Deep vertical rotary tillage increases the diversity of bacterial communities and alters the bacterial network structure in soil planted to corn

Authors: Xia, Wanyu, Ren, Xiaoyue, and Chen, Yanyun

Source: Canadian Journal of Soil Science, 102(4) : 946-958

Published By: Canadian Science Publishing

URL: https://doi.org/10.1139/cjss-2021-0208

Canadian Journal of Soil Science

Article

Deep vertical rotary tillage increases the diversity of bacterial communities and alters the bacterial network structure in soil planted to corn

Wanyu Xia[a], **Xiaoyue Ren**[a], **and Yanyun Chen**[b]

[a]School of Ecological Environment, Ningxia University, Yinchuan, Ningxia 750021, China; [b]School of Life Sciences, Ningxia University, Yinchuan, Ningxia 750021, China

Corresponding author: **Yanyun Chen** (email: nxchenyy@163.com)

Abstract

Deep vertical rotary tillage (DVRT) is a novel technique; however, its influence on soil bacterial diversity and community structure remains unclear. Herein, it was hypothesized that this tillage method significantly improves the bacterial diversity and alters the bacterial community structure and therefore it supports enhanced soil ecosystem functions in cultivated land. We investigated the soil bacterial communities and performed molecular ecological network analysis of cultivated land soils under different tillage regimes using high-throughput 16S rRNA gene Illumina sequencing. Soil samples were collected from the experimental field under 2 treatments: DVRT and conventional rotary tillage (CT) in Shizuishan City, Ningxia, China, in a 2-year field experiment. The α-diversity indices showed that DVRT resulted in higher bacterial diversity. In addition, the principal coordinate analysis results revealed a clear separation among the groups of cultivated land soils under the 2 treatment regimes. The key physicochemical factors that significantly influenced bacterial diversity and community structure were pH and total potassium concentration. The network analysis indicated that the bacterial network of DVRT soils consisted of more functionally interrelated bacterial modules than that of soils under CT, and the topological roles of characteristic bacteria and key bacteria were also different. In relation to CT, the relative abundances of organisms belonging to the functional groups of "Xenobiotics biodegradation and metabolism", "Signal transduction", and "Metabolism of cofactors and vitamins" were significantly increased in cultivated land soils under DVRT. It was concluded that DVRT treatment could improve bacterial diversity, alter the bacterial network structure, and enhance potential ecosystem functions in soils of cultivated land.

Key words: deep vertical rotary tillage, bacterial diversity, Tax4Fun, soil properties, network analysis

Résumé

Le labour rotatif vertical en profondeur (LRVP) est une nouvelle technique dont on connaît mal l'incidence sur la diversité et la structure de la microflore tellurique. Les auteurs ont formulé l'hypothèse que cette pratique améliore la diversité des bactéries et modifie la structure de leur population, donc améliore le fonctionnement de l'écosystème dans les terres cultivées. Pour le vérifier, ils ont examiné la microflore du sol et procédé à une analyse moléculaire du réseau écologique dans les terres cultivées selon différents régimes de travail du sol. L'analyse reposait sur la méthode Illumina de séquençage à haut débit des gènes de l'ARNr 16S. Les échantillons de sol venaient d'une parcelle soumise à deux régimes (LRVP et binage rotatif classique ou BR) dans le cadre d'une expérience sur le terrain de deux ans à Shizuishan, dans la régions autonome du Ningxia, en Chine. Les indices alpha de la diversité montrent que le LRVP accroît la diversité de la population bactérienne. En outre, les résultats de l'analyse en coordonnées principales révèlent une nette distinction entre les groupes présents dans les sols soumis aux deux régimes. Les principaux facteurs physicochimiques qui exercent une influence notable sur la diversité et la structure de la microflore sont le pH et la concentration totale de potassium (KT). L'analyse du réseau indique que le réseau de bactéries dans les sols LRVP se compose de modules bactériens plus étroitement liés sur le plan fonctionnel que dans les sols BR, et que les bactéries caractéristiques et les bactéries essentielles ont des rôles topologiquement différents. Les organismes appartenant aux groupes fonctionnels « biodégradation et métabolisme des xénobiotiques », « transduction des signaux » et « métabolisme des cofacteurs et des vitamines » était nettement plus abondants dans les sols cultivés soumis au régime LRVP que dans ceux assujettis au régime BR. On en conclut que le LRVP pourrait améliorer la diversité de la microflore, modifier la structure de cette dernière et rehausser les fonctions potentielles de l'écosystème dans le sol des terres agricoles. [Traduit par la Rédaction]

