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Aditi Sengupta

Janani Hariharan

Parwinder Grewal
The University of Texas Rio Grande Valley

Warren A. Dick

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Bacterial community dissimilarity in soils is driven by long-term land-use practices

Aditi Sengupta[1,3] | **Janani Hariharan**[1] | **Parwinder S. Grewal**[2] | **Warren A. Dick**[1]

[1]School of Environment and Natural Resources, Hayden Hall, Ohio Agricultural Research and Development Center, The Ohio State Univ., 1680, Madison Avenue, Wooster, OH 44691, USA

[2]College of Sciences, Univ. of Texas Rio Grande Valley, Edinburg, TX 78539, USA

[3]Current address: Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, P.O. Box, 999, MS J4-18, 902, Battelle Boulevard, Richland, WA 99352, USA

Correspondence
Aditi Sengupta, School of Environment and Natural Resources, Hayden Hall, Ohio Agricultural Research and Development Center, The Ohio State Univ., 1680 Madison Avenue, Wooster, OH 44691, USA.
Email: aditi.sengupta@pnnl.gov

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Abstract

Land-use practices impact soil microbial functionality and biodiversity, with reports suggesting that anthropogenic activities potentially result in reduced microbial functions and loss of species. The objective of this study was to assess the effect of long-term (>50 yr) land use (natural forest and grassland, and agricultural land) on soil bacterial community structure. A high-throughput sequencing-by-synthesis approach of the 16S rRNA gene was used to study bacterial community and predicted functional profiles of Alfisols, as affected by variables including land-use (forest, grass, agricultural) and soil/crop management (rotation and tillage) in long-term experimental plots in Hoytville, OH. The distribution of the abundant phyla was different across samples. No-till soils showed higher diversity indices than the plow-till (PT) soils. Ordinations across locations suggested that no-till soils had distinctly different community structure compared with plow-till soils, while crop rotation within the no-till plot had highest number of taxa. Overall land use (forest, grass, agronomic treatment) and tillage (within agricultural soils) were found to be significant when evaluating bacterial community dissimilarity. Predictive functional profiles showed that the forest soil had greatest proportion of PICRUSt-assignable gene functions followed by the no-till and grassland soils whereas plow-till soils had the lowest predicted gene abundances across all samples. The results provide a view of soil bacterial diversity and predictive functional capacity in long-term land-use and soil/crop management practices, with a potential to inform future experiments to increase our understanding of long-term impacts of land use on microbial community structure and function.

1 | INTRODUCTION

Soil microorganisms contribute to crucial ecological processes like decomposition of organic matter, regulation of greenhouse gas fluxes, breakdown of xenobiotic compounds, biogeochemical cycling of nutrients, plant disease suppression, and plant growth (Garbeva, van Veen, & van Elsas, 2004; Nannipieri et al., 2003). Activities in the soil surficial layer such as tillage-dependent fragmentation of soil

Abbreviations: CC, continuous corn; CS, corn–soybean; DNA, deoxyribonucleic acid; FOR, forest; GRA, grassland; NT, no-till; PCR, polymerase chain reaction; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; PT, plow-till; RNA, ribonucleic acid.

structure (Babujia, Silva, Nogueira, & Hungria, 2014; Potthoff et al., 2006), crop residue accumulation from no-tillage (Ceja-Navarro et al., 2010; Mathew, Feng, Githinji, Ankumah, & Balkcom, 2012), leaf litter decomposition from forests (Chapman, Newman, Hart, Schweitzer, & Koch, 2013; Purahong et al., 2014), and biomass accumulated on surface of grasslands (Garbeva, Postma, van Veen, & van Elsas, 2006; Lienhard et al., 2012; McCaig, Glover, & Prosser, 2001; Singh, Munro, Potts, & Millard, 2007) affect soil microbial community structure, diversity, biomass, and activity. Reports suggest that anthropogenic activities potentially result in reduced microbial species and function (Brown, Hungria, Oliviera, Bunning, & Montanez, 2002; Smith, 1995; The Royal Society of Chemistry, 2011). Therefore, it is critical to evaluate the impacts of land-use practices on soil microbial diversity and function.

Globally, implementation of soil conservation practices in agriculture has become crucial. Studies estimate that land-use and land-management practices account for 20–24% of total anthropogenic greenhouse gas emissions (Smith, Bustamante, Ahammad, Clark, & Dong, 2014). Conventional agriculture practices involve plowing and sowing, whereas conservation practices like no-tillage are characterized by sowing directly into the soil, while maintaining 30% crop residue present on the surface (Claasen, Bowman, McFadden, Smith, & Wallander, 2018). Worldwide, no-tillage systems account for 117 million ha with about 27 million ha of no-tillage farming in the United States in 2007 (Huggins & Reganold, 2008). Based on estimates reported in 2012, about 40% of the total cropland (1.8 million ha) in Ohio is continuous no-tillage (Lessiter Media, 2014).

