Soil bacterial community dynamics in plots managed with cover crops and no-till farming in the Lower Mississippi Alluvial Valley, USA

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Abstract

Aims: Assess bacterial community changes over time in soybean (*Glycine max*) crop fields following cover crop (CC) and no-till (NT) implementation under natural abiotic stressors.

Method and results: Soil bacterial community composition was obtained by amplifying, sequencing, and analysing the V4 region of the 16S rRNA gene. Generalized linear mixed models were used to assess the effects of tillage, CC, and time on bacterial community response. The most abundant phyla present were Acidobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia. Bacterial diversity increased in periods with abundant water. Reduced tillage (RT) increased overall bacterial diversity, but NT with a CC was not significantly different than RT treatments under drought conditions. CCs shifted abundances of Firmicutes and Bacteroidetes depending on abiotic conditions.

Conclusions: In the Lower Mississippi Alluvial Valley (LMAV), USA, NT practices lower diversity and influence long-term community changes while cover crops enact a seasonal response to environmental conditions. NT and RT management affect soil bacterial communities differently than found in other regions of the country.

Significance and impact of study

Given the environmental and productivity concerns of agricultural soils, conservation practices that improve soil health and environmental integrity are critical. In the Lower Mississippi Alluvial Valley, there is a lack of research on cover crop and no-till management's impact on soil biology that is specific to the regional nuisances. Conservation management has varying effects on soil bacterial communities and thus implementation must be uniquely applied by region.

Keywords: cover crop, no-till, agricultural soil science, bacterial genomics, community structure, conservation agriculture

Introduction

Soil health can be described as the sustained capability of soil to accept, store, and recycle water, nutrients, and energy (Gregorich et al. 1994). Soil microorganisms are a pillar of soil health and a driver of many ecosystem services connected to agricultural production, including nutrient cycling and carbon sequestration. However, it remains difficult to characterize a "healthy" soil microbiome in agricultural systems because the soil microbiome can vary tremendously depending on a combination of inherent soil properties and abiotic and biotic properties of the soil. To further complicate the matter, disturbance in the form of land management (tillage, heavy chemical use, and rotating plant communities) and weather events (flooding, drought, etc.) can also have a pronounced effect on soil bacterial communities (Guo and Gifford 2002, Jansson and Hofmockel 2019, Shanmugam et al. 2021). This raises concerns about the resiliency of beneficial soil microbial communities in agricultural soils while under the persistent threat of climate change and consistent disturbance due to conventional agriculture management practices. Therefore, investigating agricultural practices that improve the sustainability of soil biology and build resiliency in agroecosystems concomitant to achieving crop output need to be investigated and subsequently implemented when applicable.

Certain farming techniques have been developed for the purpose of enhancing cash crop fertility and manipulating microbial community composition to promote soil health. Cover crops (CCs) and no-till (NT) farming are two such practices that have shown to provide benefits to agricultural systems through weed control (Dabney et al. 2001, Leakey and Tchoundjeu 2001, Tyler and Locke 2018, Korres et al. 2019), reducing diurnal fluctuations in soil temperature (Dabney et al. 2001), surface runoff (Reddy et al. 1995, Krutz et al. 2009), and herbicide losses (Krutz et al. 2009). Additionally, soils managed with CC are higher in microbial diversity (Venter et al. 2016), functional diversity (Nivelle et al. 2019), microbial biomass carbon (dos Santos Soares et al. 2019),

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microbial biomass nitrogen (Brennan and Acosta-Martinez 2017), fungal biomass that promotes decomposition (Barel et al. 2019), and disease suppressive bacteria (Peralta et al. 2018).

Adoption of conservation management practices is a high priority in the Lower Mississippi Alluvial Valley (LMAV) because of the region's proximity to the Mississippi River and high agricultural production landscape, making the LMAV a contributor of nonpoint source pollution into surrounding water bodies and annual hypoxic zones in the Gulf of Mexico (Risal and Parajuli 2019). Although available scientific literature documenting the positive impact CC and NT have on soil microbial populations is extensive, it is generally specific to the Midwest and Northeastern regions of the United States. In Mississippi, the adoption of these practices is low, despite National Resources Conservation Service (NRCS) financial assistance programs for CCs (CTIC, SARE, and ASTA 2016). Only a handful of studies have addressed CC benefits to soil health in the Mississippi, and fewer have examined the impact CC can have on soil bacterial communities, which will be sensitive to Mississippi's high rainfall, above-freezing winter temperatures, and high clay content in alluvial soils.

Of the studies addressing soil biology in the region, Brooks et al. (2018) found no effect on soil microbial population structure, richness, or diversity following CC management; however, results concluded that CCs select for microbial community members in high abundance, as opposed to selecting for new populations. Locke et al. (2013) found increased microbial activity and diversity under CC treatments, but no effect of tillage on soil-macro-organisms (i.e. nematodes and earthworms) was observed. Shanmugam et al. (2021) found that increasing tillage intensity decreased microbial diversity, but Tyler (2021) found the opposite, with increased diversity with increased tillage intensity.