Mots-clés : labour rotatif vertical en profondeur, diversité des bactéries, Tax4Fun, propriétés du sol, analyse de réseau

Introduction

Corn (*Zea mays* L.), as an annual herbaceous plant in the family Gramineae, is an important food and feed crop and the most abundant crop in the world. At present, the conventional rotary tillage (CT) is widespread in China, and this kind of tillage is mostly used for corn cultivation in the Yellow River Irrigation Area of Ningxia. However, conventional farm management leads to accelerated soil erosion, environmental pollution, and soil degradation, and severely affects ecosystem functions (Montgomery 2007; Choudhury et al. 2014). Y. Zhang et al. (2018) proposed that traditional tillage results in a compact and closed plough pan, which hinders the circulation of soil water, fertilizer, and heat in the deep soil layer. To address the above issues, improve soil fertility, and increase crop production, agricultural researchers have explored a variety of tillage methods, such as zero tillage, rotary tillage, and deep vertical rotary tillage (DVRT) (Afzalinia et al. 2012; Zhai et al. 2017; Anand et al. 2018; Tang et al. 2021). DVRT is a recently implemented tillage method in China, which is widely used in cultivated soils in different climatic zones. DVRT is a deep-tillage technology that combines the advantages of subsoiling, deep tillage, rotation tillage, and vertical tillage (Wu et al. 2021). It is carried out by an innovative powerful machine equipped with 6 vertical spiral drills, and allows compacted plough pans to be broken up and soil layers to be loosened without disturbing soil horizons and producing a new hardpan (Wei 2017). Wu et al. (2021) concluded that compared with traditional shallow rotary tillage, DVRT had stronger beneficial effects on farmland soil quality, water storage capacity, winter wheat (*Triticum aestivum* L.) grain yield, and water and nitrogen use efficiency. In another study, DVRT could alleviate subsoil compaction and create a more favorable soil environment for crop growth (Li et al. 2020). However, it was concluded that the same tillage methods lead to increased soil compaction in the long term, which results in increased soil bulk density, decreased soil porosity and impedes root penetration. Asargew et al. (2021) studied the effects of 2 tillage practices (reduced tillage and conventional tillage), 2 planting methods (row planting and broadcast planting), and 2 compaction options (with and without trampling) on soil loss and teff yields in a split–split plot arrangement and concluded that conventional tillage practices and integrated planting systems increased soil losses and reduced teff productivity.

Soil microorganisms play vital roles in maintaining biodiversity, improving soil carbon sequestration, providing ecosystem functions, and enabling nutrient cycling in both natural and agricultural ecosystems (Hinsinger et al. 2009). Soil microbial communities are influenced by various factors, including tillage method, climate, fertilization, and pH (Essel 2019; Wang et al. 2019; Jat et al. 2020). Significant improvements in soil microbial biomass and favorable changes in microbial diversity have been observed in agricultural soils in response to DVRT (Zhou et al. 2020; Lao et al. 2021). In contrast, the application of conventional tillage could lead to a decrease in soil microbial diversity, which is commonly linked to reduced soil fertility (Franzluebbers and Arshad 1997). At present, information on the influence of DVRT on the soil bacterial community and ecosystem function of cultivated land is lacking.

Network analyses have often revealed non-random covariation patterns that may reflect community organization, such as direct interactions or shared guilds/niches, and provide a tool for investigating ecological concepts that are difficult to assess in microbial communities (Shi et al. 2016). The analysis of taxonomic co-occurrence network patterns offers new insights into keystone populations, as well as into significant module memberships in biotic communities (Chen et al. 2017); many studies have used this method to detect co-occurrence relationships among microbial communities (Deng et al. 2012; Banerjee et al. 2016). Network analysis can also be used to explore changes in microbial interactions or responses in intercropping soils (Wu et al. 2020).

