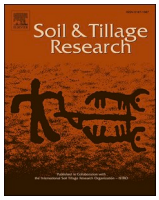
Soil & Tillage Research 236 (2024) 105920

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still



Comparative analysis of rhizobial and bacterial communities in experimental cotton fields: Impacts of conventional and conservation soil management in the Texas High Plains

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ARTICLE INFO

Keywords:
Personalized soil management
Rhizobial diversity
rpoB amplicon sequencing
Conservation agriculture
Tillage and cover crop
Machine learning model
Indigenous rhizobia

ABSTRACT

Conservative agricultural management strategies pursue long-term ecological benefits through practices such as no-tillage, cover crop, and inherent soil properties management. Farmers, however, are often hesitant to adopt such practices due to lack of experience, initial expense, and concern for low crop productivity. Overcoming this barrier requires novel approaches, such as effectively managing the soil microbiome to attain high productivity at a low cost, especially in a semi-arid region. To study the potential of conservation agriculture, we investigated components of soil bacterial community and rhizobial diversity in long-term experimental cotton fields divided into conventional tillage monoculture systems with winter fallow (CT) and no-tillage with mixed cover crop (M-NT) system on the Texas High Plain (THP). We conducted next-generation amplicon sequencing targeting *rpoB* gene with collected soil samples from different soil managements and seasons. Our research revealed that although CT had significantly greater bacterial diversity and species richness than the M-NT management, rhizobial diversity and species richness were higher in M-NT than in CT management. Both bacterial and rhizobial diversity and richness were greater in summer than in fall. The abundance of the order Rhizobiales was consistently high in M-NT than in CT fields in both seasons. Soil management altered the dominant rhizobial genus associated with cotton production systems; *Rhizobium* and *Pararhizobium* dominated M-NT management, while *Bradyrhizobium* and *Sinorhizobium* were dominant under CT management. These outcomes suggest that incorporating legumes into a cover crop in this semi-arid cotton-growing region can initiate beneficial changes to the dynamics of the indigenous rhizobial assemblage. The high prediction accuracy of our machine learning model using bacterial community data classifying the managements as CT or M-NT validates the possibilities of a strong underlying relationship between soil management and the bacterial diversity in the soil.

1. Introduction

The Texas High Plains (THP), a subregion of the Southern Great Plains, is widely known for upland cotton (*Gossypium hirsutum L.*) production, contributing $2.47 billion to the Texas economy and accounting for between 45% and 60% of the yearly Texas cotton crop (Lewis et al., 2018). However, cotton yields from year to year on THP can be extremely variable as a consequence of the high variability in precipitation frequency and amounts, increasing frequency of short-term drought, and high variability in the daily temperature within and across growing seasons (Combs, 2012; Mishra et al., 2009; Nielsen-Gammon, 2011). These climate impacts occur in conjunction with increasing groundwater depletion for irrigation and soil degradation from tillage (Hillel, 2011; Steward and Allen, 2016). Moreover, monoculture cotton production coupled with conventional tillage for several decades has resulted in poor soil health across this semi-arid region by reducing soil organic matter inputs, bulk density, and water holding capacity while increasing microbial decomposition of soil

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https://doi.org/10.1016/j.still.2023.105920
Received 31 January 2023; Received in revised form 19 August 2023; Accepted 11 October 2023
Available online 4 November 2023

organic matter stocks through disturbances resulting from tillage practices (Acosta-Martínez et al., 2004; Blanco-Canqui et al., 2009; Franzluebbers et al., 2012). Consequently, conventionally managed cotton fields are incapable of promoting and maintaining microbial processes necessary for nutrient retention, nitrogen mineralization, and building slow soil carbon and soil organic matter (Acosta-Martínez et al., 2010; Wright et al., 2008). To reverse these negative consequences requires implementing soil management practices across the region that can facilitate the development of a functionally diverse and structurally complex microbial community for addressing the stress from increasing climatic variability.

Conservation agriculture that employs minimum soil disturbance through no-tillage, crop rotation, residue retention, or planting of a seasonal or permanent cover crop has been found to ameliorate such degradation by increasing soil organic matter, soil aggregation, soil moisture, nutrient cycling, carbon storage, and biological activity (Acosta-Martínez et al., 2010; Bordovsky et al., 1994; Feng et al., 2003; Luo et al., 2020). Conservation agriculture strategies are also reported to reduce soil temperature extremes and soil susceptibility to wind and water erosion (Awe et al., 2015; Huggins and Reganold, 2008). Alternation in these physical and chemical characteristics of soil impacts the microbial populations in the soil that play critical roles in soil processes, such as nutrient capture and cycling, carbon transformation, soil structure maintenance, and participation in soil organic matter dynamics (Nannipieri et al., 2003; Kibblewhite et al., 2008).

While bacterial diversity is considered an essential metric for evaluating soil health (Castellanos et al., 2009; Shi et al., 2021; Trivedi et al., 2016), understanding how bacterial diversity contributes to soil health mechanistically is still unclear. In a recent study (Fierer et al., 2021), it is highlighted that enhanced soil bacterial diversity doesn't necessarily lead to optimal results. The study suggests that specific microbial taxa or their functional traits can act as indicators for distinct soil attributes. These indicators offer a means to track shifts in soil conditions across time, space, or due to changes in management approaches. Thus gaining a more precise comprehension of particular bacterial taxa and their diversity's connection to various soil functional processes is crucial. However, we also acknowledge that this comprehension may vary based on context.

Plant Growth Promoting Rhizobacteria (PGPR) is the specific group of bacteria that have been shown to improve plant and soil health through its contribution to biological nitrogen fixation (BNF), phytopathogen resistance, plant innate immunity, and phytohormone secretion (Ahemad and Kibret, 2014; Ahmad et al., 2006; Avis et al., 2008; Burdman et al., 2000; Kloepper, 1978; Kloepper et al., 1989; Kousar et al., 2020; Podile and Kishore, 2007). The PGPRs have also been discovered to facilitate the mineralization of organic matter in soil by releasing hydrolytic enzymes (Ollivier et al., 2011; Pii et al., 2015). They have the ability to produce various types of biosurfactants. These biosurfactants enhance the adsorption of hydrocarbons in the soil, immobilize them, and facilitate their conversion into less toxic substances through mineralization (Saeed et al., 2022).

Among the PGPR, the symbiotic relationship between rhizobia and legumes has been extensively studied because of their extraordinary capability of fixing atmospheric nitrogen into plant-available N_2 (Burris, 1994; Dixon and Wheeler, 1986). Farmers carefully consider several factors when opting to integrate legumes as cover crops or main crops. These considerations encompass financial aspects, environmental implications, and cultural factors. Subsequently, they may opt to introduce compatible rhizobia strains to enhance the symbiotic relationship with the specific legume. Conversely, in some cases, they may choose not to employ rhizobia. The selection of appropriate rhizobial inoculum is the most critical in obtaining maximum benefit from BNF. A rhizobial strain should be adopted to that environment and soil type to be considered a suitable inoculant. Moreover, the strain should be able to compete with the indigenous rhizobial pool that exists within the soil for nodule formation and subsequent BNF (Biate et al., 2014; Pohajda et al., 2016; Thies et al., 1991; Yates et al., 2011). Several studies have shown that the inoculum prepared from the indigenous rhizobial assemblage is more capable of nodulation and providing effective BNF even in semi-arid agricultural systems (Nabintu et al., 2019; Ouma et al., 2016). Therefore, prior knowledge of the indigenous rhizobial assemblages associated with crop production agriculture could facilitate efforts to boost soil health with improved yield in semi-arid regions.

To facilitate our understanding of the bacterial communities and indigenous rhizobial assemblages associated with cotton production on the THP under tillage and no-tillage stubble-managed cotton production systems, this study utilized a long-term soil management project that provided conventional tillage with winter fallow (CT), and no-tillage with mixed species cover crops (M-NT) over two growing seasons fall and Summer. Our specific goals were: (1) Evaluation of bacterial diversity as well as indigenous rhizobial assemblages of these two types of soil management practices CT and M-NT during fall (after cotton harvest in November 2019) and summer (before cotton planting in July 2020) (2) Identify indigenous rhizobia associated with these soil management practices and assess the potential for managing rhizobial diversity to enhance the benefits of legumes within a mixed species cover crop scenario when transitioning from conventionally tilled to no-tilled management and in future years (3) Determine the critical bacterial taxa that could differentiate CT and M-NT using supervised machine learning. The project employed *rpoB* amplicon sequencing instead of 16S rRNA to differentiate closely related rhizobial taxa with more resolution. Characterization of soil bacterial communities and specific groups using the 16S rRNA gene often fails to capture differences among closely related taxa. (Větrovský and Baldrian, 2013; Vos et al., 2012). The single copy *rpoB* gene that encodes the beta subunit of RNA polymerase has recently been used in several studies (Wang et al., 2021, 2018; Zhang et al., 2017) as a marker gene to analyze rhizobial diversity at the intraspecific level. In addition, the approach of analyzing bacterial community by Amplicon Sequence Variant (ASV's) before any taxonomy assignment approach instead of analyzing them with Operational Taxonomic Unit (OTUs) to species level offers better insight into the ecological significance of groups of soil bacteria (Callahan et al., 2017; Maruyama et al., 2020).

2. Materials and methods

2.1. Experimental fields

This study was conducted at the Agricultural Complex for Advanced Research and Extension Systems (AG-CARES), a cooperative experimental farm between the Texas A&M AgriLife Research and Extension Center at Lubbock and the Lamesa Cotton Growers near Lamesa, TX (32°46′22″ N, 101°56′18″ W). This area is semi-arid with an annual average temperature and precipitation of 16°C and 486 mm, respectively. The average annual temperature at the research site was 16.7, 16.4, and 17.2°C in 2018, 2019, and 2020, respectively, with observed temperatures exceeding the 30-year average by 5%, 4%, and 8%. Annual precipitation for 2018, 2019, and 2020 was 342 mm, 300 mm, and 195 mm, respectively, with September and October 2019 experiencing the highest monthly precipitation of 97 mm and 105 mm, respectively. These variations in precipitation during the growing season necessitated adjustments in irrigation. Consequently, irrigation amounts of 231.1 mm, 274.3 mm, and 289.6 mm were applied during the cotton growing seasons of 2018, 2019, and 2020, respectively, to meet crop water demand based on estimated evapotranspiration (ET) at the study site (Burke et al., 2022). The dominant soil at the site is an Amarillo fine sandy loam (fine-loamy, mixed, super active, thermic Aridic Paleustalfs) with a pH level of 7.5, a standard soil series across the THP (USDA-NRCS, 2016). Before 1998 all experimental fields were CT. In 1998, conservation tillage and fall rye (*Secale cereale* L.) cover crop was implemented in some CT fields for comparing conventional tillage soil management to no-tillage with a cover crop soil management. In 2014,

half of the no-tillage with rye cover crop management area was seeded with a mixed species cover crop, including rye, hairy vetch (*Vicia villosa Roth* L.), Diakon radish (*Raphanus sativus* L.), and winter pea (*Pisum sativum* L.) that we named M-NT management in our study. Here, we evaluated bacterial and rhizobial assemblage associated with the CT and M-NT management practices having three replications of each treatment assigned in a randomized block design with 16 rows for each replication (1239 m^2). Using a no-till drill, cover crops were sown (45 kg ha^{-1}) after harvesting cotton each year. The CT plots are tilled to a depth of 15 cm with a chisel plow after cutting cotton stalks. More information on experimental sites, fertilization, irrigation, soil, and cropping management are available (Burke et al., 2021; Lewis et al., 2018).