To that end, there are considerable knowledge gaps in the mechanisms underlying microbial community functioning and a substantial portion of soil microbial life remains unexplored. The degree that soil microbial communities respond to CC will depend on the type of cover crop planted (Fanin et al. 2015, Aronsson et al. 2016), climate conditions (Marinari et al. 2015), and if other soil health best management practices (BMPs) have been implemented in tandem to CCs (Nivelle et al. 2016, Bakker et al. 2018, Schmidt et al. 2018). The complexity of the soil microbiome creates challenges in assessing and comparing soil microbial metrics across systems, as well as implementing practices to maximize beneficial microbial interactions. However, high-throughput sequencing techniques create opportunities to analyse soil microbial community structure and functioning at a finer resolution than culture-based or physiological techniques have previously allowed

The purpose of this study was to use high-throughput sequencing to assess bacterial community changes in soybean (*Glycine max*) row crop fields in northwest Mississippi, USA following CC and NT implementation. Divergent weather patterns during study years created a unique opportunity to additionally examine how bacterial communities, under different management treatments, respond to atypical abiotic stressors. As climate change is predicated to increase the incidence of extreme weather events (IPCC 2021), the results of this study provide valuable information related to the effect of management on soil bacterial community dynamics and the relative resiliency of conservation management systems.

Materials and methods

Study design

The study was conducted in 2019 and 2020 at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA. In fall 2000, experimental plots were established under cotton (Gossypium hirsutum L.) production in a splitblock design as described in Locke et al. (2013). Within each of four blocks, six treatment plots (n = 24) were constructed as 8 m wide and 32 m long (0.026 ha) with eight 1-m wide raised-bed rows in each plot. Soil type was identified as a fine silty loam, silty clay loam, and dowling clay. Soil clay content increased with increasing block number. No-tillage or reduced tillage (NT or RT) treatments were implemented in 2000 as the main split-block effect. The cover crop treatments for this study were applied in 2017 with elbon rye (Secale cerale), rye + crimson clover (Trifolium incarnatum) mix, or no cover (RY, RC, and NC) (Tyler 2019, 2021). From spring of 2007 to present, Asgrow AG4632 variety soybean (Glycine max) was planted as the cash crop. A compete cropping description can be found in Locket et al. (2013) and Tyler et al. (2019, 2021) as well as in Supplementary Table S1. The combinations of treatments in the present study were as follows NT-RY, NT-RC, NT-NC, RT-RY, RT-RC, and RT-NC.

In late April of 2019 and 2020 when CC reached peak growth, the CC was terminated with two applications of Gramoxone SL2.0 (Syngenta, Greensboro, NC, USA), 2 weeks apart, at a rate of 0.77 kg ha⁻¹ and then rolled for planting. Twenty-four hours after termination (approximately mid-May), 150 g of CC biomass was cut and placed in litterbags measuring 20.23 × 20.23 cm made from fiberglass mesh screen (0.508-mm mesh size). No cover treatment litterbags contained volunteer vegetation from the plot. Two litterbags containing CC biomass (or volunteer vegetation) from their respective plots were stapled closed and then secured on the soil surface. There was 2 litterbags per plot, for a total of 48 litterbags in the study area.

Soil sampling

Litterbags were used to ensure a standardized amount of decomposing material over each sampling location. Soil residing under litterbags was tested at intervals over the growing season for differences in bacterial community composition between and among treatments. Two soil samples per plot were taken, one from each litterbag location with soil cores (3.8-4 cm diameter \times 10 cm depth) at the date of litterbag implementation and then alternating months until harvest of soybeans in the fall (approximately May, July, and September 2019 and 2020). Cores from the same plot were consolidated into one composite sample per plot for a total of 24 samples per sampling event, comprising 72 total samples over the growing season per sampling year (144 samples total). The initial sampling event (in May after CC termination) occurred before litterbags were secured to the soil surface; all subsequently collected samples were taken from the soil directly under the litterbag. Samples from each plot were placed in a sterile plastic bag and stored on ice ($\sim 4^{\circ}$ C) for transport back to USDA Genetics and Sustainable Agriculture Unit in Starkville, MS, USA. Ten grams of each soil sample was sepa-

Table 1. Summary of microbial community statistical analyses.