In this study, we analyzed the effect of DVRT on the bacterial community and ecosystem function in a field experiment in soils of cultivated land using high-throughput 16S rRNA gene Illumina sequencing and molecular ecological network analysis. The objectives of our research were to (*i*) study the differences in bacterial diversity and community structure between cultivated land soils under DVRT and CT; (*ii*) analyze the correlation between soil physical and chemical properties and bacterial community distribution and diversity in cultivated land soil under different tillage methods; and (*iii*) reveal the influence of DVRT treatment on the changes of bacterial network structure, key bacterial organisms, and functional groups in the bacterial community of cultivated land soils compared with CT treatment. We hypothesized that DVRT treatment would significantly improve the bacterial diversity and alter the bacterial network structure and potential function, leading to the enhancement of additional soil ecosystem functions in cultivated land.

Materials and methods

Site description and sample collection

The field experiment was conducted in Touzha Village (38°55′52″N, 106°39′39″E), Pingluo County, Shizuishan City, Ningxia Hui Autonomous Region, China, between April and September 2019, and April and September 2020. The climate is temperate continental with an altitude of 1091 m, annual evaporation of 1755 mm, average wind speed of 2–3 m s^{-1}, annual frost-free period of 171 days, annual rainfall of 184 mm, and annual mean temperature of 8.21 °C. The soil in the test area is anthropogenic-alluvial soil. Before 2019, the experimental plots were planted with wheat, and the method used was CT.

In this study, 2 treatments were evaluated, namely CT and DVRT. These 2 treatments were replicated 6 times each in a complete block design, giving a total of 12 plots. Each plot had an area of 75 m^2 (6 m × 12.5 m), and was marked with protection lines all around.

A single corn variety (Dika 5) was used in the experiment. Vertical rotary tillage was carried out on April 25, 2019 and April 11, 2020. The machine used was a suspended ridge machine provided by Wuzhong Yihe Agricultural Machinery Operation Service Co., Ltd. At the same time, conven-

tional tillage operations were carried out with rotary tiller. A commercial compound fertilizer ($N:P_2O_5:K_2O \approx 12\%:25\%:5\%$; Kangsheng Fertilizer Industry Co., Ltd., Shizuishan City, Ningxia Province, China) was applied at 450 kg ha^{-1}. All fields were treated using identical fertilization and field management practices. On April 25, 2019, and April 11, 2020, the seeds were planted mechanically, with a row spacing of 0.5 m and a plant spacing of 0.25 m. In 2019, the first irrigation was carried out on June 10 and the second irrigation on July 12. In 2020, the first irrigation was carried out on June 22, and the second irrigation on July 15.

Soil samples (0–20 cm soil layer) were collected from 5 random locations within each replicate and mixed together to form composite samples. The soil samples to represent environmental background values were collected on April 8, 2019, bringing the number of collected samples to 6, and the soil samples from DVRT and CT treatments were collected during the corn harvest period on September 24, 2019 and September 15, 2020, making the total number of collected samples 24. The sterile bag containing the samples was transported back to the lab, and then samples were sieved indoors through a 2 mm mesh into 2 parts: 1 was used to determine the physicochemical properties, and other was stored at −80 °C for later DNA extraction.

Soil physical and chemical analysis

After the soil samples were air dried, the following soil physicochemical properties were determined: soil water content (WC) was determined by the drying method, pH was measured with a pH meter (pHS-3C), electrical conductivity (EC) was measured by a conductivity meter (DDS-308A), total organic carbon (TOC) was determined by a TOC instrument (multi N/C 2100S, Analytik Jena), total nitrogen (TN) was quantified by a Kjeldahl nitrogen analyzer, alkali-hydrolyzed nitrogen (AN) was determined by alkaline–hydrolysis diffusion method, total phosphorus (TP) was measured by the perchloric acid-concentrated sulfuric acid and molybdenum blue colorimetric method, available phosphorus (AP) was determined by sodium bicarbonate leaching in the molybdenum antimony anti-colorimetric method, total potassium (TK) was determined by using hydrofluoric acid in the perchloric acid digestion flame photometer method, and available potassium (AK) was extracted with neutral ammonium acetate and determined by flame photometry (Lu 2000). The soil environmental background values were as follows: WC, 12.38%; pH, 8.53; EC, 1.38 mS cm^{-1}; TOC, 20.63 g kg^{-1}; TN, 0.86 g kg^{-1}; AN, 38.38 mg kg^{-1}; TP, 0.58 g kg^{-1}; AP, 34.69 mg kg^{-1}; TK, 20.63 g kg^{-1}; and AK, 42.81 mg kg^{-1}.

