

Conservation tillage regulates soil bacterial community assemblies, network structures and ecological functions in black soils

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Abstract

Aims

Conventional tillage is a serious threat to the stability of soil ecosystems. Understanding the response mechanisms of soil microbial community assemblies to anthropogenic activities is a major topic of ecological research.

Methods

Here, we investigated the bacterial community structures and assemblies in bulk and rhizosphere soils of soybeans grown with conventional tillage (moldboard plow, MP) and with conservation tillage that involved no-tillage (NT) or ridge tillage (RT) using high-throughput sequencing methods.

Results

We found that soil bacterial community compositions, structures and assembly processes were primarily altered by tillage practices. Briefly, in comparison to MP, NT and RT increased the relative abundances of the nitrogen-fixing bacteria *Mesorhizobium* sp., *Bradyrhizobium* sp. and *Burkholderia* sp., but decreased the abundance of soil carbon-degrading bacteria, especially *Blastococcus* sp., *Streptomyces* sp. and *Sphingomonas* sp. In addition, in comparison to MP, NT and RT resulted in more stable bacterial networks and more lower the relative contribution of homogenizing dispersal. Soil pH was the primary soil factor regulating both the bacterial community structures and assembly processes under the three tillage practices.

Conclusions

The altered functional bacteria under conservation tillage was mostly affiliated with biomarkers and keystone taxa, inferring that conservation tillage might contribute to biological nitrogen fixation and soil carbon sequestration.

Introduction

Black soils (Mollisols) are highly productive and play key roles in ensuring food security in China (Liu et al., 2008). Tillage practices aim to improve crop production and agricultural sustainability through soil mechanical inversion and residue return (Dang et al, 2015; Zuber and Villamil, 2016). However, the intensive mechanical interference of conventional tillage, such as moldboard plows (MPs), leads to serious soil degradation, which is a major threat to the health of agricultural ecosystems (Montgomery, 2007; Sainju et al., 2011; Zhang et al., 2012). In contrast, conservation tillage, such as the practice of no-tillage (NT), which minimizes soil erosion risks and input costs, is adopted to combat agricultural scourge

(Busari et al., 2015; Zhao et al., 2017). Despite the benefits of NT, the key disadvantages are that it causes herbicide-resistant weeds to thrive and the soil surface to become compacted, which impede normal root development, reduces the seed germination rate, and increases stubble-borne diseases (Dang et al., 2015; Steinkellner and Langer, 2004; Sun et al., 2018a). Therefore, compared with NT and MP, the conservation tillage practice of ridge tillage (RT) with low soil disturbance increases soil resilience and maximizes the positive impacts on soil quality (Alvear et al., 2005; Hobbs et al., 2008).

Tillage practices change soil properties and thus lead to variations in soil microorganisms, which play a considerable role in agroecosystems by mediating soil biogeochemical processes and plant nutrient uptake (Falkowski et al., 2008; Fierer, 2017). However, most studies on the influences of tillage practices on soil microorganisms have focused on microbial community composition and diversity, but a few have focused on microbial ecological functions. The results of long-term local experiments have revealed that conservational tillage enhances soil biological nitrogen fixation by increasing the abundances of *Pseudomonas* spp. and *Agrobacterium* spp. (Hoflich et al., 1999; Wang et al., 2020a), but other studies have found that conservation tillage increases denitrifiers, which in turn promotes soil nitrogen loss (Johnson and Hoyt, 1999; Puerta et al., 2019). In addition, it is still not well understood how tillage practices impact soil carbon cycling with shifts in microbial communities between conventional and conservation tillage. Evidence has revealed that NT increases the abundance of anaerobes with low carbon utilization efficiency and reduces the abundance of cellulose-degrading groups, thus encouraging soil carbon sequestration (Allison et al., 2010; Sun et al., 2018b; Wang et al., 2020b). However, de Vries et al. (2015) documented that there is no obvious variation in cellulose-degrading communities among different tillage practices based on metagenomic analyses. Therefore, it is important to comprehensively understand the ecological functions of soil microorganisms under different tillage practices to improve the productivity and sustainable development of agricultural ecosystems. However, studies of soil microbial functions are not easy because of the complex interactions between microbes. Recently, microbial networks and community assembly processes were used to determine these complex relationships while providing insight into the response of soil microbiomes to anthropogenic and environmental changes (Banerjee et al., 2019; Ye et al., 2021; Zhou and Ning, 2017).

It is widely accepted that stochastic (neutral theory) and deterministic (niche-based theory) processes both simultaneously influence microbial community assemblies (Feng et al., 2018; Stegen et al., 2012). Nevertheless, the importance of stochastic and deterministic processes to microbial community assembly is still debated. A conceptual framework dividing ecological processes into five fundamental processes, including homogenizing dispersal, dispersal limitation, homogeneous selection and variable selection, as well as “undominated processes”, which indicate those not dominated by any of the above single processes, has been developed and widely used to quantify the relative contributions of these processes (Stegen et al., 2015). Empirical evidence supports that community assemblies are driven by different combinations of these ecological processes. Recent studies have shown that deterministic processes determine the assembly of soil bacterial communities in wheat fields (Shi et al., 2018). Jiao and Lu (2020) found that homogeneous selection dominates the soil bacterial community assembly processes in rice and maize fields. Cheng et al. (2021) confirmed that variable selection has the greatest

contribution to soil bacterial community assembly processes in arable and forest soils. However, an understanding of the relative effects of these ecological processes on the assembly of soil bacterial communities under different soil tillage practices and their consequences for ecosystem functionality is lacking.

In this study, bulk and rhizosphere soils from soybean plants were collected to explore the compositions, network structures and assembly processes of bacterial communities under conventional tillage (MP) and conservation tillage (NT and RT) practices in Northeast China using high-throughput sequencing methods. We aimed to 1) reveal the bacterial community structures and the variations in the functional taxa in response to the three tillage practices; 2) illuminate how the network properties and keystone taxa varied among NT, RT and MP; and 3) clarify the relative contribution of ecological processes in shaping the assembly of bacterial communities under these three tillage practices.

Materials And Methods

Experimental design and soil sampling collection

A long-term experimental station was established at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin, China ($44^{\circ}59'N$, $125^{\circ}23'E$) in the fall of 2013. The average yearly precipitation at the experimental site is 614 mm, and the mean annual temperature is $6.4^{\circ}C$. The soil samples belong to black soil that is classified as Mollisols according to the soil taxonomy system of the US.

The experiment was arranged with a completely randomized block design. Three tillage treatments were selected for this study: moldboard plow (MP) tillage, no-tillage (NT) and ridge tillage (RT). Except for sowing with a KINZE-3000NT planter (Iowa, USA), the soil under NT was not disturbed. For RT, only ridging with a cultivator was conducted in June of each year. The MP site was disturbed by moldboard plowing (0–20 cm) after the harvest of the maize, during seedbed preparation in spring, and during the ridge process in June. For all treatments, a soybean and maize rotation was implemented and maize straw of approximately 30 cm in length or all soybean residues covered the soil surface after the annual harvest. For soybean, potassium (K), phosphorus (P) and nitrogen (N) fertilizers were applied at 80 kg ha^{-1} , 60 kg ha^{-1} , and 40 kg ha^{-1} , respectively, as basal fertilizers. For maize, basal fertilizer was applied at 78 kg K ha^{-1} , 45.5 kg P ha^{-1} and 100 kg N ha^{-1} , whereas an additional 50 kg N ha^{-1} was used at the V6 stage as the top dressing.

