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ORIGINAL ARTICLE

Bacterial community composition under long-term reduced tillage and no till management

Heather L. Tyler

Crop Production Systems Research Unit, USDA Agricultural Research Service, Stoneville, MS, USA

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16S rRNA gene, bacteria, community composition, diversity, microbial biomass, soil, tillage.

Correspondence
Heather L. Tyler, Crop Production Systems Research Unit, USDA Agricultural Research Service, PO Box 350, Stoneville, MS 38776, USA.
E-mail: heather.tyler@ars.usda.gov

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Abstract

Aims: The purpose of this study was to assess impacts of long-term reduced tillage and no till management on bacterial communities in agricultural field soils.

Methods and Results: Samples from surface soils were collected from field plots maintained under reduced tillage and no till conditions for 14 years. No till soils had significantly higher microbial biomass, as well as β-glucosidase activity, which is linked to organic matter breakdown. Sequencing of the 16S rRNA gene revealed most variability in bacterial community composition was observed in low abundance community members. Diversity estimates (Chao 1, ACE, and Shannon indices) were lower in no till soils, and several bacterial taxa linked to organic matter breakdown were significantly higher in no till compared to tilled soils.

Conclusions: Long-term no till management can significantly enhance the size of soil microbial communities while negatively impacting bacterial diversity, through the lack of soil disturbance and breakdown of crop residues left on soil surfaces.

Significance and Impact of the Study: This study provides insights into how no till management can influence the microbial community's contribution to soil health and suggests that long-term no till field plots may benefit from occasional tillage.

Introduction

The importance of soil health in agricultural systems has gained increased attention in recent years, as variation in a soil's chemical, physical and biological properties may impact plant growth and health, and ultimately crop yield. Tillage, a mechanical disruption of soil, is widely used in agricultural cultivation of row crops, such as corn, cotton and soybeans, as a way to prepare soil for seed planting, as well as, a method of weed control. However, this practice has several detrimental effects on soil systems. It disrupts soil structure and increases erosion (Prasuhn 2012) and nutrient losses (Jackson *et al.* 2003; Shipitalo *et al.* 2013). Tillage also negatively impacts many soil quality parameters, such as decreasing organic carbon, bulk density, water holding capacity and mineralizable nitrogen, thereby lowering a soil's functional potential (Kumar *et al.* 2012; Karlen *et al.* 2013). As such, reducing or eliminating tillage has been introduced as a way to improve soil structure and mitigate many of the detrimental effects of repeated soil disruption. The decrease in soil disturbance of no till plots increases carbon sequestration by slowing the decomposition of soil organic carbon, although the extent of these improvements is determined by several site-specific factors, including soil type and texture, drainage and the length of time no till management has been in place (Mishra *et al.* 2010). In some cases, such as Faba bean (*Vicia faba* L.) fields, no till management has been found to increase yields relative to conventionally tilled plots (Badagliacca *et al.* 2018).

Effects of tillage on soil microbial populations vary, with the potential to decrease microbial biomass, activity and community composition relative to no till plots.

Numerous studies have reported higher soil enzyme activities in no till plots (Acosta-Martínez *et al.* 2007; Zhang *et al.* 2014; Mbuthia *et al.* 2015). Effects on microbial biomass appear to be more varied, with some reporting elevated microbial biomass in no till plots (Feng *et al.* 2003; Helgason *et al.* 2010; Zhang *et al.* 2014), while others report no significant difference between tilled and no till soils (Acosta-Martínez *et al.* 2007; Cookson *et al.* 2008; Mbuthia *et al.* 2015). Examinations of microbial community composition between soils under various tillage management practices yield varying results. Several studies have reported differences in soil microbial community composition between till and no till treatments from a variety of geographically and climatically diverse locations. However, the effect of tillage on soil communities can differ within similar geographic regions. For instance, Acosta-Martínez *et al.* (2003) found that tillage did not have a significant effect on microbial profiles in soils in the plains of western Texas, while Acosta-Martínez *et al.* (2007) reported significant effects of tillage on soil fungal communities in the Central Great Plains of the United States.

Studies on tillage have been widespread, and in some cases, long-standing, for many decades. However, research on the effects of tillage on soil microbial communities in the Mississippi Delta (northwest Mississippi) region have been more limited. Much of the research on tillage in this region centres around its role in weed management, with a focus on minimizing downstream environmental impacts due to contaminants in run-off (Locke *et al.* 2008; Krutz *et al.* 2009) and the degradation of herbicides in soil (Locke *et al.* 1996; Zablotowicz *et al.* 2007). General hydrolytic activity, as determined by fluorescein diacetate (FDA) hydrolysis, has been found to be elevated in no till cotton (Zablotowicz *et al.* 2000, 2007) and soybean (Reddy *et al.* 2003; Zablotowicz *et al.* 2010), while enzymes linked to carbon and phosphate cycling have been greater in fields under no till management of some varieties of corn (Jenkins *et al.* 2017). Regarding microbial community composition, a 4-year field study found differences in microbial community composition indicated a greater effect of tillage than cover crop (Zablotowicz *et al.* 2010). In contrast, Locke *et al.* (2013) found cover crop, but not tillage, had a significant impact on microbial community structure after 6 years of differing tillage treatment. With such a variability observed in response to tillage, there is still much to learn about how this practice can influence soil microbial communities.