DNA extraction and PCR amplification

The bacterial community genomic DNA was extracted from 24 samples using the FastDNA® SPIN for soil kit (MP Biomedicals, Solon, USA) according to the manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined by a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified using the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Zhang et al. 2017) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR amplification of 16S rRNA genes was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, single extension at 72 °C for 10 min, and holding at 4 °C. The PCR mixtures contained 5× 4 μL TransStart FastPfu buffer, 2 μL 2.5 mmol/L dNTPs, 0.8 μL forward primer (5 μm), 0.8 μL reverse primer (5 μm), 0.4 μL TransStart FastPfu DNA Polymerase, 10 ng template DNA, and finally ddH_2O to complement to 20 μL. The PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions, and quantified using a Quantus™ Fluorometer (Promega, USA).

Illumina MiSeq sequencing and processing of sequencing data

The purified amplicons from 24 samples were pooled in equimolar quantities and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0, and merged by FLASH version 1.2.7 (Magoč and Salzberg 2011). The operational taxonomic units (OTUs) with 97% similarity cutoff values (Stackebrand and Goebel 1994; Edgar 2013) were clustered using UPARSE version 7.1 (Edgar 2013), and any chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al. 2007) against the 16S rRNA database (Silva v132) using a confidence threshold of 0.7. All OTUs classified as mitochondria, chloroplast, or unclassified were removed before the analysis. Phylogenetic trees were calculated using maximum likelihood estimation in MEGA (version 10.0) (Chen et al. 2016).

Ecological and statistical analyses

The R software package (version 4.0.5) was utilized to perform the statistical analyses. The bacterial Shannon index and ACE index were calculated by QIIME (Quantitative Insights into Microbial Ecology, Version 1.9.1) (Caporaso et al. 2010), and the plot, boxplot, and qplot packages of the R software were employed to draw boxplot diagrams (Coker et al. 2017). The vegan and ggplot2 packages of the R software were employed to draw rarefaction curve (Quintanilla et al. 2018). Subsequently, QIIME software was used to calculate the Bray–Curtis distance, and the WGCNA, stats, and ggplot2 packages of the R software were used to draw principal coordinate analysis (PCoA) diagrams (Quintanilla et al. 2018). Distance-based redundancy analysis (db-RDA) was plotted using the R packages vegan and ggplot2 (Mack et al. 2016). The vegan package of R software was used to test the differences in the bacterial community structure among different treatments through PERMANOVA (Quintanilla et al. 2018). Additionally,

Table 1. Physicochemical properties and yield of cultivated soil under DVRT and CT treatments.

	2020		2019	
Soil properties	DVRT	CT	DVRT	CT
WC (%)	17.29 ± 0.36a	14.67 ± 0.64b	21.23 ± 0.46b	22.96 ± 1.01a
pH	7.91 ± 0.04b	8.35 ± 0.02a	10.01 ± 0.08b	10.79 ± 0.36a
EC (mS cm^{-1})	1.30 ± 0.00b	1.50 ± 0.01a	1.89 ± 0.01b	2.24 ± 0.01a
TOC (g kg^{-1})	23.54 ± 0.29a	22.23 ± 0.42b	28.70 ± 0.35a	28.75 ± 0.54a
TN (g kg^{-1})	1.15 ± 0.02a	1.07 ± 0.01b	1.17 ± 0.01a	1.17 ± 0.02a
AN (mg kg^{-1})	58.22 ± 1.25a	53.81 ± 0.02b	54.26 ± 0.81a	53.60 ± 1.71a
TP (g kg^{-1})	0.67 ± 0.01a	0.60 ± 0.02b	0.81 ± 0.02a	0.82 ± 0.03a
AP (mg kg^{-1})	55.14 ± 2.18a	40.76 ± 1.62b	54.98 ± 1.52a	55.65 ± 1.54a
TK (g kg^{-1})	23.44 ± 0.35a	19.54 ± 0.07b	27.16 ± 0.37b	27.80 ± 0.12a
AK (mg kg^{-1})	48.53 ± 0.00a	49.36 ± 1.29a	53.14 ± 0.26a	52.35 ± 1.58a
yield (t ha^{-1})	12.51 ± 0.49a	9.63 ± 0.64b	8.61 ± 0.26a	6.58 ± 0.32b

Note: Data are presented as mean ± SD, $n = 6$; the different letters in rows indicate significant differences ($P < 0.05$) according to the Student's t test for both cultivars. DVRT, deep vertical rotary tillage; CT, conventional rotary tillage. These notes also apply to Table 2 below.