A total of 48 soil samples (bulk and rhizosphere, three treatments \times eight replicates) were collected on July 25, 2017, at the soybean podding stage. Soil (0–20 cm) was randomly collected from each soybean plot ($25\text{ m} \times 7.8\text{ m}$) as bulk soil. To collect the rhizosphere soil samples, ten soybean plants in each plot were shoveled out. After shaking off the loose adhesive soil on the root, the tightly bonded soil on the root surface was brushed down as rhizosphere soil (Philippot et al., 2013). The collected soils were kept in $-80^{\circ}C$ and $4^{\circ}C$ refrigerators for soil DNA extraction and chemical property analysis, respectively.

Soil chemical properties analysis

Soil chemical properties were assayed according to the description by Lu (1999) after being passed through a 2 mm sieve. The specific data on soil pH, nitrate nitrogen (NO_3^- -N), ammonium nitrogen (NH_4^+ -N), available phosphorus (AP), available potassium (AK), total nitrogen (TN), total carbon (TC), total potassium (TK) and total phosphorus (TP) are shown in Table S1. Briefly, in comparison to the bulk soil, the rhizosphere soil had higher TC, TN and available nutrients (NO_3^- -N, NH_4^+ -N, AK and AP). In addition, in comparison to NT and RT, MP significantly increased NO_3^- -N and pH in the bulk and rhizosphere soils and AP, TP and TC in the rhizosphere soil.

Soil DNA extraction and sequencing

Soil microbial total DNA was extracted (0.5 g fresh soil per sample) using the Fast DNA® Spin Kit for Soil (MP Biomedicals, USA). The primer pair 338F/806R (Castrillo et al., 2017), with a sample-specific barcode at the 5' end, was used to amplify the V3-V4 region of the soil bacterial 16S rRNA gene. The PCR system and amplification conditions were described previously (Liu et al., 2020). The PCR products were further pooled and purified by an agarose gel DNA purification kit (TaKaRa, Dalian, China). The purified PCR products were sequenced on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Bioinformatic analysis

The QIIME pipeline (version 1.9.1) was used to perform raw FASTAQ data analysis (Caporaso et al., 2010). First, low-quality sequences with an average quality score < 20 and/or length < 200 bp were removed (Aronesty, 2011). The UCHIME algorithm was used to filter chimeric sequences (Edgar et al., 2011). Then, the obtained high-quality sequences were clustered into operational taxonomic units (OTUs) with a 97% similarity level by UPARSE (Edgar, 2010). The OTU representative sequences were identified taxonomically using the RDP classifier based on the SILVA database for bacterial species classification (Cole et al., 2005). Our raw sequences were submitted to the NCBI Sequence Read Archive (SRA) database with the accession number PRJNA644753.

Community assembly process analysis

A null model was employed to evaluate the bacterial community assembly processes. Phylogenetic conservatism of communities was tested using the function 'mantel.correlog' in the "vegan" package of R (version 3.6.2) (R Development Core Team, 2016) before building the null model (Stegen et al., 2013). To identify the bacterial community assembly processes within each sample, the standardized effect size measure of the mean nearest taxon distance (ses.MNTD) was calculated using the "picante" package in the R environment ('taxa.labels' function, 999 randomization). The positive and negative values of ses.MNTD indicated phylogenetic overdispersion and phylogenetic clustering, respectively (Webb et al., 2002).

We quantified the influences of stochastic and deterministic processes on bacterial assemblage by the nearest taxon index (β NTI) ('comdistnt' function, abundance.weighted = TRUE), which is the standard deviation of the between-community mean nearest taxon distance (β MNTD) (Stegen et al., 2012). Values of $|\beta$ NTI| > 2 indicate a significant deviation between observed and expected phylogenetic turnover, which indicates the dominance of deterministic processes. β NTI values < - 2 and > + 2 denote homogeneous selection (lower phylogenetic turnover than expected) and variable selection (higher phylogenetic turnover than expected) processes, respectively (Stegen et al., 2012). Furthermore, we combined β NTI with the Bray-Curtis-based Raup-Crick (RC_{bray}) to quantify the effects of dispersal-based stochastic ecological processes (Stegen et al., 2013, 2015). RC_{bray} values < - 0.95 or > + 0.95 denote distinct divergence from the null model expectation. The homogenizing dispersal and dispersal limitation processes were quantified by $|\beta$ NTI| values < 2 but RC_{bray} < - 0.95 and RC_{bray} > + 0.95, respectively. Moreover, $|\beta$ NTI| < 2 and $|RC_{bray}| < 0.95$ were considered undominated processes (Stegen et al., 2015). The "picante" package in the R environment was used to perform the above analyses (R Development Core Team, 2016).

Co-occurrence network construction

To determine the bacterial species interactions, co-occurrence networks were constructed with OTUs with relative abundances > 0.01% to rule out spurious correlations. The topological indices of random empirical networks were summarized by 999 iterations to determine whether network properties were error prone. Ten nodes with the highest degree were defined as hub nodes (Ma et al., 2020). The role of individual nodes was divided into four categories: network hubs, connectors, module hubs and peripherals (Olesen et al., 2007). Ecologically, peripheral nodes represent specialists, while the other three represent generalists (Deng et al., 2012). The generalists and hub nodes together were considered keystone taxa in this study. Gephi (version 0.9.2) was applied to visualize the co-occurrence network.

Statistical analyses

In this study, we randomly selected 21,900 bacterial sequences from each sample to compare the relative variation between samples. One-way ANOVA was conducted to test the divergences in the measured variables among the treatments. The changes in the bacterial community composition and abundance at the phylum, genus and OTU levels among the tillage practices were determined with heatmaps, circles and ternary plots, which were displayed in the R environment with the "pheatmap", "circlize" and "ggtern" packages, respectively (R Development Core Team, 2016). In addition, the non-parametric multivariate analysis of variance (Adonis) and principal coordinate analysis (PCoA) were carried out using the "vegan" package in the R environment to reveal the influence of different disturbance degrees on bacterial community structures. The correlation between bacterial communities and soil chemical properties was performed by the Mantel test and distance-based redundancy analysis (db-RDA) using the "vegan" package in the R environment. The ecological function of the bacterial communities was predicted using FAPROTAX (Louca et al., 2016). To identify the biomarkers, random forest modeling was performed using the "randomForest" package in the R environment.

Results

Bacterial community composition

In this study, 1,740,398 high-quality bacterial sequences were obtained from 48 soil samples (30,182 – 44,915 per sample), which were clustered into 4,649 OTUs belonging to 38 phyla. The dominant bacterial phyla/classes (with average relative abundances higher than 5%) were Alphaproteobacteria (23.25%), Actinobacteria (20.07%), Acidobacteria (11.45%), Betaproteobacteria (9.95%), Chloroflexi (8.09%) and Bacteroidetes (6.13%) across all samples (Fig. 1, Table S2). In the bulk soil, in comparison to MP, NT and RT significantly increased ($P < 0.05$) the relative abundance of Nitrospirae while reducing the abundance of Saccharibacteria. Additionally, the relative abundances of Gemmatimonadetes and Actinobacteria were significantly lower ($P < 0.05$) in NT and RT than in MP in the rhizosphere soils (Table S2).

Fifty-two genera were observed with mean relative abundances higher than 0.5% in at least one treatment. Among them, *Bradyrhizobium* (6.81%) and *Sphingomonas* (3.22%) were the most dominant genera. In the bulk soils, in comparison to MP, NT and RT significantly increased ($P < 0.05$) the relative abundances of three genera, including *Bacillus*, *Mesorhizobium* and *RB41*, while they decreased the abundances of 12 genera, including *Blastococcus*, *Sphingomonas* and *Methylobacterium*. In the rhizosphere soils, *Burkholderia*, *Mesorhizobium* and *Aquicella* significantly increased ($P < 0.05$), while nine genera, including *Mycobacterium*, *Methylotenera* and *Gemmatimonas*, decreased in NT and RT compared with in MP (Table S2).