Many prior studies of tillage's effects on microbial communities in soil used fatty acid methyl ester (FAME) or phospholipid fatty acid analyses, techniques commonly employed in assessments of soil community composition (Drijber *et al.* 2000; Feng *et al.* 2003; Zablotowicz *et al.* 2010; Locke *et al.* 2013). However, these techniques may not have a high enough resolution to discern shifts in low abundance community members at finer taxonomic scales, as it is estimated that a single gram of soil can contain several thousand bacterial species (Schloss and Handelsman 2006; Roesch *et al.* 2007). As such, many studies in recent years have utilized various high-throughput sequencing approaches, including 16S rRNA amplicon sequencing, to elucidate effects of different agricultural management practices on microbial soil communities in greater taxonomic detail (Sengupta and Dick 2015; Dong *et al.* 2017; Sun *et al.* 2018). Experimental plots established by Locke *et al.* (2013) have been maintained under the same tillage (reduced tillage) and no till conditions since the conclusion of that study, providing the opportunity to determine if changes in the soil microbial community are more apparent after a longer period of no till management. The objective of the current study was to assess the impact of long-term tillage on soil microbes in Mississippi Delta field soils by assessing plots that have been under reduced tillage and no till management for 14 years, with an emphasis on community composition using a 16S rRNA sequencing approach.

Materials and methods

Site history and sample collection

A total of 24 plots (12 replicates of reduced tillage and 12 replicates of no till) were established as part of a cover crop study and maintained under cotton (*Gossypium hirsutum* L.) production for 6 years as described in Locke *et al.* (2013). Briefly, each plot measures 8·12 × 32 m (26·7 × 105 ft) in size. All plots were initially plowed in the fall of 2000. No till plots have not been plowed since that time, while reduced till plots were disked each fall after harvest. Original cover crop treatments included Balansa clover (*Trifolium michelianum*), Abruzzi rye (*Secale cereale*) or no cover. After completion of the original experiment (Fall of 2006), cover crop treatments ceased, but plots continued to be maintained under reduced tillage and no till conditions, but planted in continuous soybean (*Glycine max* L.) crop annually instead of cotton. Soil in field plots is classified as Commerce very fine sandy loam, Commerce silty clay loam or Dowling clay in the USDA-NRCS Web Soil Survey (Soil Survery Staff 2012). Surface soil samples (0–5 cm) were collected from each plot in the fall of 2014 using a soil probe with a 1·8 cm diameter. Eight subsamples were taken from each plot and combined to form one composite sample per plot. Aliquots from each sample were frozen at −80°C for later DNA extraction. All soils were

passed through a 2-mm sieve and stored field moist at 4°C until all other analyses.

Enzymatic activity

Soil samples were assayed for the activities of β-glucosidase, cellobiohydrolase, N-acetylglucosaminidase (NAGase) and phosphatase using pNP-linked assays in a 96-well plate format as described by Jackson *et al.* (2013). Fluorescein diacetate hydrolysis was assayed using a protocol modified from Schnürer and Rosswall (1982). Briefly, 2 g of soil was transferred into 50-ml polypropylene centrifuge tubes and 5 ml of 0·05 mol l^{-1} potassium phosphate buffer (pH 7·6) was added. Tubes were vortexed and 150 μl were pipetted into six wells per sample in a deep well block. Next, 150 μl KPO_4 2X-FDA buffer was added to four sample wells and 150 μl of 0·05 mol l^{-1} phosphate buffer without FDA were added to the last two wells to serve as sample blanks. Ninety-six-well blocks were incubated at 30°C for 1 h with shaking (200 rev min^{-1}). Reactions were stopped by the addition of 300 μl acetone, shaken for an additional 3 min, and centrifuged at 3000 ***g*** for 15 min. Supernatant (300 μl) was withdrawn from each well and transferred to a clean 96-well plate, and absorbance was measured at 490 nm on a Synergy HT Microplate Reader (BioTek Instruments, Winooski, VT). FDA concentration was calculated by comparison to a standard curve.

Microbial biomass and soil organic matter

Microbial biomass was determined by the chloroform fumigation extraction method; 12 g fresh weight of each soil sample was fumigated as described by Horwath and Paul (1994). Fumigated and unfumigated (12 g) soil samples from each plot were extracted with 50 ml of 0·5 mol l^{-1} K_2SO_4 for 1 h with shaking (200 rev min^{-1}). Extracts were gravity filtered through Whatman GF/F filter paper (GE Healthcare, Pittsburgh, PA). Total organic carbon (TOC) and total nitrogen (TN) concentrations in extracts were determined on a Shimadzu TOC-L analyzer with TNM-L module. The TOC and TN levels in fumigated and unfumigated soils were calculated from extract concentrations, adjusting for the dry weight of soil extracted from each sample. These values were used to calculate microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) using the following equations:

$$\mathrm{MBC} = \mathrm{EC} \div \mathrm{kEC}$$

$$\mathrm{MBN} = \mathrm{EN} \div \mathrm{kEN}$$

where EC and EN are the difference in TOC and TN between chloroform fumigated and unfumigated soil, and kEC and kEN are constants of 0·35 and 0·68 respectively (Horwath and Paul 1994). Soil organic matter (SOM) content of soil samples was determined by ashing oven dried soil samples in a muffle furnace at 500°C for 2 h and is reported as per cent organic matter per g dry weight of soil.