PERMDISP was used to test for the homogeneity of multivariate dispersions between sampling groups (Quintanilla et al. 2018). The Mantel test was applied to evaluate the correlations between bacterial communities with environmental variables using the Mantel procedure in the R package vegan (Zaneveld et al. 2016). The correlations between bacterial phylum levels and predicted functional groups were then plotted using the heatmap package of R software (Gu et al. 2019). The bacterial ecological function profiles were predicted by Tax4Fun (Aßhauer et al. 2015). SPSS software (version 22.0.0) was used to conduct a Student's t test of independent samples to determine the functional group, bacterial diversity index, and soil physicochemical properties differences between treatments, and to analyze the correlation between bacterial α-diversity and soil physical and chemical properties. The variance inflation factor (VIF) of SPSS was used to analyze the environmental factors. The correlation analysis between bacterial diversity index, soil physicochemical properties, and yield was carried out by SPSS. When the environmental factor VIF > 10, the colinearity was obvious, and the environmental factor needed to be reduced. All results were presented as the mean ± standard deviation (SD).

Network construction and analyses

The below steps were followed for the ecological network construction using MENAP (http://129.15.40.240/mena/). (*i*) A relative abundance (RA) matrix and an OTU annotation file were prepared as per the pipeline guidelines. (*ii*) The RA matrix was submitted for network construction. A cutoff value (similarity threshold, st) for the similarity matrix was automatically generated using default settings. (*iii*) The calculations of "global network properties", "individual nodes' centrality", and "module separation and modularity" were performed (Deng et al. 2012). (*iv*) The "output for Cytoscape visualization" procedure was carried out in "greedy modularity optimization mode". The data network was then exported for visualization using Cytoscape software (Shannon et al. 2003). (*v*) The "randomize the network structure and then calculate network" procedure was conducted using the Maslov–Sneppen procedure to calculate random network properties while maintaining the same number of nodes and links as the empirical networks (Lu et al. 2013).

Results

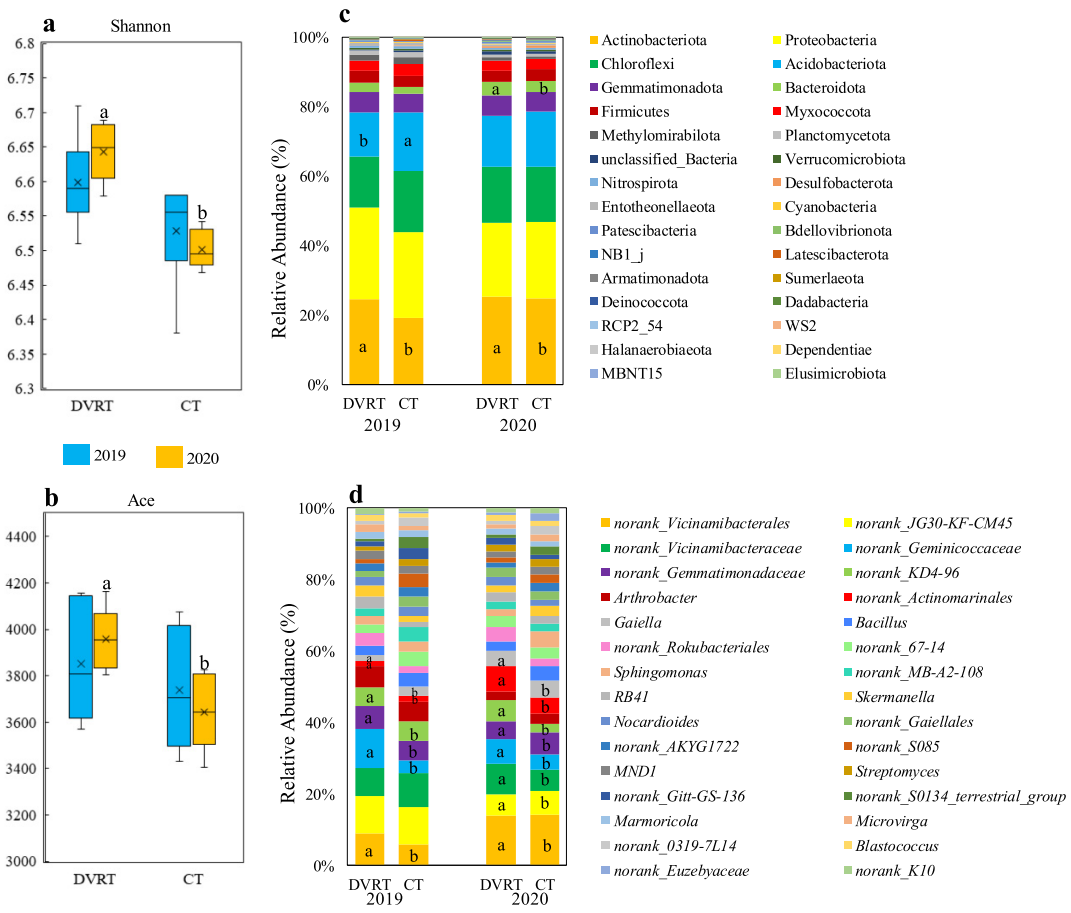
Effects of different tillage regimes on soil properties

