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ORIGINAL PAPER

Shifts in bacterial community in response to conservation management practices within a soybean production system

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Abstract

A 3-year field study was conducted to assess effects of two winter cover crops, Elbon rye (*Secale cereal* L.) and Crimson clover (*Trifolium incarnatum* L.), under till and no till management, on bacterial community composition in soybean (*Glycine max* L.) field soils using high-throughput sequencing of the 16S rRNA gene. Effects of tillage and cover crop on bacterial composition at the phylum level were minor, with most significant differences between treatments occurring at finer taxonomic levels. Tilled plots displayed higher levels of Xanthomonadaceae, while cover cropped soils had greater *Bradyrhizobium* abundances. Functional gene prediction indicated that genes associated with decomposition of C and P compounds, as well as biocontrol agents, were elevated in tilled plots, genes associated with root growth promotion were elevated in cover crop treatments, and nitrate reductase and denitrification genes were elevated in both no till and cover crop plots. While valuable functional insights can be gained from sequence analyses, not all differences observed at the sequencing level will translate into functional differences due to variation in gene expression, and further study is needed to validate which functions can be predicted from sequencing data.

Keywords Tillage · Cover crop · 16S rRNA sequencing · Bacteria · Soil · Community composition

Introduction

There is a growing emphasis on implementing conservation practices in row crop production systems, such as corn, cotton, and soybean, to increase sustainability. Each practice can have both beneficial and detrimental effects on soil quality, and careful consideration should be made when selecting which practices to use in order to maintain optimal conditions for crop growth. No till management can help combat loss of soil structure and erosion (Prasuhn 2012), as well as nutrient losses in runoff (Locke et al. 2015), but complete elimination of tillage can result in higher weed populations requiring herbicide application or occasional tillage events (Peixoto et al. 2020). Planting of cover crops instead of leaving the land fallow during the winter can also protect the soil surface from erosion (Prasuhn 2012), promote the retention of soil moisture (Zablotowicz et al. 2010), and increase soil organic matter and nutrient levels in fields (Zablotowicz et al. 2010). Increasing the diversity of cover crop species planted also results in higher carbon (C) inputs to the rhizosphere, thereby increasing microbial biomass in soil (Gentsch et al. 2020). However, cover crops can serve as hosts for disease organisms (Bakker et al. 2016), and depending on the timing of cover crop residue breakdown, they may potentially tie up nutrient availability when it is needed by developing summer crops.

Agricultural management practices also have the potential to impact the microorganisms in soils that carry out key functional roles important to maintain healthy crops (Van Der Heijden et al. 2008). Soil microorganisms mineralize plant residues (Hobara et al. 2014) and are involved in the cycling of key nutrients, such as nitrogen (N) (Schimel and Bennett 2004) and phosphorus (P) (Richardson and Simpson 2011), necessary for plant productivity. The soil microbial community also serves as a source for beneficial bacteria, including symbiotic rhizobia, that provide fixed N to legumes (Masson-Boivin and Sachs 2018). Additionally, there are many species of rhizosphere bacteria that produce siderophores that function to protect plants from pathogens (Kramer et al. 2020) or are capable of promoting plant growth through secretion of the hormone auxin (Ali et al. 2009) or producing enzymes that

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interfere with the synthesis of the hormone ethylene (Glick 2014). The soil can also serve as a reservoir for plant pathogens as well as biocontrol agents, such that variation in bacterial community composition and diversity can result in suppression of plant diseases (Garbeva et al. 2004). Given the many roles that soil microorganisms play in maintaining plant health, it is important to assess how different tillage practices and cover crop species may impact the composition of the communities in field soils.

Effects of different conservation management practices on microbial community composition can be variable. Some studies note that tillage management significantly impacts community composition (Acosta-Martínez et al. 2007; Chávez-Romero et al. 2016; Cookson et al. 2008; Dong et al. 2017; Helgason et al. 2010), while others found it to have little to no effect (Acosta-Martínez et al. 2003). The effects of cover crops are often minor compared to differences by location and can vary by timepoint (Fernandez et al. 2016). Within the lower Mississippi River Basin, winter cover with cereal rye and Balansa clover was found to have significant effects on microbial community composition compared to tillage (Locke et al. 2013). By contrast, Zablotowicz et al. (2010) found that tillage had a greater impact on community composition than rye or hairy vetch. Zablotowicz et al. (2010) also found that sampling date had significant effects on community composition. The studies by Locke et al. (2013) and Zablotowicz et al. (2010) were performed in two different cropping systems (cotton and soybean), emphasizing the need for further research to determine how differences in summer crop can impact how cover crop and tillage treatments interact to alter microbial communities in soils. As these prior studies used fatty acid methyl ester (FAME) analysis to assess the composition of main microbial groups, the current study chose to utilize the greater sensitivity of high-throughput sequencing of the 16S rRNA gene with the purpose of gaining more insight into how these conservation practices interact to influence bacterial community composition in soil under a soybean production system. Multiple sampling timepoints were collected in accordance with the guidelines of Nannipieri et al. (2019) in order to account for shifts in bacterial communities due to seasonal differences that may obscure treatment based effects.

Materials and methods

Study site and sample collection The study was conducted on the experimental research farm located in Stoneville, MS, USA. Field plots were set up in a randomized complete block design, with four replicate till blocks and four replicate no till blocks (total of eight), each containing three plots (32 m × 8.4 m) planted with either Elbon rye, Crimson clover, or no cover crop during the winter. Tillage treatments were established in October 2000 for a previous study on cover crops in a cotton production system (Locke et al. 2013). The current study was established with the planting of cover crops in Fall 2014 and concluded with soybean harvest in Fall 2017. Soil pH and soil organic matter fluctuated slightly in the plots over the course of the study period, ranging from 5.6 to 7.08 and 4.7–7.1%, respectively (Online Resource 1). Soil texture formed a gradient across the field, and the randomized complete block design was arranged so that the replicates of each treatment included a similar soil texture distribution, with two replicate plots per treatment having a Commerce very fine sandy loam, one replicate plot per treatment having a Commerce silty clay loam, and one replicate plot per treatment being half Commerce silty clay loam and half Dowling clay. Plot management involved plowing tilled plots in the fall after soybean harvest, with winter cover crop treatments planted in mid-October of each year. Winter vegetation in both cover crop and non-cover plots was terminated each April by two applications of Gramoxone SL2.0 (Syngenta, Greensboro, NC, USA) 2 weeks apart at a rate 0.77 kg ha^{-1} paraquat dichloride active ingredient per application. Soybean (Asgrow AG4632), the summer crop, was planted in May and harvested in September.

Soil samples were collected prior to cover crop termination in April, at soybean flower stage in June, and post-harvest in September of each year. Eight replicate subsamples (0–5 cm) were collected per plot using 1.8-cm-diameter soil probes and pooled to make one composite sample per plot and timepoint. April 2016 and 2017 samples were collected at both 0–5 cm and 5–15 cm depths to encompass the "plow zone" disturbed by tillage. Soil samples were sealed in plastic bags in the field and transported back to the laboratory on ice. Subsamples were mixed in the bags to break up clumps and form composite samples, transferred to 14 mL Falcon tubes, and stored at –20 °C prior to DNA extraction.