DNA extraction and 16S rRNA gene sequencing

DNA was extracted from soil samples using MoBio PowerSoil DNA Isolation kits (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's instructions. DNA extracts were submitted to MR DNA (Shallowater, TX) for sequencing. The V4 hypervariable region of the 16S rRNA gene was amplified using primers 515F (5′ GTGCCAGCMGCCGCGGTAA 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 intensity of bands. Multiple samples were pooled in equal proportions based on their molecular weight and DNA concentrations and purified using calibrated Ampure XP beads. Pooled and purified PCR product was used for library preparation according to the Illumina TruSeq DNA library protocol. Paired-end 2 × 300 sequencing reactions were performed on an Illumina (San Diego, CA) MiSeq analyzer following manufacturer's guidelines. The sequence data generated have been deposited in the NCBI Short Read Archive under bioproject accession number PRJNA492265.

Sequence analysis

The FASTQ files from each sample were processed using the bioinformatics software MOTHUR (ver. 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 (ver. 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. Diversity estimates, including numbers of observed OTUs per sample, Shannon index, abundance-based coverage estimator (ACE) and Chao 1 were calculated by subsampling 25 840 sequences from each soil sample 1000 times to normalize for differences

in read numbers between samples. Nonmetric multidimensional scaling (NMDS) ordination was performed to visualize differences in community structure between reduced tillage and no till plots. Significant differences in community composition between treatments observed in NMDS ordination plots were determined using analysis of molecular variance (AMOVA) and impact of soil properties on ordination plots were calculated using Pearson correlations. OTUs that were differentially abundant between no till and reduced tillage treatments were identified using linear discriminant analysis effect size (LEfSe) analysis (Segata *et al.* 2011) in MOTHUR. Correlations between individual OTU abundance and soil parameters were determined by Pearson correlations in MOTHUR.

Statistics

Comparison of enzyme activities, microbial biomass, abundance of bacterial taxa and diversity indices were performed in JMP ver. 11.2.0. (SAS Institute Inc., Carey, NC). Two-way analysis of variance (ANOVA) was conducted to determine if there were any residual effects of past cover crop treatments from previous experiments at the field site using the fit model function and full factorial analysis with factors of tillage (no tillage or reduced tillage) and cover crop (clover, rye, or no cover) treatments from the original experiment. Once a lack of residual effects was confirmed, subsequent comparisons of soil pH, organic matter, microbial biomass, enzyme activities and diversity scores between reduced tillage and no till treatments were performed by Student's *t*-test. Pearson correlations were conducted to determine relationships between soil characteristics and bacterial diversity. All analyses were assessed using an α of 0·05.

Results

Chemical and biological characteristics

Two-way ANOVA demonstrated that there were no significant differences between former cover crop treatments, indicating no residual effect of cover crops from 8 years prior. Therefore, statistical analyses reported here focus on comparisons between the two tillage treatments. No till plots had slightly lower soil pH ($P = 0{\cdot}0175$), while neither soil moisture content ($P = 0{\cdot}983$) nor SOM differed significantly between tillage treatments ($P = 0{\cdot}884$) (Table 1). However, MBC ($P = 0{\cdot}0053$) and MBN ($P = 0{\cdot}025$) were both greater in no till, indicating a larger microbial population in those soils (Table 1). Of the five assays for soil activities, FDA hydrolysis ($P = 0{\cdot}009$), phosphatase ($P = 0{\cdot}0016$), β-glucosidase ($P = 0{\cdot}0024$) and cellobiohydrolase ($P = 0{\cdot}0368$) were all higher in no till soil, indicating these 0–5 cm surface soils had a greater potential for phosphate mineralization and organic matter turn over (Table 1). Meanwhile, NAGase activity, while appearing slightly higher in no till, did not differ significantly from reduced tillage soil.

16S rRNA sequence analysis

A total of 1 673 362 16S rRNA gene sequencing reads >245 bp were generated from Miseq sequencing of the 24 no till and reduced tillage soil samples. These sequences were processed for the removal of ambiguous bp (85 367), homopolymers (306 994), chimeras (123 312) and non-bacterial sequences (85 216), leaving a total of 1 072 473 sequences for further analysis. There was an average of 44 686 reads per sample ranging from 25 840 to 110 486. Subsequent analyses on OTUs, diversity and community structure were performed by subsampling 25 840 sequences from each soil sample 1000 times to normalize for differences in read numbers between samples.

Effects of tillage on bacterial community composition

A total of 25 phyla were identified in the 16S rRNA sequences, most of which were present in both reduced tillage and no till soils. Of these phyla, Proteobacteria and Acidobacteria were the most abundant in all samples. Few differences were apparent between tillage treatments among the most abundant bacterial phyla, but significant differences between treatments were observed in lower abundance phyla (Fig. 1). Bacteroidetes ($P = 0{\cdot}042$), Firmicutes ($P < 0{\cdot}0001$), Armatimonadetes ($P = 0{\cdot}017$) and