Long-term, no-till practices lead to increased soil organic matter content, improved soil physical properties, and optimum soil moisture levels (Campbell, Chen, Dygert, & Dick, 2014; Triplett & Dick, 2008) whereas conventional tillage over time reduces soil organic matter content, increases soil compaction due to use of heavy machinery, and disrupts pockets of microbial metabolic activity (Triplett & Dick, 2008). There are conflicting reports on microbial diversity in soils under different management practices. Comparisons between agricultural and grassland soils showed decreased microbial species richness in agricultural soils (Steenwerth et al., 2014). In other agricultural soils, Rodrigues et al., 2013 reported that conversion of the Amazon rainforest in South America to cultivation resulted in an increase in microbial alpha diversity but resulted in the loss of beta diversity. Within agricultural practices, alpha and beta diversity may be differentially as seen in decreased beta diversity in soybean monoculture when compared with crop rotation management (Figuerola et al., 2012). Other studies have indicated that tilled soil may or may not contain greater bacterial diversity than no-tilled soil (Ferreira et al., 2000; Torsvik & Øvreås, 2002; Upchurch et al., 2008), but the Frey, Elliott, & Paustian (1999) study reported no consistent effects on bacterial abundance or biomass in a 30-yr tillage practice. Although diversity indices may not be ideal for deciphering ecological drivers of microbial community patterns (Shade, 2017), evaluating diversity patterns in response to environmental changes helps generate ecologically relevant follow-up hypotheses and experimentation that may collectively contribute to deciphering long-term impacts of environmental changes. A lack of consensus on how long-term land-use/management impacts soil microbial diversity signatures therefore presents a necessity to examine impact of long-term land-use/management on soil microbial diversity in greater detail.

Core Ideas

- Long-term land-use practices impact soil microbial functionality and biodiversity.
- Forest, grassland, and continuously maintained till and no-till soils were evaluated.
- Land use and tillage drove soil bacterial dissimilarity.
- Predictive functional-gene abundances were higher in no-till than plow-till soils.

To the best of our knowledge, only a handful of studies have attempted to study the effect of long-term agricultural practices and land use change on soil microbiomes using high-throughput 16S rRNA sequencing. These include investigations of soil microbiome in Argentinean pampas (Carbonetto, Rascovan, Álvarez, Mentaberry, & Vázquez, 2014; Figuerola et al., 2012), soil microbial diversity and composition studies of a long-term agricultural experiment field in southern Brazil (Dorr de Quadros et al., 2012), a comparison of microbial diversity and composition following grassland to arable conversion (French, Tkacz, & Turnbull, 2017), effect of land use intensification on the diversity of soil bacteria in the Brazilian Amazon (de Carvalho, da C. Jesus, Barlow, Gardner, & Soares, 2016), and a pilot study of soil bacterial community in long-term till and no-till plots in Ohio (Sengupta & Dick, 2015).

The 2015 pilot study that we conducted (Sengupta & Dick, 2015) focused on just two treatments, was a proof of concept for our research objectives, and was conducted using the 454 pyrosequencing platform known to have lower sequencing depth and higher error rates compared with the Illumina platform (Loman et al., 2012). This study is unique as it evaluates the impact of long-term land-use and soil-management practices on soil bacterial diversity across six treatments, including the longest maintained tilled and no-tilled plots (> 52 yr) in the world. Very few places in the world have no-tillage cropping been continuously maintained for such a long time. Thus, the treatments are firmly established and the results from this

study are considered representative of a well-established bacterial community as impacted by land management.

All practices were established on a Hoytville clay loam (fine, illitic, mesic Mollic Epiaqualfs) Alfisol soil with plots located near Hoytville, OH. We hypothesized that soil bacterial diversity in these soils would differ significantly with respect to their long-term land-use and soil/crop-management practices. Categorical variables such as crop rotation, tillage, and land use were tested for their influence on community structure and diversity. Additionally, we employed an in silico tool, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)(Langille et al., 2013), to predict functional potential of the soil microbial communities in response to long-term land-use management. Results showed that land management followed by tillage practices significantly affected bacterial community structure, and functionally, forest soil followed by no-till soils had highest predicted gene abundances across the most abundant predicted functions.

2 | MATERIAL AND METHODS

2.1 | Description of the field sites, and soil sampling and processing

Soil samples were collected from long-term experimental field sites, at the Northwest Agricultural Research Station of the Ohio Agricultural Research and Development Center (OARDC) located near Hoytville, OH. Detailed site information and plot treatments are provided in Sengupta and Dick (2017). Briefly, the following treatments were studied: (a) no-till continuous corn (*Zea mays* L.; NTCC); (b) no-till corn–soybean [*Glycine max* (L.) Merr., NTCS]; (c) plow-till continuous corn (PTCC); (d) plow-till corn–soybean (PTCS); (e) grassland (GRA); and (f) forest, FOR. The plots of the corn–soybean rotation had corn growing in the previous season. Rotation plots were selected to maintain uniformity in the crop planted in the previous growing season (in this case corn). Since the plots have been maintained for >50 yr, we expect microbial community characteristic to be a cumulative reflection of the corn–soybean rotation in the rotation plots. Adjacent grass and forest sites within meters accompany the agricultural plots were also sampled in this study. Samples were collected from three replicated plots per treatment in spring 2013, before the planting season. Three sub-samples (0–10 cm) were collected using a hand-held soil auger (35-mm i.d.) from each replicated treatment. A composite soil sample was prepared for each replicate plot by pooling the sub-samples. After thorough mixing, each replicate sample was split in half to be used for chemical analyses after passing the soil through a 2-mm sieve, and the other half was used for microbial DNA extraction.