DNA extraction and 16S rRNA gene sequencing DNA was extracted from soil samples using DNeasy PowerSoil kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA extracts were submitted to Molecular Research LP (Shallowater, TX, USA) for amplicon sequencing of the 16S rRNA V4 hypervariable region as described by Caporaso et al. (2011) with some modifications. The region was amplified using primers 515F (5′ GTGCCAGC MGCCGCGGTAA 3′) and 806R (5′ GGACTACHVGGGTWTCTAAT 3′) with the barcode on the forward primer using the HotStartTaq Plus Master Mix Kit (Qiagen, Hilden, Germany) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s, and 72 °C for 1 min, with a final elongation step of 72 °C for 5 min. PCR products were run on a 2% agarose gel to check for successful amplification and relative band intensity. Samples were pooled in equamolar proportions, purified using Ampure XP beads, and used for library preparation following the Illumina TruSeq DNA library protocol. Paired-end 2 × 300 sequencing was performed on an

Illumina (San Diego, CA) MiSeq analyzer following manufacturer guidelines. Sequencing reads have been deposited in the NCBI Short Read Archive under BioProject accession number PRJNA663701.

Sequence analysis FASTQ files from each sample were processed using the bioinformatics software Mothur (v.1.39.5) (Schloss et al. 2009) following procedures outlined by Kozich et al. (2013). Briefly, paired-end reads were joined, depleted of barcodes, trimmed, and had chimeras removed. Sequences were classified against the Ribosomal Database Project (RDP) 16S rRNA gene training set (v. 14). Sequences unable to be classified at the kingdom level, as well as those classified as Chloroplast, Mitochondria, Archaea, or Eukaryota, were removed. Sequences were assigned to operational taxonomic units (OTUs) based on 97% similarity. Inverse Simpson diversity index was calculated by subsampling 30,425 sequences from each soil sample 1000 times to normalize for differences in read numbers between samples. Statistically significant differences in community composition were determined using analysis of molecular variance (AMOVA) with a Bonferroni correction of 0.01667 for pairwise comparisons between cover crop treatments. OTUs with differential abundance between treatments at each timepoint were identified by linear discriminant analysis effect size (LEfSe) analysis (Segata et al. 2011) in Mothur. Sequences were classified into phylotypes against the geengenes database (version 13_5) using a 97% cutoff in Mothur. Representative fasta sequences from each phylotype were uploaded to myphyloDB (Manter et al. 2016), where predicted functional genes were determined using PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) (Langille et al. 2013). Analysis of co-variance (ANcOVA) was performed in myphyloDB to determine differences in predicted gene function between treatments.

Statistics Analyses were performed in JMP version 11.2.0 (SAS Institute Inc., Carey, NC). Comparisons of inverse Simpson diversity score and relative abundance of selected bacterial taxa between tillage and cover crop treatments were performed using two-way repeated measures analysis of variance (ANOVA) on data from all timepoints for each depth. Individual two-way ANOVAs followed by Tukey's honestly significant difference (HSD) test were also performed for each depth and timepoint. All analyses were assessed using an α of 0.05.

Results

Community profiles at the phylum level remained largely similar between treatments, with few notable differences (Fig. 1). The most abundant bacterial phyla across all soils, Proteobacteria and Acidobacteria, did not differ consistently between tillage or cover crop treatments, only varying across timepoints ($p < 0.0001$). The third most abundant phylum, Bacteroidetes, was elevated in reduced till plots for the first 2 years of the study ($p = 0.001$), while cover crop had no significant impact. At finer taxonomic levels, bacterial genera responsible for symbiotic root nodule formation in clover and soybean were both impacted by conservation treatments. The relative abundance of *Rhizobium* was higher in tilled soils ($p = 0.0098$; Fig. 2b) but not impacted by cover crop in 0–5 cm soil except for April 2017 ($p < 0.0001$) and June 2017 ($p = 0.0373$), where it was higher in clover plots (Fig. 2f). At the 5–15 cm depths, cover cropped plots tended to have higher *Rhizobium* abundance ($p = 0.0066$; Table 1). By contrast, *Bradyrhizobium* was significantly impacted by cover crop ($p < 0.0001$; Fig. 2e), but not tillage (Fig. 2a), in 0–5 cm, while tillage ($p < 0.0071$), but not cover crop, was significant in 5–15 cm soils. Both cover crops displayed greater relative abundance compared to no cover plots in 0–5 cm soils across most timepoints (Fig. 2e). Of the two, rye plots had the highest levels of *Bradyrhizobium*, while the level in clover was intermediate and did not always differ significantly from no cover plots. Abundances of *Pseudomonas* and Xanthomonadaceae were significantly higher in tilled plots (Fig. 2c, d; $p \leq 0.0007$), while unaffected by cover crop in the 0–5 cm soil (Fig. 2g, h). Xanthomonadaceae was also elevated in the tilled 5–15 cm soils ($p = 0.0003$), while *Pseudomonas* was only higher in April 2016 at this depth (Table 1).

Effects of cover crop and tillage on bacterial diversity in soils were relatively minor, with inverse Simpson scores ranging from 181 in April 2017 5–15 cm no till rye soils to 443 in April 2015 0–5 cm tilled rye soil (Fig. 3 and Table 1). Two-way repeated measures ANOVA indicated that there was no interaction between cover and tillage treatments, although diversity was significantly higher in tilled soils ($p = 0.0011$), while no consistent cover crop effects were observed. Non-metric multi-dimensional scaling (NMDS) plots and AMOVA indicated that community composition differed significantly between tilled and no till plots throughout all 3 years in both 0–5 (Fig. 4) and 5–15 cm soils (Table 2). Cover crop also significantly influenced community composition, with rye differing from no cover plots in early 2015 and all of 2017, while clover only differed from no cover plots in April of 2017 (Table 2). After successive timepoints, communities from the tilled no cover treatment tended to cluster together, while clustering of communities under treatments with at least one conservation management practice was less distinct (Fig. 4).

Evaluation of the bacterial community at the OTU level provided more insight into community shifts between treatments over time. In April 2015, 270 OTUs were differentially abundant between till and no till soils, compared to only 67 OTUs between cover crop treatments. However, the numbers of OTUs responding to tillage and cover crop shifted over the course of the experiment, with cover crop–associated OTUs exceeding those associated with tillage treatments by the final timepoint (Fig. 5a). Of these OTUs, similar numbers were affected by till

Table 1 Diversity and relative abundance of select taxonomic groups in till and no till soybean plots with rye, clover, or unplanted winter cover crop, 5–15 cm soils[1]

	No till			Till		
	Rye	Clover	Unplanted	Rye	Clover	Unplanted
	2016					
Inverse Simpson	200 ± 12	230 ± 14	194 ± 26	251 ± 21	243 ± 20	305 ± 12
Bradyrhizobium	0.58 ± 0.15	0.38 ± 0.04	0.31 ± 0.07	0.34 ± 0.05	0.32 ± 0.02	0.27 ± 0.06
Rhizobium	0.15 ± 0.04	0.41 ± 0.17	0.15 ± 0.02	0.13 ± 0.03	0.39 ± 0.21	0.1 ± 0.02
Pseudomonas	0.18 ± 0.03	0.23 ± 0.07	0.32 ± 0.05	0.1 ± 0.03	0.11 ± 0.01	0.1 ± 0.01
Xanthomonadaceae	0.44 ± 0.07	0.36 ± 0.01	0.52 ± 0.05	0.3 ± 0.02	0.35 ± 0.04	0.29 ± 0.04
	2017					
Inverse Simpson	181 ± 16	215 ± 18	233 ± 14	212 ± 24	270 ± 28	264 ± 18
Bradyrhizobium	0.45 ± 0.06	0.42 ± 0.04	0.36 ± 0.06	0.29 ± 0.06	0.29 ± 0.07	0.27 ± 0.05
Rhizobium	0.15 ± 0.04	0.31 ± 0.06	0.11 ± 0.02	0.11 ± 0.02	0.23 ± 0.02	0.14 ± 0.03
Pseudomonas	0.17 ± 0.04	0.35 ± 0.08	0.14 ± 0.02	0.15 ± 0.03	0.49 ± 0.35	0.22 ± 0.09
Xanthomonadaceae	0.36 ± 0.03	0.53 ± 0.09	0.44 ± 0.03	0.33 ± 0.04	0.35 ± 0.05	0.35 ± 0.02