At the OTU level, the numbers of unique and shared OTUs among the three tillage practices in the bulk and rhizosphere soils were displayed by a Venn diagram (Fig. 2a and b). In general, in comparison to the bulk soils (5.95%), the rhizosphere soils (10.78%) had more unique OTUs, irrespective of the tillage practice. Additionally, the differences in the relative abundance of soil bacterial OTUs among the three tillage practices were further revealed by ternary plots (Fig. 2c and d). The results showed that there were more OTUs altered with specific tillage practices in the bulk soils (158 OTUs) than in the rhizosphere soils (72 OTUs). Specifically, in comparison to MP, NT and RT significantly increased ($P < 0.05$) the relative abundances of OTU2114 (*Mesorhizobium*) and OTU1206 (*Dehalogenimonas*) while decreasing the abundances of seven OTUs, including OTU1829 (*Blastococcus*), OTU846 (*Sphingomonas*) and OTU3866 (*Gemmatio*), in the bulk soils. Six OTUs, including OTU4404 (*Bradyrhizobium*), OTU2109 (*Rhizobium*) and OTU3445 (*Burkholderia*), remarkably increased ($P < 0.05$), and six OTUs, including OTU1829 (*Blastococcus*), OTU846 (*Sphingomonas*) and OTU3141 (*Methylotenera*), decreased in the rhizosphere soils with NT and RT compared with those with MP (Fig. 2c and d, Table S3).

Soil bacterial community structure

The PCoA plot based on the Bray-Curtis distance clearly showed that the bacterial communities were mainly divided into two major groups with the bulk and rhizosphere soil samples (Adonis tests, $P < 0.001$) (Fig. 3a). In addition, the separated PCoA plots based on the bulk and rhizosphere soils individually

showed that the three tillage treatments significantly altered the bacterial communities (Adonis tests, $P < 0.01$) (Fig. 3b and c, Table S4).

The results of the Mantel test revealed that soil pH, C/N, TC, TN, TP, AP, AK, NH_4^+ -N and NO_3^- -N were significantly correlated with the bacterial community structures ($P < 0.05$) (Fig. S1). After variance inflation factor (VIF) screening, db-RDA showed that NH_4^+ -N played the most important role ($R^2 = 0.740$, $P < 0.001$) in regulating the bacterial community structures under three tillage practices in combination with the bulk and rhizosphere soils (Fig. 3d, Table S5). Furthermore, the separated db-RDA plots revealed that of the factors, pH contributed the most to the variations in the bacterial community structures under different tillage practices in both the bulk ($R^2 = 0.728$, $P < 0.001$) and rhizosphere ($R^2 = 0.698$, $P < 0.001$) soils (Fig. 3e and f, Table S5).

Functional annotation and biomarker identification

In this study, we observed that the dominant functional groups (mean relative abundances higher than 1%) were associated with the carbon cycles, including aerobic chemoheterotrophy, chemoheterotrophy and aromatic compound degradation, based on FAPROTAX annotation. The relative abundance of the aforementioned groups was significantly decreased in RT compared with that in MP in bulk soils. In comparison to MP, NT and RT distinctly reduced the relative abundance of aromatic compound degradation in the rhizosphere soils (Fig. 4). In addition, the dominant functional groups were involved in the nitrogen cycles that involved nitrogen fixation, ureolysis, nitrification, nitrate reduction and aerobic ammonia oxidation. Compared with MP, NT and RT significantly increased the relative abundances of nitrification, aerobic ammonia oxidation and nitrate reduction in the bulk soils. Compared to MP, NT and RT increased the relative abundance of nitrogen fixation, and NT increased nitrification and aerobic ammonia oxidation in the rhizosphere soils (Fig. 4).

Random forest analysis based on machine learning was carried out to identify biomarkers (Fig. 5a). A tenfold cross-validation was performed to assess the importance of biomarkers since the error rate of cross-validation tended to stabilize when the 10 most relevant genera were selected. Ten genera were defined as biomarkers: *Methylotenera*, *Gemmatimonas*, *Burkholderia*, *Segetibacter*, *Gemmatio*, *Luteibacter*, *Noviherbspirillum*, *Methylobacterium*, *Blastococcus* and *Mycobacterium*. The relative abundance of the biomarkers in the different treatments was further illustrated by a heatmap, which showed that most of the biomarkers, such as *Methylotenera*, *Blastococcus* and *Gemmatimonas*, had lower relative abundance values, while only *Burkholderia* had a relatively higher abundance in the rhizosphere soils of NT and RT than those of MP (Fig. 5b).

Network analysis

To determine the interactions of soil bacterial communities and their potential roles in response to tillage practices, six bacterial co-occurrence networks were constructed for three tillage practices in the bulk and rhizosphere soils (Fig. 6, Table S6). The bacterial co-occurrence networks displayed differences in

network structures and topological properties among the different tillage practices. The number of edges was higher values with NT than with RT and MP in the bulk and rhizosphere soils. The number of nodes increased with NT and RT in the bulk and rhizosphere soils, respectively, in comparison to that with MP. Additionally, the average clustering coefficient (avgCC) and average connectivity (avgK) of the network were greater with NT than with RT and MP, whereas in comparison to NT and MP, RT resulted in greater modularity (M) and average path distance (GD).

We observed that the degree of hub nodes and the number of generalists were higher with NT than with RT and MP in both the bulk and rhizosphere soils. In the bulk soils, most of the hub nodes were affiliated with Acidobacteria under the three tillage practices. The majority of the hub nodes that occurred with MP belonged to Acidobacteria, while the hub nodes of Actinobacteria and Proteobacteria (Alpha-, Beta- and Gamma-) were dominant when NT and RT were used in the rhizosphere soils (Table S7). Intriguingly, some functional OTUs, such as OTU2160 (*Brevundimonas*) and OTU3445 (*Burkholderia*) in NT and OTU2736 (*Rhizobacter*) and OTU2500 (*Nitrosospira*), were shared by hub nodes and generalists with the use of RT in the rhizosphere soils. The other OTUs which were shared by hub nodes and generalists belonged to Acidobacteria, including OTU2346 (*Stenotrophobacter*) with in the rhizosphere soils with MP and OTU2900 (*Vicinamibacter*), OTU567 (*Stenotrophobacter*) and OTU3322 (*Unclassified*) in the bulk soils with the three tillage practices (Tables S7 and S8).

Soil bacterial community assembly processes

The values of ses.MNTD were significantly less than zero across all samples and was lower in the bulk soils than in the rhizosphere soils ($P < 0.05$) (Fig. 7a), suggesting that the bacterial communities were more phylogenetically clustered in the bulk soils. Additionally, a significant phylogenetic signal was observed across short phylogenetic distances by the phylogenetic mantel correlogram (Fig. 7b). The combined results of β NTI and RC_{bray} showed that no homogeneous selection was observed in the deterministic processes, while only homogenizing dispersal and undominated processes were detected for the stochastic processes (Fig. 7c). Variable selection dominated the bacterial community assembly processes across all the tillage practices, while it played a greater role in the bulk soils than in the rhizosphere soils. Homogenizing dispersal contributed more to the bacterial community assembly processes in the bulk and rhizosphere soils with MP than in those with NT and RT. In comparison to RT, NT increased the importance of stochastic processes in the bulk and rhizosphere soils. Additionally, the Mantel test showed that soil pH was significantly correlated ($P < 0.05$) with β NTI (Fig. S1).

Discussion

Bacterial community structures significantly affected by tillage practices

In this study, irrespective of the tillage practice, soil bacterial communities were divided into two major groups according to the bulk and rhizosphere soil samples, suggesting that rhizosphere effort rather than tillage practice was the dominant factor in regulating the bacterial communities (Fig. 3a). However, the

bacterial communities were also significantly affected by the tillage practices in the bulk and rhizosphere soils (Fig. 3b and c). This finding is corroborated by Tyler (2019) and Xia et al. (2019), who observed that underlying soil properties varied under different tillage practices and were the dominant factors in shifting microbial communities. Intriguingly, we observed that the variations in the bacterial community structures in the bulk soils were larger than those in the rhizosphere soils, which were likely because the stronger determinant of root exudates released from the roots onto the bacterial communities concealed the variation between tillage practices (Philippot et al., 2013).