Response variable	Statistical test	Purpose
Soil temperature	Linear model with <i>post-hoc</i> Tukey's pairwise comparisons	Determine the effect of tillage, cover, month, and year on response variable. Assess differences between treatment groups.
Soil gravimetric moisture	GLMM with <i>post-hoc</i> Tukey's pairwise comparisons	
OTU richness	-	
Shannon diversity		
Bacterial phyla		
Firmicutes to Bacteroides ratio		
Beta diversity	PERMANOVA with Bray–Curtis Distance and homogeneity of dispersion test	Measure the dissimilarity of microbial communities and evaluate differences in the variability of community assemblages among treatments.

rated for soil moisture content analysis using the gravimetric method. Fresh soil samples were temporarily stored in the laboratory at 4°C and subsequently transferred to -20°C prior to further extraction and assay.

High-throughput sequencing

Soil samples were extracted for total microbial DNA (0.25 gsoil) using MP Biomedical FastDNA Spin Kits on a FastPrep-24 homogenizer. DNA was quantified and subjected to serial dilution(s) (10- to 100-fold) to avoid inhibitory cocontaminants. We submitted soil microbial genomic DNA from the 2019 sampling event to Novogene (Sacramento, CA, USA; outsourced to China); however, due to USA policy on utilizing foreign companies with ties to China during the COVID-19 pandemic, the 2020 sampling event was submitted to Microbiome Insights (Vancouver, BC, Canada) for 16S rRNA library preparation and sequencing via the Illumina MiSeq DNA sequencing platform. Both followed the same library prep and sequencing protocol. Briefly, the MiSeq standard operating procedure proposed by Kozich et al. (2013) was followed, after library preparation and quality assurance, and control (Kozich et al. 2013). V4 region of the 16S rRNA gene was amplified, sequenced, and analysed. Bioinformatic 16S rRNA sequence analysis was performed using the Mothur platform (Version 1.44.2.5) following the MiSeq standard operating procedure as outlined on the Mothur website (http://www.mothur.org/wiki/miseq_sop; Kozich et al. 2013). Libraries were contiged and curated to reduce errors and low-quality sequences. Sequences were aligned to the most current Silva alignment reference files (Release 138.1) and taxonomically classified using the most current Ribosomal Database Project reference files (RDP, Version 18). We used commands "screen," "filter," and "classify" in Mothur (Kozich et al. 2013) to clean the database, including removing 18S rRNA gene fragments and 16S rRNA Archaea, chloroplast, and mitochondria. Operational taxonomic units (OTUs) were assigned at a 3% dissimilarity, and taxonomy-based analyses were conducted at the genus taxon. Prior to analysis, libraries were rarefied to minimum library size.

Environmental conditions

Hobo sensors were installed within four treatments plots (NTcover, NT-NC, RT-cover, and RT-NC). Soil temperature was measured at 15 second intervals beginning in May and continuing through September in 2019 and 2020, when they were removed at the last sampling event of the year, just before harvest. Soil moisture was determined from each sample using the gravimetric method. Total monthly rainfall data were sourced from NOAA National Center for Environmental Data at the Leland, MS, USA weather station for May–September of 2019 and 2020 (NOAA 2020).

Statistical analysis

A summary of all statistical tests can be found in Table 1. OTU-based analyses were conducted using R packages Phyloseq and Vegan (R Core Team 2020). To determine the effect of tillage, CC, month, and year on alpha diversity metrics (richness and Shannon), we used generalized linear mixed models (GLMMs), followed by Tukey's pairwise post-hoc comparisons to assess differences between groups. The interaction of tillage, CC, month, and year were used as fixed effects. Block was used as a random effect to account for the gradient of soil types observed in the study area. GLMMs with a tillage, cover, month, and year interaction term were also used to model soil gravimetric moisture and Firmicutes to Bacteroidetes ratio (Ochoa-Hueso 2017), followed by Tukey's post-hoc comparisons. The Firmicutes to Bacteroides ratio was calculated by dividing the relative abundances of the two phyla. Because soil temperature was measured at a singular treatment location, there was no need for a random effect. A linear model was used followed by Tukey's pairwise comparisons to assess the effect of tillage, CC, month, and year on soil temperature. Each model was checked for goodness of fit (homogeneity of variance, normality of residuals, residual distribution, normality of random effect, and collinearity). Differences in bacterial phyla between treatments were determined with GLMMs, with a tillage, cover, year interaction term as a fixed effect and block as a random effect.

Beta diversity was assessed with a permutational analysis of variance (PERMANOVA) using bray dissimilarity distances (999 permutations) to test whether microbial community assemblage was influenced by tillage and/or cover treatments within each year. To evaluate differences in the variability of community assemblages among treatments, homogeneity of dispersion test ANOVA was conducted to compare the mean distance-to-centroid of microbial community among treatments (tillage or cover). Significant ANOVAs were followed by Tukey's *post-hoc* test to identify pairwise differences in microbial community dispersion between management treatments. Nonmetric dimensional scaling (NMDS) using Bray– Curtis distances was performed for an analysis of community assemblage between treatments.