2.2. Soil sampling

Five soil samples were randomly collected from the three replicates of CT (conventional tillage) and M-NT (no-tillage) management. The sampling was conducted in the fall (F) of November 2019, after the cotton harvest, and in the summer (S) of July 2020, prior to cotton planting. All soil samples were collected from a 0–15 cm depth of bulk soil, placed in a 15 ml falcon tube, and stored in a cooler for transport. In the lab, they were stored at − 80˚C until DNA extraction was performed. From now on, soil samples collected from CT and M-NT during fall and summer will be called CT. F, M-NT. F, CT. S, and M-NT. S, respectively.

2.3. DNA extraction and rpoB amplicon sequencing

Total microbial DNA was extracted from 250 mg of soil using the DNeasy PowerSoil Pro Kit (Qiagen, France). DNA quality was assessed by a nanodrop spectrophotometer. A part of the *rpoB* locus was amplified using the primer pair of rpoB1479-F (50-GAT CGA RAC GCCGGA AGG-30) and rpoB1831-R (50-TGC ATG TTC GARCCC AT-30) with specified PCR conditions (Zhang et al., 2018). It is worth noting that the *rpoB* locus we used here is unable to amplify Acidobacteria.

2.4. Raw data processing and statistical analysis

Paired-end reads were generated by the Illumina MiSeq platform in RTL Genomics (Lubbock, Texas). Raw sequences obtained from RTL were demultiplexed, filtered, merged, and clustered into amplicon sequence variants (ASVs) using the pipeline of Quantitative Insights into Microbial Ecology (QIIME2, version 2020.02) (Bokulich et al., 2018). Afterward, ASVs were assigned to rhizobial species by *rpoB* reference database using classify-consensus-blast (–p-perc-identity 0.759 –p-maxaccepts 1 –p-min-consensus 0.80) which maintains 97.7% sequence similarities among different species with a DDH value 70% and an ANI value 94.3%(Wang et al., 2021). To evaluate how soil management practice shaped overall bacterial and rhizobial community structure ASV's were further assigned to Genus, Family, Order, Class, and Phylum levels, maintaining an identity of 91.7%, 85.9%, 81.8%, 78.8% and 75.9% respectively maintaining the median value obtained by sequence similarity of the *rpoB* reference database for each sampling season.

Statistical and descriptive analyses were conducted in R version 4.0.3 (2020–10–10) (R Core Team, 2020). Rank abundance plots (Zak and Willig, 2004) using ASV were created by the "abuocc" function of the labdsv package (Roberts and Roberts, 2016) to examine the overall structure of the bacterial community in response to soil management practices and season. Alpha diversity analyses of bacterial and rhizobial communities were carried out using the phyloseq package of R to calculate Observed richness, Pielou's evenness, and Shannon index (Lahti et al., 2017; McMurdie and Holmes, 2013). Alpha diversity metrics were assessed for normality and heterogeneity of variance via the function "shapiro. test" and "var. test" respectively embedded in R. Before conducting Analyses of Variance (ANOVA), the numbers of Shannon diversity for bacteria and Observed richness for rhizobia were log-transformed. Two-Way ANOVAs were conducted to examine Species richness, Evenness, and Shannon diversity of the total bacteria and rhizobia as a function of soil management practices and sampling seasons. Significant interactions or main effects were analyzed using Tukey's Honest Significant Difference (HSD) post hoc tests. The Wilcox Rank Sum Test tested differences in relative abundances of different taxa in soil management practices and sampling seasons with False Discovery Rate (FDR) (Nie et al., 2020; Reynders et al., 2020). Beta-diversity of bacteria and rhizobia was calculated by Bray-Curtis distance matrix based on the abundances of ASV and rhizobial species and then visualized using non-metric multidimensional scaling (NMDS)(Kruskal, 1964) and Principal Component Analysis (PCoA) respectively using the phyloseq ordinate () function in R (McMurdie and Holmes, 2013). The Bray-Curtis distance metric(Roberts and Roberts, 2016) measured the dissimilarity between groups (soil management practices, sampling seasons) based on the relative abundances of ASVs and rhizobial species. A permutational ANOVA (PERMANOVA) was used to see whether the overall bacterial as well as rhizobial community compositions were influenced by soil management practices or sampling seasons or their interactions using the adonis() function in the vegan package (Jari Oksanen et al., 2018). Since PERMANOVA is sensitive to group dispersion, we also performed an analysis of multivariate homogeneity (PERMDISP) after PERMANOVA using the betadisper() function in R to test if groups differed in dispersion (Anderson and Walsh, 2013).

A supervised machine learning model was utilized to predict the metadata groups (M-NT, CT) using QIIME2 to confirm the links between the applied soil management practices and subsequent bacterial communities. In our investigation, the 58 samples were divided randomly into the train and test sets, each of which had 46 and 12 samples, respectively. The model is first trained with the labeled training set of 46 samples for the machine learning workflow to recognize the underlying patterns and correlations. The training is needed to optimize the model parameters. The test set was verified using the model created with the training set to see the model's prediction accuracy. The ASVs critical for group prediction have also been identified as biomarkers(Bokulich et al., 2018; Bolyen et al., 2019). ASVs that were not identified using the *rpoB* reference database were blasted against the NCBI database and were assigned to Species, Genus, Family, Order, Class, and Phylum levels, maintaining an identity of 94.3%, 91.7%, 85.9%, 81.8%, 78.8%, and 75.9% respectively with the best-matched bacteria; also the option "Distance tree of results" was verified before assigning them in probable taxa.

3. Results

The *rpoB* amplicon sequencing yielded 2090,819 high-quality reads with a maximum frequency of 63,555, a minimum frequency of 11,163, and a mean frequency of 36,048. The total number of ASVs from 58 samples (2 samples were lost during merging forward and reverse reads) across the two crop management systems and sample dates was 19,662. Since the lowest frequency reads for a sample was 11,163 and all samples reached their plateau by that depth, samples were rarefied at an 11,000-frequency sequencing depth (Figure S1).

3.1. Rank abundance plot of bacteria

The structure of the soil bacterial communities as determined by the densities of specific ASVs for irrigated cotton production systems under CT and M-NT management during both fall and summer samples were best described by the lognormal distribution (Fig. 1). The log-normal distribution models indicate that the structure of the bacterial communities was not influenced by season or soil management practices. For each soil management approach and sampling season, the soil bacterial community was characterized by a few abundant taxa, many moderately abundant taxa, and a small group of rare species.

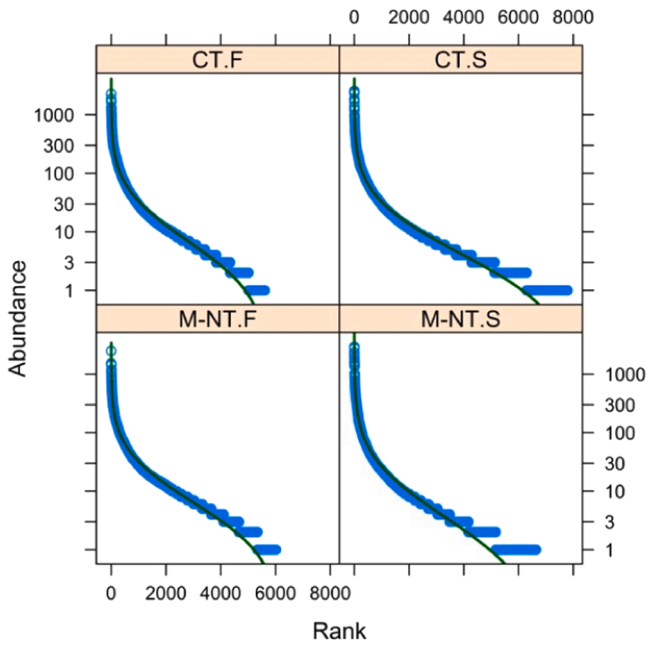

Fig. 1. Rank Abundance Plots of bacterial communities found in two different soil management practices during two growing seasons of the Texas High Plains. The X-axis represents species rank, from most abundant species to low abundant species, and the Y-axis indicates relative abundance measured in log scale. Abbreviations: CT. F = Conventional tillage with winter fallow management during fall (2019); M-NT. F = No-tillage with mixed species cover crop management during Fall (2019); CT. S = Conventional tillage with winter fallow management during Summer (2020); M-NT. S = No-tillage with mixed species cover crop management during Summer (2020).

3.2. Alpha diversity of bacteria

The two-way ANOVA results (Table S1, S2, and S3) of bacterial alpha diversity indices indicated no significant interaction effect of soil management practices and sampling seasons on the Observed richness ($p = 0.60$), the Pielou evenness ($p = 0.62$), and Shannon diversity ($p = 0.77$) for the bacterial communities associated with CT and M-NT. The main effects of soil management practices and seasons influenced the alpha diversity of bacteria. The soil management had significant effects on Observed richness ($p = 0.016$); Pielou's evenness values ($p = 8.98e\text{-}08$) and Shannon diversity indices ($p = 2.27e\text{-}05$) irrespective of sampling seasons. These three metrics of bacterial alpha diversity were greater in the CT practices than in the M-NT practice, reflecting the increased bacterial diversity in tilled systems (Fig. 2A–C). Seasons also influenced bacterial alpha diversity irrespective of soil management practices with significantly increased Observed richness ($p = 5.99e\text{-}10$) and Shannon diversity ($p = 0.008$) in summer than in fall. However, Pielou's evenness was significantly greater in fall than in summer ($p = 0.001$).