Table 1 Soil Properties in reduced tillage and no till field plots*

	Reduced tillage	No till
pH	6·5 ± 0·08a	6·2 ± 0·06b
Moisture content	9·63 ± 2·17a	10·90 ± 1·74a
Organic matter	4·79 ± 0·22a	4·83 ± 0·17a
Microbial biomass C	473·87 ± 19·71a	581·13 ± 28·55b
Microbial biomass N	24·91 ± 0·97a	31·05 ± 2·4b
β-glucosidase	164·6 ± 4·1a	192·6 ± 7·1b
N-acetylglucosaminidase	37·8 ± 1·7a	43·9 ± 3·1a
Phosphatase	690·5 ± 48·6a	951·8 ± 54·3b
Cellobiohydrolase	44·7 ± 1·9a	52·4 ± 2·9a
FDA hydrolysis	34 ± 4·2a	59·7 ± 7·9b

*Values represent mean ± standard error ($n = 12$). Values that share the same letter are not significantly different ($P \leq 0{\cdot}05$). Moisture content and organic matter reported as per cent (%) dry weight of soil. Microbial biomass is reported as mg of carbon (C) or nitrogen (N) per kg dry weight of soil. Enzyme activities (β-glucosidase, N-acetylglucosaminidase, phosphatase, cellobiohydrolase and fluorescein diacetate (FDA) hydrolysis) are reported as nmole of pNP or fluorescein generated per g dry weight soil per h.

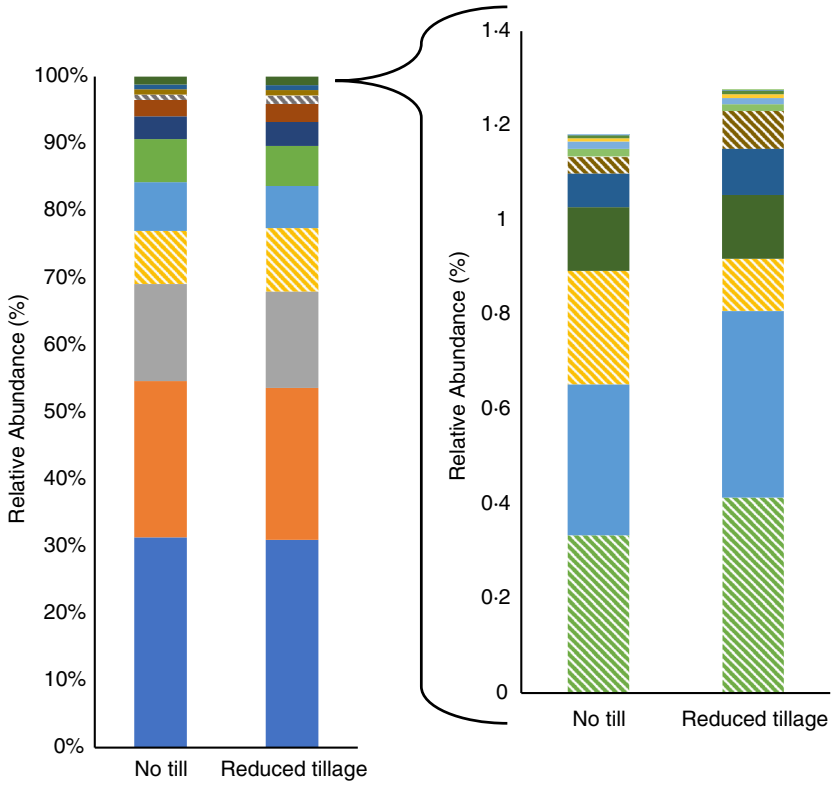

Figure 1 Relative abundance of bacterial phyla composing the soil communities of reduced tillage and no till plots. Phyla represented with hatch filled bars (Bacteroidetese, Firmicutes, Armatimonadetes, Latescibacteria and Parcubacteria) are significantly different between treatments ($P < 0{\cdot}05$). Bacterial phyla in the left panel compose at least 0·5% of the bacterial community and are shown top (least abundant) to bottom (most abundant) as Other bacteria, candidate division WPS-2, Nitrospirae, Firmicutes, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, Actinobacteria, Bacteroidetes, unclassified bacteria, Acidobacteria and Proteobacteria. "Other bacteria" in the left panel represents the sum of bacterial phyla that individually compose <0·5% of the bacterial community and are shown in the right panel top (least abundant) to bottom (most abundant) as Synergistetes, Ignavibacteriae, Hydrogenedentes, Deinococcus–Thermus, Candidatus Saccharibacteria, Spirochaetes, Microgenomates, BRC1, Parcubacteria, candidate division WPS-1, Chlamydiae, Latescibacteria, Chloroflexi and Armatimonadetes. [Colour figure can be viewed at wileyonlinelibrary.com]