[1] Values represent mean ± standard error

and no till treatment at each timepoint (Fig. 5b). However, fewer OTUs were in greater abundance in clover compared to rye and no till plots (Fig. 5c). This difference appears to be driven largely by higher numbers of Chitinophagaceae and Acidobacteria OTUs associated with rye and no cover plots (Online Resource 2). Other bacterial families frequently identified among OTUs with differential abundance between tillage treatments include Azospirillaceae, Chthoniobacteraceae, and Geobacteraceae in no till plots, and Chitinophagaceae, Gemmatimonadaceae, Nitrosomonadaceae, Xanthobacteraceae, and Xanthomonadaceae in till plots (Online Resource 2).

Functional gene prediction via PICRUSt revealed 32 genes or KEGG orthologies associated with plant growth promotion, biocontrol, or nutrient cycling that differed between treatments for at least one timepoint (Table 3, Online Resource 3). Individually, these functional genes were in relatively low abundance and associated with less than 1% of reads from any given sample. The most notable of these predicted functions were those associated with nutrient cycling and root growth. Genes encoding beta-glucosidase (*bglB* or *bglX*) were significantly higher in till than in no till plots for all timepoints except April and June in 2017 ($p \leq 0.04$). Both acid and alkaline phosphatase

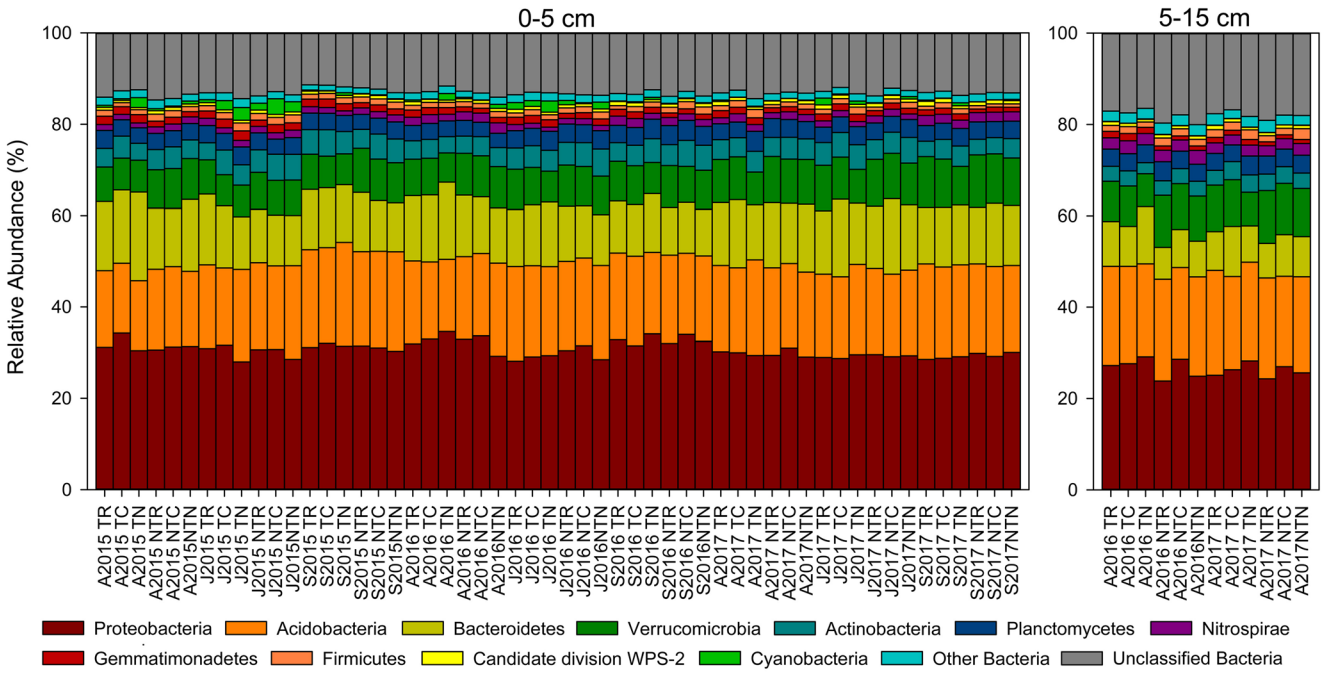

Fig. 1 Phylum level community profiles in April (A), June (J), and September (S) timepoints from 2015 to 2017 in till (T) and not till (NT) soybean plots planted with rye (R), clover (C), or no (N) winter cover crop in 0–5 cm and 5–15 cm soil