Soil chemical properties had significant effects on bacterial community structure in the bulk and rhizosphere soils (Fig. S1). Soil NH_4^+ -N played a dominant role in driving differentiation across all soil samples (Fig. 3d). Soil available nitrogen observably affects plant growth, and its uptake by plants is strongly dependent on rhizosphere microbial guilds (Moreau et al., 2019). In this study, the NH_4^+ -N content ranged from an average of 1.75 mg kg^{-1} in the bulk soils to 4.36 mg kg^{-1} in the rhizosphere soils (Table S1); thus, it is reasonable that a large variation in soil NH_4^+ -N contributed the most to shaping the bacterial community structures of all the samples across the bulk and rhizosphere soils. Additionally, soil pH had contributed the most to regulating the bacterial community structures in the bulk and rhizosphere soils (Fig. 3e and f), although soil pH varied by only 0.3 units among the three tillage practices. This finding suggested that even a small pH range change induced by tillage practices was still the most important factor determining soil bacterial community compositions (Degrune et al., 2015). This result occurred because of the narrow optimum soil pH range for bacteria, and when pH values exceed a certain range, they can directly cause a physiological constraint on soil bacteria that will change the competition outcomes or decrease the net growth of the individual groups that cannot survive (Lauber et al., 2009).

Variations in soil bacterial community compositions induced by different tillage practices

We discovered that the tillage practices displayed species-specific effects on the bacterial communities in both the bulk and rhizosphere soils. Although the degree of differentiation in the soil bacterial community structures among the tillage practices in the bulk soils was larger than that in the rhizosphere soils, more unique species induced by tillage practices occurred in the rhizosphere soils than in the bulk soils (Fig. 2a and b). This result is understandable because the rhizosphere has a more resource-enriched environment owing to the variety of carbon and nutrients provided by root exudates, which reduces microbial competition and thus allows more species to maintain their lives freely (Costello et al., 2012). However, fewer changes in OTU numbers were observed in the rhizosphere soils than in the bulk soils (Fig. 2c and d). The results are consistent with those of Thebault and Fontaine (2010), who showed that in comparison to bulk soils, rhizosphere soils have greater ecological stability. This may be explained by plant roots only selectively enriching microbes with specific functions (Berendsen et al., 2012; Yan et al., 2017), which leads to a strong carbon mineralization capacity and rapid nutrient cycling in rhizosphere soils (Fan et al., 2017; Turner et al., 2013).

The responses of the functional bacteria involved in carbon and nitrogen cycling to different soil disturbances were diverse (Tables S2 and S3). NT and RT in the bulk and rhizosphere soils significantly

reduced the relative abundances of OTU1829 and OTU846, which were affiliated with *Blastococcus* that can degrade soil organic matter (Wang et al., 2020c) and *Sphingomonas* that can degrade polycyclic aromatic hydrocarbons (Macchi et al., 2018). OTU3343 (*Streptomyces adustus*) and OTU3407 (*Streptomyces yanglinensis*) can effectively degrade lignocellulose (Noda et al., 2012) and had lower abundances in the bulk soils with RT and in the rhizosphere soils with NT. These results indicate that intensive disturbance may accelerate the decomposition of carbon sources (Wang et al., 2020c) because the increased disturbance would increase the soil surface area by forming more microaggregates (Six et al., 2000).

In contrast, Nitrospirae, which contains many nitrifiers, was enriched in the bulk soils with NT and RT and likely enhanced the nitrogen turnover capacity of conservation tillage (Xue et al., 2020). In brief, the representative sequences of OTU3445 and OTU2114 had 97.12% and 99.53% similarity to *Burkholderia singularis* and *Mesorhizobium gobiense*, respectively. *Burkholderia* and *Mesorhizobium* have been widely reported to have nitrogen fixation capabilities (Fan et al., 2019; Soe et al., 2020), and their abundances were significantly higher in the rhizosphere soils with NT and RT than in those with MP. OTU2806 (*Nitrosospira multiformis*) and OTU3942 (*Nitrosospira tenuis*), which play a pivotal role in the nitrification process (Lin et al., 2018), were enriched in the bulk soils with RT and rhizosphere soils with NT. OTU4404 (*Bradyrhizobium japonicum*) and OTU2109 (*Rhizobium etli*) are widely documented to facilitate nodulation and biological nitrogen fixation of legumes (Kalita and Malek, 2020; Rivas et al., 2009; Roper et al., 2020), and they increased in the rhizosphere soils with NT and RT. Therefore, given the changes in the relative abundances of the bacterial taxa associated with carbon and nitrogen cycling in the soils with NT and RT compared with that with MP, we speculate that conservation tillage might enhance the soil nitrogen cycle and weaken soil carbon degradation. This concept was reconfirmed by the FAPROTAX annotation; NT and RT reduced the relative abundance of the functional groups associated with aromatic compound degradation while increasing nitrogen fixation in the rhizosphere soils (Fig. 4).

Tillage practices affect the stability of the soil bacterial network and the ecological function of keystone taxa and candidate biomarkers

Network analysis offers deep insight into the complex interactions of bacterial communities under soil perturbations (Barberan et al., 2012). Our study observed that tillage practices had impacts on bacterial network structures (Fig. 6, Table S6). The network of NT with the largest edge displayed more complex interactions among the OTUs in the bulk and rhizosphere soils, even though the NT network possessed relatively fewer nodes than the MP and RT network in the rhizosphere soils. This finding was in line with the results of Banerjee et al. (2019), who observed that the complexity of microbial networks is determined by the number of associations rather than the number of taxa. Additionally, the highest avgK of the NT network in the bulk and rhizosphere soils also demonstrated that in comparison to MP, NT created a higher density of interactions among OTUs (Ma et al., 2020). Bouizgarne et al. (2014) showed that closely coupled OTUs share the same habitat preferences, and their simultaneous occurrence markedly alleviates environmental stresses and stimulates crop growth. Additionally, a network with a greater average path distance (GD) can enable environmental fluctuations to propagate more slowly to

the whole network, thus playing a better role in buffering environmental perturbations (Wang et al., 2014). Simultaneously, higher modularity (M) was conducive to enhancing the stability of network structures (Kitano, 2004; Krause et al., 2003). Therefore, the higher values of GD and M in the bulk and rhizosphere soils of the RT network indicated that in comparison to MP, RT was more robust to environmental disturbances.

As gatekeepers of ecosystem function, keystone species provide important contributions to biogeochemical cycling (Banerjee et al., 2018; Jiang et al., 2017). It has been widely reported that generalists with important topological roles and hub nodes with the highest degree exert a considerable influence on microbial network structure and function (Banerjee et al., 2019; Herren and McMahon, 2018; Shi et al., 2020). In this study, it was common for the Acidobacteria OTUs to be identified as keystone taxa because Acidobacteria can occupy a broad niche and have traits related to degrading soil organic carbon (Jiang et al., 2017; Mannisto et al., 2013). Additionally, half of the biomarkers were also tied to the soil carbon cycle, including *Methylotenera*, *Gemmatimonas*, *Methylobacterium*, *Blastococcus* and *Mycobacterium* (Guo et al., 2016; Kalyuzhnaya et al., 2012; Ren et al., 2017; Sanjenbam et al., 2020; Wang et al., 2020c). More notably, some keystone taxa were simultaneously identified by generalists and hub nodes, which might be explained by their particularly important ecological functions. For instance, the keystone taxa OTU2160 (*Brevundimonas*) and OTU3445 (*Burkholderia*) in rhizosphere soils with NT have been reported to be diazotrophic and can enhance crop growth and root colonization (Fan et al., 2019; Naqqash et al., 2020). The OTU2500 and OTU2736 in the rhizosphere soils with RT were classified as *Nitrosospira* and *Rhizobacter*, respectively, and these bacteria are well known to play a crucial role in nitrification and nitrogen fixation (Lin et al., 2018; Tong et al., 2020). We also found that the nitrogen-fixing bacterium *Burkholderia* was identified as a biomarker based on random forest analysis (Fig. 5). Overall, the soil bacteria closely involved in soil carbon and nitrogen cycling were integrated as keystone taxa and biomarkers, indicating that tillage practices changed microbial-mediated carbon and nitrogen cycling, thereby affecting ecological functions.