Parcubacteria ($P = 0{\cdot}0028$) had higher relative abundances in tilled plots, while Latescibacteria were higher in no till treatments ($P = 0{\cdot}0002$). As the most abundant phylum identified (approximately 30% of sequences), Proteobacteria was further broken down to the subphylum level to elucidate potential shifts in this portion of the bacterial community. Of these subphyla, Alphaproteobacteria abundance was greater in tillage plots ($P = 0{\cdot}028$) while Betaproteobacteria were more abundant in no till plots ($P = 0{\cdot}0121$; Fig. 2a). Breaking down the second most abundant phylum, Acidobacteria, to its component subgroups revealed fewer insights. Of the 22 Acidobacteria subgroups identified, the only three that differed significantly between treatments were in very low relative abundance, including Gp2 ($0{\cdot}07 \pm 0{\cdot}0005$ in no till *vs* $0{\cdot}05 \pm 0{\cdot}0006$ in reduced till), Gp11 ($0{\cdot}05 \pm 0{\cdot}006$ in not till *vs* $0{\cdot}03 \pm 0{\cdot}005$ in reduced till) and Gp22 ($0{\cdot}23 \pm 0{\cdot}02$ in no till and $0{\cdot}13 \pm 0{\cdot}01$ in reduced till). At finer taxonomic scales, differences in abundance between treatments included *Bacillus* ($P < 0{\cdot}0001$), Paenibacilliaceae ($P = 0{\cdot}0023$), Rhizobiales ($P = 0{\cdot}024$) and *Sphingomonas* ($P = 0{\cdot}039$), which were significantly higher in reduced tillage, and *Azoarcus* ($P < 0{\cdot}0001$), which was higher in soil from no till plots (Fig. 2b). These groups make up a small proportion of the total bacterial community, each comprising <5%, and in the case of *Bacillus*, *Azoarcus* and Paenibacillaceae, <1% of the total sequences identified.

Differences between tillage and no till were most apparent after assigning sequences to OTUs. A total of 35 231 OTUs were identified in all soil samples, of which 13 418 were unique to reduced tillage, 9439 unique to no till and 12 374 shared between the two. Unique OTUs were all relatively low abundance, ranging from 1–69 sequences per OTU in reduced till and 1–145 in no till. The taxonomic classifications of these unique OTUs were also similar between reduced till and no till treatments, with the majority identified as unclassified bacteria (4519

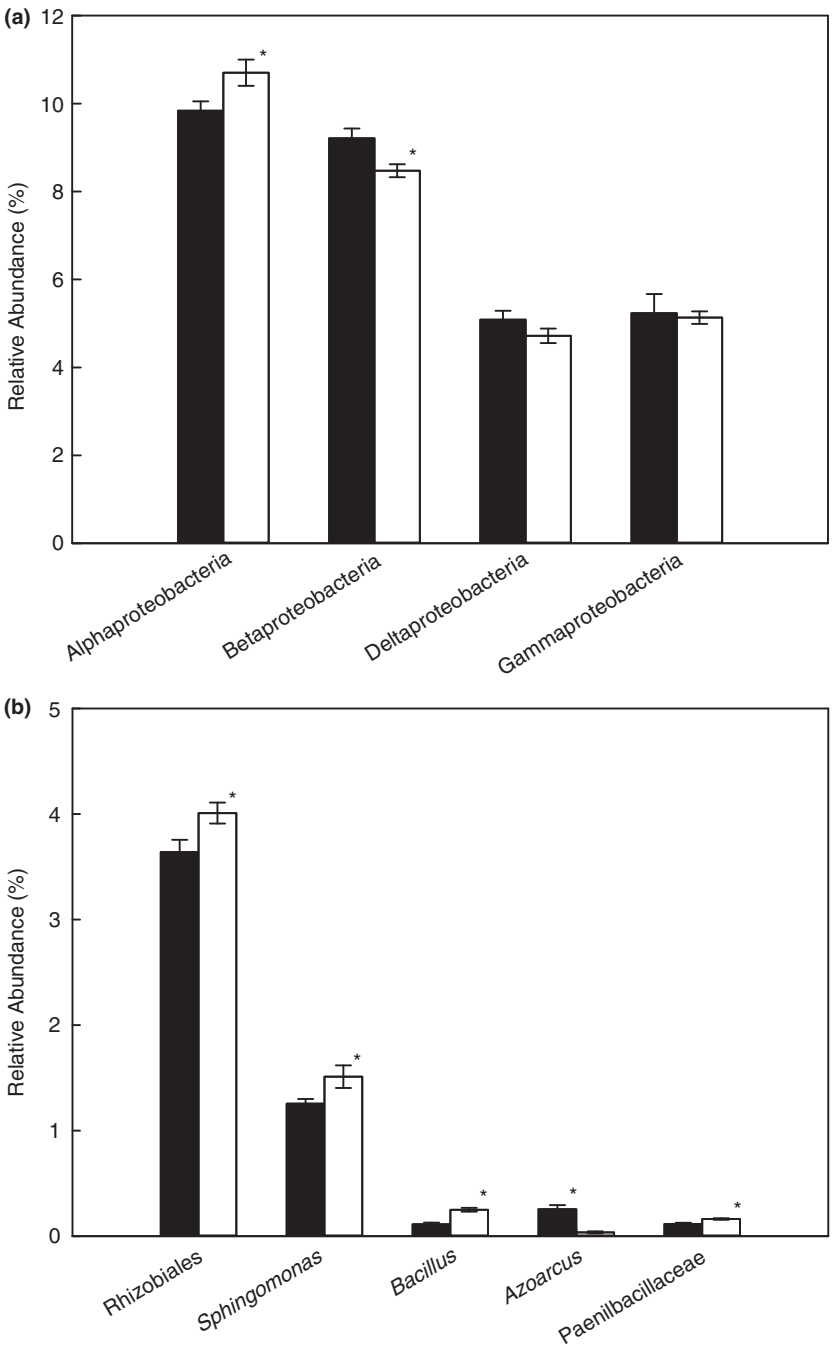

Figure 2 Relative abundance of Proteobacteria subphyla (a) and selected bacterial taxonomic groups (b) in reduced tillage (open bar) and no till (closed bar) plots. Significant differences between reduced tillage and no till treatments are noted by asterisks (*). Values represent mean ± standard error (n = 12).