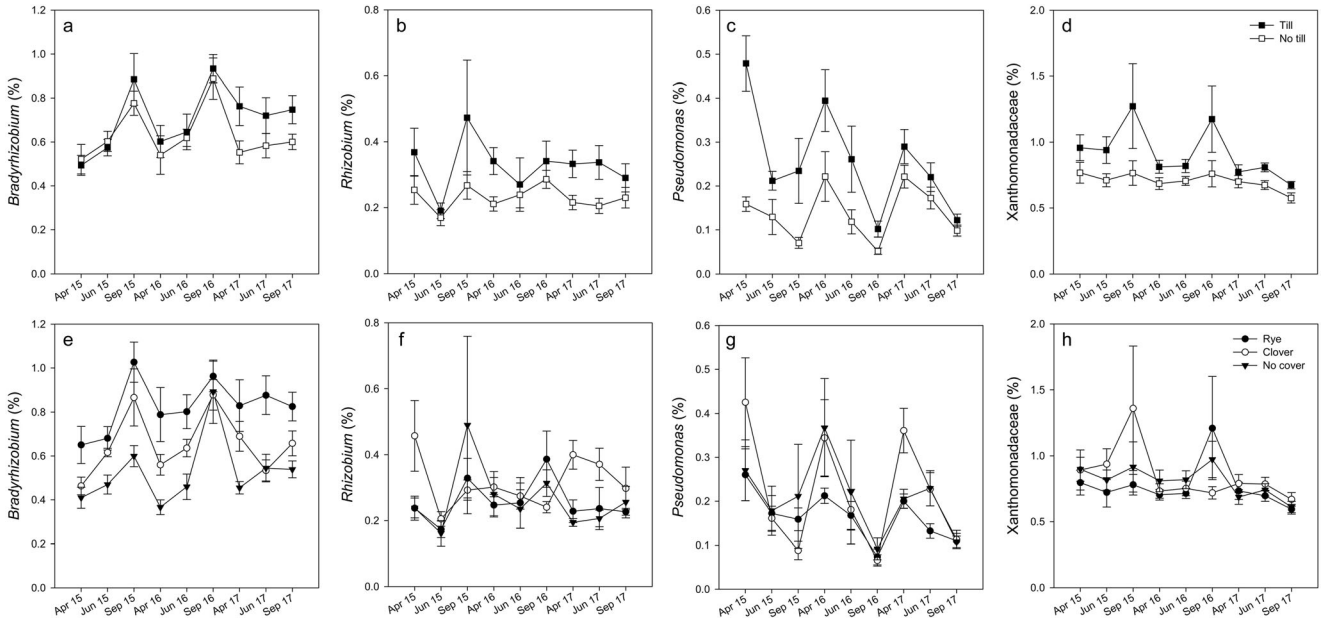

Fig. 2 Relative abundance of *Bradyrhizobium* (**a**, **e**), *Rhizobium* (**b**, **f**), *Pseudomonas* (**c**, **g**), and Xanthomonadaceae (**d**, **h**) in 0–5 cm field soil from 2015 to 2017 broken down by tillage (**a**, **b**, **c**, **d**) and cover crop (**e**, **f**, **g**, **h**) treatments. Tillage treatments ($n = 12$) include till (closed square) or no till (open square) ($n = 12$), and cover treatments ($n = 8$) include rye cover (closed circle), clover cover (open circle), or no cover (inverted triangle). Values represent mean ± standard error

genes were differentially abundant between tillage treatments, with tilled plots having higher levels of *phoD* (alkaline phosphatase; $p \leq 0.034$) and/or *phoN* (acid phosphatase; $p \leq 0.046$), while no till plots tended to have higher levels of *phoA* (alkaline phosphatase; $p \leq 0.015$) across multiple, but not all timepoints, with no apparent seasonal trends. Nitrogen fixation genes (*nifDK* and *nifH*) were higher in clover ($p \leq 0.009$) and no cover ($p \leq 0.048$) than in rye in June 2015 and greater in tilled plots in 0–5 cm soil from April ($p \leq 0.003$) and September ($p = 0.0152$) of 2017. Genes associated with various stages in the N cycle, including ammonification (*ureC*), nitrification (*hao*), nitrate reduction (*nrfA*), and denitrification (*nirk*, *norBC*), tended to be higher in no till plots, particularly in the first year of the study. Bacterial genes associated with root growth promotion (*acdS* and *ipdC*) were predicted across all samples and timepoints, with *ipdC* being significantly higher in no till plots for the entire study period ($p \leq 0.019$), while *acdS* abundance tended to be greater in tilled soil ($p \leq 0.023$) in 2015 and 2016. Generally,

Table 2 Analysis of molecular variance (AMOVA) of community composition between tillage and cover crop treatments[1]

Year	Month	Depth (cm)	Tillage	Cover crop	No cover–rye	No cover–clover	Rye–clover
2015	April	0–5	< 0.001*	0.045*	0.014*	0.06	0.586
	June	0–5	0.007*	0.003*	< 0.001*	0.076	0.019
	September	0–5	< 0.001*	0.358			
2016	April	0–5	< 0.001*	0.041*	0.048	0.092	0.35
		5–15	< 0.001*	0.112			
	June	0–5	< 0.001*	0.06			
	September	0–5	0.008*	0.032*	0.018	0.264	0.254
2017	April	0–5	0.003*	0.001*	0.005*	0.014*	0.091
		5–15	0.004*	0.017*	0.007*	0.11	0.079
	June	0–5	0.039*	< 0.001*	0.001*	0.042	0.043
	September	0–5	0.001*	0.002*	0.002*	0.02	0.352

[1] Values represent *p*-values of each comparison. Statistically significant differences are indicated by asterisks (*). Tillage and cover crop analyses for each timepoint use an α of 0.05. When a significant cover crop effect was calculated, pair-wise comparisons between cover crop treatments were performed, with a Bonferroni correction of 0.01667. Individual pair-wise comparisons between cover crops were not performed if no significant differences were found in the initial cover crop analysis

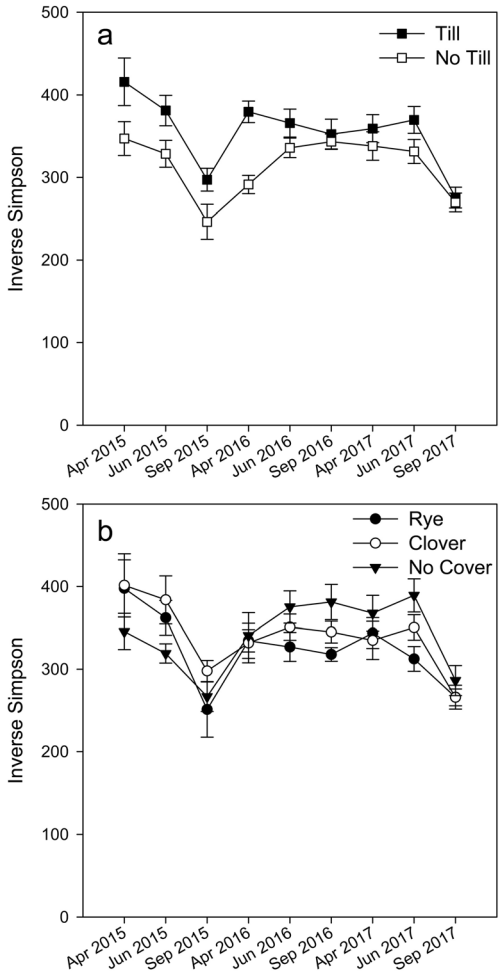

Fig. 3 Inverse Simpson diversity scores in 0–5 cm soils from field plots broken down by tillage (**a**) and cover crop (**b**) treatments from 2015 to 2017. The two tillage treatments ($n = 12$) include till (closed square) or no till (open square). The three cover treatments ($n = 8$) include rye cover (closed circle), clover cover (open circle), or no cover (inverted triangle). Values represent mean ± standard error

significant differences in predicted functions between cover crop treatments were less frequent than for tillage. However, both cover crops tended to have more functions associated with them than no cover plots, with rye tending to have greater abundances of *ipdC*, acid and alkaline phosphatase, phosphate solubility, and nitrate reductase genes across multiple timepoints, while clover had greater abundances of genes for ammonification, siderophore production, nitrification, and nitrate reductases.

Discussion

Effects of tillage

Relative effects of different conservation management practices on community composition in soils can vary between studies, often due to regional differences in climate as well as soil physical and chemical traits. In the current study, the influence of tillage on bacterial community composition was apparent throughout the entire 3-year study period, likely a result of the 17-year history of tillage vs. no tillage treatments at the study site. Tillage effects were most evident in AMOVA and NMDS clustering between till and no till soils, although tilled soils also displayed elevated diversity and increased abundances of certain bacterial taxa (*Pseudomonas* and Xanthomonadaceae), which is consistent with observations made at the field site prior to initiation of the current study (Tyler 2019) and confirms that the higher diversity observed in tilled soil prior to cover crop planting persists over multiple seasons and years. Souza et al. (2013) reported similar results, finding tillage had a greater impact on microbial community composition in soils than crop rotation, with conventionally tilled plots displaying greater abundances of organisms linked to the breakdown of plant residues, as well as C and N cycling (including members of Xanthomonadales), and no till plots having more N fixers (including Rhizobiales). No consistent differences in *Bradyrhizobium* between tillage treatments were observed here, which is in contrast to Ferreira et al. (2000), who found them to be elevated in no till soils. While both studies examined sites that had been under long-term no till management (13–17 years), the sites have been in soybean production for very different lengths of time (8–10 years in the current study vs. 30+ years in Ferreira et al. 2000), suggesting that crop history may influence soil community members' responsiveness to differences in tillage. Additionally, the current study utilized DNA sequencing which could have detected unculturable bacteria cells missed by the culturing-based approach employed by Ferreira et al. (2000), suggesting another potential cause for the disparity between these two studies.