The Analysis of variance (ANOVA) showed significant differences in soil physicochemical properties between different treatments ($P < 0.05$), as shown in Table 1. In 2019, the concentrations of WC, EC, pH, and TK in the samples under CT treatment were higher. In 2020, the concentrations of WC, TOC, TN, TP, AN, AP, and TK in the samples under DVRT treatment were higher, but the EC and pH values were lower compared with samples under CT treatment. In 2019 and 2020, the corn yield of DVRT treatment was significantly higher than that of CT treatment, and the yield increase rates were 30.72% and 29.85%. The 2020 DVRT treatment increased the corn yield by 45.33% compared with the 2019 DVRT corn yield.

Effects of different tillage treatments on the diversity, structure, and composition of bacterial communities

Based on the Illumina MiSeq sequencing results, the total raw reads of the 24 samples were 2 883 850, the clean reads were 2 726 681, and the OTUs were 6474. The rarefaction curves tended to approach a saturation plateau, suggesting that the sequencing depths were sufficient for downstream analysis (Fig. S1). The Shannon (Fig. 1*a*) and Ace (Fig. 1*b*) indices were determined to assess the α-diversity of soil bacterial communities in cultivated land under different tillage methods. According to the box diagram of the 2 diversity indices, there was a little difference between treatments in 2019. In 2020, the level of bacterial diversity in DVRT was significantly higher than the one in CT.

Fig. 1. Alpha diversity and structural composition of bacterial community. Ace (*a*) and Shannon (*b*) indices of the bacterial community. Top 30 bacterial phyla (*c*) and genera (*d*) of soil under DVRT and CT. The different letters indicate statistically significant differences at $P < 0.05$ as determined by the Student's *t* test for independent samples, $n = 6$. DVRT, deep vertical rotary tillage; CT, conventional rotary tillage. [Color online]



All OTUs were divided into 1005 genera and 44 phyla. Figure 1*c* shows the top 30 bacterial phyla (relative sequence abundance > 1%) in all samples. They included Actinobacteria (23.52%), Proteobacteria (23.60%), Chlorella (16.06%), Acidobacteria (15.02%), Gemmatimonadota (5.73%), Bacteroidota (2.93%), Firmicutes (3.27%), and Myxococcota (3.02%), accounting for 93.14% of bacterial sequences. Figure 1*d* shows the top 30 bacterial genera (relative sequence abundance > 1%) detected in all samples. Most bacterial genera were unclassified and untapped, with the most abundant groups of *norank_Vicinamibacterales* (10.69%), *norank_JG30-KF-CM45* (8.44%), *norank_Vicinamibacteraceae* (7.88%), *norank_Geminicoccaceae* (6.44%), *norank_Gemmatimonadaceae* (5.77%), *norank_KD4-96* (4.73%), *Arthrobacter* (4.21%), *norank_Actinomarinales* (3.65%), *Gaiella* (3.34%), and *Bacillus* (3.30%), the top 10 genera accounting for 58.44% of bacterial sequences. When comparing the abundance of bacterial phyla between DVRT and CT, that of Actinobacteriota was significantly higher in DVRT than in CT. Acidobacteria were significantly more abundant in CT than in DVRT in 2019, but the difference between DVRT and CT treatments in 2020 was not significant. Bacteroidota had significantly higher abundance in DVRT than in CT in 2020. When comparing the top 10 abundance of bacterial genera between DVRT and CT, it was found that that of 6 genera was significantly higher than CT treatment in 2019 and 2020, namely *norank_Vicinamibacterales*, *norank_Geminicoccaceae*, *norank_Gemmatimonadaceae*, *norank_KD4-96*, *norank_Actinomarinales*, and *Gaiella*. The abundances of *norank_JG30-KF-CM45* and *norank_Vicinamibacteraceae* were significantly higher in DVRT treatment than in CT treatment in 2020. In short, the DVRT and CT treatments showed a significant divergence in the composition and relative abundance of soil bacterial community, with a more positive impact of DVRT in 2020 on bacterial community structure.