Assembly of soil bacterial communities influenced by tillage practices

In this study, we revealed that stochastic and deterministic processes played a role in the assembly of bacterial communities by null models (Ofiteru et al., 2010). Soil bacterial communities were more closely phylogenetically clustered in the bulk soils than in the rhizosphere soils (Fig. 7a). The weak phylogenetic clustering in the rhizosphere soils might be tied to the reduced relative contribution of deterministic processes, owing to less variable environmental filtering in the rhizosphere soils, which can cause phylogenetic overdispersion of bacterial communities (Fan et al., 2017; Goberna et al., 2014). Additionally, the increasing relative importance of stochastic processes in rhizosphere soils was well associated with higher soil fertility, which can weaken niche selection by promoting the growth of soil microorganisms and reducing competition for resources (Chen et al., 2017; Zhou et al., 2014). We found that stochastic processes, especially homogenizing dispersal, had a higher relative contribution to bacterial community assembly processes in the bulk and rhizosphere soils with MP than in those with NT and RT (Fig. 7c). This could be explained by the strong soil disturbance in the soils with MP, which could have led to high

dispersal rates and thus tended to be more similar to the soil bacterial communities (Stegen et al., 2015; Zaneveld et al., 2017).

The results of the Mantel test also showed that soil pH was markedly correlated with bacterial community assembly processes (Fig. S1), which was consistent with previous reports (Tripathi et al., 2018). The closer to neutral pH of both the bulk and rhizosphere soils with MP may have reduced the selection pressure from environmental filters (Tripathi et al., 2018) since a neutral pH environment is more suitable for the growth of most bacteria (Madigan, 2012). Moreover, niche-based processes are influenced by not only abiotic factors (environmental filtering) but also biotic interactions (e.g., competition, predation and mutualism) (Zhou and Ning, 2017). Therefore, the soil nutrients and soil pH in NT were not significantly higher than those in the soils with RT, but NT increased the relative importance of stochastic processes because more microbial interactions in soils with NT could lead to alleviation of environmental selection pressure (Bouizgarne et al., 2014; Jiao et al., 2020).

Conclusions

Our study showed that the compositions, structures and assemblies of bacterial communities in the bulk and rhizosphere soils were significantly affected by tillage practices. Specifically, the relative abundance of nitrogen-fixing bacteria increased, while the bacteria associated with carbon degradation decreased in the soils with NT and RT compared with those with MP. Additionally, the bacterial networks in soils with NT and RT were more robust to environmental disturbances than those in soils with MP. The deterministic processes played a predominant role in the assemblage of bacterial communities under the three tillage practices, and the relative contribution of the homogenizing dispersal process was greater in both the bulk and rhizosphere soils with MP than in those with NT and RT. Soil pH played a major role in mediating bacterial community structures and assembly processes under the different tillage practices. Overall, this study provides a variety of evidence that in comparison to conventional tillage practices, conservation tillage practices were more conducive to biological nitrogen fixation and soil carbon sequestration.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figures

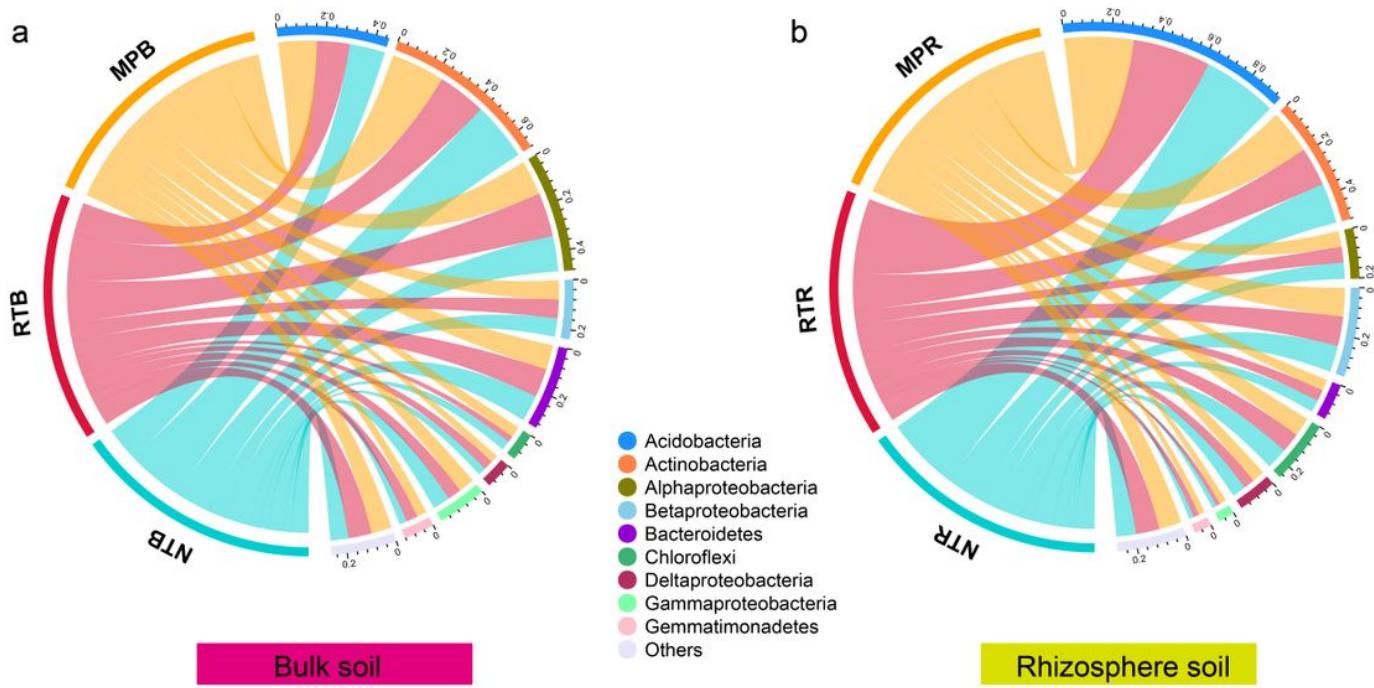


Figure 1

The bacterial taxonomic distribution is shown as a circos plot at the phylum/class level in the bulk (a) and rhizosphere (b) soils under the different tillage practices. The thickness of each ribbon represents the relative abundances of bacterial phyla/classes. NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil. For example, NTB represents soil samples collected under no-tillage in bulk soils.