Genomic DNA of 18 samples was extracted from approximately 0.25 g of field-moist soil immediately after sampling by using an UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc.) following the manufacturer's instructions. The extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and quality confirmed on 1% agarose gel with 1× TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). The DNA extracted from the replicates were pooled together to obtain six samples representing six treatments.

2.2 | Illumina library generation and sequencing

Sample preparation was performed according to an in-house two-step PCR (polymerase chain reaction) amplification protocol targeting partial region of the 16S rRNA gene, with sample depth coverage being ~180 bp (V3 region), following protocol presented in Sengupta and Dick (2017). Briefly, the first round of PCR reaction was conducted using modified primers 341F (5′-TCGTCGGCAGCGTCAGATGTG TATAAGAGACAG-CCTACGGGAGGCAGCAG-3′) and 518R (5′-GTCTCGTGGGCTCGG AGATGTGTATAA GAGACAG-ATTACCGCGGCTGCTGG -3′) as per Lynch, Bartram, & Neufeld (2012) with PCR conditions involving an initial denaturation step at 94 °C for 1 min followed by 20 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min in a Bio-Rad C1000 Touch Thermocycler (Hercules, CA).

The second PCR step was identical to description provided in Sengupta and Dick (2017), and involved attaching complementary primers to the Illumina forward, reverse, and multiplex sequencing primers with the forward and reverse primer also containing a unique 8-bp read index allowing for multiplexing (detailed barcode information provided in Sengupta & Dick, 2017). Amplified samples were pooled and loaded onto 1.5% agarose Pippin Prep (Sage Science) instrument for targeted size selection of the pooled fragment (180 bp). The pooled sample was extracted and quantified again using Qubit Fluorometer and submitted to Molecular and Cellular Imaging Center (MCIC) housed at OARDC for sequencing. Sequencing was performed using the Illumina MiSeq instrument with MiSeq Reagent Kit v3 and MiSeq Control Software and Reporter v2.4.1 (Illumina, Inc.). The pooled sample was sequenced as 2 × 150 paired-end reads and two 8 bp index reads. Data obtained from sequencing were processed with an in-house data analysis pipeline as described below. Sequence data is archived at NCBI's Sequence Read Archive (SRP129028, Bioproject PRJNA429691).

Initial quality filtering steps were like those discussed in Sengupta and Dick (2017), with exception that TrimGalore

parameters included: min length = 150. The rest of the steps outlined below were performed on the Linux terminal using Mothur v.1.33.3 (Schloss et al., 2009). The quality trimmed reads were joined using default parameters of 'make.contigs' into a single fasta file containing sample-wise merged sequences. The sequences were screened to ensure stringent quality using the following parameters: minlength = 150, maxlength = 180, maxambig = 0, and maxhomop = 10. The sequences were then split sample-wise to obtain individual sample fasta files for further downstream analysis.

2.3 | Sequence classification and operational taxonomic unit picking

The Quantitative Insights Into Microbial Ecology (QIIME) software suit (Caporaso, Kuczynski, Stombaugh, Bittinger, & Bushman, 2010) was used for sequence processing. Briefly, the pick_open_reference.py operational taxonomic unit (OTU) picking protocol in QIIME was used with 97% sequence similarity against the reference database for OTU assignment. Taxonomy was assigned to all OTUs using the RDP classifier within QIIME using the Greengenes database (DeSantis et al., 2006). Chimeric sequences were checked and filtered out using parallel_identify_chimeric_seqs.py and filter_fasta.py. The sequences were then aligned, followed by generation of phylogenetic tree using make_phylogeny.py. The sequences that failed to align were filtered out, followed by generation of a new OTU table used for downstream analysis.

2.4 | Data analysis for open reference operational taxonomic unit picking

Multiple rarefactions were performed (multiple_rarefy.py) to determine alpha-diversity at different depths followed by collating the multiple rarefactions using collate-alpha.py to give average alpha diversity metrics including number of observed phylotypes and Shannon's H′ index were calculated. The results were then imported into JMP (v11.0, SAS, Cary NC; SAS Institute, 2013) and were subjected to analyses of variance using the general linear model in JMP. All subsequent analyses were performed in R (R Core Team, 2014) using output files generated in QIIME. The OTU table containing read counts for each OTU in each sample, taxonomy information for each OTU, sample metadata, representative sequences, and representative tree were exported from QIIME, and imported into R using Phyloseq (McMurdie & Holmes, 2013). Sequences observed with very low frequency, that is OTUs representing <0.001% of the total number of sequences, were removed. Absolute OTU abundances were normalized by transforming to fractional abundance, where fraction of OTU = [OTU/sum(all OTUs)] (McMurdie & Holmes, 2013). After the normalization step, relative abundances of the OTUs at each taxonomic rank and in each sample were studied to determine community dissimilarity of samples.

To explore whether bacterial community composition clustered according to land use, non-metric multidimensional scaling (NMDS) of the Bray-Curtis dissimilarity index was used. These results were further evaluated with adonis (Permutation Multivariate Analysis of Variance using Distance Matrices) using Vegan package (Oksanen, Blanchet, Kindt, Legendre, & Minchin, 2015) in R. Relationship between community composition and categorical variables (rotation, tillage, land use) were analyzed. Raw OTU tables were grouped based on abundant rank orders and formatted for the DESeq2 package in R (Love, Anders, & Huber, 2014). Differential abundance of OTUs by sample type was determined using DESeq2, using forest (FOR) and grassland (GRA) samples (served as controls) vs. agricultural samples (NTCC, NTCS, PTCC, PTCS) as treatment.