in reduced till *vs* 3140 in no till that could not be classified to finer taxonomic levels), Proteobacteria (3372 *vs* 2347 in reduced till and no till respectively) and Acidobacteria (1387 *vs* 935 in reduced till and no till respectively). Generally, reduced till soils had more unique OTUs from each phylum, with the exceptions of Deinococcus–Thermus (0 reduced till *vs* 3 no till), Latescibacteria (5 reduced till *vs* 9 no till) and Spirochaetes (1 reduced till *vs* 6 no till). At finer taxonomic scales, there were similar numbers of Bradyrhizobiaceae (17 *vs* 14) and Xanthomonadaceae (58 *vs* 46) in both treatments. LEFSe analysis identified 161 OTUs that were differentially abundant between treatments, with 94 being more abundant in no till plots and 67 OTUs being higher in reduced tillage plots (Fig. 3, Table S1). No till appears to have a greater effect on differentially abundant OTUs, as evidenced by higher LDA scores of 2·0016–3·2679 (mean of 2·3597) compared to 2·0004–2·7341 (mean of

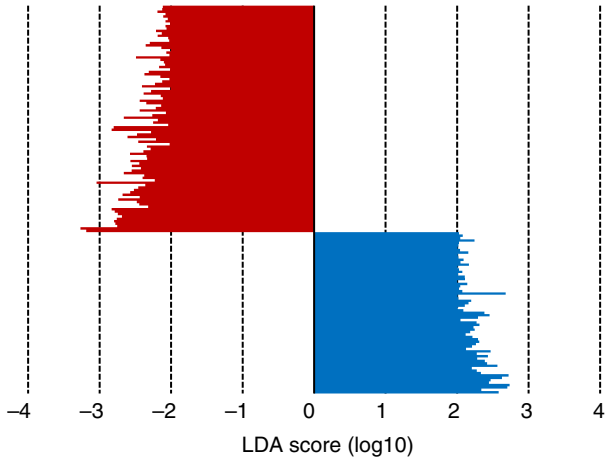

Figure 3 Differentially abundant operational taxonomic units (OTUs) in reduced till and no till soils as determined by the linear discriminant analysis (LDA) effect size (LEFSe) method. LDA scores of OTUs in greater abundance within reduced tillage soils are represented by positive (blue) LDA scores and OTUs in greater abundance within no till soils are represented by negative (red) LDA scores. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Diversity of reduced tillage and no till soils as determined by 16S rRNA sequence analysis*

	Reduced tillage	No till
OTUs	5182 ± 45a	4926 ± 69b
Shannon	7·44 ± 0·01a	7·36 ± 0·02b
Chao 1	10 412 ± 169a	9797 ± 177b
ACE	14 478 ± 313a	13 507 ± 284b

*Values represent mean ± standard error (n = 12) of diversity statistics (number of observed operational taxonomic units (OTUs), Shannon index, Chao 1 and abundance-based coverage estimator (ACE)) calculated for each sample. Values that share the same letter are not significantly different ($P \leq 0{\cdot}05$).

2·2249) for reduced tillage. Among those OTUs of greater abundance in no till soils were 14 Betaproteobacteria, 11 Actinobacteria, seven Verrucomicrobia, two Latescibacteria and two *Azoarcus*. In comparison, only six Betaproteobacteria, one Actinobacteria and none of the other three above listed taxa were identified among OTUs that were more abundant in reduced tillage soil.

Bacterial diversity effected by tillage

Reduced tillage soils tended to have slightly greater diversity than no till soils, with significantly higher observed OTUs, Shannon, Chao 1 and ACE diversity scores (Table 2). These four diversity estimates all significantly correlated with pH, soil moisture, SOM and MBC (Table 3). Enzyme activities linked to organic matter breakdown, however, only correlated with observed OTUs and Shannon index (Table 3). The NMDS plots show bacterial communities from reduced tillage plots cluster together within no till soils, with the exception of two outliers (Fig. 4). These are from plots in the same region of the field, indicating variation in soil characteristics within the study site might contribute to their differences. Examination of the USDA-NRCS's soil survey revealed the outliers (plots 16 and 17) are in a region of the field intersected by a vein of Dowling clay (Fig. S1), although these plots did not cluster in the NMDS with others that contained Dowling soils. These outlier plots did not have the highest diversity scores relative to the other reduced till plots, indicating they were not driving the above mentioned differences in diversity between reduced till and no till treatments. AMOVA indicated the clustering of reduced tillage and no till communities was significant (P = 0·002). Pearson correlations with soil metadata indicated SOM was a driving factor of these differences in community composition (Fig. 4). In fact, 3577 of the 35 231 OTUs (10·2%) identified were significantly correlated with SOM ($P < 0{\cdot}05$; Table 4). MBC, MBN and pH also correlated significantly with shifts in bacterial community composition and diversity between samples (Fig. 4, Tables 3 and 4).