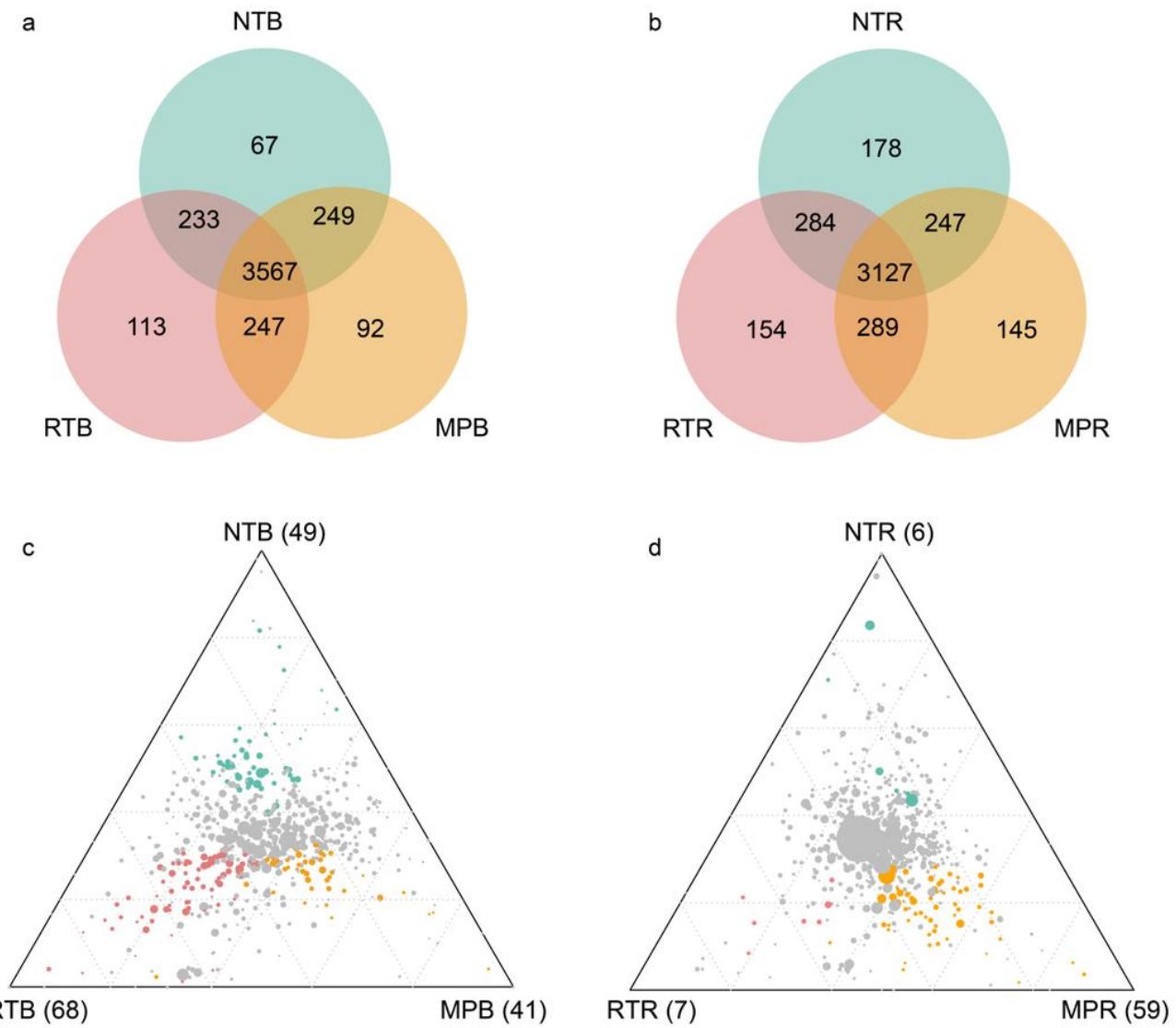


Figure 2

Venn diagrams showing the number of shared and unique bacterial OTUs in the bulk (a) and rhizosphere (b) soils under the different tillage practices. Ternary plot showing the enriched bacterial OTUs in the bulk (c) and rhizosphere (d) soils. Each circle represents one OTU, and the sizes of each circle represent its relative abundance. The treatment description is same as Figure 1. Green, red and orange circles mark OTUs significantly enriched in soils with NT, RT and MP, respectively. The values in the brackets indicate the number of enriched OTUs.

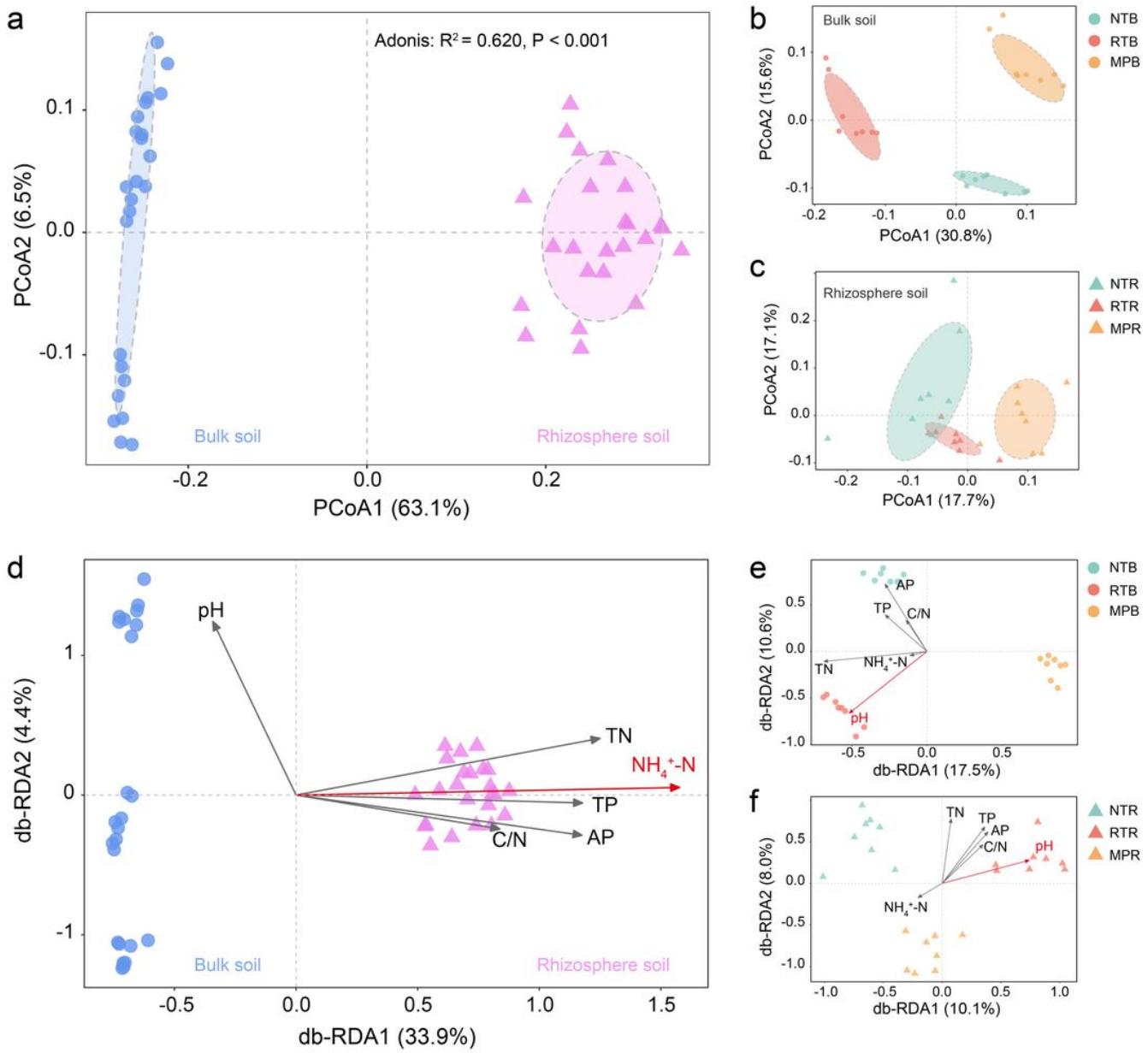


Figure 3

Principal coordination analysis (PCoA) of soil bacterial community structures and distance-based redundancy analysis (db-RDA) of the relationships between soil environmental factors and soil bacterial communities in the bulk and rhizosphere soils among the three tillage practices based on Bray-Curtis distance. All 48 soil samples (a and d) and 24 bulk soil (b and e) and rhizosphere soil samples (c and f). NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil.