2.5 | PICRUSt data analysis

Quality filtered sequences from Step 2.3 were used for closed reference OTU picking in QIIME. Briefly, the closed-reference OTU picking involved clustering to obtain OTUs (UCLUST) (Edgar, 2010), sequence alignment with PyNAST (Caporaso et al., 2010), removal of chimeric sequences using ChimeraSlayer (Haas et al., 2011) and taxonomy assignment using UCLUST with 99% sequence similarity to the Greengenes database. The OTUs with known Greengenes IDs from the previous step were run through the PICRUSt workflow (copy number prediction, normalization, and metagenome prediction). Detailed information on gene counts for each OTU were obtained using the "metagenome_contributions.py" script. Genes were mapped to their protein products using the KEGG pathway database. Output of the KEGG database mapping was classified into functional categories such as energy metabolism, amino acid metabolism, hydrocarbon degradation, transcription and translation, replication and repair, membrane transport, signal transduction, cell motility, etc. Predictive functional comparisons were made between the treatments followed by confidence estimation of these predictions using Nearest Sequenced Taxon Index (NSTI), which is the sum of phylogenetic distances for each organism in the OTU table to its nearest relatives with a sequenced reference genome, measured in terms of substitutions per site in the 16S rRNA gene and weighted by the frequency of that organism in the OTU table (Langille et al., 2013). Additionally, a Welch's *t*-test was conducted to evaluate the differences produced by tillage and crop rotation on the predicted functional potential

TABLE 1 Number of sequences after processing, observed operational taxonomic units (OTUs) (richness estimator), and Shannon's H′ (diversity estimator) for soil samples

Samples	Sequences after processing	Observed OTUs	Shannon's H′
NTCC	233,706	13,788	7.5
NTCS	367,053	18,145	7.5
PTCC	240,512	14,169	7.3
PTCS	180,487	11,978	7.2
GRA	364,930	15,326	7.0
FOR	275,705	13,764	7.3

Note. NTCC, no-till continuous corn; NTCS, no-till corn–soybean; PTCC, plow-till continuous corn; PTCS, plow-till corn–soybean; GRA, grassland, FOR, forest.

of the no-till vs. tilled soils. Here, "predicted functional potential" was quantified as the sum of predicted gene abundances for each enzyme (identified through its gene) in that particular sample, which included contributions from all OTUs in that sample which would have the potential to produce that enzyme. Data files detailing output of PICRUSt can be accessed at https://doi.org/10.5281/zenodo.1161547.

3 | RESULTS AND DISCUSSION

3.1 | Soil chemical properties

Chemical properties of these soils including pH and percentage organic matter is provided in detail in another study evaluating methanotrophic bacterial characteristic of the soils (Sengupta & Dick, 2017) with significant differences ($p < .005$) observed as a result of land use. Briefly, the pH of the soils was in the acidic range (4.9–5.8). The NTCC, NTCS, and GRA soils (5.3, 5.4, and 5.3, respectively) were significantly different from the forest soil (4.9) as were the PTCC and PTCS soils (5.8 and 5.6, respectively). The percentage organic matter content was higher for the non-agricultural soils, FOR = 5.7 and GRA = 5.0. Within the agricultural soils, no-till soils had higher percentage organic matter (NTCC = 4.8, NTCS = 4.1) compared with the PT soils (PTCC = 3.5, PTCS = 3.3).

3.2 | Observed richness and diversity trends

Pre-processing of sequences resulted in 1.7 million sequences from 2.1 million combined reads. A total of 30,517 OTUs were obtained with OTUs ranging from 11,978 to 18,145 per sample (Table 1). The original library size of the samples was preserved by not performing rarefactions, as has been recommended in recent studies (Debenport et al., 2015; McMurdie & Holmes, 2014) to avoid losing sparse OTUs. Rarefaction is not an ideal normalization method (Weiss et al., 2017) as it leads to losing rare OTUs, but researchers also acknowledge that alternatives to rarefying have not been sufficiently developed. The NTCC and NTCS had highest alpha diversity whereas GRA exhibited lowest Shannon diversity. Since biological replicates were pooled, the results must be treated with caution but, nonetheless, provide preliminary information on the impact of land-use and land-management practices on soil microbial diversity.

Shannon's diversity index diversity accounts for both richness (the number of members) and evenness (how evenly those members are distributed) of a community (Gosselin, 2006; Wilsey & Stirling, 2007). Thus, even though a community might have higher number of species, the diversity may be low if those species are distributed less evenly. No-till soils, irrespective of rotation, showed highest diversity among all samples in agreement with other studies as has been reported in multiple studies (Carbonetto et al., 2014; Lienhard et al., 2012; Schmidt, Gravuer, Bossange, Mitchell, & Scow, 2018) where no-till soils were found to have significantly higher diversity than tilled soils. However, NTCC showed lower richness estimates compared with NTCS. With respect to rotation as well, no-till (NTCS) had higher diversity and richness compared with plow-till (PTCS) in contrast to studies that show periodic disturbances like plowing provide dynamic habitats for soil microorganisms and therefore may be expected to exhibit higher diversity (Souza, Cantão, Vasconcelos, Nogueira, & Hungria, 2013; Szoboszlay, Dohrmann, Poeplau, Don, & Tebbe, 2017). Therefore, crop rotation with a no-till approach appeared to increase both diversity and richness of microbial communities. Grassland had the lowest diversity despite second highest observed OTUs in the dataset, which may be due to a more evenly distributed community of many members.