3.3. Beta diversity of bacteria

Beta diversity analysis using NMDS ordination plot based on Bray - Curtis's distance matrix demonstrated bacterial community structure across two growing seasons was clustered according to soil management practices and sampling seasons (Fig. 3A). The unique bacterial community composition in response to soil management practices and sampling seasons was supported by the PERMANOVA analysis (Table S4). In addition, the dispersion of the data was higher under the M-NT management, indicating a more varied bacterial community composition than what was observed under CT management. Moreover, the CT management exhibited less seasonal variation as compared with the M-NT fields (Fig. 3B). The analysis of homogeneity of variance for the bacterial communities in response to soil management practices and seasons indicated significant differences in the centroids (betadisper, $F = 4.7$, $p = 0.029$) on bacterial community composition.

3.4. Composition of soil microbiome

We found no differences in bacterial abundances by phylum for either soil management practices or seasons (Fig. 4A, and Table S5). The predominant class found was Alphaproteobacteria accounting for almost 80% of the total bacterial community regardless of soil management practices and seasons. A significantly higher abundance of Gammaproteobacteria was observed in the CT than in the M-NT

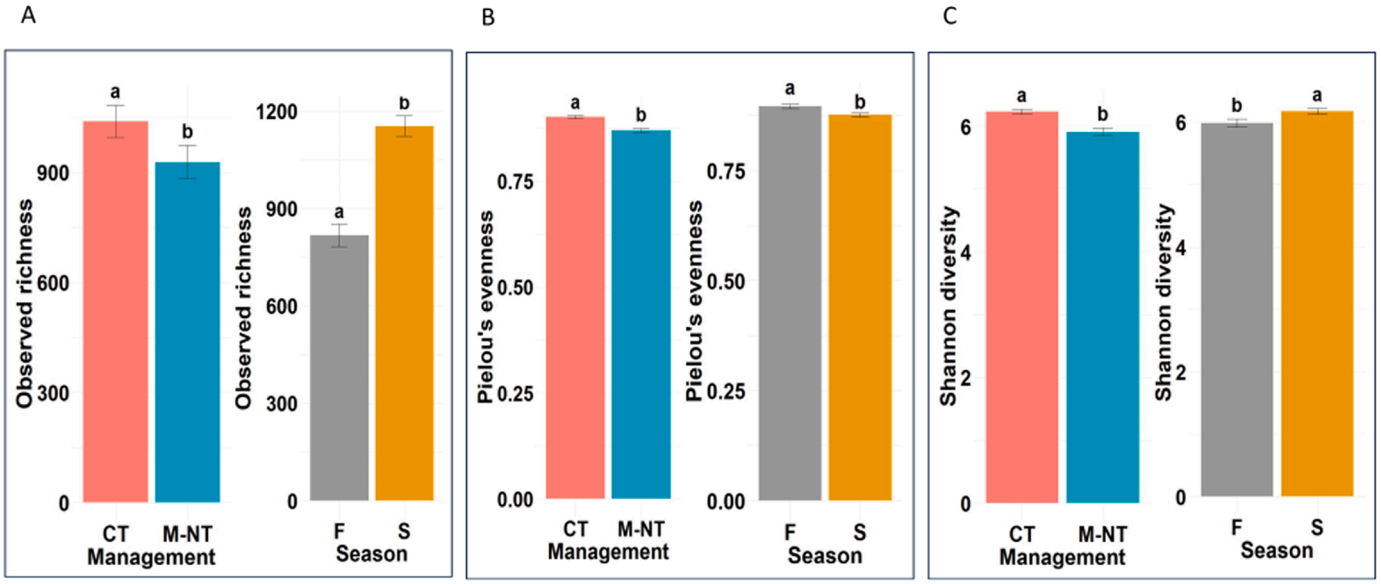

Fig. 2. Alpha-diversity indices of bacterial community found in irrigated conventional tillage with winter fallow and no-tillage with mixed species cover crop soil management practices of the Texas High Plain during fall (2019) and summer (2020) (A) Observed richness (B) Pielou's evenness (C) Shannon diversity. Diversity indices are means + / − Standard Errors. Significant differences between bar heights ($p \leq 0.05$) are indicated by different letters above bars. Abbreviations: CT = Conventional tillage with winter fallow; M-NT = No-tillage with mixed species cover crops; F = Fall, S = Summer.

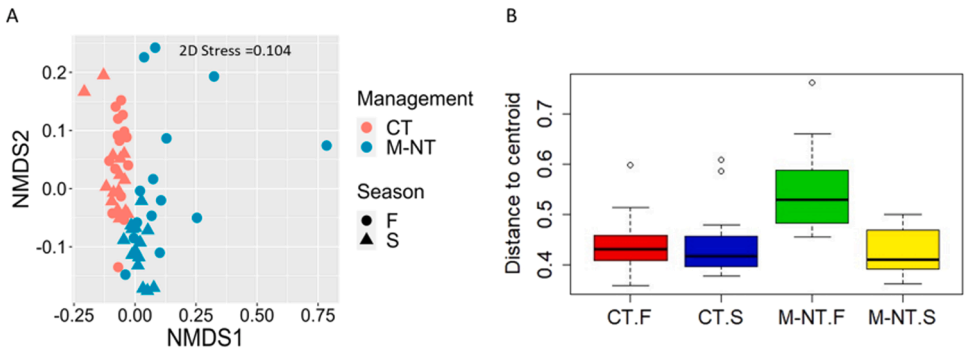

Fig. 3. Beta diversity and multivariate homogeneity of groups dispersions analysis. (A)Non-metric multidimensional scaling (NMDS) of bacterial amplicon sequence variant associated with conventional tillage with winter fallow (CT) and no-tillage with mixed species cover crop systems (M-NT) in Fall (F) and Summer (S). (B) Box plots of distances among the treatments and the centroid (heterogeneity) of each treatment. The horizontal lines inside the box plots indicate the median, and the boundaries of the box plots indicate the 25th and 75th percentiles. Abbreviations: CT. F = Conventional tillage with winter fallow management during Fall (2019); M-NT. F = No-tillage with mixed species cover crop management during Fall (2019); CT. S = Conventional tillage with winter fallow management during Summer (2020); M-NT. S = No-tillage with mixed species cover crop management during Summer (2020).

management in both fall and summer. Summer increased the abundance of Gammaproteobacteria significantly than fall in both soil management practices (Fig. 4B, and Table S5). In addition, a significant difference in the composition of the bacterial community was also observed at the Order level. The relative abundance of Rhizobiales was highest in the M-NT management for both summer and fall when compared to the CT management (Fig. 4C, and Table S5). Soil management practices and sampling seasons also resulted in changes in the relative abundance of certain families. We found a higher abundance of the family Rhizobiaceae in M-NT compared to CT management in both fall and summer; the relative abundance of Bradyrhizobiaceae was higher in CT than in M-NT management during summer (Fig. 4D, and Table S5). The relative abundances of dominant genera *Pararhizobium*, *Sinorhizobium*, *Rhizobium* were significantly different in response to soil management practices (Fig. 4E, and Table S6). Rhizobial species *Pararhizobium giardinii* increased 138% in M-NT compared to CT and 98% in M-NT compared to CT management during fall and summer, respectively. However, rhizobial species *Sinorhizobium meliloti* decreased by 79% in M-NT to CT and 87% in M-NT to CT during fall and summer, respectively (Fig. 4F, and Table S6).

3.5. Principal component analysis of bacterial class and family

A principal component analysis (PCA) exploring the relationship between soil management practices and bacterial class indicated that each management shifted slightly from the other (CT, M-NT) along the PC1 and PC2 axes. Class Gammaproteobacteria showed to be positively associated with CT management (Fig. 5A). Similar separation was also observed at the family level on soil management practices (CT, M-NT). The family Bradyrhizobiaceae and Hypomicrobeaceae are strongly associated with CT management, whereas family Rhizobiaceae is associated with M-NT management (Fig. 5B).

3.6. Alpha diversity of rhizobial assemblages

A two-way ANOVA (Table S7, S8 and S9) on alpha diversity matrices of rhizobial assemblages indicated no significant interaction effect of soil management practices and sampling seasons on Observed richness ($p = 0.09$) and Pielou's evenness ($p = 0.05$). However, a significant interaction effect has been observed on Shannon diversity ($p = 0.03$). The observed richness of rhizobia was affected by soil management practices ($p = 0.0001$) and sampling seasons ($p = 0.02$) separately; M-NT fields had increased rhizobial Observed species richness than CT management. Also, summer harbored more rhizobia than fall. A similar significant effect of soil management practices ($p = 0.009$) and sampling seasons ($p = 0.02$) separately was found on Shannon diversity of rhizobial assemblage. The M-NT management during summer had the greatest Shannon diversity than other management and season. The evenness of rhizobial assemblages showed no differences based on soil management practice ($p = 0.79$) and sampling seasons ($p = 0.29$) (Fig. 6A–C)).

3.7. Beta diversity of rhizobial assemblages

The analysis of β-diversity demonstrated that the composition of the rhizobial assemblage is highly dependent on soil management practices than on sample seasons. Each management had unique rhizobial assemblages (Fig. 7A). The PERMANOVA analysis also indicated that soil management was the main factor responsible for rhizobial community composition, explaining 26% of rhizobial community variance, while sampling season only accounted for 4% of the rhizobial community variance (Table S10). No difference in the centroids (levels of dispersion) of the group was found (Fig. 7B).

3.8. Machine learning model for sample prediction

The model created by supervised machine learning technique using the *rpoB* amplicon data exhibited a high prediction accuracy for sample prediction (Fig. 8A); overall model accuracy was approximately 95%the with some fluctuations (Fig. 8B). We found 30 ASVs that are differentially expressed in response to soil management practices associated with a long-term cotton production system in semi-arid THP. Among them, only *Pararhizobium giardinii* and*Rhizobium etli* were identified to species level (Fig. 8C). Additional critical ASVs were identified to the best plausible taxa for the diagnosis of soil management (Table S11).