Total Monthly Precipitation



Figure 1. Total monthly precipitation. Total monthly precipitation (mm) during the growing season of 2019 and 2020. Data sourced from NOAA National Center for Environmental Data at Leland, MS, USA weather station. The black line indicates the 10-year average of monthly rainfall in Leland, MS, USA.

Results

Environmental variables

Data sourced from NOAA of total monthly rainfall during the study periods of 2019 and 2020 showed contrasting patterns between years, particularly in the months of May and September (Fig. 1). Although not tested statistically, both years were observed to deviate from the past 10-year monthly average (10-year May mean: 5.25 mm, June mean: 5.49 mm, July mean: 4.32 mm, August mean: 4.88 mm, and September mean: 2.76 mm; Fig. 1). Mean soil temperatures for 2019 and 2020 additionally showed significant differences in treatments, months, and years ($F_{39,143}$ $_{130} = 1203$, $R^2 = 0.24$, P < 0.05). Soil gravimetric water content was significantly higher in 2019 than 2020 and higher in NT than RT ($R^2 = 0.85$, P < 0.05; Table S2), but not different between cover crop treatments.

16S rRNA high-throughput sequencing

Mixed model effect sizes for all phyla can be found in Table S3. Tukey's pairwise comparisons revealed significant differences in the abundance of several phyla between years and between treatments. The most abundant phyla present in both sampling years were Acidobacteria, Actinobacteria, Bacteroidetes, and Verrumomicobia (Fig. 2). In 2019, there were six phyla present that were not detected in 2020: Chlamydiae, Cyanobacteria, TM7, Spirochates, OD1, and BRC1, although their relative abundances were <1% of the community composition. Proteobacteria, Bacteroidetes, and Firmicutes were greater in abundance in 2019 (P < 0.05), while Acidobacteria, Verrucomicrobia, and Planctomycetes were more abundant in 2020 (P < 0.05). Acidobacteria abundance was greater in RY (P < 0.03) and there was a significant interaction between RT and both CC treatments in 2020. Overall, Bacteroidetes

abundance was significantly greater in NT plots (P < 0.05). Cover treatments (RY and RC) were greater in abundance in 2019, but in 2020, NC had greater abundances (P < 0.05). The greatest abundances of Verrucomicrobia were observed in the combination of NT and plots with cover (RY and RC). Reduced till treatments had significantly greater abundances of Firmicutes in both years. In 2019, NC plots showed the highest abundance of Firmicutes, but in 2020 abundances were greatest in RC, followed by RY, with lowest abundances observed in NC (P < 0.05). Predicted values of relative abundances for each taxa within each treatment can be found in Fig. 2.

Tillage had a positive effect on F:B and 2020 had a negative effect on F:B ($R^2 = 0.85$, Table S4). NT had a significantly lower F:B than RT (P < 0.05) and there was a trend of higher F:B in 2019 than 2020 (P = 0.15). NT behaved similarly across all CC treatments between years, but RT plots with CCs behaved differently than RT-NC plots between years (Fig. 3).

When assessing the effect of tillage, cover, month, and year on richness, tillage and RTxSeptx2020 had a positive effect, while 2020 and Septx2020 had a negative effect (R^2 : 0.95, Table S5). When assessing the effect of tillage, cover, month, and year on Shannon Diversity, tillage, Septx2020 and RTxSeptx2020 had significant positive effects, while 2020 had a significant negative effect. Tillage, cover, month, and year, as well as the interaction, were significant in the model $(R^2: 0.63;$ Table S6). Both alpha diversity metrics indicated that 2019 was more diverse than 2020. Tukey's post-hoc test showed a marginal difference in richness between RT and NT (P = 0.07; Fig. 4), and significant differences were found in 2019 between NT \times NC and RT \times NC in the months of May and September and between NT \times RC and RT \times RC in September 2019 (P < 0.05). Using the Shannon Diversity Index, RT was significantly more diverse than NT



Phylum Percent Relative Abundance

Figure 2. Bacterial phyla percent relative abundance. Percent abundances for soil bacterial communities in study plots at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA. Abundances are calculated for each bacterial phyla within each treatment combination (tillage treatments: NT = no-till, RT = reduced till, and cover treatments: no cover, RC = rye + clover, RY = rye) within each year (2019 and 2020). Darker color indicates greater % relative abundance. Numbers in columns are the indicated phyla % abundance of total community.



Firmicutes to Bacteroidetes Ratio

Figure 3. Yearly shifts in firmicutes to bacteroidetes ratio. Model predicted values of Firmicutes to Bacteroidetes ratio in study plots at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA. Ratio is calculated for each treatment type based off relative abundance in bacterial community. Different cover treatments are denoted by line type (NC = solid line, RC = small dashes, RY = large dashes) and shape (NC = open circle, RC = cross, RY = filled triangle). Error bars indicate standard error of model predicted values.