3.3 | Bacterial community composition and ecological significance

Overall, 80% of all reads could be assigned to phylotypes with a total of 44 bacterial phyla identified. The dominant phylum was *Proteobacteria* (~25% in NTCC, NTCS, PTCC, PTCS, and FOR) followed by *Actinobacteria* (~30% in GRA), as are commonly present in most soils (Fierer et al., 2012). The 10 most abundant phyla represented about 95% of the total distribution in each sample (Figure 1), with agricultural soils (NTCC, NTCS, PTCC, PTCS) representing a similar relative abundance distribution when compared with the grass and forest soils. For example, *Verrucomicrobia* and *Planctomycetes* were higher in abundance in FOR than the other treatments. These lignocellulolytic phyla are known to be commonly found in deciduous forest soils, and are slow-growing, serving to degrade cellulose and hemicellulosic inputs in forest soils

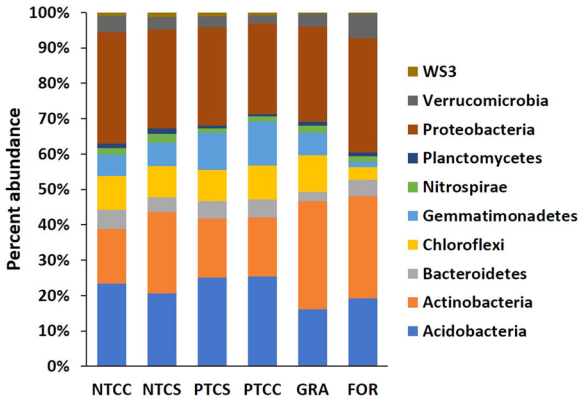

FIGURE 1 Relative percent abundance of top 10 phyla. Sample nomenclature: NTCC (no-till continuous corn), NTCS (no-till corn–soybean), PTCC (plow-till continuous corn), PTCS (plow-till corn–soybean), GRA (grassland), FOR (forest)

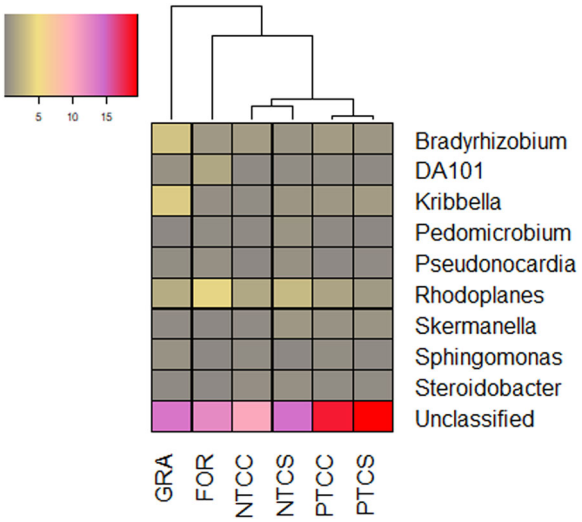

FIGURE 2 Heatmap showing relative percent abundance of top 10 genera

(López-Mondéjar, Voříšková, Větrovský, & Baldrian, 2015; Wilhelm, Singh, Eltis, & Mohn, 2019). Although *Chloroflexi* has also been identified to have cellulose-degrading capability, the phylum was lower in abundance in FOR than the other treatments where the abundance (~5%) suggested that residue inputs can drive selection of different phyla with similar function. *Acidobacteria* was lower in abundance in FOR and GRA compared with agricultural soils. This phyla is dominant in soil environments and is considered to be correlated to pH (Jones et al., 2009), in contrast to the pattern observed in our results and which reported some acidobacterial subgroups did not occur in low pH soils (Barns, Takala, & Kuske, 1999), thereby suggesting broad metabolic and physiological adaptations. *Gemmatimonadetes* abundance was highest in PT soils (~10%), followed by NT soils and GRA (~5%), and very low abundance in FOR soil (~0.5%), a pattern consistent with another study reported by DeBruyn, Nixon, Fawaz, Johnson, and Radosevich (2011)). This phyla has been suggested to be adapted to low moisture conditions (DeBruyn et al., 2011), which aligns with their prevalence in conventionally tilled soils, which are known to contain less moisture compared with other conservation tillage and naturally managed land.

It has been reported that members of *Actinobacteria* are among the most important litter decomposers in soil (Kopecky et al., 2011) as observed with relatively high percentage in GRA, FOR, and NTCS soils. *Actinobacteria* are particularly critical to soil health since they play an important role in biogeochemical cycling of nutrients, improve the availability of nutrients, enhance the production of metabolites, and promote plant growth promoters (Bhatti, Haq, & Bhat, 2017; Zhang et al., 2019). Plow-till soils evidently had low abundance of *Actinobacteria* (~10%, Figure 1), likely due to low residue-input compared with grassland and forest soils while also suggesting that within NT soils, crop rotation (and therefore variation in residue input) increases the abundance of this phylum.