Effects of cover crop

A shift in the relative effects of cover crop and tillage on bacterial community composition occurred over the course of the study. Cover crop–associated differences were minor at the start of the experiment, when four times as many OTUs were associated with tillage than cover treatments. However, after three successive years, over twice as many OTUs were associated with cover crop than tillage treatments. This difference appears to be driven by rye and no cover, since the number of OTUs associated with these treatments increased almost fourfold by the end of the study. In comparison, the number of OTUs enriched in clover plots fluctuated but did not increase to the extent observed in either rye or no cover. It is unclear why fewer OTUs responded to clover compared to rye or even no cover crop treatments, although differences in root exudate composition may be a contributing factor (Haichar et al. 2008). The interaction between plant roots and bacteria in the rhizosphere is complex, with plants secreting compounds with

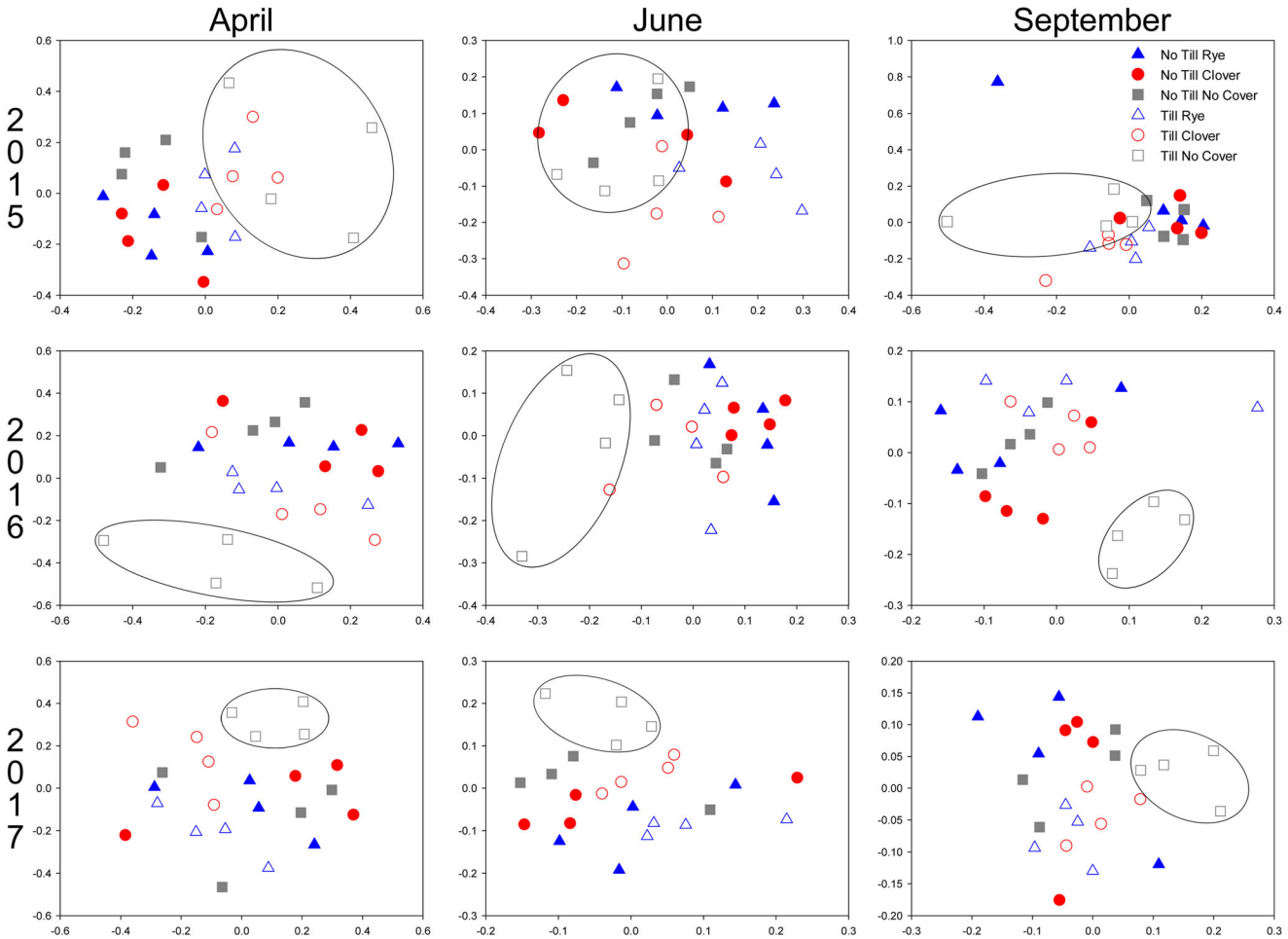

Fig. 4 Non-metric multi-dimensional scaling (NMDS) plots of community composition in no till (closed symbol) and till (open symbol) plots planted in winter cover crop treatments of rye (triangle), clover (circle), or no cover (square). No till, no cover plots are circled, indicating clustering over successive timepoints

growth-promoting or anti-microbial properties depending on environmental stimuli, including the presence of pathogens and pests (Olanrewaju et al. 2019). Having a legume crop in plots year-round (soybean in summer and clover in winter) may have resulted in the proliferation of pathogens and/or pests that triggered secretion of anti-microbial compounds in clover plots, suggesting a potential mechanism for the differential response of OTUs to rye and no cover relative to clover winter cover crop treatments.

Brennan and Acosta-Martinez (2017) reported that the type of cover crop impacted *Pseudomonas* and *Agromyces* abundances in soil. By contrast, the current study noted that *Pseudomonas* differed across tillage rather than cover crop treatment but did observe other notable effects at the genus level. *Bradyrhizobium*, the genus responsible for nodulation in soybeans, was in greater abundance in rye cover treatments, suggesting another potential mechanism for cover crop–based improvements to plant nutrition beyond increasing soil organic matter and nutrient levels in field soils (Zablotowicz et al. 2010).

Potential impacts on soil function

The composition of microbial communities in soil can have a direct impact on soil function as they carry out nutrient cycling and organic matter turn over in soils. Fernandez et al. (2016) found that the bacterial community composition at phylum and family levels was predictive of soil function in bulk soils. By contrast, the current study found the bacterial community was relatively similar between plots at the phylum level, with treatment-specific differences being more evident at finer taxonomic scales. Similarly, Brennan and Acosta-Martinez (2017) did not note any dramatic differences between cover crop treatments in soil microbial communities at the phylum level. This difference in phylum level responsiveness to cover crop treatments between studies may be due to the relative locations of the plots assessed within each study. While the current study and Brennan and Acosta-Martinez (2017) compared multiple cover crop treatments at single sites, Fernandez et al. (2016) made comparisons across field sites at multiple locations with differing soil textures. These observations