4. Discussion

The rank abundance plots offer a unified perception of the underlying ecological rules governing microbial diversity and abundance in a microbial ecology community (McGill et al., 2007; Prosser et al., 2007). However, because of the extensive complexity of these bacterial communities, accompanied by the inability to culture the vast majority of bacteria, the work necessary to obtain rank abundance plots is challenging (Curtis et al., 2006; Gans et al., 2005). Although 16S rRNA genes are widely used to characterize the structure and composition of microbial communities, having multiple copies often results in over-estimation of certain bacterial taxa, which lowers the ability to effectively enumerate bacterial community using bacterial species abundance distributions (Doroghazi and Buckley, 2008; Schloss and Handelsman, 2006; Sloan et al., 2007). Using *rpoB* locus and enumerating the ASVs to create rank abundance plots reduced this inherent bias

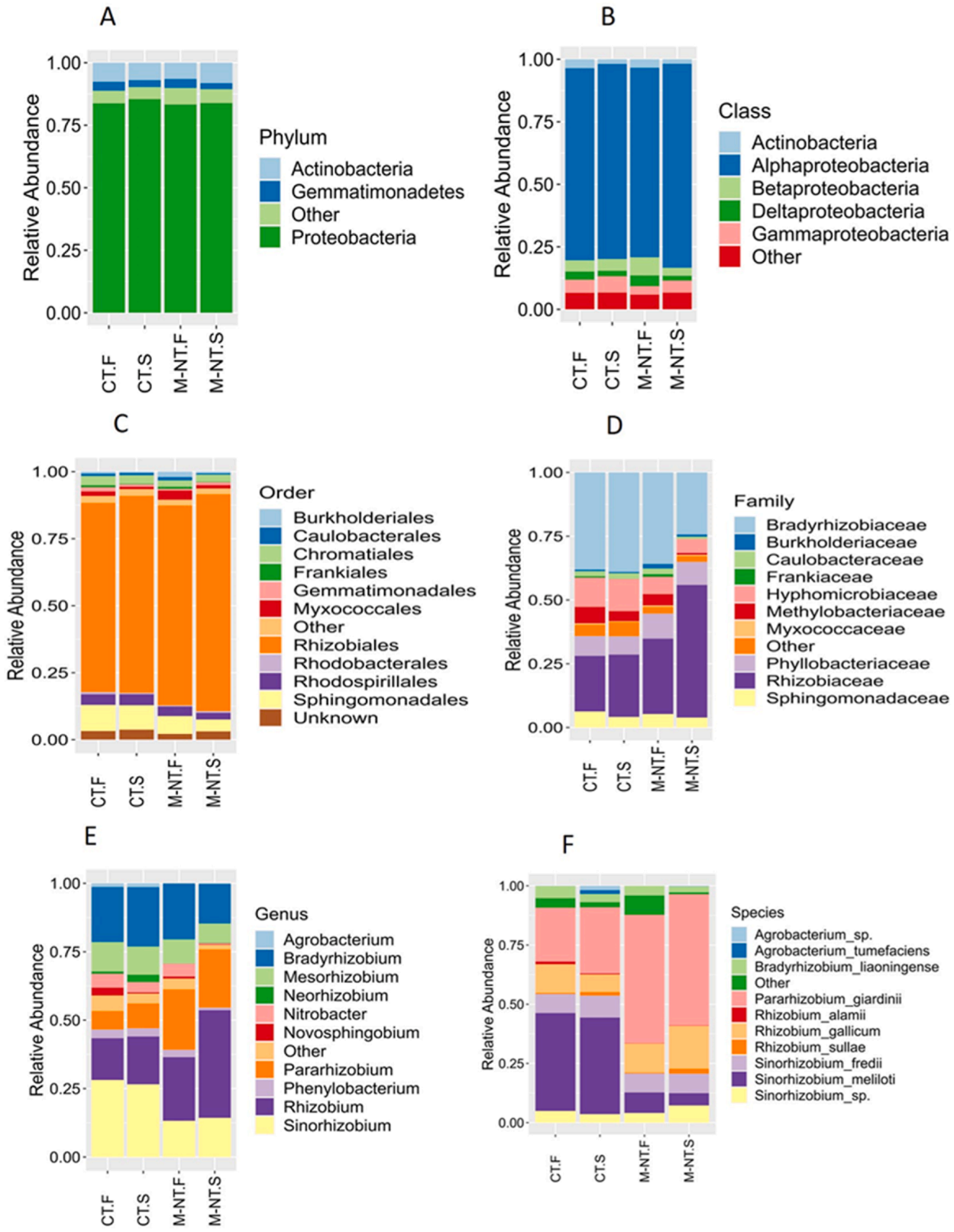

Fig. 4. Relative abundance of most abundant bacterial taxa associated with two soil management practices for irrigated cotton production in the Texas High Plains during two growing seasons. (A) Top 3 phyla (B) Top 5 classes (C) Top 10 families (D) Top 10 orders (E) Top 10 genera (F) Top 10 species. Abbreviations: CT. F = Conventional tillage with winter fallow management during fall (2019); M-NT. F = No-tillage with mixed species cover Crops management during Fall (2019); CT. S = Conventional tillage with winter fallow management during Summer (2020); M-NT. S = No-tillage with mixed species cover crop management during Summer (2020).

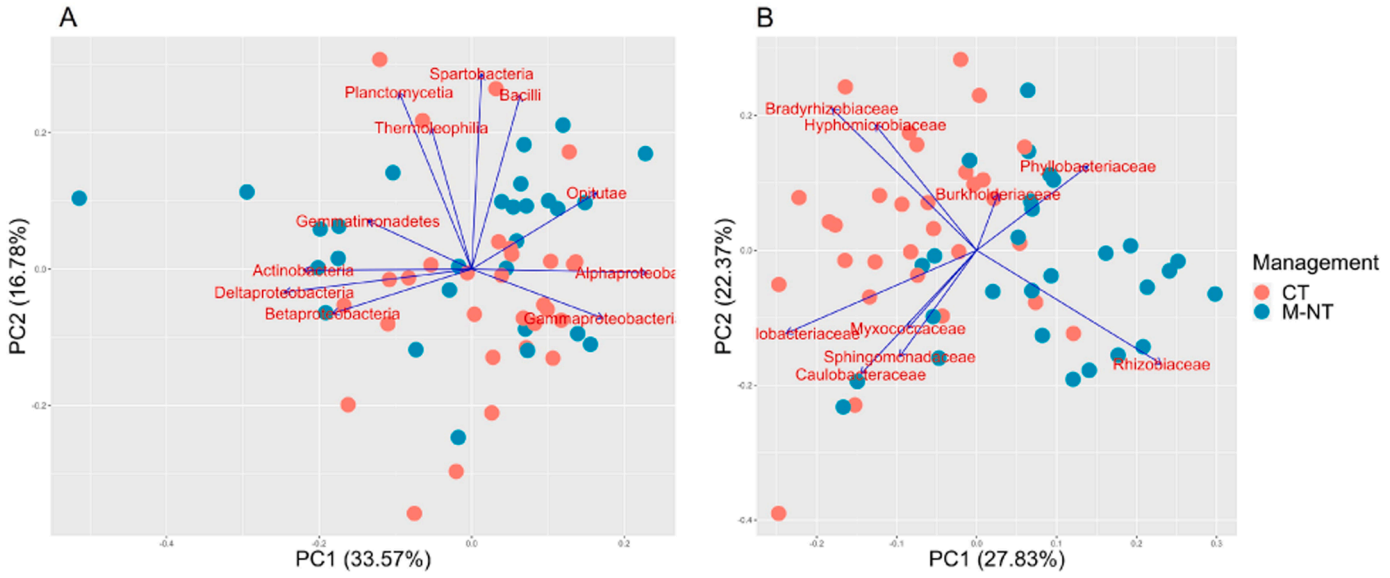

Fig. 5. Principal component analysis (PCA) of soil bacteria at the (A) Class (B) Genus level found in two different soil management practices of semi-arid Texas High Plain. Abbreviations: CT = Conventional tillage with winter fallow; M-NT = No-tillage with mixed species cover crop.

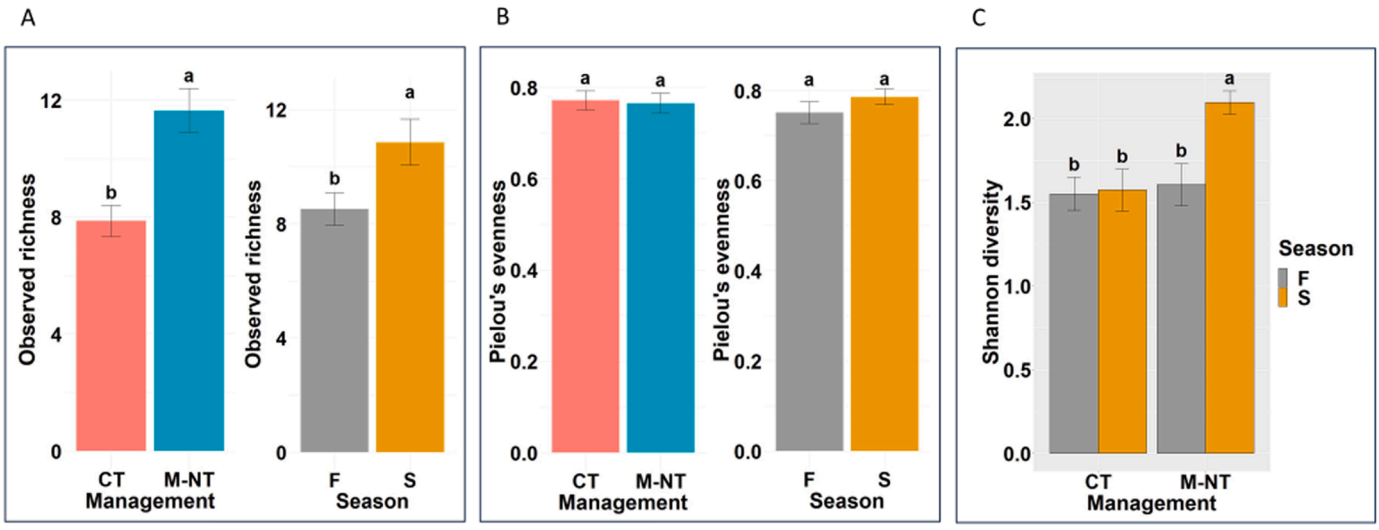

Fig. 6. Alpha-diversity indices reflecting rhizobial diversity found in conventional tillage with winter fallow and no-tillage with mixed species cover crop practice during fall and summer (A) Observed richness, (B) Pielou evenness, and (C) Shannon diversity. The averages were compared by ANOVA and Tukey test ($p \leq 0.05$) and indicated by the small letters. Abbreviations: CT = Conventional tillage with winter fallow; M-NT = No-tillage with mixed species cover crop, F = Fall, S = Summer.

in quantifying bacterial community composition and structure. Our results indicate that regardless of soil management practices and sampling seasons for irrigated cotton in semi-arid west Texas, the bacterial communities are best described by a lognormal distribution, which agrees with other previous research stating soil bacterial community generally fits in the lognormal model (Doroghazi and Buckley, 2008; Dunbar et al., 2002). The existing bacterial communities in these agricultural soils are, hence, deemed to be more influenced by other processes from environmental factors such as climate, soil type, soil moisture, temperature etc. than by factors of soil management practices or sampling season.

