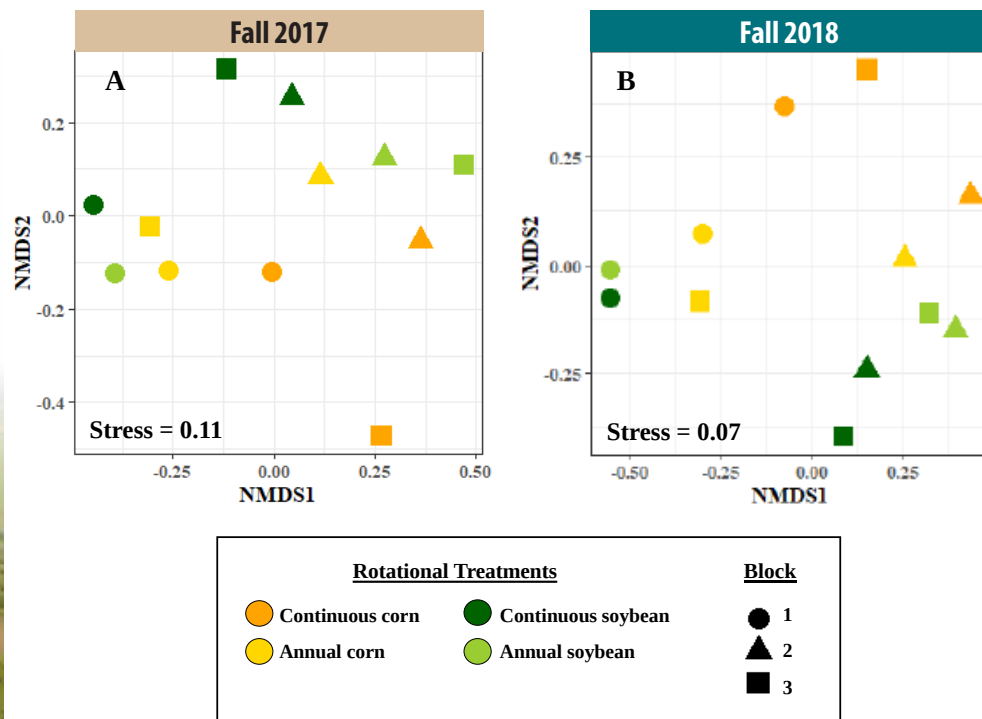
soybean. Both phases of the annual rotation were similar to the continuous rotations. This relationship between the four treatments is consistent in both fall and spring (Fig. 2). Crop rotational treatment explained 28% of the variation in the observed bacterial communities in fall 2017 and 30% in spring 2018 (Fig. 3, Table 1). Points in the ordination plot are also grouped by block (Fig. 2), which is explained in part by differences in pH (Table 1). In fall 2017, 22% of community structure variation between whole plots is explained by pH, and 29% in spring 2018. There is a range in whole plot average pH of 6.0 to 6.9 across whole plots in the fall, and 5.8 to 6.8 in the spring (data not shown). This will be discussed further in a later section, but it is important to acknowledge that plant community is not as important for bacterial community structure as chemical factors like pH (Fierer, 2017).

One biological explanation for the distinct bacterial communities observed between continuous corn and continuous soybean may be soil organic matter quantity and quality. Higher levels of organic matter were observed in the spring in continuous corn, over continuous soybean (Table 2). Not only the quantity, but the quality of organic matter should be considered. A corn crop (harvested for grain only) leaves behind about three times more plant residue than soybean after harvest (Buyanovsky and Wagner, 1986). Furthermore, corn biomass has a higher carbon to nitrogen ratio than soybean, meaning corn residue takes longer to decompose (Kaboneka et al., 1997). On many farms, corn residue is removed from the field after grain harvest. Lehman et al. found decreased fungal to bacterial ratios when corn residue was removed (2014) indicating a response of microbial communities to crop residue inputs. Differences in crop residue may explain the rotation effects we observed; the soil microbiota decompose residues from each crop, and differences in the amount and quality of residue (both from the previous year's crop and historical residues) differentially select for microorganisms (McDaniel et al., 2014).