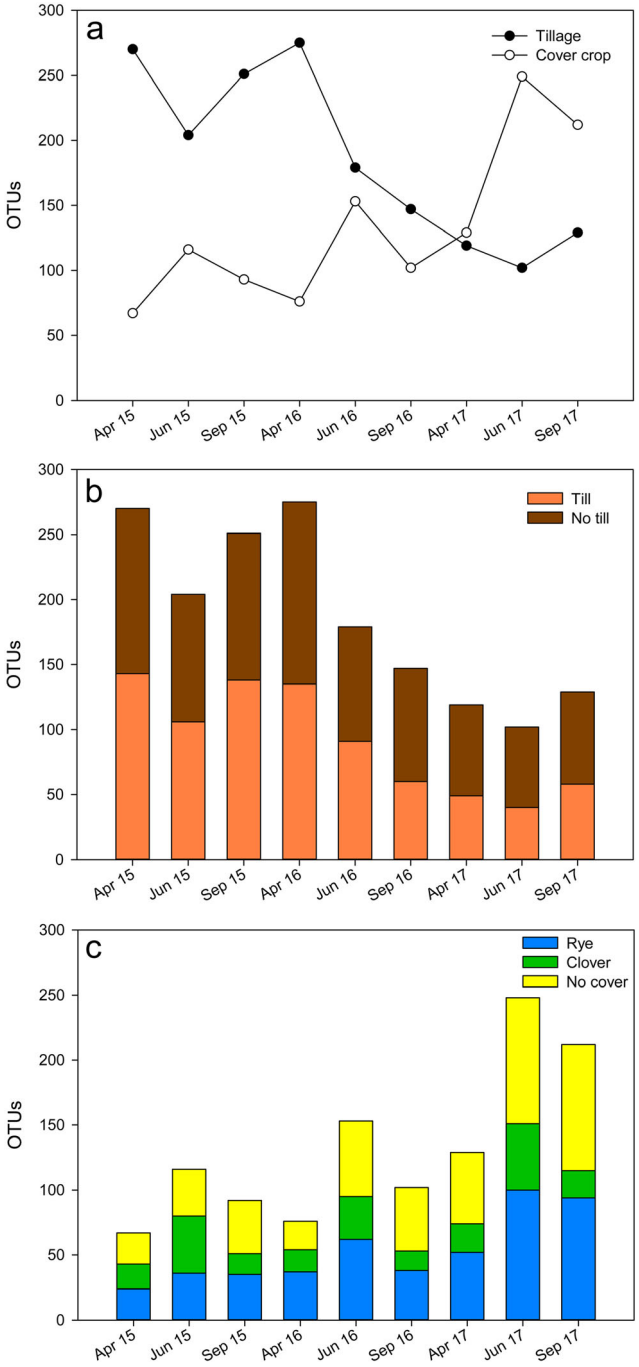

Fig. 5 Operational taxonomic units (OTUs) with differential abundance between treatments as determined by linear discriminant analysis effect size (LEfSe) analysis (α = 0.05) presented as **a** total OTUs associated with tillage (closed circle) and cover crop (open circle) plots, **b** number of OTUs significantly greater in till and no till plots, and **c** number of OTUs significantly greater in rye, clover, and no cover plots

suggest the importance of using replicate plots at the same site for assessing the potential impacts of tillage and cover crop practices on the soil community.

Examining differentially abundant OTUs between treatments provided some insight into how tillage and cover crop–induced community shifts may potentially alter ecosystem functions. For example, a large number of OTUs classified as *Geobacter* were elevated in no till, but not in tilled plots. As this is a genus of anaerobic bacteria capable of metabolizing humic materials in soil (Voordeckers et al. 2010), its increased detection in no till soil suggests that they were more prone to anaerobic conditions, likely due to poor drainage in no till plots. Tilled plots had 18 times more OTUs classified as Xanthomonadaceae compared to no till plots. Several of these OTUs are members of *Lysobacter*, a genus known for suppressing fungal plant diseases (Folman et al. 2003; Postma et al. 2008; Sullivan et al. 2003), suggesting that tillage may promote disease suppressive conditions in the soil.

Given the large number of bacterial species present in any given soil sample and the functional redundancy between soil bacteria, elucidating potential effects of bacterial composition on soil function can be overwhelming when looking at individual taxa. As such, PICRUSt, a computational tool that uses phylogenetic information and reference genomes to predict functional gene profiles (Langille et al. 2013), was used to assess differential abundances of nutrient cycling and plant-beneficial bacterial gene functions, with the caveat that the presence of a gene in a bacterial genome does not indicate whether it is being actively expressed under field conditions. Among the genes predicted to be differentially abundant were those associated with root growth, organic matter turn-over, phosphate decomposition, N fixation, N cycling, disease response, and siderophore production. Of the plant root–promoting genes, *ipdC* was predicted to be elevated in no till plots, while elevated *acdS* was predicted in tilled plots, suggesting that till vs. no till favors plant growth-promoting bacteria that employ different modes of action. While *ipdC* is an auxin synthesis gene, *acdS* encodes an ACC deaminase that breaks down the precursor of the plant hormone ethylene, a hormone known to inhibit root elongation (Abeles et al. 1992). Bacteria expressing this enzyme are capable of lowering levels of this hormone in plant tissue, thereby promoting root growth (Glick 2005). As ethylene is also involved in plant defense pathways and stress response (Glick 2005; Pieterse et al. 1998), *acdS* expressing bacteria are more competitive in colonizing plant tissue (Ma et al. 2004). The elevated levels of *acdS* predicted in tilled plots indicate that bacterial populations in those soils could include a greater number of efficient plant colonizers. Multiple genes (endo-1,3(4)-beta-gulcanase and glycine dehydrogenase) associated with biocontrol control agents that limit plant disease were also predicted to be higher in tilled plots across multiple timepoints. This is consistent with the observation that OTUs of the biocontrol agent *Lysobacteria* were higher in tilled soils. Whether this is a case of tillage directly promoting conditions that lower disease pressure or a response to higher number of soybean pathogens (many of

Table 3 Gene functions predicted to be higher in till, no till, clover, rye, or no cover crop treatments from 2015 to 2017[1]

Date	Higher in till	Higher in no till	Higher in clover	Higher in rye	Higher in no cover
2015					
April	*acdS*, *bglX*, E,3.2.1.6, *hcnA*, *pchB*, *nirBD*, *nosZ*	*ipdC*, *hao*, *nirA*, *nrfA*, *nirK*, *norBC*	*entA*, *pchB*, *narGH*		
June	*acdS*, *phoN*, *bglX*, *nosZ*	*ipdC*, *ureC*, *entA*, *hao*, *nirA*, *nrfA*, *nirK*	*ureC*, *phzE*, *entA*, *mbtI*, *nifDK*, *nifH*, *nirBD*	*acdS*, *phoA*, *nirBD*	*nifDK*, *nifH*
September	*amiE*, *phoD*, *phoN*, *bglX*	*ipdC*, *hao*, *nirA*, *nrfA*, *nirK*, *pqqC*		*pqqC*	
2016					
April	*acdS*, *amiE*, *phoD*, *phoN*+, *bglB**, *bglX*, E,3.2.1.6*, *hcnA**, *pchB**, *nirBD*, *nosZ*	*ipdC*, *ureC**, E,3.1.3.2+, *phoA**, *budA*, *hao*, *nirA*, *nrfA*, *nirK*, *norBC*+, *pqqC**	*ureC**, *narGH**, *nirA**, *nrfA*, *norBC**	*ipdC**, *nirA**, *nrfA*, *nirK**, *pqqC**	*bglX*+, *mbtI*+, *norBC**
June	*acdS*, *amiE*, *phoN*, *bglX*, E,3.2.1.14*, *nosZ*	*ipdC*, *ureC*, *entA*, *hao*, *nrfA*, *nirK*, *norBC*	*nrfA*	*nrfA*, *nirK*	*pmoA-amoA*
September	*acdS*, *phoN*, *bglX*, E,3.2.1.14, *hcnA*	*ipdC*, *budA*, *hao*, *narGH*, *nrfA*, *norBC*		*ipdC*, E,3.1.3.2, *nrfA*, *nirK*	*hcnA*
2017					
April	*amiE**, *phoD**, *phoN*+, *bglB**, E,3.2.1.6, *pchB**, *narGH**, *nifDK*+, *nifH*+, *nosZ**	*ipdC*, *phoA*, *nirA**, *nrfA*, *nirK*, *pqqC**	*ureC*, *hcnA*+, *narGH**, *nirBD**, *nirK*+, *norBC**, *pqqC**	*ureC**, *appA**, *nirA**, *pqqC*	*narGH**
June	*pchB*	*ipdC*, *nrfA*, *nirK*, *norBC*	*acdS*, *bglX*, *nirBD*, *nirK*, *nosZ*	*ipdC*, *phoA*, *bglX*, *nrfA*, *nirK*, *pqqC*	*phoD*, E,3.2.1.6
September	*phoN*, *bglX*, E,3.2.1.6, *nifH*	*ipdC*, *phoA*, *nrfA*, *nirK*		E,3.1.3.2, *phoA*, *nirA*, *nrfA*, *nirK*, *pqqC*	*narGH*, *norBC*