In the next taxonomic level, 144 classes were identified, with an average of ~60% phylotypes represented by the 10 most abundant classes. The lower ranks included 307 orders, 477 families, and 758 genus classifications. A consistent trend observed was with agricultural soils (NTCC, NTCS, PTCC, and PTCS) representing greater percentage of sequences unclassified compared with forest and grassland soils (e.g., about 40% of reads represented in agricultural soils whereas GRA had 60% and FOR had 50% of reads represented by the 10 most abundant orders). The genus *Rhodoplanes* was higher in FOR and NTCS than others (Figure 2). This genus is known to be affiliated with plant growth-promoting bacteria (Gkarmiri et al., 2017), which likely explains their higher relative abundance in soils with diverse aboveground vegetation. The PT soils had the highest abundance of unclassified genera, which could be an outcome of diverse microhabitats introduced by plowing leading to diverse organisms with no cultured representatives. Differential abundance of genera showed that 7 out of the top 10 identified genera were significantly differentially abundant in the agricultural soils compared with forest and grass (alpha = .05) (Figure 3). These included *Steroidibacter*, *Sphingomonas*, *Skermanella*, *Rhodoplanes*, *Pseudonocardia*, *Pedomicrobium*, and DA 101. These may represent core microbial taxa for these agricultural soils, as has been suggested in studies (Hartman et al., 2018; Jiao, Xu, Zhang, Hao, & Lu, 2019; Pérez-Jaramillo et al., 2019) that show development and/or presence of core microbiota dependent on land-use and land-management practices. Interestingly, out of the total 29 that were differentially abundant, more than 20 did not feature in the 10 abundant genera, and yet recorded significantly different log2fold changes in

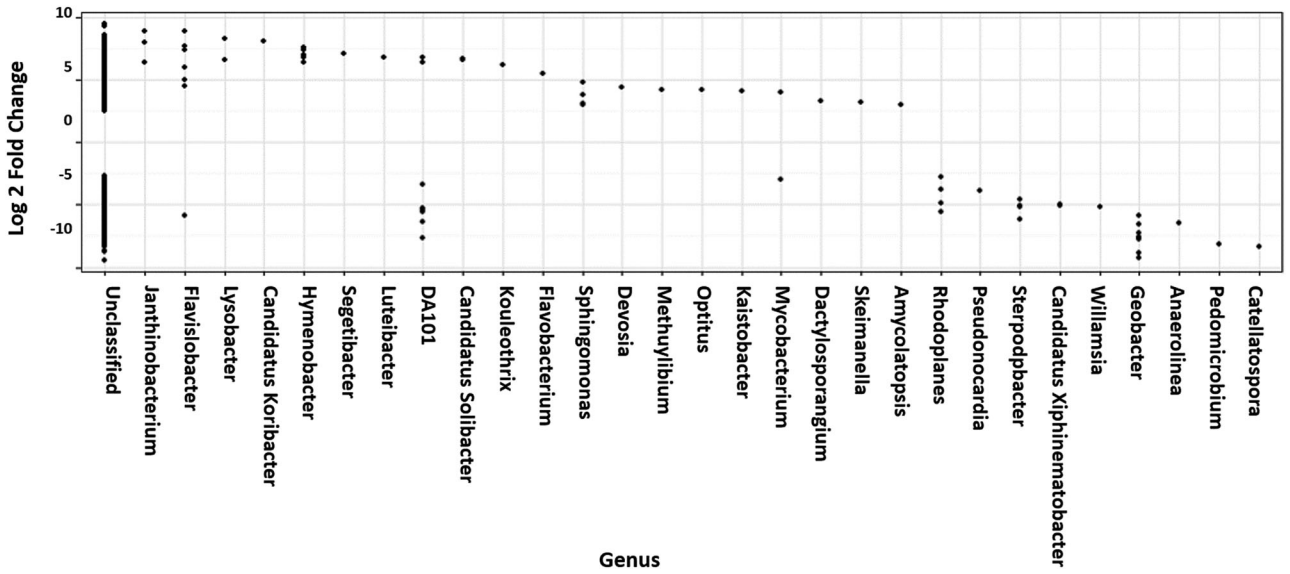

FIGURE 3 Differential abundance of genera across samples, where forest (FOR) and grassland (GRA) communities served as the control (0) against which cumulative counts of agronomic soils taxa (treatment) were evaluated to be either differentially more abundant in the treatment (positive log2fold change) or less abundant than the control (negative log2fold change)

their count abundance. Since Deseq2 uses log-normalization approach, differential abundance of low abundant genera is clearer to observe and not masked by highly abundant groups. Indeed, it may be likely that rare OTUs disproportionately impact community richness and evenness and lead to variation in community structure than abundant groups, which may be less likely to change in response to environmental changes (Karpinets et al., 2018; Skopina, Vasileva, Pershina, & Pinevich, 2016). This suggests that even at low abundances, OTUs are differentially abundant as a function of land use.

3.4 | Similarity and differences in community structure between samples

Overall similarities and differences in community structure between soil samples was visualized by calculating pairwise Bray-Curtis dissimilarity, and ordinating the matrix in two-dimensional nonmetric multidimensional scaling (NMDS) plot (Figure 4). Predominantly, samples were grouped according to the land use and land-management with GRA and FOR samples distinctly separated from agricultural soils. Plow-till soils (PTCC and PTCS) were clustered closely together. The relative abundance of phyla (Figure 1) support this observation. The abundance of phyla *Proteobacteria* (~25%), *Acidobacteria* (~20%), *Actinobacteria* and *Gemmatimonadetes* (~10%), *Chloroflexi* (~8%), and other low abundance phyla were similar in the PT soils. Although dominant phyla in NTCC were more similar in abundance to the PT soils than the NTCS sample (Figure 1), NTCC was distinctly separate in beta-diversity. This suggests that more than abundance, the evenness of the communities in NTCC may be driving the beta diversity shift. The distinct separation of GRA and FOR soils from the agriculture soils shows that these soils are highly dissimilar compared with the agricultural ones, therefore proving that long-term land-management practices strongly drive soil microbial diversity.