Table 1. Results of permutational multivariate analysis of variance (PERMANOVA) to determine the significance of crop rotational treatment, organic matter, and pH on bacterial Bray-Curtis dissimilarity for each main plot in this field study. In this type of analysis, R^2 may be interpreted as percent of variation explained by each factor in the model. Each factor was tested alone in the model, so the estimated effects should not be summed.

	Fall 2017		Spring 2018	
	R^2	P-value	R^2	P-value
Rotation Treatment	0.29	.016	0.30	0.003
Organic Matter %	0.17	.077	0.22	0.088
pH	0.22	.005	0.29	0.001

Figure 3. Nonmetric multidimensional scaling (NMDS) ordination plot of bacterial Bray-Curtis dissimilarity colored by crop rotational treatments from two time points: fall 2017 (A, left) and spring 2018 (B, right). Shape indicates block (replication) within the field study. Separation of treatments indicates that crop rotational treatment has an impact on bulk soil bacterial community structure. Differences between continuous soybean and continuous corn are most notable.

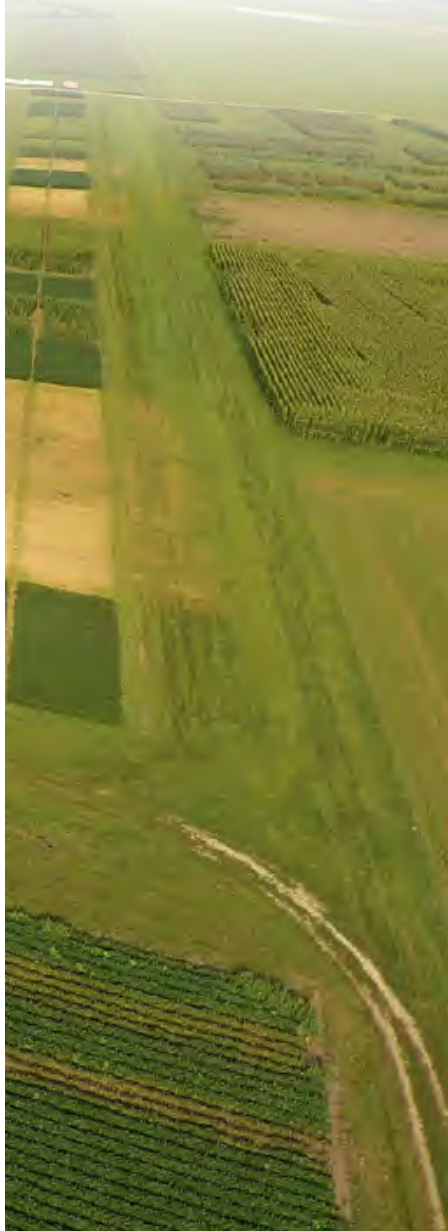


Grass cover crops did not impact soil bacterial communities.

We did not observe changes in soil bacterial communities under different grass cover crop treatments, likely due to limited growth in the first season of cover cropping (Fig. 4). Southern Wisconsin experienced below normal fall and spring temperatures the year this study was conducted (data not shown), thus none of the cover crop treatments flourished. The broadcast cereal rye or oat treatments reached no more than 15% ground cover before the first frost (Fig. 5). In the spring of 2018, the cereal rye treatments continued to grow, but as oat cannot survive winter in Wisconsin, no additional oat biomass was produced. Cover crops were first established in this study in 2017, so treatments in the spring only reflect a single season of cover cropping. Bulk soil microbiota are somewhat robust to change, tending to cycle more slowly than gut or rhizosphere communities (Fierer, 2017; Foo et al., 2017). This short period of cover cropping with limited growth was not sufficient to impact bacterial communities.