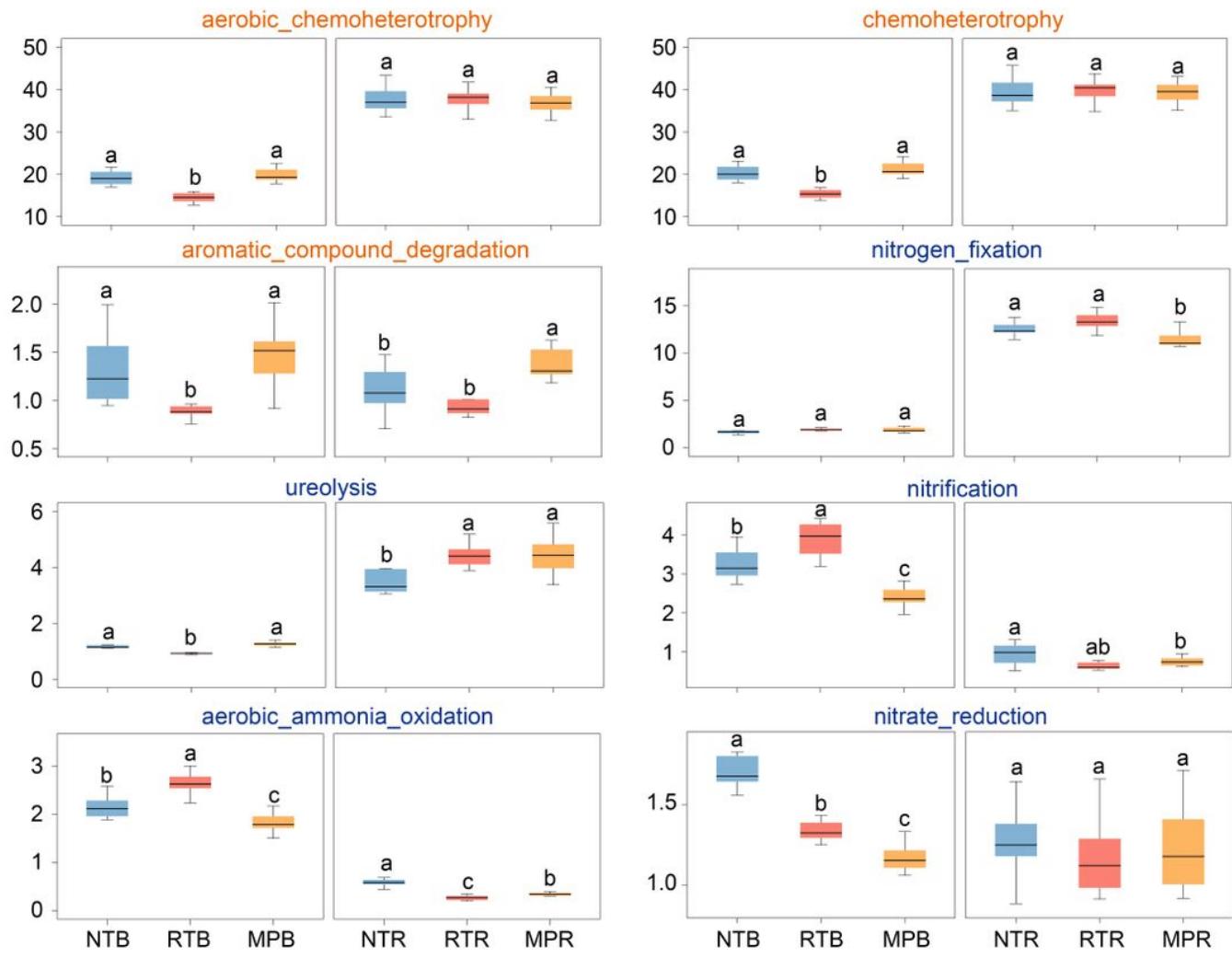


Figure 4

Functional prediction of bacterial communities in the bulk and rhizosphere soils under the different tillage practices using FAPROTAX. Different letters indicate significant differences among the three tillage treatments at the $P < 0.05$ level. The dominant functional groups (mean relative abundance $> 1\%$ in at least one treatment) associated with the soil carbon cycle and nitrogen cycle are highlighted in orange and blue font, respectively. NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil.

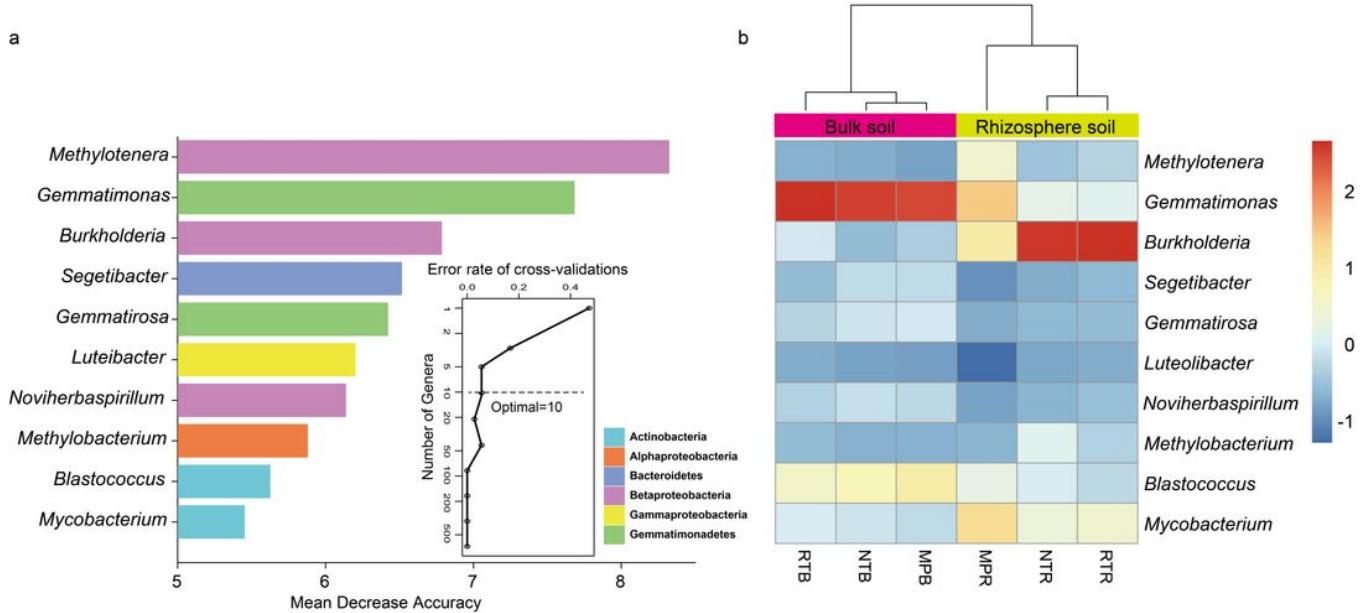


Figure 5

The bacterial biomarkers were identified at the genus level by random forest analysis. The top 10 biomarkers were ranked in descending order of importance (a). Heatmap showing the variations in the relative abundances of the biomarkers in the bulk and rhizosphere soils under the different tillage practices (b). NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil.