The impacts of tillage on the diversity of soil bacterial communities have been variable, with studies reporting a positive response (Degrune et al., 2016; Smith et al., 2016a), a negative response (Dorr de Quadros et al., 2012; Schmidt et al., 2018), and no response (Schmidt et al., 2022; Wang et al., 2020). Our data demonstrated a decreased richness, evenness, and diversity of bacteria in soils under M-NT when compared with the CT management. The observed decline in bacterial diversity under M-NT management could be attributed to the potential soil compaction resulting from the absence of tillage practices, especially in semi-arid regions, as found in some previous studies (Blanco-Canqui and Ruis, 2018; Peixoto et al., 2019; Catania et al., 2018). Such high compaction conditions may have adverse effects on bacterial diversity, given that many bacterial species thrive and flourish in oxygen-rich environments (Schnurr-Pütz et al., 2006), which are hindered under compacted soil conditions. On the other hand, tillage might have fostered bacterial diversity by speeding up oxygen diffusion and more energy sources available for bacterial growth (Hartmann et al., 2014; Pastorelli et al., 2009; Zhang et al., 2018). Additionally, our observations align with previous studies that have noted higher bacterial diversity during summer than in fall (Pastorelli et al., 2009, 2013). The warm conditions and adequate moisture from irrigation were likely to facilitate the greater bacterial diversity in West Texas during summer than in fall.

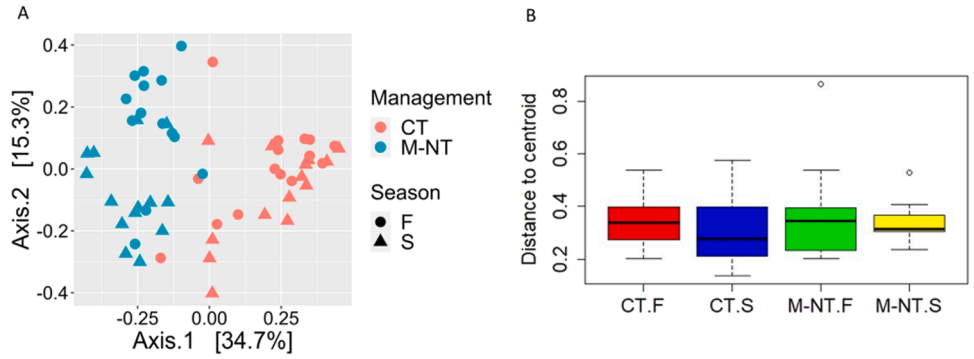

Fig. 7. Beta diversity and homogeneity of group dispersion analysis of rhizobial assemblages (A) Principal co-ordinate analysis (PCoA) plot showing differences in the composition of rhizobial assemblages (using Bray Curtis distance matrix) at two different soil management practices comprising conventional tillage with winter fallow (CT) and no-tillage with mixed species cover crop (M-NT) during fall(F) and summer(S) (B) Box plots of distances among the groups (CT.F, CT.S, M-NT.F, M-NT.S) and the centroid (heterogeneity) of each group. The horizontal lines inside the box plots indicate the median and the boundaries of the box plots indicate the 25th and 75th percentiles. Abbreviations: CT. F = Conventional tillage with winter fallow management during fall (2019); M-NT. F = No-tillage with mixed species cover Crops management during Fall(2019); CT. S = Conventional tillage with winter fallow management during Summer (2020); M-NT. S = No-tillage with mixed species cover Crop management during Summer (2020).

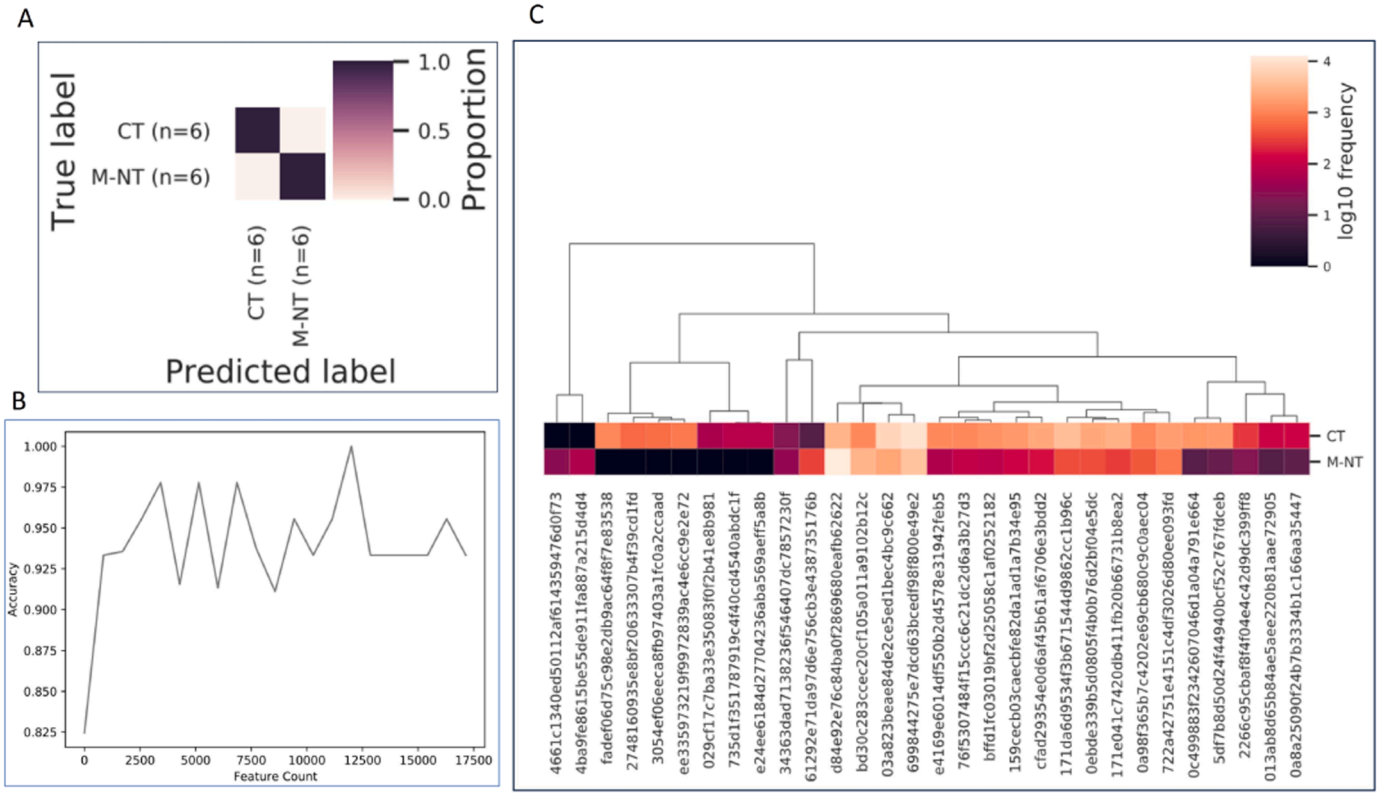

Fig. 8. Soil sample prediction by supervised machine learning model. A. "Confusion matrix" indicating the accuracy of result when generated by obtained ASVs, row labels indicating the true class, column labels indicating the predicted class and proportion compares the color key B. A line graph representing the relationship between the number of different features and the prediction accuracy. C. Heat map of important features showing ASVs that are critical for sample prediction based upon phylogeny. Abbreviations: CT = Conventional tillage with winter fallow; M-NT = No-tillage with mixed species cover crop.

Consistent with earlier research, our findings highlight the significant influence of soil management practices on bacterial β-diversity (Carbonetto et al., 2014; Degrune et al., 2017, 2016; Schmidt et al., 2018). However, bacterial group alterations do not uniformly align across studies. For example, in our cotton production systems, we noticed no significant variation at the phylum level in response to soil management practices. In contrast, (Carbonetto et al., 2014) discovered a greater relative abundance of Actinobacteria in a no-tillage system during their investigation of a 34-year-old experimental field at Balcarce in the Southern Pampas region. Conversely, (Degrune et al., 2017) found a higher relative abundance of Actinobacteria in a conventional tillage system compared to a reduced tillage system. In our investigation, we observed higher levels of class Gammaproteobacteria and family Bradyrhizobiaceae abundance in CT fields compared to M-NT. Another study on the same cotton fields indicated that CT led to greater cotton yield than M-NT (Lewis et al., 2018). Interestingly, this observation complies with recent findings from a Metagenome-Wide Association Study (MWAS) that suggested a positive connection between elevated productivity and increased levels of class Gammaproteobacteria and family Bradyrhizobiaceae in the soil (Chang et al., 2017). Our study

further substantiates prior discoveries indicating that the Rhizobiales order is more abundant in no-tillage practices when compared to conventional tillage methods (Smith et al., 2016b; Souza et al., 2013. This order encompasses numerous beneficial bacteria, including those involved in legume nodulation, nitrogen fixation, methanotrophy, and microsymbiosis, which contribute to plant growth by supplying hormones, nutrients, and precursors for vital plant metabolites (Delmotte et al., 2009; Garrity et al., 2004; Ivanova et al., 2000; Verginer et al., 2010). The differences in the relative abundance of *Pararhizobium*, *Sinorhizobium*, and *Rhizobium* under contrasting soil management practices CT and M-NT, have not been definitively elucidated, and no studies to date have demonstrated their specific alternation or response to management practices. Therefore, further investigations are required to understand the underlying drivers behind these observed differences comprehensively. Nevertheless, it is plausible that the shift in taxa dominance between CT and M-NT could be associated with alterations in the physical and chemical conditions resulting from tillage and the use of the cover crop. Our study revealed that M-NT management supported a more heterogenous bacterial community in the fall. The availability of diverse and abundant substrates in the soil through mixed cover crops and uninterrupted management practice of no-tillage may provide heterogeneous habitat niches occupied by diverse bacterial communities. The decreased heterogeneity of CT management could be a sign of biotic homogenization, a process that increases the similarity of community composition (Olden et al., 2004; Montecchia et al., 2015). Agricultural practices that include monoculture, intensive use of agrochemicals, and heavy fertilization, result in lower ecological niches diversity with a subsequent disturbance that results in a homogeneous microbial community and their functional gene pool, lowering ecosystem services (Constancias et al., 2014; Figuerola et al., 2015; Lupatini et al., 2017; Olden et al., 2004).