(P < 0.05; Fig. 5). In 2020, NT × NC and NT × RC were significantly different from the other combinations of treatments (P < 0.05); see Figs. 4 and 5 for trends in diversity metrics.

In 2019, beta diversity analysis showed tillage regime influenced community assemblage (PERMANOVA, $F_{1,70} = 4.55$, P = 0.001; Fig. 6a). However, the assumption of homogeneity of dispersion was rejected, that is, certain com-



Figure 4. OTU richness in 2019 and 2020. Model predicted values for the change in OTU richness in study plots at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA in 2019 (a) and 2020 (b). Treatment richness varied through the season. NT is represented with a solid line, RT is represented as dashed line. Error bars indicate standard error of model predicted values. Note different scales for *y*-axis.



Figure 5. Shannon diversity in 2019 and 2020. Model predicted values for the change in Shannon diversity in study plots at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA in 2019 (a) and 2020 (b). Treatment Shannon diversity (H) varied through the season. NT is represented with a solid line, RT is represented as dashed line. Error bars indicate standard error of model predicted values. Note different scales for *y*-axis.

munities had more variability than others ("betadisper," ANOVA, $F_{1,70} = 4.613$, P = 0.035). Tukey's *post-hoc* comparison showed communities within NT were more dispersed than in RT (95% CI= -0.016 to -0.031, P < 0.01). Cover treatments in 2019 likewise showed an effect on community assemblage (PERMANOVA, $F_{2, 69} = 2.48$, P = 0.001, NMDS stress = 0.205; Fig. 7a). Dispersion of community assemblages showed marginal differences within cover treatments ("betadisper," $F_{2, 69} = 2.91$,

P = 0.06). Tukey's *post-hoc* comparison indicated RC was more dispersed than RY (95% CI= -0.001 to 0.043, P = 0.06).

Similarly, in 2020, PERMANOVA showed tillage to influence community assemblage ($F_{1,70} = 3.95$, P = 0.001; Fig. 6b). The dispersion of communities between tillage treatments differed significantly ("betadisper," ANOVA, $F_{1,70} = 4.386$, P = 0.03). Specifically, bacterial communities in NT treatments were more variable than in RT treatments (Tukey's



Figure 6. Tillage beta diversity NMDS in 2019 and 2020. Beta diversity using NMDS Bray–Curtis distances of tillage treatments in 2019 (a) and 2020 (b) at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA. The dispersion of communities between tillage treatments differed significantly in both years (2019: NMDS stress = 0.205, P = 0.001; 2020: NMDS stress = 0.191, P = 0.03).



Figure 7. Cover crop beta diversity NMDS in 2019 and 2020. Beta diversity using NMDS Bray–Curtis distances of cover crop treatments in 2019 (a) and 2020 (b) at the USDA-ARS Crop Productions Systems Research Unit farm near Stoneville, MS, USA. In 2019, RC was more dispersed than RY (P = 0.06; NMDS stress = 0.205). In 2020, NC communities were more dissimilar to RC and RY, while RC and RY were less dissimilar to each other (NMDS stress = 0.191, P = 0.001).

HSD, 95% CI= -0.023 to -0.045, P = 0.03). Nonmetric dimension scaling (NMDS, stress = 0.191) of 2020 tillage communities showed a similar configuration to that of 2019. Cover in 2020 had an effect on community assemblage (PERMANOVA, $F_{2,69} = 34.99$, P = 0.001; Fig. 7b), with NC communities being more dissimilar to RC and RY, while RC and RY were less dissimilar to each other (Fig. 7b). There were no differences in dispersion of microbial communities within cover treatments (ANOVA, $F_{2, 69} = 0.491$, P = 0.613).

Discussion

While it was the original objective of this study to understand the impact of long-term implementation of conservation management practices (NT and CC) on soil bacterial community composition and function in row-cropping systems of the LMAV, contrasting weather patterns over the sampling years presented the opportunity to examine the dynamics of soil bacteria communities when exposed to abiotic stressors (extreme rainfall and drought). Results of this study found both abiotic stressors and management to have a pronounced effect on soil bacterial communities. Conservation management also influenced how microbial communities responded to changing environmental conditions.

Abiotic stressors and microbial response

Adverse climatic conditions creating abiotic stresses are among the principal limiting factors for decline in agricultural productivity. Climate change models predict increases in rainfall, temperature fluctuations, and extreme weather events that can tax a crop's ability to produce (Wang et al. 2003, IPCC 2021). Of the two years that this study took place, 2019 was the second wettest year on record in the United States (NOAA 2020, 2021), while drought conditions were reported in Mississippi in May and July of 2020 (NOAA 2020, 2021). The NOAA rainfall reports were reflected in gravimetric soil moisture content between years, with the soil in 2019 having a higher moisture content on average than 2020.