The β-diversity of soil bacterial communities in cultivated land under different tillage methods was further analyzed. The PCoA map of the bacterial community indicated that both the DVRT and CT treatments significantly affected the soil bacterial community in 2019 and 2020 (Fig. 2*a*). Moreover, significant differences in community compositions were inferred between these 4 samples (PERMANOVA, $P < 0.05$, Table S1), and nonsignificant differences in the homogeneity of multivariate dispersions were also inferred (PERMDISP, $P > 0.05$, Table S1). To study the relationship between soil properties and bacterial community structure, Bray–Curtis

Fig. 2. Analysis of similarity and environmental factors of bacterial community. (*a*) Principal coordinate analysis (PCoA) of bacterial community composition under DVRT and CT. (*b*) Distance-based redundancy analysis (db-RDA) of 16S rRNA genes and soil characteristics. DVRT-2019, deep vertical rotary tillage in 2019; CT-2019, conventional rotary tillage in 2019; DVRT-2020, deep vertical rotary tillage in 2020; CT-2020, conventional rotary tillage in 2020. [Color online.]

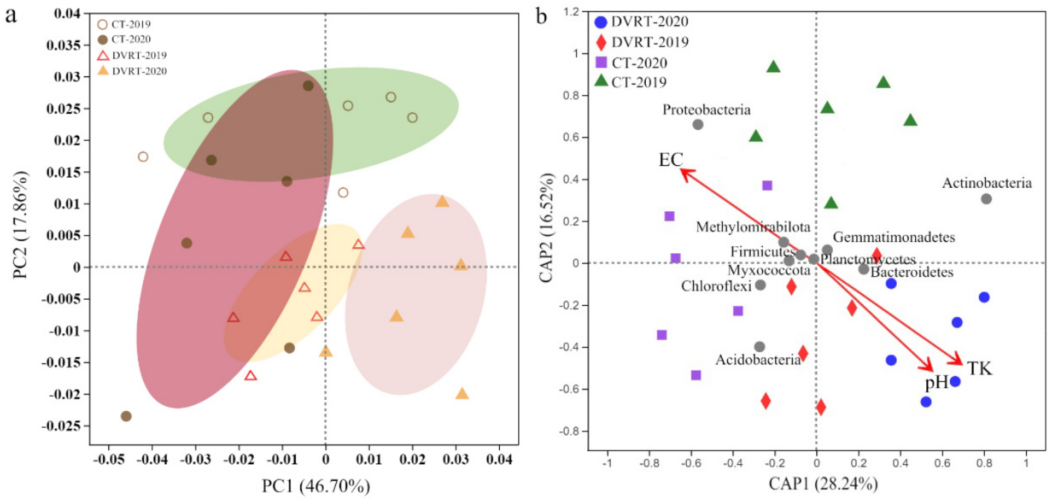


Table 2. Correlation analysis between bacterial α-diversity with soil physicochemical properties and yield.