3.5 | Distance measure of operational taxonomic units with respect to variables

Ordinations in Figure 4 suggest that bacterial community structure differed with respect to land use and land management. The distance matrix was analyzed using Permutation Multivariate Analysis of Variance using Distance Matrices (also called as "adonis" in Vegan package in R). The Bray-Curtis measure of dissimilarity showed land use had a significant effect ($P < .05$) on the measure of distance between the communities. Within the agricultural soils, tillage was significant ($P = .001$) but not rotation ($P = .06$).

Multivariate analyses of the treatment variables, along with NMDS plots in Figure 4, therefore indicates that land use was significantly impacting community patterns composition followed by soil management in terms of tillage practices employed. Secondly, crop rotations did not appear to have a significant effect on the distribution of bacterial community. However, in the absence of replicates, the low-resolution of this study needs to be taken into consideration, especially with literature evidence showing crop rotation does impact soil microbial community

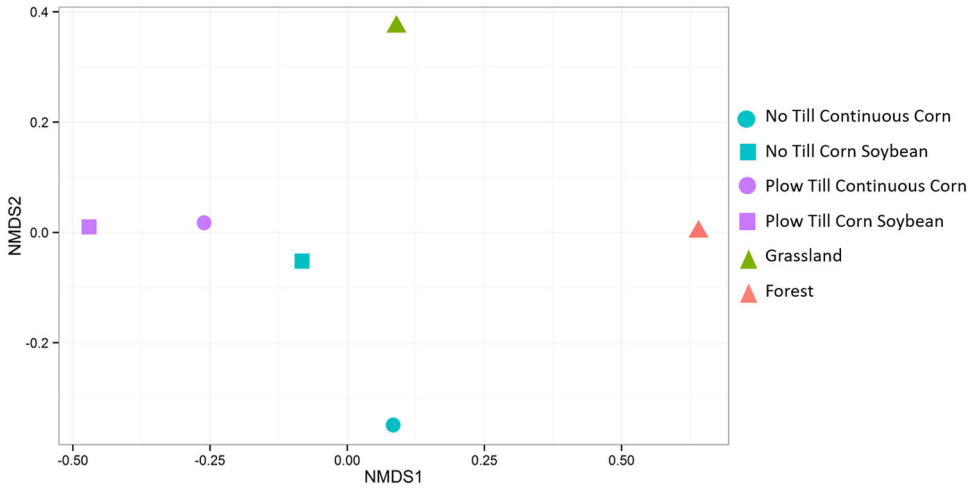

FIGURE 4 Nonmetric multidimensional scaling (NMDS) plots derived from pairwise Bray-Curtis dissimilarity measure of bacterial community composition of Hoytville soils

composition (Ashworth, DeBruyn, Allen, Radosevich, & Owens, 2017; D'Acunto, Andrade, Poggio, & Semmartin, 2018; Maarastawi, Frindte, Linnartz, & Knief, 2018). Furthermore, the long-term impact of crop rotation and tillage practices on microbial community composition may be different from short-term adaption strategies exhibited by soil microbes to the management practices, and therefore need to be monitored.

3.6 | Predictive functional profiling

Closed reference OTU picking resulted in 13,214 OTUs, which were classified into various predictive functional categories. The most abundant of these predicted functions were mapped to membrane transport, DNA repair and recombination, signal transduction, purine metabolism, translation-related protein processing, oxidative phosphorylation, and bacterial motility protein secretion (top five functions shown in Figure 5). Most of these functions are related to maintenance of cell function and structure, and therefore would be performed by every species in the community. The forest soil had highest predicted gene abundances across the top five most abundant functions, aligning with studies reporting high microbial metabolic potential in secondary forest soil (Zhang et al., 2014), followed by the NTCS, and NTCC and GRA samples (Figure 5). Both PT soils showed the lowest predicted gene abundances across all samples (overall and by individual function). These results followed the pattern soil organic matter percentages, which therefore suggests that soils with higher organic matter may have higher functional potential compared with soils with low organic matter. The predictive abundances indicate that functionally, forest soil, and crop rotation on a no-till management had highest gene abundances. Within PT,