There is a direct relationship between the amount of moisture present and the abundance and diversity of microbial life (Greaves and Carter 1920). Generally, increases in moisture content increase biological activity and diversity. This was observed in the drastic increases of alpha diversity metrics of bacteria present in 2019 compared to 2020. Decreases in the abundance of the phylum Proteobacteria in response to moisture limitations is well documented in literature (Bouskill et al. 2013, Acosta-Martínez et al. 2014, Curiel Yuste et al. 2014), and further supported by this study's decrease in Proteobacteria abundance in 2020. The significant increase in Acidobacteria in 2020 concurs with findings of others concerning Acidobacteria's association with droughted roots (Curiel Yuste et al. 2014, Desgarennes et al. 2014). Likewise, the six phyla present in 2019 that did not appear in 2020 (Chlamvdiae, Cyanobacteria, TM7, Spirochates, OD1, and BRC1) all have members that are facultatively or obligately anaerobic and associated with water (Konopka 2009, Vincent 2009, Winsley et al. 2014, Taylor-Brown et al. 2015, Schwab et al. 2017, Martínez-Gómez 2019). Contrary to what was found in this study, several studies have documented little impact of drought on bacterial phylogenetic diversity of soil communities (Bachar et al. 2010, Acosta-Martínez et al. 2014, Armstrong et al. 2016, Tóth et al. 2017). The impact may be context dependent, however, as Bouskill et al. reported a 40% decrease in phylogenetic alpha-diversity after drought stress (Bouskill et al. 2013).

Typically, greater diversity of soil bacteria is equated with a heathier crop-soil system (McNaughton 1977, McCann et al. 1998). However, crop yield was lower in 2019, a higher bacteria diversity year, than in 2020 (Firth 2022). When comparing monthly soil moisture between years, 2019 had the highest moisture in May and declined through the growing season, while 2020 had the lowest moisture in May and increased through the growing season. We hypothesize that the lower yield in 2019 was caused by the proliferation of disease causing bacteria and an increase in the incidence of root-rot at the time of seed germination (Ahmed et al. 2013). This is supported by the detection of phylum Cyanobacteria in 2019, which has members known to contribute to pathogen suppression (Domracheva et al. 2010, Huang et al. 2019).

It should be noted that the switch in sequencing companies could have affected the differences observed in diversity between years. However, in an accompanying study, Firth (2022) used quantitative polymerase chain reaction to compare 16S relative abundances between years. Bacterial abundance in 2019 was greater than 2020 and reflect similar trends as this study's differences in diversity. Thus, differences found mostly likely reflect the soil environmental conditions, and not the quality of library preparation.

Influence of management on soil community diversity and function

CCs and NT management are two common conservation agriculture practices that have shown to have a positive effect on bacterial diversity, activity and system functioning. In this study, the effects of implementing NT and CC had varying effects on soil bacterial community dynamics, indicating that system responses to conservation management are location and context dependent.

Tillage

Many studies have documented the negative impact tillage has on microbial diversity (Kaurin et al. 2015, Chávez-Romero et al. 2016, Wang et al. 2016, Schmidt et al. 2018, Shanmugam et al. 2021). Tillage homogenizes soil microhabitats which reduces the types of habitat available for bacteria to exploit, leading to a reduction in bacterial diversity (Schimel and Schaeffer 2012, Sengupta and Dick 2015). NT environments leave roots intact and minimize disturbance creating porous microhabitats that allow opportunity for unique microhabitat development (Carson et al. 2010). Here, it was found that RT and NT were similar in community composition, but NT was more variable. Therefore, we conclude that higher incidence of unique microhabitats created by NT will select for already abundant species that can survive in the context of microhabitat setting.

Contrary to most studies, NT exhibited overall lower species richness and Shannon Diversity. These findings are consistent with previous observations made at the field site (Tyler 2019, 2021). NT management in heavy clay soils can create low-O₂ conditions that would not be present in soils with more silty or sandy textures because O₂ diffusion rates are slower in soils with small particle sizes (Khan 1996). In effect, microhabitats created by NT management select for low-O₂ tolerant bacteria. This is further supported by previous observations of the study fields by Tyler (2021) where an abundance of OTUs classified as *Geobacter* were detected occupying NT, but not in RT (Tyler 2021). *Geobacter* is a genus of anaerobic bacteria capable of metabolizing humic materials in soil (Voordeckers et al. 2010).