In the current study, the fall cover crop used in the M-NT field included two legumes, hairy vetch, and winter pea, into the mixed species cover crop planted each year following the cotton harvest in November. However, Our study did not find the appropriate rhizobia required to nodulate these two legumes (Figure S2). To get the maximum benefit of legume inclusion, we should develop a targeted soil management approach to maximize the rhizobial benefit either by changing the choice of legumes in the cover crop that would utilize the indigenous rhizobia or by inoculating the compatible rhizobia for the chosen legume. Inoculation of legumes with compatible rhizobial strains will not only increase nitrogen addition to the soil through BNF but can also assist in increasing soil health (Padilla and Pugnaire, 2006; Pérez-Fernández et al., 2016; Rodríguez-Echeverría et al., 2003; Thrall et al., 2011). Determining the rhizobial diversity conveys better insight into the functionality of soil. Understanding how tillage systems influence the beneficial soil microbial community will facilitate the development of more productive and sustainable systems. Our result confirms M-NT increased rhizobial diversity. Other previous studies also indicated that rhizobial assemblages in agroecosystems could be impacted by crop management (Depret et al., 2004, tillage (Ferreira et al., 2000; Kaschuk et al., 2006; Souza et al., 1997, 2013), and legume cultivation history (Andrade et al., 2002; Parker, 1999). In addition, rhizobia are very stable in soil; even in the absence of a host plant, they survive for a long period (Silva Batista et al., 2007; Vargas et al., 2017). It has been reported that Bradyrhizobia stayed in a field for 30 years in the absence of host plants and was even able to nodulate soybean (Domit et al., 1990)subsequently. Therefore, incorporating appropriate legumes to facilitate the growth of indigenous rhizobia as a part of the cover crop or crop rotation holds great potential in terms of maintaining beneficial bacteria.

Rhizobia has also been found to play a crucial role in promoting non-legume growth and improving crop quality through various mechanisms, such as phosphorus solubilization and the production of siderophore, IAA, cytokinin, gibberellin, and ethylene. Numerous studies have demonstrated the positive effects of Rhizobium on plant growth, height, and yield in diverse crops such as rice, corn, maize, and spinach (García-Fraile et al., 2012; Fahde et al., 2023; Gutiérrez-Zamora and Martınez-Romero, 2001; Jiménez-Gómez et al., 2018). Thus, the rhizobia strains identified in our study exhibit promising potential as biofertilizers in this region, particularly due to their better adaptability. However, it is important to acknowledge that further experiments and field trials are necessary to validate their efficacy in practical applications. While personalized biofertilizers in agriculture are a relatively novel concept, the utilization of personalized probiotics in animal health has already been explored and experimented with (Mishra et al., 2022; Celiberto et al., 2018). Recent research indicates that personalized probiotics might offer advantages over conventional probiotics when treating dysbiosis-related conditions, likely owing to their direct derivation from the host's own microbiota (Abid and Koh, 2019). Therefore, promoting the endemic PGPRs hold promise for enhancing soil health and crop productivity while decreasing fertilizer application in a sustainable way.

In recent times, the use of microbiome data from agricultural fields in supervised machine learning algorithms is becoming an increasingly effective method for gaining a deeper understanding of the important microbial taxa present in various soil management practices (De Souza et al., 2020; Egenriether, 2021; Wilhelm et al., 2022). An MWAS study found that the bulk soil microbiome is the main factor in crop productivity in agricultural soil, rather than other soil characteristics such as location, composition, and pH. No correlation was found between crop productivity and these factors. Productivity in that area was accurately predicted (75%) using a machine learning model incorporating microbiome data (Chang et al., 2017). One aspect of our approach for undertaking this study was to test if machine learning in association with whole bacterial community analysis could allow producers and consultants to obtain real-time data for visualizing the bacterial and rhizobial community before and after conversion and to determine where on a soil health continuum that field was located compared to other fields in the region. Using the *rpoB* marker gene instead of using the traditional 16 s rRNA gene approach gave us better resolution at the species level of rhizobia, but the limitation of the reference database and lack of information about all bacterial genome sequences prevented us from categorizing several identified ASVs to the species level, which is necessary for establishing important biomarker for soil health management. However, the growing number of studies involving soil microbiomes and the advancement of bioinformatic tools would create more opportunities to identify those bacteria and would enable us to understand their impact on soil health and crop yield better in the future. The high prediction accuracy of our machine learning model identifying farming strategy indicates bacterial communities are indeed shaped by soil management practices- and can be used as a low-cost tool for monitoring sustainable agriculture in this region as in other agroecosystems (Matteoli et al., 2022).

5. Conclusion

In semi-arid agroecosystems, such as the Texas High Plains of the United States, no-till approaches to soil management along with winter and or summer cover crops are now considered critical management tools necessary for increasing soil organic matter levels, increasing rainfall infiltration, decreasing erosion, while improving overall soil health and addressing increasing climate variability. A major question for producers as they implement these management tools concerns the impacts of these approaches on specific components of the soil microbiome that are recognized as critical for plant growth and the ability to maintain and improve on positive changes. Specifically, to what degree can we tailor soil microbiome components through soil management practices under semiarid growing conditions? This study provides an initial assessment of the feasibility of personalized soil microbiome management using targeted primers for assessing specific taxon biodiversity in response to soil management and growing season climatic

impacts. Our results showed that long-term use of cover crops and no-till alters rhizobial taxa's biodiversity and species richness compared to traditional tillage approaches to cotton production. The increased species richness and shift in rhizobial genera and species composition under no-till can provide the microbial information required to understand and potentially tailor changes in soil microbiome diversity and composition to specific cropping system production outcomes. This study lays the groundwork for future research to investigate the integration of indigenous rhizobia into crop management practices, aiming to foster more sustainable agriculture. Given the rapid decrease in the cost of microbiome sequencing with associated data analysis, these molecular tools can provide producers the ability to assess their soil microbiome response to management practices and track long-term soil health trajectories.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The raw data presented in this study have been made available to the public through their deposition in the Sequence Read Archive database under study accession number PRJNA1033680.

Acknowledgement

We acknowledge the support of Cotton Inc. and South Central Climate Adaptation Science Center for funding the project.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2023.105920.

References

Abid, M.B., Koh, C.J., 2019. Probiotics in health and disease: fooling mother nature? Infection 47, 911–917.
Acosta-Martínez, V., Zobeck, T.M., Allen, V., 2004. Soil microbial, chemical and physical properties in continuous cotton and integrated crop-livestock systems. Soil Sci. Soc. Am. J. 68, 1875–1884.
Acosta-Martínez, V., Burow, G., Zobeck, T.M., Allen, V.G., 2010. Soil microbial communities and function in alternative systems to continuous cotton. Soil Sci. Soc. Am. J. 74, 1181–1192.
Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud. Univ. - Sci. 26, 1–20.
Ahmad, F., Ahmad, I., Aqil, F., Ahmed Wani, A., Sousche, Y.S., 2006. Plant growth promoting potential of free-living diazotrophs and other rhizobacteria isolated from northern indian soil. Biotechnol. J.: Healthc. Nutr. Technol. 1, 1112–1123.
Anderson, M.J., Walsh, D.C.I., 2013. Permanova, anosim, and the mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol. Monogr. 83, 557–574.
Andrade, D., Murphy, P., Giller, K., 2002. Effects of liming and legume/cereal cropping on populations of indigenous rhizobia in an acid brazilian oxisol. Soil Biol. Biochem. 34, 477–485.
Avis, T.J., Gravel, V., Antoun, H., Tweddell, R.J., 2008. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol. Biochem. 40, 1733–1740.
Awe, G.O., Reichert, J.M., Wendroth, O.O., 2015. Temporal variability and covariance structures of soil temperature in a sugarcane field under different management practices in southern brazil. Soil Tillage Res. 150, 93–106.
Biate, D.L., Kumar, L.V., Ramadoss, D., Kumari, A., Naik, S., Reddy, K.K., Annapurna, K., 2014. Genetic diversity of soybean root nodulating bacteria. Bact. Divers. Sustain. Agric. 131–145.
Blanco-Canqui, H., Ruis, S.J., 2018. No-tillage and soil physical environment. Geoderma 326, 164–200.
Blanco-Canqui, H., Stone, L., Schlegel, A.J., Lyon, D., Vigil, M., Mikha, M., Stahlman, P., Rice, C., 2009. No-till induced increase in organic carbon reduces maximum bulk density of soils. Soil Sci. Soc. Am. J. 73, 1871–1879.
Bokulich, N.A., Dillon, M., Bolyen, E., Kaehler, B.D., Huttley, G.A., Caporaso, J.G., 2018. q2-sample-classifier: machine-learning tools for microbiome classification and regression. bioRxiv, 306167.
Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., DaSilva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., vonHippel, M., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. Nat. Biotechnol. 37, 852–857.
Bordovsky, J.P., Lyle, W.M., Keeling, J.W., 1994. Crop rotation and tillage effects on soil water and cotton yield. Agron. J. 86, 1–6.
Burdman, S., Jurkevitch, E., Okon, Y., 2000. Recent advances in the use of plant growth promoting rhizobacteria (pgpr) in agriculture. Microb. Interact. Agric. For. (Vol. II) 229–250.
Burke, J.A., Lewis, K.L., Ritchie, G.L., DeLaune, P.B., Keeling, J.W., Acosta-Martinez, V., Moore, J.M., McLendon, T., 2021. Net positive soil water content following cover crops with no tillage in irrigated semi-arid cotton production. Soil Tillage Res. 208, 104869.
Burke, J.A., Lewis, K.L., DeLaune, P.B., Cobos, C.J., Keeling, J.W., 2022. Soil water dynamics and cotton production following cover crop use in a semi-arid ecoregion. Agronomy 12, 1306.
Burris, R.H., 1994. Biological nitrogen fixation—past and future. Nitrogen fixation with nonlegumes. The American University in Cairo Press,, Cairo, Egypt, pp. 1–11.
Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639.
Carbonetto, B., Rascovan, N., Álvarez, R., Mentaberry, A., Vázquez, M.P., 2014. Structure, composition and metagenomic profile of soil microbiomes associated to agricultural land use and tillage systems in argentine pampas. PLoS One 9, e99949.
Castellanos, T., Dohrmann, A.B., Imfeld, G., Baumgarte, S., Tebbe, C.C., 2009. Search of environmental descriptors to explain the variability of the bacterial diversity from maize rhizospheres across a regional scale. Eur. J. Soil Biol. 45, 383–393.
Catania, P., Badalucco, L., Laudicina, V.A., Vallone, M., 2018. Effects of tilling methods on soil penetration resistance, organic carbon and water stable aggregates in a vineyard of semiarid mediterranean environment. Environ. Earth Sci. 77, 1–9.
Celiberto, L.S., Pinto, R.A., Rossi, E.A., Vallance, B.A., Cavallini, D.C., 2018. Isolation and characterization of potentially probiotic bacterial strains from mice: proof of concept for personalized probiotics. Nutrients 10, 1684.
Chang, H.-X., Haudenshield, J.S., Bowen, C.R., Hartman, G.L., 2017. Metagenome-wide association study and machine learning prediction of bulk soil microbiome and crop productivity. Front. Microbiol. 8.
Combs, S. (2012). The impact of the 2011 drought and beyond.Texas Comptroller of Public Accounts Special Rep., Publ, 96, 16.
Constancias, F., Prévost-Bouré, N.C., Terrat, S., Aussems, S., Nowak, V., Guillemin, J.-P., Bonnotte, A., Biju-Duval, L., Navel, A., Martins, J.M., 2014. Microscale evidence for a high decrease of soil bacterial density and diversity by cropping. Agron. Sustain. Dev. 34, 831–840.
Curtis, T.P., Head, I.M., Lunn, M., Woodcock, S., Schloss, P.D., Sloan, W.T., 2006. What is the extent of prokaryotic diversity? Philos. Trans. R. Soc. B: Biol. Sci. 361, 2023–2037.
De Souza, R.S.C., Armanhi, J.S.L., Arruda, P., 2020. From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. Front. Plant Sci. 11, 1179.
Degrune, F., Theodorakopoulos, N., Dufrêne, M., Colinet, G., Bodson, B., Hiel, M.-P., Taminiau, B., Nezer, C., Daube, G., Vandenbol, M., 2016. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (belgium). Agric. Ecosyst. Environ. 224, 12–21.
Degrune, F., Theodorakopoulos, N., Colinet, G., Hiel, M., Bodson, B., Taminiau, B., Daube, G., Vandenbol, M., Hartmann, M., 2017. Temporal dynamics of soil microbial communities below the seedbed under two contrasting tillage regimes. Front. Microbiol 8, 1127.
Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., vonMering, C., Vorholt, J.A., 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc. Natl. Acad. Sci. USA 106, 16428–16433.
Depret, G., Houot, S., Allard, M.-R., Breuil, M.-C., Nouaïm, R., Laguerre, G., 2004. Long-term effects of crop management on rhizobium leguminosarum biovar viciae populations. FEMS Microbiol. Ecol. 51, 87–97.
Dixon, R., Wheeler, C.T., 1986. Nitrogen Fixation in Plants. Blackie and Son, GLASGOW (UK), p. 1986.
Domit, L., Costa, J., Vidor, C., Pereira, J., 1990. Inoculation of cereal seeds with bradyrhizobium japonicum and its effect on soyabeans grown in succession. Rev. Bras. De. Ciência do Solo 14, 313–319.
Doroghazi, J.R., Buckley, D.H., 2008. Evidence from gc-trflp that bacterial communities in soil are lognormally distributed. PLoS One 3, e2910.