Variation in soil properties had an impact on soil bacteria.

Although these samples originated from a single study site, there was some variation both in nutrient availability, soil organic matter content, and pH. Organic matter differed by crop rotational treatment (in the spring only) with the highest level in continuous corn, and the lowest in continuous soybean (Table 2). The rest of the factors summarized in Table 3 did not differ by crop rotational treatment (data not shown). Variation was noted between blocks as well; organic matter was highest in Block 2, while pH was highest in Block 1 (Table 4). These differences in pH and organic matter explain some of the variation in the bulk soil bacterial community (Table 3). Nutrient availability for phosphorus (P), ammonium (NH_4), and nitrate (NO_3) all significantly explain a small portion of bulk soil bacterial community variation in the spring and fall. Potassium (K) explained community differences in the spring but not in the fall



(Table 3). Of the abiotic factors measured, pH explains the most variation in bacterial communities, followed by soil organic matter (Table 3). In the first block, pH was somewhat higher than Blocks 2 and 3 (Table 4). This is likely the result of a limestone driveway near Block 1 of the field study. The pH differences between blocks may have contributed to Block 1 samples grouping together in NMDS visualizations of Bray-Curtis dissimilarity (Fig. 1).

Factors like pH, soil organic matter, moisture, and nutrient availability tend to have more impact on bacterial community composition than plant community (Fierer, 2017). The importance of these factors is part of the reason a long-term crop rotational experiment on a research farm was used for this study, since abiotic factors would differ so much between locations, fields, or farms. However, some variation in these factors is inevitable in any field-scale experiment. The impact of variation in a small-scale study like this emphasizes the importance of collecting information on soil properties that may confound results of soil microbiome research.

Table 2. Mean and standard error of organic matter content and pH for each crop rotational treatment. Groups with the same letter are statistically similar, as determined by Tukey's HSD post hoc ($P < 0.05$). Note that statistical tests were conducted for each time point separately. Organic matter differed between crop rotational treatments in the spring of 2018, but not in the fall of 2017. There was no effect by rotation on pH.

	Treatment	pH	SE	OM %	SE
Fall 2017	Continuous Corn	6.4	0.074	a	3.1
	Annual Corn	6.6	0.070	a	2.9
	Continuous Soybean	6.5	0.085	a	2.8
	Annual Soybean	6.3	0.087	a	3.2
Spring 2018	Continuous Corn	6.0	0.076	a	3.3
	Annual Corn	6.4	0.083	a	2.9
	Continuous Soybean	6.4	0.089	a	2.8
	Annual Soybean	6.2	0.107	a	3.2

Figure 4. Nonmetric multidimensional scaling (NMDS) plots of bacterial Bray-Curtis dissimilarity colored by cover crop treatment from two time points: fall 2017 (left panel) and spring 2018 (right panel). Cover crop treatments were the same after corn or soybean, but separate NMDS plots were generated for soybean rotations (top panel) and corn rotations (bottom panel) for clarity. Shape of points indicates a continuous or annually rotated treatment. No patterns in cover crop treatment were observed, which results in the conclusion that cover crop treatment did not impact soil bacterial communities. This result was confirmed with a permutational multivariate analysis of variance ($P > 0.05$).