The Intermediate Disturbance Hypothesis (IDH) suggests that diversity is maximized in environments that introduce "intermediate" levels of disturbance (Hutchinson 1961, Grime 1973, Connell 1978). Environments with low- or highdisturbance rates will have low diversity, but at intermediate levels, there is a balance of colonization and competition that allows most species to exist together (Hutchinson 1961, Grime 1973, Connell 1978). While the IDH is widely criticized amongst ecologists because of the vagueness of "frequent disturbance" and general variability in environmental responses (Fox 2013), the hypothesis offers explanation of bacterial community diversities in heavy clay soils. Reduced tillage involves a one-time tillage event in the fall, as opposed to conventional tillage that tills soil both preplanting and postharvest. Reduced tillage qualifies as the intermediate of the two extremes (NT or conventional tillage), where diversity is maximized. Tilling soil only once a year may partially conserve soil microhabitats and additionally provide O_2 to clay soils, leading to increased opportunities for a diverse array of species to colonize, while decreasing the frequency of tillage increases species' ability to compete and persist in a soil environment.

An occasional tillage event, for example tilling soils once every 5–10 years, may optimize tillage benefits observed. A less frequent tillage event would periodically introduce O₂ into the system thereby increasing diversity, yet be infrequent enough to maintain other documented benefits of NT, for example, the promotion of fungal populations (Blanco-Canqui and Wortmann 2020). Future studies should address the applicability of the IDH by testing frequencies of tillage events that optimize both diversity and function in clay soils of the LMAV.

Cover crops

Verrucomicrobia, Acidobacteria, and Planctomycetes are common in low-nutrient environments and associated with conservation agricultural practices (Navarro-Noya et al. 2013, Ramirez-Villanueva et al. 2015). All were greater in CCs (RY and/or RC) than in NC, although in some cases the difference depended on the year. The phylum Verrucomicrobia has many important members associated with the plant rhizosphere that thrive on compounds released by plant roots in the form of exudates, mucilage, or sloughed-off cells (Chow et al. 2002, Nunes Da Rocha et al. 2011). Jones et al. and Navarrett et al. found a positive correlation between Acidobacteria abundance and organic carbon availability, which is supported by the increased soil organic carbon found in CC plots (Jones et al. 2009, Navarrete et al. 2015, Firth et al. 2022). Likewise, Planctomycetes possess several members that are responsible for anaerobic oxidation of ammonium. Abundance is correlated with spatial heterogeneity of nitrate suggesting that the addition of CC increases soil nitrogen (Buckley et al. 2006).

Differences in vegetation establishment, abiotic factors or a combination thereof account for the observed differences in CC community composition between years. Planting two species in a CC mix will cause a certain amount of heterogeneity in vegetative cover, accounting for the variability seen in RC beta diversity. Interestingly, the Shannon Diversity Index of NT-RY in 2020 was as diverse as all the RT cover treatments, indicating that the influence of CCs will change under different abiotic conditions. Romdhane et al. found that management of CC had more effect on the abundance of certain community members than the cover mixes themselves (Romdhane et al. 2019). Different degrees of CC soil contact with rolling or tilling may shift the decomposition, nutrient mineralization, and community composition more dramatically than was observed in this study (Parr et al. 2014). Additionally, because samples were collected during the soybean growing season, the activity of soybean roots could be a stronger selective agent on a short-term basis, while CCs influence community structure and functioning through long-term carbon buildup. This highlights the importance of considering production goals when choosing CC mixes. The effect of a legume (crimson clover) CC may be minimized if followed by a legume cash-crop. This is akin to applying nitrogen fertilizer to a legume cash-crop, which is an unnecessary expenditure because legumes can fix atmospheric nitrogen. Choosing

Abiotic factors effect on bacteria communities under different management

Within a bacteria community, there is a diversity of responses that can be deployed when under external pressure. This study only compared 2 years of community response to extreme rain and varying soil moisture, but the treatments were long term, allowing a certain degree in confidence to address the management practices' potential to provide resiliency. Tillage management had the largest effect on overall community structure, while the combination of tillage regime and CC enacted a seasonal response to environmental conditions.

In periods of high moisture, soil pore space will be O_2 limited, further compounding the effects of NT's selective low O₂ environment. This is seen in the early months of 2019 and in the later months of 2020, where RT plots have higher Shannon diversity and richness across all cover treatments. As moisture declines, the gap in RT and NT diversity becomes less pronounced. This is particularly apparent in the latter months of 2019, when bacterial richness of NT-RC and NT-NC is greater than RT and in the early months of 2020, when Shannon diversity and richness in NT-RC and NT-RY were not significantly different from each other. In general, both NT and RT plots that included a CC were more similar in diversity than those without, but the effect is strongest under drought conditions. Roots from CC can provide an extra carbon source and create more opportunities for bacteria habitat via pore space. In times of limited moisture, a rve CC will provide fibrous and quick growing roots to aid in bolstering soil O₂ in NT plots, leading to increases in diversity. Furthermore, NT and CCs can promote the establishment of fungal hyphal networks (Beare et al. 1997), which facilitate access to water and nutrients in times of drought (Allen 2007, Six 2012, Guhr et al. 2015).