[1] Genes and pathways involved in root growth (*acdS* and *ipdC*), biocontrol (*budA*, *hcnA*, *phzE*, E3.2.1.14, and E3.2.1.6), siderophore production (*entA*, *mbtI*, and *pchB*), C decomposition (*bglB*, *bglX*, and E3.2.1.21), N fixation (*nifDK* and *nifH*), denitrification (*nirK*, *norBC*, and *nosZ*), ammonification (*amiE* and *ureC*), nitrification (*hao*, *narGH*, and *pmoA-amoA*), nitrate reduction (*nirA*, *nirBD*, and *nrfA*), P decomposition (*appA*, E3.1.3.2, *phoA*, *phoD*, and *phoN*), and phosphate solubility (*pqqC*)

+Only higher in 0–5 cm depth

*Only higher in 5–15 cm depth

which are fungal in nature) in tilled plots will require further study to elucidate.

The relative abundance predicted for nitrogenase genes (*nifDK* and *nifH*) was not consistently impacted by either tillage or cover crop treatment. Given that clover is a legume colonized by N-fixing *Rhizobium*, their levels were expected to be higher in clover plots. Despite this, elevation of N fixation gene abundances was only observed in June of 2015. This does not correspond to either of the timepoints when *Rhizobium* abundance was significantly higher in clover plots but does correspond to the only timepoint where LEfSe analysis indicated that more OTUs were enriched in clover than in either rye or no cover plots, suggesting that other N-fixing bacteria may have responded to the clover winter cover. The lack of cover crop effect on nitrogenase genes at the other timepoints may be due to potential inhibitory factors in clover plots mentioned above.

While genes encoding beta-glucosidase, one of the enzymes involved in cellulose degradation, were predicted to be slightly elevated in tilled plots across multiple years, prior assessment of these soils demonstrated that this did not equate to higher beta-glucosidase activities in tilled soils. In fact, the opposite trend was observed, where glucosidase activities recorded during the study period were actually higher in no till plots (Tyler 2020). Similarly, while an acid phosphatase gene (*phoN*) was greater in abundance in till plots of the current study at certain timepoints, prior analyses demonstrated that the acid phosphatase was consistently higher in no till soils across all timepoints (Tyler 2020). Along those same lines, Liu et al. (2021) reported that the abundance of rare taxa carrying *phoD* had a greater association with phosphatase activity than *phoD* gene copy number. These contradictions between predicted and actual soil functions are likely a result of differential gene expression due to differing conditions between plots or higher expression levels in low abundance taxa. The presence of DNA from dead cells that are no longer metabolically active may also skew functional predictions. These observations highlight the limitation in relying on functional gene prediction in soil metagenomic studies and suggest that caution should be taken when using predicted gene levels to estimate potential shifts in some soil functions.

Conclusion

Tillage tended to have a greater and more consistent effect on bacterial community composition, while the effects of cover crops were variable but increased in frequency and intensity over the course of the study, suggesting that more than 3 years of cover crop treatment may be necessary before effects of winter cover crops can be fully realized. Shifts in abundance at the genus and OTU level revealed potential functional changes due to management practices, with winter cover crops appearing to shift bacterial community composition in favor of genera important for crop nodulation, root growth, ammonification, and nitrate reduction, while tillage appeared to promote taxa containing biocontrol agents and those optimized for plant colonization, and no till promotes denitrifying bacteria. However, these shifts were small in nature, and the magnitude of any functional shifts will depend on gene expression, which can vary according to environmental factors. While fewer OTUs were associated with clover than rye or no cover treatments, the greater number of predicted functions in clover compared to no cover treatments suggests that this may be a result of clover selecting for certain bacterial groups rather than a general inhibitory effect. Interestingly, beneficial shifts in community composition were not limited to conservation practices, highlighting the need for careful consideration when choosing how to implement these practices in a field, with soybean fields potentially benefiting from greater root-nodulating bacteria with rye winter cover, or plots with higher disease incidence benefiting from incorporating tillage instead of being maintained as no till. Further study comparing community composition–based functional predictions to actual activities is needed before sequencing technology can be used as a reliable indicator of soil function in agricultural fields. However, gene prediction can still provide insight for functions that are not easily determined via laboratory or field-based assessments.

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Code availability Not applicable

Declarations

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References

Abeles FB, Morgan PW, Saltveit ME (1992) Chapter 3 - The biosynthesis of ethylene. In: Abeles FB, Morgan PW, Saltveit ME (eds) Ethylene in plant biology, Second edn. Academic Press, New York, pp 26–55. https://doi.org/10.1016/B978-0-08-091628-6.50009-6

Acosta-Martínez V, Mikha MM, Vigil MF (2007) Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat-fallow for the Central Great Plains. Appl Soil Ecol 37:41–52. https://doi.org/10.1016/j.apsoil.2007.03.009

Acosta-Martínez V, Zobeck TM, Gill TE, Kennedy AC (2003) Enzyme activities and microbial community structure in semiarid agricultural soils. Biol Fertil Soils 38:216–227. https://doi.org/10.1007/s00374-003-0626-1

Ali B, Sabri A, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. Lett Appl Microbiol 48:542–547. https://doi.org/10.1111/j.1472-765X.2009.02565.x

Bakker MG, Acharya J, Moorman TB, Robertson AE, Kaspar TC (2016) The potential for cereal rye cover crops to host corn seedling pathogens. Phytopathology 106:591–601. https://doi.org/10.1094/phyto-09-15-0214-r

Brennan EB, Acosta-Martinez V (2017) Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. Soil Biol Biochem 109:188–204. https://doi.org/10.1016/j.soilbio.2017.01.014

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108:4516–4522. https://doi.org/10.1073/pnas.1000080107

Chávez-Romero Y, Navarro-Noya YE, Reynoso-Martínez SC, Sarria-Guzmán Y, Govaerts B, Verhulst N, Dendooven L, Luna-Guido M (2016) 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. Soil Tillage Res 159:1–8. https://doi.org/10.1016/j.still.2016.01.007