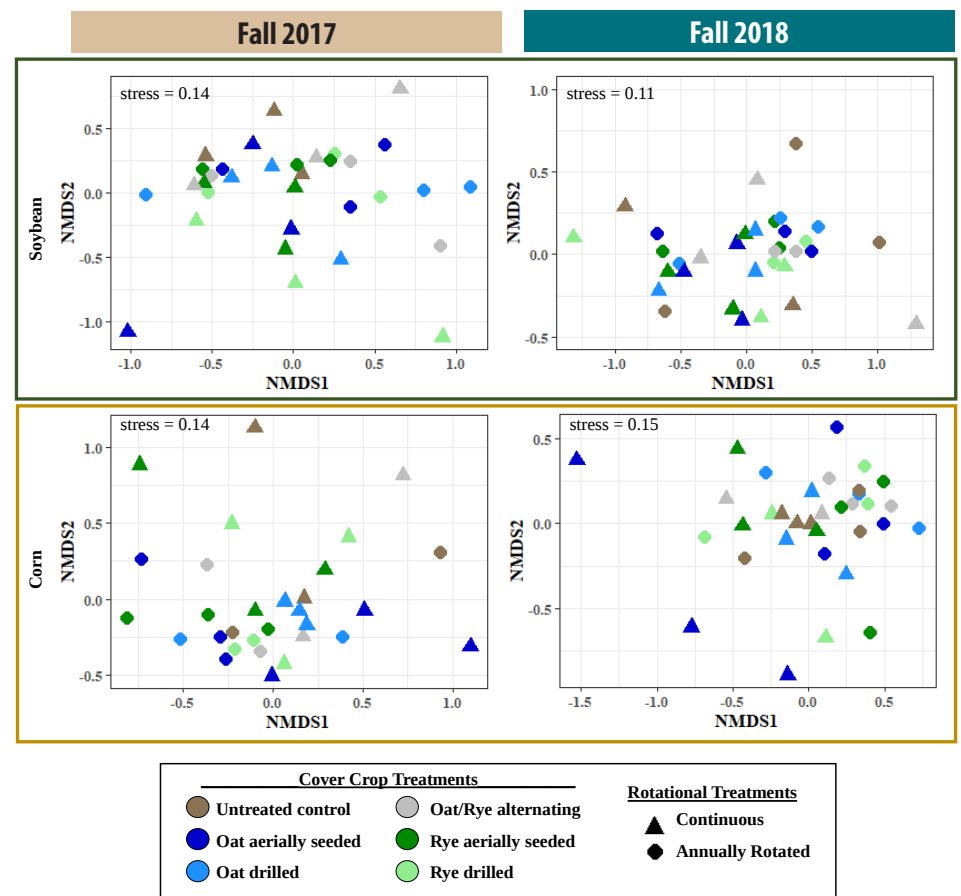


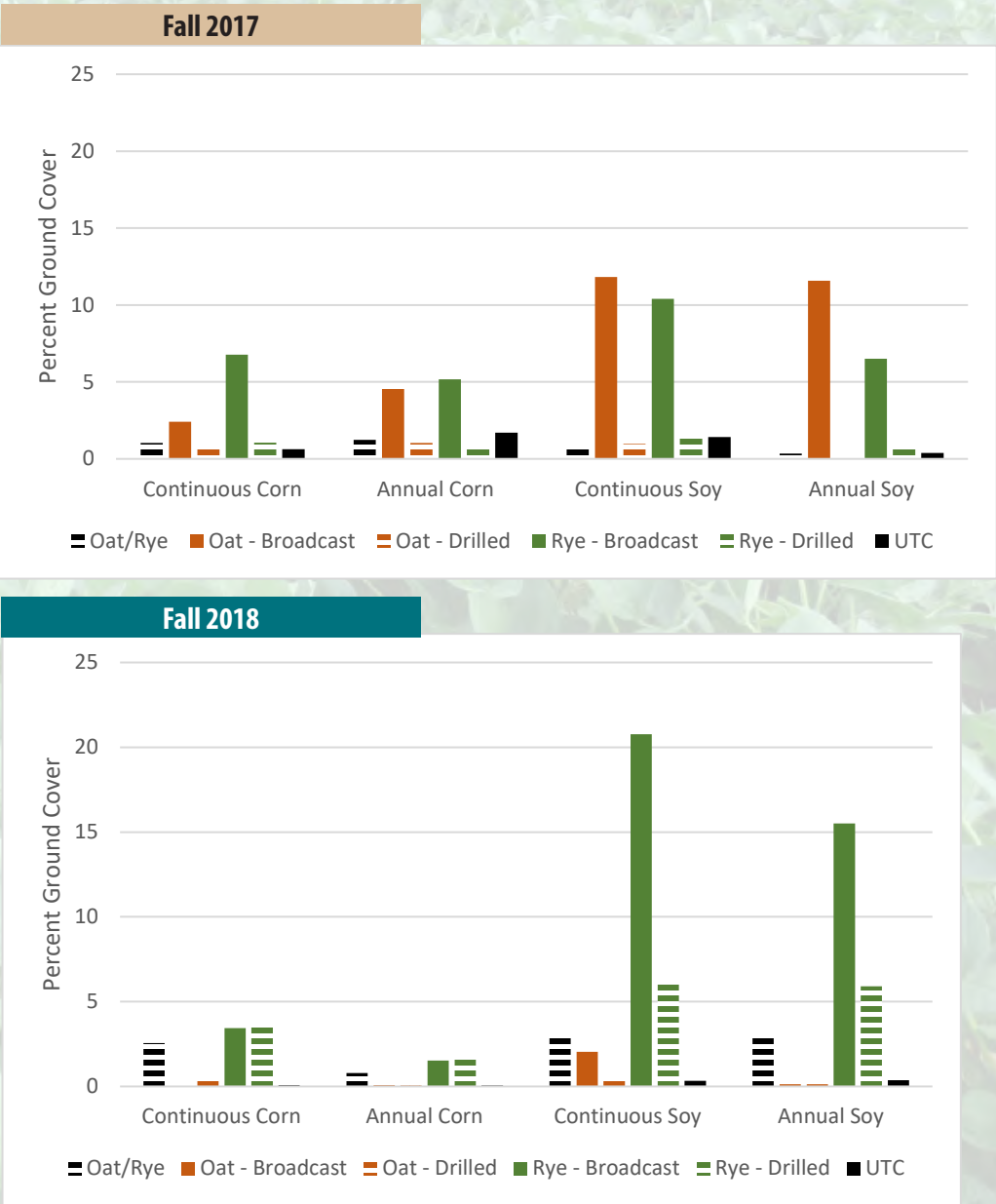
Table 3. Results of permutational multivariate analysis of variance (PERMANOVA) identifying significant effects and percent community variation explained (R^2) by pH, organic matter, and macronutrients Each factor was tested alone in the model, so the estimated effects should not be summed.

	Fall 2017		Spring 2018	
	R^2	p-value	R^2	p-value
pH	0.083	0.001	0.124	0.001
OM %	0.052	0.001	0.083	0.001
P	--	0.555	0.024	0.023
K	0.026	0.008	0.027	0.010
NH ₄	0.023	0.050	0.031	0.005
NO ₃	0.031	0.002	0.063	0.001

Table 4. Mean and standard error of organic matter content and pH for each field study block (replication).

	Block	pH	SE	OM %	SE
Fall 2017	1	6.8	0.037	2.6	0.057
	2	6.2	0.043	3.3	0.054
	3	6.4	0.080	3.1	0.061
Spring 2018	1	6.6	0.069	2.8	0.039
	2	6.0	0.056	3.3	0.048
	3	6.1	0.079	3.0	0.067

Figure 5. Bar plot of percentage ground cover before the first frost in the fall of 2017 and before chemical termination in spring 2018 for six cover crop treatments within four crop rotation treatments. Groups with the same letter are statistically similar ($P < 0.05$), as determined by Tukey's HSD post hoc.





CONCLUSION

Crop rotation was found to have an impact on the structure of bacterial communities in the bulk soil resulting in distinct communities associated with continuously cropped corn and continuously cropped soybean. Cover crop treatment had no impact on the structure of bacterial communities, likely due to limited growth of the cover crops. Over time, cropping practices do have an impact on soil microbiota, especially those that alter soil properties.

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