Dorr de Quadros, P., Zhalnina, K., Davis-Richardson, A., Fagen, J.R., Drew, J., Bayer, C., Camargo, F.A., Triplett, E.W., 2012. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical acrisol. Diversity 4, 375–395.
Dunbar, J., Barns, S.M., Ticknor, L.O., Kuske, C.R., 2002. Empirical and theoretical bacterial diversity in four arizona soils. Appl. Environ. Microbiol. 68, 3035–3045.
Egenriether, S.M. (2021). Management, microbiology, and machine learning: A systems approach to evaluating the nitrogen cycling microbiome in agricultural soils. Thesis.
Fahde, S., Boughribil, S., Sijilmassi, B., Amri, A., 2023. Rhizobia: A promising source of plant growth-promoting molecules and their non-legume interactions: examining applications and mechanisms. Agriculture 13, 1279.
Feng, Y., Motta, A.C., Reeves, D.W., Burmester, C.H., van Santen, E., Osborne, J.A., 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biol. Biochem. 35, 1693–1703.
Ferreira, M.C., Andrade, D.d.S., Chueire, L.M.d.O., Takemura, S.M., 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.
Fierer, N., Wood, S.A., de Mesquita, C.P.B., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108111.
Figuerola, E.L., Guerrero, L.D., Türkowsky, D., Wall, L.G., Erijman, L., 2015. Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. Environ. Microbiol. 17, 678–688.
Franzluebbers, A., Hubbs, M., Norfleet, M., 2012. Evaluating soil organic carbon sequestration potential in the cotton belt with the soil conditioning index. J. Soil Water Conserv. 67, 378–389.
Gans, J., Wolinsky, M., Dunbar, J., 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309, 1387–1390.
García-Fraile, P., Carro, L., Robledo, M., Ramirez-Bahena, M.-H., Flores-Felix, J.-D., Fernández, M.T., Mateos, P.F., Rivas, R., Igual, J.M., Martínez-Molina, E., et al., 2012. Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7, e38122.
Garrity, G.M., Bell, J.A., and Lilburn, T.G. (2004). Taxonomic outline of the prokaryotes. bergey's manual of systematic bacteriology. New York.
Gutiérrez-Zamora, M.T., Martınez-Romero, E., 2001. Natural endophytic association between rhizobium etli and maize (Zea mays l.). J. Biotechnol. 91, 117–126.
Hartmann, M., Niklaus, P.A., Zimmermann, S., Schmutz, S., Kremer, J., Abarenkov, K., Lüscher, P., Widmer, F., Frey, B., 2014. Resistance and resilience of the forest soil microbiome to logging-associated compaction. ISME J. 8, 226–244.
Hillel, D., 2011. An overview of soil and water management: the challenge of enhancing productivity and sustainability. Soil Management: Building A Stable Base for Agriculture 1–11.
Huggins, D.R., Reganold, J.P., 2008. No-till: the quiet revolution. Sci. Am. 299, 70–77.
Ivanova, E., Doronina, N., Shepelyakovskaya, A., Laman, A., Brovko, F., Trotsenko, Y.A., 2000. Facultative and obligate aerobic methylobacteria synthesize cytokinins. Microbiology 69, 646–651.
Jari Oksanen, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R., Simpson, G.L., Solymos, P., and Stevens, M.H.H. (2018). Vegan: community ecology package. R package version, 2.
Jiménez-Gómez, A., Flores-Félix, J.D., García-Fraile, P., Mateos, P.F., Menéndez, E., Velázquez, E., Rivas, R., 2018. Probiotic activities of rhizobium laguerreae on growth and quality of spinach. Sci. Rep. 8, 295.
Kaschuk, G., Hungria, M., Andrade, D., Campo, R., 2006. Genetic diversity of rhizobia associated with common bean (Phaseolus vulgaris l.) grown under no-tillage and conventional systems in southern brazil. Appl. Soil Ecol. 32, 210–220.
Kibblewhite, M., Ritz, K., Swift, M., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. B: Biol. Sci. 363, 685–701.
Kloepper, J.W. (1978). Plant growth-promoting rhizobacteria on radishes.In: Proc. of the 4th Internet. Conf. on Plant Pathogenic Bacter, Station de Pathologie Vegetale et Phytobacteriologie, INRA, Angers, France, 1978, 879–882.volume 2.
Kloepper, J.W., Lifshitz, R., Zablotowicz, R.M., 1989. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7, 39–44.
Kousar, B., Bano, A., Khan, N., 2020. Pgpr modulation of secondary metabolites in tomato infested with Spodoptera litura. Agronomy 10, 778.
Kruskal, J.B., 1964. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29, 1–27.
Lahti, L., Shetty, S., Blake, T., Salojarvi, J., 2017. Tools for microbiome analysis in r. Version 1, 28.
Lewis, K.L., Burke, J.A., Keeling, W.S., McCallister, D.M., DeLaune, P.B., Keeling, J.W., 2018. Soil benefits and yield limitations of cover crop use in texas high plains cotton. Agron. J. 110, 1616–1623.
Luo, Y., Iqbal, A., He, L., Zhao, Q., Wei, S., Ali, I., Ullah, S., Yan, B., Jiang, L., 2020. Long-term no-tillage and straw retention management enhances soil bacterial community diversity and soil properties in southern china. Agronomy 10, 1233.
Lupatini, M., Korthals, G.W., De Hollander, M., Janssens, T.K., Kuramae, E.E., 2017. Soil microbiome is more heterogeneous in organic than in conventional farming system. Front. Microbiol. 7, 2064.
Maruyama, H., Masago, A., Nambu, T., Mashimo, C., Okinaga, T., 2020. Amplicon sequence variant-based oral microbiome analysis using qiime 2. J. Osaka Dent. Univ. 54, 273–281.
Matteoli, F.P., Silva, A.M., Feiler, H.P., de Araújo, V.L., Cardoso, E.J., 2022. Predicting soil farming system and attributes based on soil bacterial community. Appl. Soil Ecol. 171, 104335.
McGill, B.J., Etienne, R.S., Gray, J.S., Alonso, D., Anderson, M.J., Benecha, H.K., Dornelas, M., Enquist, B.J., Green, J.L., He, F., 2007. Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. Ecol. Lett. 10, 995–1015.
McMurdie, P.J., Holmes, S., 2013. phyloseq: an r package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8, e61217.
Mishra, A.K., Özger, M., Singh, V.P., 2009. An entropy-based investigation into the variability of precipitation. J. Hydrol. 370, 139–154.
Mishra, J., Stubbs, M., Kuang, L., Vara, N., Kumar, P., Kumar, N., et al., 2022. Inflammatory bowel disease therapeutics: A focus on probiotic engineering. Mediat. Inflamm. 2022.
Montecchia, M.S., Tosi, M., Soria, M.A., Vogrig, J.A., Sydorenko, O., Correa, O.S., 2015. Pyrosequencing reveals changes in soil bacterial communities after conversion of yungas forests to agriculture. PloS One 10, e0119426.
Nabintu, N.B., Ndemo, O.R., Sharwasi, N.L., Gustave, M.N., Esther, M.R., Okoth, K.S., 2019. Indigenous rhizobia strains: The silver bullet for enhanced biological nitrogen fixation and soybean (Glycine max (l.) merr.) yield under different soil conditions in south kivu province, democratic republic of congo. Afr. J. Agric. Res. 14, 2038–2047.
Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54, 655–670.
Nie, X., Chen, J., Ma, X., Ni, Y., Shen, Y., Yu, H., Panagiotou, G., Bao, Y., 2020. A metagenome-wide association study of gut microbiome and visceral fat accumulation. Comput. Struct. Biotechnol. J. 18, 2596–2609.
Nielsen-Gammon, J. (2011). The changing climate of texas (austin, tx.
Olden, J.D., Poff, N.L., Douglas, M.R., Douglas, M.E., Fausch, K.D., 2004. Ecological and evolutionary consequences of biotic homogenization. Trends Ecol. Evol. 19, 18–24.
Ollivier, J., Töwe, S., Bannert, A., Hai, B., Kastl, E.-M., Meyer, A., Su, M.X., Kleineidam, K., Schloter, M., 2011. Nitrogen turnover in soil and global change. FEMS Microbiol. Ecol. 78, 3–16.
Ouma, E.W., Asango, A.M., Maingi, J., Njeru, E.M., 2016. Elucidating the potential of native rhizobial isolates to improve biological nitrogen fixation and growth of common bean and soybean in smallholder farming systems of kenya. Int. J. Agron. 2016, 4569241.
Padilla, F.M., Pugnaire, F.I., 2006. The role of nurse plants in the restoration of degraded environments. Front. Ecol. Environ. 4, 196–202.
Parker, M.A., 1999. Relationships of bradyrhizobia from the legumes Apios americana and Desmodium glutinosum. Appl. Environ. Microbiol. 65, 4914–4920.
Pastorelli, R., Piccolo, R., Landi, S., 2009. Changes in active microbial soil communities in agricultural managments: from anthropic to natural. Agrochimica 53, 386–397.
Pastorelli, R., Vignozzi, N., Landi, S., Piccolo, R., Orsini, R., Seddaiu, G., Roggero, P.P., Pagliai, M., 2013. Consequences on macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of central italy. Soil Biol. Biochem. 66, 78–93.
Peixoto, D.S., Silva, B.M., de Oliveira, G.C., Moreira, S.G., daSilva, F., Curi, N., 2019. A soil compaction diagnosis method for occasional tillage recommendation under continuous no tillage system in brazil. Soil Tillage Res. 194, 104307.
Pérez-Fernández, M.A., Calvo-Magro, E., Valentine, A., 2016. Benefits of the symbiotic association of shrubby legumes for the rehabilitation of degraded soils under mediterranean climatic conditions. Land Degrad. Dev. 27, 395–405.
Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., Crecchio, C., 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. a review. Biol. Fertil. Soils 51, 403–415.
Podile, A.R., Kishore, G.K., 2007. Plant growth-promoting rhizobacteria. Plant-associated bacteria. Springer,, pp. 195–230.
Pohajda, I., Babić, K.H., Rajnović, I., Kajić, S., Sikora, S., 2016. Genetic diversity and symbiotic efficiency of indigenous common bean rhizobia in croatia. Food Technol. Biotechnol. 54, 468–474, 28115905[pmid] PMC5253983[pmcid] FTB-54-468[PII].
Prosser, J.I., Bohannan, B.J., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J.J., 2007. The role of ecological theory in microbial ecology. Nat. Rev. Microbiol. 5, 384–392.
R Core Team (2020). R: A Language and Environment for Statistical Computing.R Foundation for Statistical Computing Vienna, Austria.
Reynders, T., Devolder, L., Valles-Colomer, M., Van Remoortel, A., Joossens, M., De Keyser, J., Nagels, G., D'hooghe, M., Raes, J., 2020. Gut microbiome variation is associated to multiple sclerosis phenotypic subtypes. Ann. Clin. Transl. Neurol. 7, 406–419.
Roberts, D.W., and Roberts, M.D.W. (2016). Package 'labdsv'. Ordination and multivariate, 775.
Rodríguez-Echeverría, S., Pérez-Fernández, M., Vlaar, S., Finnan, T., 2003. Analysis of the legume-rhizobia symbiosis in shrubs from central western spain. J. Appl. Microbiol. 95, 1367–1374.
Saeed, M., Ilyas, N., Jayachandran, K., Shabir, S., Akhtar, N., Shahzad, A., Sayyed, R., Bano, A., 2022. Advances in biochar and pgpr engineering system for hydrocarbon degradation: A promising strategy for environmental remediation. Environ. Pollut. 305, 119282.
Schloss, P.D., Handelsman, J., 2006. Toward a census of bacteria in soil. PLoS Comput. Biol. 2, e92.
Schmidt, D.E., Dlott, G., Cavigelli, M., Yarwood, S., Maul, J.E., 2022. Soil microbiomes in three farming systems more affected by depth than farming system. Appl. Soil Ecol. 173, 104396.
Schmidt, R., Gravuer, K., Bossange, A.V., Mitchell, J., Scow, K., 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PloS One 13, e0192953.
Schnurr-Pütz, S., Bååth, E., Guggenberger, G., Drake, H.L., Kirsten, K., 2006. Compaction of forest soil by logging machinery favours occurrence of prokaryotes. FEMS Microbiol. Ecol. 58, 503–516.