Cookson WR, Murphy DV, Roper MM (2008) Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biol Biochem 40:763–777. https://doi.org/10.1016/j.soilbio.2007.10.011

Dong W, Liu E, Yan C, Tian J, Zhang H, Zhang Y (2017) Impact of no tillage vs. conventional tillage on the soil bacterial community structure in a winter wheat cropping succession in northern China. Eur J Soil Biol 80:35–42. https://doi.org/10.1016/j.ejsobi.2017.03.001

Fernandez AL, Sheaffer CC, Wyse DL, Staley C, Gould TJ, Sadowsky MJ (2016) Associations between soil bacterial community structure and nutrient cycling functions in long-term organic farm soils following cover crop and organic fertilizer amendment. Sci Total Environ 566-567:949–959. https://doi.org/10.1016/j.scitotenv.2016.05.073

Ferreira MC, Andrade DS, De OCLM, Takemura SM, Hungria M (2000) Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean. Soil Biol Biochem 32:627–637. https://doi.org/10.1016/s0038-0717(99)00189-3

Folman LB, Postma J, van Veen JA (2003) Characterisation of *Lysobacter enzymogenes* (Christensen and Cook 1978) strain 3.1T8, a powerful antagonist of fungal diseases of cucumber. Microbiol Res 158:107–115. https://doi.org/10.1078/0944-5013-00185

Garbeva P, Van Veen JA, Van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270. https://doi.org/10.1146/annurev.phyto.42.012604.135455

Gentsch N, Boy J, Batalla JDK, Heuermann D, von Wirén N, Schweneker D, Feuerstein U, Groß J, Bauer B, Reinhold-Hurek B, Hurek T, Céspedes FC, Guggenberger G (2020) Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. Biol Fertil Soils 56:943–957. https://doi.org/10.1007/s00374-020-01475-8

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7. https://doi.org/10.1016/j.femsle.2005.07.030

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39. https://doi.org/10.1016/j.micres.2013.09.009

Haichar FZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W (2008) Plant host habitat and root exudates shape soil bacterial community structure. ISME J 2:1221–1230. https://doi.org/10.1038/ismej.2008.80

Helgason BL, Walley FL, Germida JJ (2010) No-till soil management increases microbial biomass and alters community profiles in soil aggregates. Appl Soil Ecol 46:390–397. https://doi.org/10.1016/j.apsoil.2010.10.002

Hobara S, Osono T, Hirose D, Noro K, Hirota M, Benner R (2014) The roles of microorganisms in litter decomposition and soil formation. Biogeochemistry 118:471–486. https://doi.org/10.1007/s10533-013-9912-7

Kozich J, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–5120. https://doi.org/10.1128/aem.01043-13

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676

Liu S, Zhang X, Dungait JAJ, Quine TA, Razavi BS (2021) Rare microbial taxa rather than *phoD* gene abundance determine hotspots of alkaline phosphomonoesterase activity in the karst rhizosphere soil. Biol Fertil Soils 57:257–268. https://doi.org/10.1007/s00374-020-01522-4

Locke MA, Krutz LJ, Steinriede RW Jr, Testa S III (2015) Conservation management improves runoff water quality: Implications for environmental sustainability in a glyphosate-resistant cotton production system. Soil Sci Soc Am J 79:660–671. https://doi.org/10.2136/sssaj2014.09.0389

Locke MA, Zablotowicz RM, Steinriede RW Jr, Testa S, Reddy KN (2013) Conservation management in cotton production: long-term soil biological, chemical, and physical changes. Soil Sci Soc Am J 77:974–984. https://doi.org/10.2136/sssaj2012.0325

Ma W, Charles TC, Glick BR (2004) Expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in *Sinorhizobium meliloti* increases its ability to nodulate alfalfa. Appl Environ Microbiol 70:5891–5897. https://doi.org/10.1128/AEM.70.10.5891-5897.2004

Manter DK, Korsa M, Tebbe C, Delgado JA (2016) MyPhyloDB: A local web server for the storage and analysis of metagenomic data. Database 2016:baw039. https://doi.org/10.1093/database/baw037

Masson-Boivin C, Sachs JL (2018) Symbiotic nitrogen fixation by rhizobia— the roots of a success story. Curr Opin Plant Biol 44:7–15. https://doi.org/10.1016/j.pbi.2017.12.001

Nannipieri P, Penton CR, Purahong W, Schloter M, van Elsas JD (2019) Recommendations for soil microbiome analyses. Biol Fertil Soils 55:765–766. https://doi.org/10.1007/s00374-019-01409-z

Olanrewaju OS, Ayangbenro AS, Glick BR, Babalola OO (2019) Plant health: feedback effect of root exudates-rhizobiome interactions. Appl Microbiol Biotechnol 103:1155–1166. https://doi.org/10.1007/s00253-018-9556-6

Peixoto DS, Silva LDCMD, Melo LBBD, Azevedo RP, Araújo BCL, Carvalho TSD, Moreira SG, Curi N, Silva BM (2020) Occasional tillage in no-tillage systems: a global meta-analysis. Sci Total

Environ 745:140887. https://doi.org/10.1016/j.scitotenv.2020.140887

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in arabidopsis. Plant Cell 10:1571–1580. https://doi.org/10.1105/tpc.10.9.1571

Postma J, Schilder MT, Bloem J, van Leeuwen-Haagsma WK (2008) Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. Soil Biol Biochem 40:2394–2406. https://doi.org/10.1016/j.soilbio.2008.05.023

Prasuhn V (2012) On-farm effects of tillage and crops on soil erosion measured over 10 years in Switzerland. Soil Tillage Res 120:137–146. https://doi.org/10.1016/j.still.2012.01.002

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996. https://doi.org/10.1104/pp.111.175448

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. https://doi.org/10.1186/gb-2011-12-6-r60

Souza RC, Cantão ME, Vasconcelos ATR, Nogueira MA, Hungria M (2013) Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Appl Soil Ecol 72:49–61. https://doi.org/10.1016/j.apsoil.2013.05.021

Sullivan RF, Holtman MA, Zylstra GJ, White JF Jr, Kobayashi DY (2003) Taxonomic positioning of two biological control agents for plant diseases as *Lysobacter enzymogenes* based on phylogenetic analysis of 16S rDNA, fatty acid composition and phenotypic characteristics. J Appl Microbiol 94:1079–1086. https://doi.org/10.1046/j.1365-2672.2003.01932.x

Tyler HL (2019) Bacterial community composition under long-term reduced tillage and no till management. J Appl Microbiol 126:1797–1807. https://doi.org/10.1111/jam.14267

Tyler HL (2020) Winter cover crops and no till management enhance enzyme activities in soybean field soils. Pedobiologia 81-82:150666. https://doi.org/10.1016/j.pedobi.2020.150666

Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Voordeckers JW, Kim BC, Izallalen M, Lovley DR (2010) Role of *Geobacter sulfurreducens* outer surface c-type cytochromes in reduction of soil humic acid and anthraquinone-2,6-disulfonate. Appl Environ Microbiol 76:2371–2375. https://doi.org/10.1128/AEM.02250-09

Zablotowicz RM, Reddy KN, Weaver MA, Mengistu A, Krutz LJ, Gordon RE, Bellaloui N (2010) Cover crops, tillage, and glyphosate effects on chemical and biological properties of a lower Mississippi Delta soil and soybean yield. Environ Res J 4:227–251