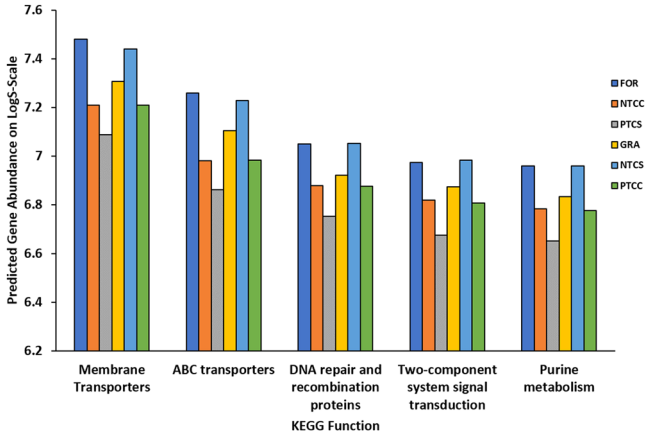

FIGURE 5 Predicted functional categories of the top five functions

continuous corn (PTCC) practice had higher predictive gene abundances than the crop rotation regime (PTCS). PICRUSt predicted NSTI (Nearest Sequenced Taxon Index) values between 0.17 and 0.20 for the soil samples, a range similar to soil test datasets in Langille et al. (Langille et al., 2013) and the NSTI values obtained in a previous study by our group (0.17–0.19) (Hariharan, Sengupta, Grewal, & Dick, 2017). The FOR soil had the lowest NSTI, whereas NTCS, PTCS, and GRA had the highest values. Welch's *t*-test revealed significant pairwise differences for C metabolism predictions ($p < .05$) between (a) GRA and three agricultural samples (NTCC, PTCS, and PTCC), (b) NTCS and the agricultural soils (NTCC, PTCS, and PTCC), and (c) between FOR and PTCS soils. Additionally, significant difference was observed for xenobiotic degradation for pairwise comparisons between PTCS and GRA, and PTCS and NTCS. Therefore, the agricultural soils primarily appeared to differ from each other functionally with respect to C metabolism.

Nitrogen, S, and methane metabolism did not show any significant differences in the predicted functions between the samples.

As noted in Hariharan et al. (Hariharan et al., 2017), a large proportion of the PICRUSt functional predictions were found to be unclassified (around 13% of the reference OTUs were not mapped to any protein/function). Given that a high percentage of OTUs remained unclassified at the Family and Genus level, which in turn were dropped from PICRUSt evaluations, it is difficult to accurately estimate bacterial functional capacity of the soils. Since PICRUSt is a predictive estimate, does not consider horizontal gene transfer, is representative of only those species whose genome have completely been sequenced, and drops unclassified sequences from the analysis, the results must be treated with caution. However, these results do provide a platform to generate new hypotheses regarding microbial functional dynamics under different land-use and land-management practices, which in turn can be tested through targeted gene abundance and expression analyses including functional gene-specific qPCR and metatranscriptomics. For example, high predicted gene abundances across the top five most abundant functions in the FOR soil may be related to diverse leaf-litter characteristics when compared with agricultural soils whereas the lowest predicted gene abundances associated with PT soils, irrespective of rotation practices, may indicate that plowing reduces soil microbial functional potential.

This study evaluates influences of long-term land-use and management practices on soil bacterial community diversity. The novelty of the study lies in the experimental set-up where soils from long-term practices (>52 yr) were accessed and sampled for bacterial community composition trends and predictive functional potential capacity. The legacy impact of long-term management practices influences strategies for long-term soil conservation and management practices, in addition to informing plant productivity and resiliency to global climate change scenarios (Hartmann, Frey, Mayer, Mäder, & Widmer, 2015; Webb, Marshall, Stringer, Reed, & Chappell, 2017) The vast body of literature on this topic suggests variability in trends associated with land-use and land-management impacts on soil microbial diversity (Szoboszlay et al., 2017), therefore suggesting a need for evaluating additional sites/location/practices, with the goal of advancing site-, time-, and intensity-specific land-use and land-management practices (Liebig et al., 2017). However, it is evident that land use history is predominant and more important than aboveground vegetation and soil properties in influencing soil microbial community composition (Jangid et al., 2011). The results from the current study add to that knowledge base; but due to lack of replication, should be treated as preliminary and used to develop informed hypotheses.

4 | CONCLUSION

Phylogenetic approaches coupled with functional potential highlighted the effect of long-term land-use and soil-management practices on microbial communities. We found that bacterial communities in agricultural soils, forest, and grass areas were diverse and impacted by long-term land use, despite the soil types being similar across all the land use practices. Differences in community composition were attributed to land use. Within the agricultural soils no-till soils showed higher diversity compared with PT soils. The results are in accordance with vast previous research that show land-use impacts soil microbial diversity. In light of the long-term experimental set-up, these results provide critical preliminary information about impact of agricultural land-management practices and seem to suggest that physical disturbance to the soil (tillage vs. no-tillage) may influence soil microbial community dynamics more than residue-type (continuous-corn vs. corn–soybean). The choice of tillage and rotation for an area often depends on the climate, type of soil, and crop (Kumar, Kadono, Lal, & Dick, 2012). Based on published research, it is widely accepted that tillage destroys the soil structure and reduces concentrations of soil organic matter when practiced long term, leading to deterioration in soil health. We found that for the soils evaluated in this study, crop rotation combined, with a no-till soil- management approach, appeared to represent the highest bacterial diversity and predictive functional capacity under long-term agricultural use. Moreover, soil microbial functional potential overall may be lost when forest lands are converted to agricultural fields. Finally, the effect of land-use history on soil microbial communities needs to be assessed both from microbial diversity and functional potential signatures and may likely be influential than aboveground vegetation and soil-properties on a long-term scale.

DATA AVAILABILITY

Microbial sequences are available in Sequence data and is archived at NCBI's Sequence Read Archive (SRP129028, Bioproject PRJNA429691). All other data are provided in the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Aditi Sengupta https://orcid.org/0000-0002-0815-4464

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