Shi, G., Sun, H., Calderón-Urrea, A., Li, M., Yang, H., Wang, W., Su, G., 2021. Bacterial communities as indicators of soil health under a continuous cropping system. Land Degrad. Dev. 32, 2393–2408.
Silva Batista, Hungria, M., Barcellos, F.G., Ferreira, M.C., Mendes, I.C., 2007. Variability in Bradyrhizobium japonicum and B. elkanii seven years after introduction of both the exotic microsymbiont and the soybean host in a Cerrados soil. Microbial ecology 53, 270–284.
Sloan, W.T., Woodcock, S., Lunn, M., Head, I.M., Curtis, T.P., 2007. Modeling taxa-abundance distributions in microbial communities using environmental sequence data. Microb. Ecol. 53, 443–455.
Smith, C.R., Blair, P.L., Boyd, C., Cody, B., Hazel, A., Hedrick, A., Kathuria, H., Khurana, P., Kramer, B., Muterspaw, K., Peck, C., Sells, E., Skinner, J., Tegeler, C., Wolfe, Z., 2016a. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecol. Evol. 6, 8075–8084, 7878079[pmid] PMC5108259[pmcid] ECE32553[PII].
Smith, C.R., Blair, P.L., Boyd, C., Cody, B., Hazel, A., Hedrick, A., Kathuria, H., Khurana, P., Kramer, B., Muterspaw, K., Peck, C., Sells, E., Skinner, J., Tegeler, C., Wolfe, Z., 2016b. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecol. Evol. 6, 8075–8084, 27878079[pmid] PMC5108259[pmcid] ECE32553[PII].
Souza, R.C., Cantão, M.E., Vasconcelos, A.T.R., Nogueira, M.A., Hungria, M., 2013. Soil metagenomics reveals differences under conventional and no-tillage with crop rotation or succession. Appl. Soil Ecol. 72, 49–61.
Souza, V., Bain, J., Silva, C., Bouchet, V., Valera, A., Marquez, E., Eguiarte, L.E., 1997. Ethnomicrobiology: do agricultural practices modify the population structure of the nitrogen fixing bacteria rhizobium etli biovar phaseoli. J. Ethnobiol. 17, 4.
Steward, D.R., Allen, A.J., 2016. Peak groundwater depletion in the high plains aquifer, projections from 1930 to 2110. Agric. Water Manag. 170, 36–48.
Thies, J.E., Singleton, P.W., Bohlool, B.B., 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. 57, 19–28.
Thrall, P.H., Laine, A.-L., Broadhurst, L.M., Bagnall, D.J., Brockwell, J., 2011. Symbiotic effectiveness of rhizobial mutualists varies in interactions with native australian legume genera. PloS One 6, e23545.
Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of soil properties and microbial communities to agriculture: Implications for primary productivity and soil health indicators. Front. Plant Sci. 7.
USDA-NRCS (2016). Amarillo soil series. U.S. Department of Agriculture, Natural Resource Conservation Service, Washington, DC. Technical Report U.S. Department of Agriculture, Natural Resource Conservation Service, Washington, DC. (Accessed 24 Nov. 2017).
Vargas, L.K., Volpiano, C.G., Lisboa, B.B., Giongo, A., Beneduzi, A., Passaglia, L.M.P., 2017. *Potential of rhizobia as plant growth-promoting rhizobacteria.* Springer International Publishing, pp. 153–174.
Verginer, M., Siegmund, B., Cardinale, M., Müller, H., Choi, Y., Míguez, C.B., Leitner, E., Berg, G., 2010. Monitoring the plant epiphyte methylobacterium extorquens dsm 21961 by real-time pcr and its influence on the strawberry flavor. FEMS Microbiol. Ecol. 74, 136–145.
Větrovský, T., Baldrian, P., 2013. The variability of the 16s rrna gene in bacterial genomes and its consequences for bacterial community analyses. PLOS ONE 8, e57923.
Vos, M., Quince, C., Pijl, A.S., de Hollander, M., Kowalchuk, G.A., 2012. A comparison of rpob and 16s rrna as markers in pyrosequencing studies of bacterial diversity. PLoS ONE 7, e30600. PONE-D-11-18381[PII] 22355318[pmid] PLoS One.
Wang, C.H., Wu, L., Wang, Z., Alabady, M.S., Parson, D., Molumo, Z., Fankhauser, S.C., 2020. Characterizing changes in soil microbiome abundance and diversity due to different cover crop techniques. PloS One 15, e0232453.
Wang, F., Men, X., Zhang, G., Liang, K., Xin, Y., Wang, J., Li, A., Zhang, H., Liu, H., Wu, L., 2018. Assessment of 16s rrna gene primers for studying bacterial community structure and function of aging flue-cured tobaccos. AMB Express 8, 182, 30415449 [pmid] PMC6230335[pmcid] 10.1186/s13568-018-0713-1[PII] AMB Express.
Wang, X., Feng, H., Wang, Y., Wang, M., Xie, X., Chang, H., Wang, L., Qu, J., Sun, K., He, W., 2021. Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia-legume symbiosis. Mol. Plant 14, 503–516.
Wilhelm, R.C., van Es, H.M., Buckley, D.H., 2022. Predicting measures of soil health using the microbiome and supervised machine learning. Soil Biol. Biochem. 164, 108472.
Wright, A.L., Hons, F.M., Lemon, R.G., McFarland, M.L., Nichols, R.L., 2008. Microbial activity and soil c sequestration for reduced and conventional tillage cotton. Appl. Soil Ecol. 38, 168–173.
Yates, R.J., Howieson, J.G., Reeve, W.G., O'Hara, G.W., 2011. A re-appraisal of the biology and terminology describing rhizobial strain success in nodule occupancy of legumes in agriculture. Plant Soil 348, 255–267.
Zak, J.C., and Willig, M.R. (2004). Fungal biodiversity patterns.
Zhang, X., Xin, X., Zhu, A., Yang, W., Zhang, J., Ding, S., Mu, L., Shao, L., 2018. Linking macroaggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements. Soil Tillage Res. 178, 99–107.
Zhang, X.X., Guo, H.J., Jiao, J., Zhang, P., Xiong, H.Y., Chen, W.X., Tian, C.F., 2017. Pyrosequencing of rpob uncovers a significant biogeographical pattern of rhizobial species in soybean rhizosphere. J. Biogeogr. 44, 1491–1499.