Discussion

Effects of tillage on microbial community composition in Mississippi field soils tend to vary. One previous study indicated tillage did not have a significant impact on microbial community composition after 6 years of no till *vs* reduced tillage management (Locke *et al.* 2013). However, 8 years later, after a total of 14 years under different tillage management, the current study did find subtle, but significant, differences in bacterial community composition. Whether this difference is a new development that occurred over the intervening years or was the result of employing high-throughput sequencing of the 16S rRNA gene rather than the FAME approach utilized in the original study is unclear. One shortcoming of FAME is that many of the fatty acids used to identify members of the microbial community are shared between multiple groups of microbes (Cavigelli *et al.* 1995), which could serve to mask differences in communities between treatments. Differences in crop species may also have influenced the tillage effect. The original study at this field site was conducted using cotton (Locke *et al.* 2013). However, crop preference in the region shifted, and these plots have been planted in continuous soybean since the conclusion of the original study.

Time of sample collection can also influence assessment of microbial composition in soil. Drijber *et al.*

(2000) found differences in microbial community profiles were most pronounced during fallow periods. Feng *et al.* (2003) noted a similar trend, where microbial communities were different between conventional and no till treatments in February and May, but not in October, suggesting that during the growing season, microbial communities are more impacted by crop growth, which would provide an explanation for why differences are more apparent during fallow periods. Interestingly, the prior experiment conducted at our Mississippi Delta field site sampled soil in late Spring prior to crop planting, when potential differences based on tillage would theoretically be at their greatest. However, that study also examined winter cover crops, which might have obfuscated tillage-based differences between treatments. The current study was conducted when the site had been without cover crop for 8 years, allowing for a comparison of tillage treatments without the influence of differences in crop species. The current study also sampled in the fall, several months after tillage. As such, it is likely the differences in community composition may have been more pronounced earlier in the growing season. Given these observations, it is possible that a combination of longer time under tillage *vs* no till management and a lack of confounding factors, such as winter cover crop differences, account for the differences observed between these two studies.

Differences in community composition between tillage treatments have also been attributed to changes in soil pH, organic matter and microbial biomass (Cookson *et al.* 2008). Similarly, the current study noted that bacterial diversity in soils positively correlated with pH and negatively correlated with SOM and MBC. Approximately 7–10% of OTUs identified in soil samples also correlated with these parameters. Using a shotgun metagenomics approach, Souza *et al.* (2013) found differences between soil communities were associated more closely with tillage than with crop rotation, with no till soils containing

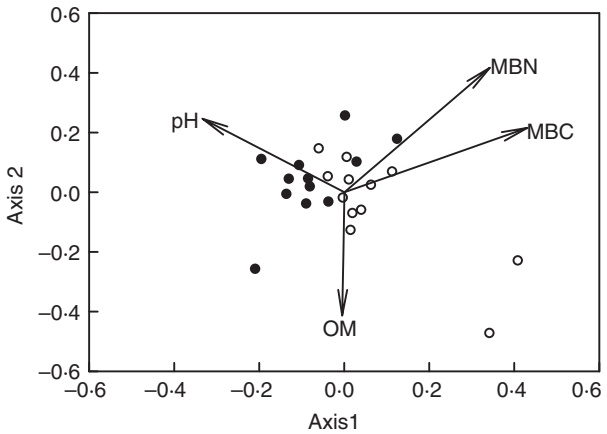

Figure 4 Nonmetric multidimensional scaling (NMDS) biplots of bacterial communities reduced till (open circle) and no till (closed circle) soils (stress = 0·1502, R^2 = 0·947678). Vectors were determined by Pearson correlations using the corr.axes command in MOTHUR and represent the strength and direction of the influence of the following soil characteristics on bacterial communities: organic matter (OM), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), pH and moisture content (MC). All vectors were significant on axis 1, with the exception of OM and MBN, which were significant on axis 2 ($P < 0·05$).

greater abundances of microbes associated with high organic matter environments. Total Proteobacteria, as well as Alpha- and Betaproteobacteria were also more abundant in tilled soil, while Deltaproteobacteria and Rhizobiales were higher in no till (Souza *et al.* 2013). The current study also noted higher Alphaproteobacteria in tilled plots, but in contrast to Souza *et al.* (2013), observed higher Betaproteobacteria and lower Rhizobiales in no till than reduced tilled soil. Since crop rotations in Souza *et al.* (2013), as well as the current study, included soybean, a legume dependent on nodulation for N fixation, the differential abundance of Rhizobiales is of interest. However, further analysis revealed that *Bradyrhizobium*,

Table 3 Correlation coefficients (*r*) between diversity and soil chemical and biological properties

	OTUs	Shannon	Chao 1	ACE
pH	0·555**	0·475*	0·598**	0·636***
Soil moisture	−0·484*	−0·405*	−0·529**	−0·569**
Organic matter	−0·630***	−0·629***	−0·641***	−0·643***
Microbial biomass carbon	−0·491*	−0·455*	−0·413*	−0·435*
Cellobiohydrolase	−0·444*	−0·480*	–	–
β-glucosidase	−0·408*	−0·413*	–	–
N-acetylglucosaminidase	−0·413*	−0·443*	–	–
Phosphatase	−0·437*	−0·422*	−0·452*	−0·448*
FDA hydrolysis	−0·549**	−0·543**	−0·525**	−0·499*

Values represent Pearson coefficients of significant correlations (*$P \leq 0·05$, **$P \leq 0·01$, ***$P \leq 0·001$) between diversity and richness indexes (number of observed operational taxonomic units (OTUs), Shannon index, Chao 1 and abundance-based coverage estimator (ACE)) and soil chemical characteristics. Blank values indicate nonsignificant correlations.