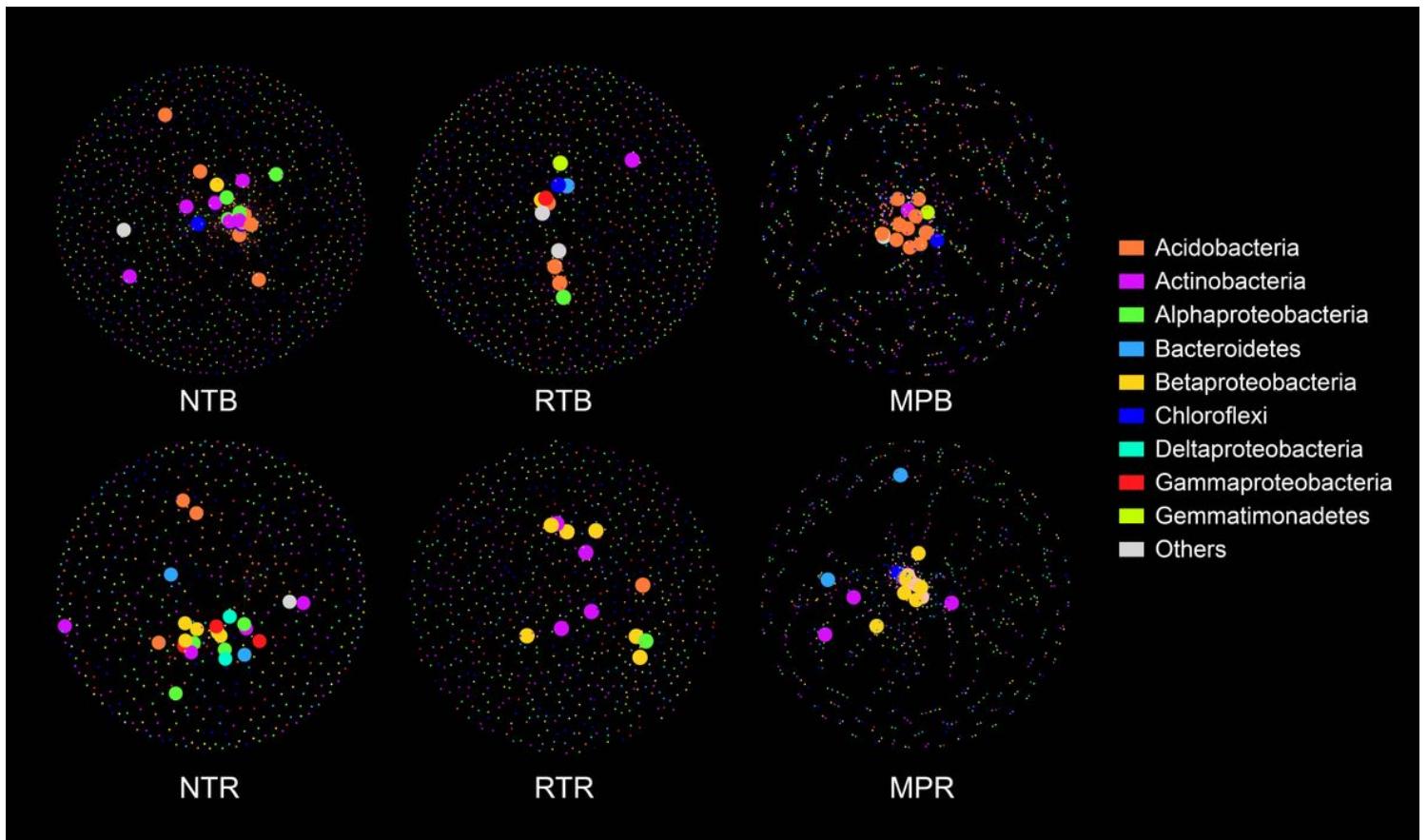


Figure 6

Bacterial ecological networks displayed at the OTU level in the bulk and rhizosphere soils under the different tillage practices. A connection indicates a strong (Spearman's $\rho > 0.6$) and significant ($P < 0.01$) correlation. Each node represents a unique OTU. The nodes colored by phyla/classes and the keystone taxa are magnified. NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil.

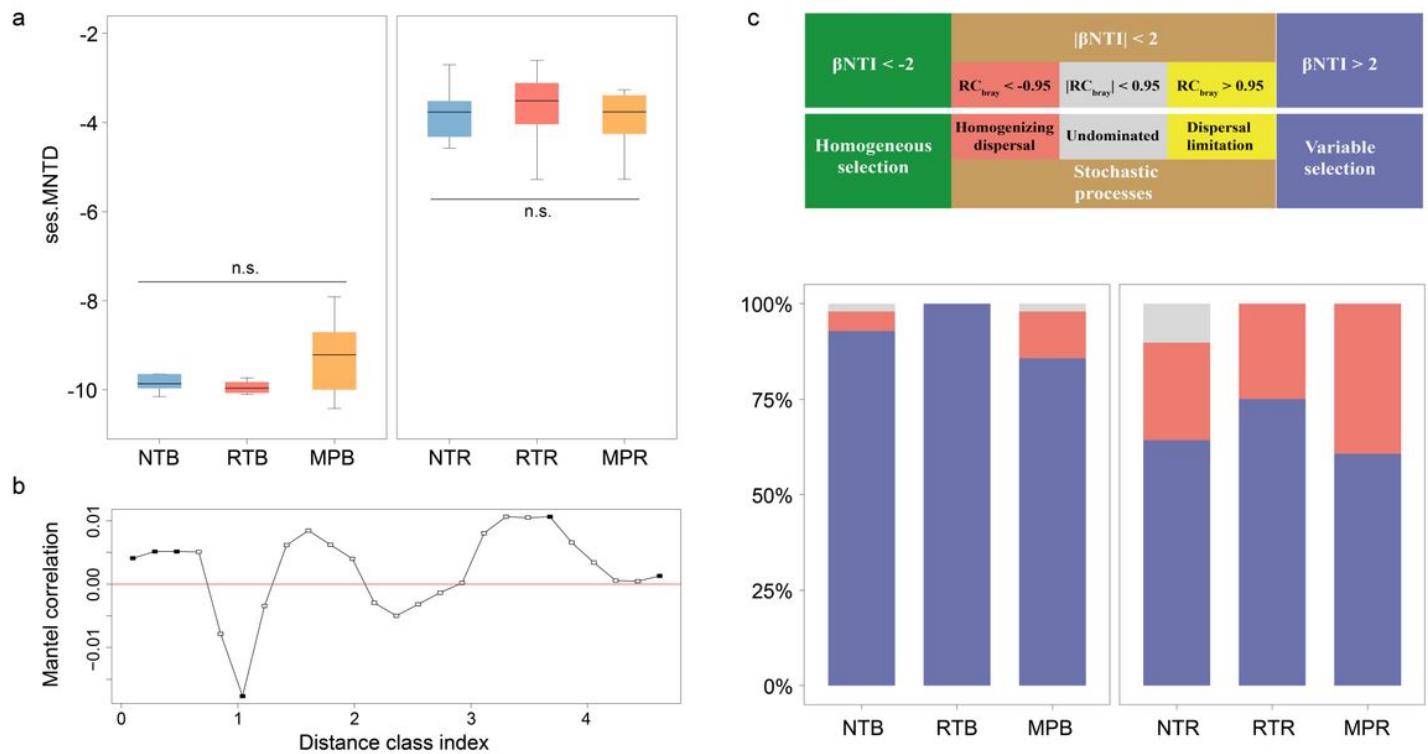


Figure 7

Relative impacts of ecological processes on bacterial community assembly under the different tillage practices in the bulk and rhizosphere soils. The standardized effect size measure of the mean nearest taxon distance (ses.MNTD) was shown in the bulk and rhizosphere soils within the different tillage practices (a), n.s. represent no significant difference ($P > 0.05$). Mantel correlograms between the phylogenetic distances of two OTUs and their niche distances. Solid points indicate significant correlations ($P < 0.05$) (b). The relative contributions of five ecological assembly processes with the three tillage practices (c). NT, RT and MP represent no-tillage, ridge tillage and moldboard plow tillage, respectively; B: bulk soil; R: rhizosphere soil.

Supplementary Files

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