At the phylum level, there were significant changes in abundances of Bacteroidetes and Firmicutes between treatments depending on the year. Soil moisture likely explains the shift in both phyla. Bacteroidetes was greater in CC plots in 2019 and decreased in NC plots in 2020. Bacteroidetes has been shown to increase in moist soils (Chodak et al. 2015) and is a copiotroph that grows quickly in the presence of high soil nutrient concentrations (Fierer et al. 2011, Fierer 2017). In wetter years, plots with CCs retained more moisture and provided greater concentrations of carbon, nitrate, and phosphorus (Firth et al., unpublished data [2022]). Phylum Firmicutes showed the opposite pattern in abundance shifts, increasing in NC in 2019, while higher in cover treatments in 2020. Between May and June of 2019, moisture in NC plots fell to \sim 15%, whereas plots with cover remained at 20%–25%. Firmicutes are a spore-forming, gram-positive bacteria that can survive and persist in stressful environments. In 2020, when conditions became more favorable and moisture levels were similar across treatments, the effect is no longer seen, with abundances averaging $\sim 2\% - 3\%$ across all treatments.

Implications of bacteria community analysis on soil health

Efforts to assess soil health and resiliency may be informed by more developed research on inflammation and digestionassociated bacteria in the human gut microbiome. It is well accepted in the medical community that alterations to entire microbial communities create system weaknesses that lead to modern diseases (Ha et al. 2014, Tilg and Adolph 2015, Chang et al. 2016, ML and CJ 2016). For example, it is the ratio of Firmicutes to Bacteroides (F:B) in the human gut that is associated with obesity at high ratios and inflammatory bowel disease at low ratios (Mathur and Barlow 2015). A similar concept was proposed by Ochoa-Hueso (Ochoa-Hueso 2017) to assess soil health; high ratios indicate inefficient nutrient cycling while low ratios indicate increased likelihood of pathogen invasion. While the classification of a healthy soil F:B will need further study, it is interesting to note that RT had higher F:B overall and ratios in cover treatments behaved differently depending on the year and tillage type. In both years, NT combined with a CC exhibited a mid-range ratio and the least dramatic change between years, suggesting that conservation management may be less vulnerable to environmental stress and promote system resiliency.

Using bacteria diversity alone as an indicator of soil health without consideration to the roles other soil microfauna play can bias assessments of soil health. Microbial communities are not only characterized by the number and composition of taxa, but also by the ecological associations among microbiome members (Banerjee et al. 2019). Wagg et al. explored the multifunctionality of the soil microbiome, reporting that bacteria diversity and fungi diversity together were better indicators of system health than when considered alone (Hines et al. 2015). Other studies have found that there is division of metabolic labor among soil microorganisms (e.g. bacteria, fungi, and archaea), leading to complementarity physiological functional properties (Kohlmeier et al. 2005, Deveau et al. 2018). In this study, soil bacterial diversity was explored, but diversity of archaea, actinomycetes, fungi, algae, protozoa, and a wide variety of larger soil fauna was not addressed. Vertical diversity, that is the diversity among guilds of organisms and the complexity of interactions between guilds, may be just as important, if not more so, for system multifunctioning (Duffy et al. 2007).

Furthermore, forays into diversity analyses to identify the presence or abundance of individuals that promote plant growth after management implementation may lead efforts to assess soil health astray. Ecosystem functioning and resilience are largely dependent on the species interactions that define the community's ability to respond to stress, not the presence of certain species per se. Given the recent surge in available literature characterizing soil microbial communities, we may be better served to launch landscape level comparative studies across a multitude of diverse "healthy" and "unhealthy" agricultural systems. Practical application of microbial community analysis should be used to identify the common community shifts and ratios of taxa that indicate system weaknesses (e.g. ideal F:B). Identification of key associations and ratios within communities could thus be used as a diagnostic tool that can target individual, site-specific production vulnerabilities, and the means to implement swift and successful management interventions toward ecosystem resilience, sustainability, and food security.

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Supplementary data

Supplementary data is available at JAMBIO online.

Conflict of interest

All authors declare no conflict of interest.

Author contributions

For the current work, author contributions are as follows: A.G.F.: conceptualization and design of experiment, collected, processed and assayed samples, data analysis, and authored the manuscript; J.P.B.: assisted in designing sampling scheme, collecting samples, determining appropriate assays, and manuscript development; M.A.L.: assisted in experiment conceptualization and manuscript development. D.J. M.: assisted in determining appropriate statistical analysis and manuscript development. A.B.: assisted in manuscript development. B.H.B.: conceptualization and design of experiment, determining appropriate analysis, and manuscript development. All authors have read and agreed to the published version of the manuscript.

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