Table 4 Number of operational taxonomic units that correlate significantly with soil parameters*

	OTUs
pH	2563
Moisture content	2110
Organic matter	3577
Microbial biomass carbon	2264
Microbial biomass nitrogen	2217

*Values represent number of operational taxonomic units (OTUs) identified in soils with a significant Pearson correlation coefficient ($P < 0{\cdot}05$) with select soil parameters.

the genus responsible for nodulation in soybean, did not differ significantly between reduced tillage and no till soils in the current study. Also of note within no till soil communities was a higher abundance of *Azoarcus*, a genus of diazotrophic, N-fixing bacteria associated with endophyte and rhizosphere communities of grasses (Reinhold-Hurek *et al.* 1993).

Multiple studies have reported an increase in diversity associated with no till soil management. Dong *et al.* (2017) found bacterial diversity, as calculated by number of OTUs, Chao (richness), ACE (evenness) and Shannon index, was higher in no till than in conventionally tilled soils. Sengupta and Dick (2015) also found bacterial richness, namely Chao 1 and ACE, were higher in no till plots, while the Shannon diversity index was higher in tilled plots. These differences were attributed to homogenization of tilled soil, reducing the number of unique microenvironments for bacteria to inhabit, and higher organic matter content in no till soils, favouring a fungal dominated microbial community (Sengupta and Dick 2015). However, their study did not include biological replicates, but instead, examined one sample each from tillage and no till treatments, which were pooled from a variety of crop rotations. This may have masked differences in tillage that varied between crop treatments.

In contrast to the above studies, results reported in the current study indicated no till plots displayed slightly lower diversity compared to tilled soils. One possible factor contributing to the differences between these studies is soil texture. Both the Dong *et al.* (2017) and Sengupta and Dick (2015) study sites had silt loam soils, while soils in the current study ranged from a very fine sandy loam at one end of the field to a silty clay loam at the other, with veins of clay soil running through 12 of the 24 plots (six no till and six reduced till). Similar to the current study, Sun *et al.* (2018) also found bacterial OTU richness was greater in conventional and rotary tillage compared to no till soil. A potential reason for this decrease in diversity could be the release of nutrients from decomposing crop residues on the soil surface, promoting the proliferation of bacterial groups involved in organic matter breakdown. All diversity and richness indicators in the current study were negatively correlated with SOM levels. In addition, several of the taxa and OTUs found to be more abundant in no till soils are known to contain organic matter degraders. Latescibacteria, a candidate phylum found to contain genes for degradation of plant polysaccharides (Farag *et al.* 2017) and believed to be involved in organic matter turnover (Youssef *et al.* 2015), was found in greater abundance in no till soils. In addition, several of the OTUs in greater abundance in no till soil were classified as Actinobacteria and Verrucomicrobia, two phyla composed primarily of chemoheterotrophs that utilize complex polysaccharides (Hedlund 2010; Barka *et al.* 2016). Consistent with these observations, microbial enzymes involved in organic matter breakdown, such as β-glucosidase, are routinely observed to be higher in no till soils (Acosta-Martínez *et al.* 2003; Zhang *et al.* 2014; Mbuthia *et al.* 2015), and were similarly elevated in no till soils of the current study. As tillage involves incorporating crop residues into soils, while no till management results in these residues remaining on the soil surface, the gradual breakdown of crop residues on the soil surface can provide a prolonged source of substrate for these microbes, thus sustaining elevated activities in no till soils.

In conclusion, the current study found that, while relative abundances of dominant phyla were similar between reduced tillage and no till plots, a more significant variation between treatments was evident in low abundance phyla and at finer taxonomic scales. As such, this study highlights the advantages of high-throughput sequencing for identifying potential shifts in soil microbial communities, allowing for the detection of differences that would not be discernible via other methods, such as FAME, which may have previously failed to detect differences based on tillage at this same study site. The discovery that no till soils possessed slightly lower bacterial diversity was unexpected, as no till management is commonly regarded to be beneficial to overall soil health, with multiple studies noting increased diversity in no till soils. However, the lower diversity observed here is consistent with the proliferation of heterotrophic bacteria in these soils, owing to the breakdown of plant residues left on the surface of no till plots. These results suggest that while no till management has some benefits on soil health, prolonged no till conditions may lead to decreases in microbial and, ultimately, functional diversity of 0–5 cm surface soils. In addition, no till soils are also prone to soil compaction, resulting in poor aeration and water infiltration (Tyler and Locke 2019), which could in turn influence the microbial community. Soil compaction has been found to have significant impacts on bacterial diversity in forestry settings (Hartmann *et al.* 2014), but more work is needed to examine its effects in agricultural fields.

As such, further research is needed to ascertain if these tillage-based effects extend to greater depths in the soil profile and to determine if occasional inclusion of tillage in predominantly no till fields may provide some benefits to sustain healthy soil microbial communities.

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Conflict of Interest

No conflict of interest declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Study site plot map denoting soil texture and reduced tillage or no till treatment by plot.

Table S1 List of operational taxonomic units identified as differentially abundant in reduced tillage and no till soil by linear discriminant analysis (LDA) effect size (LEfSe) analysis.