Soil properties	Shannon		Ace	
	r	*P*	*r*	*P*
WC	0.669	0.002**	0.774	0.000***
pH	−0.708	0.001**	−0.831	0.000***
EC	−0.619	0.006**	−0.539	0.021*
TOC	0.675	0.002**	0.741	0.000***
TN	0.513	0.030*	0.603	0.008**
AN	0.457	0.057	0.635	0.005**
TP	0.662	0.003**	0.864	0.000***
AP	0.671	0.002**	0.852	0.000***
TK	0.758	0.000***	0.810	0.000***
AK	0.291	0.241	0.418	0.085
yield	0.827	0.001**	0.775	0.003**

Note: *r* is the Pearson's correlation coefficient, $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

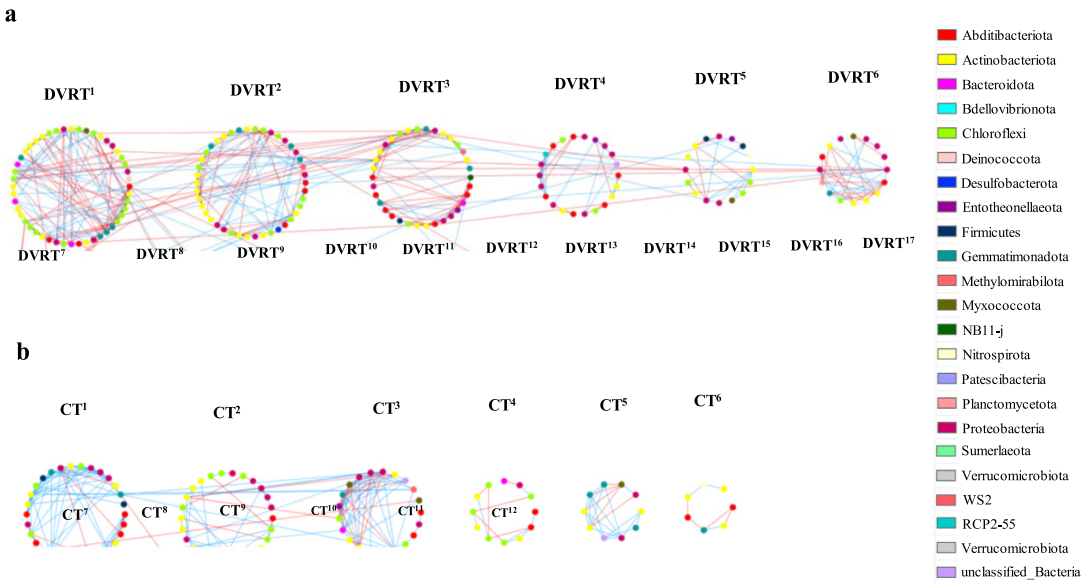
Table 3. Partial Mantel test of correlation between soil properties and bacterial community structure based on Bray–Curtis distance methods.

Soils properties	Bray–Curtis	
	r	*p*
WC	−0.056	0.750
pH	0.799	0.001***
EC	0.577	0.001***
TOC	0.327	0.001***
TN	0.705	0.001***
AN	0.779	0.001***
TP	0.201	0.021*
AP	−0.221	0.999
TK	0.254	0.016*
AK	−0.162	0.973

db-RDA was carried out. Before db-RDA, 3 environmental factors with VIF < 10 were screened by VIF: pH (VIF = 3.192), EC (VIF = 3.015), and TK (VIF = 6.265), which revealed the importance of the abovementioned soil properties and their impact on the soil bacterial community (Fig. 2*b*). The db-RDA plot clearly demonstrated that pH and TK were correlated with DVRT-2019 and DVRT-2020. Thus, pH and TK were considered to be the key physicochemical factors in the assembly of DVRT bacterial community structure.

The Pearson correlation analysis was used to evaluate the correlation between soil physicochemical properties, yield, and α-diversity index of bacterial communities (Table 2). The results showed that, except for AK concentration, the soil physicochemical properties and yield were significantly correlated with the Shannon and Ace indices. Among them, the Pearson's correlation coefficients of the 2 indices of pH value and TK concentration were all between $0.7 \leq |r| \leq 1$, which are highly linearly related soil physicochemical properties. Therefore, we consider that pH and TK are the major physicochemical factors significantly influencing the bacterial community. We further conducted Bray–Curtis Partial Mantel tests to determine the principal physicochemical factors affecting the bacterial community (Table 3). It was confirmed that the concentrations of pH and TK were significantly correlated with the bacterial communities (pH, TK, $P < 0.001$); therefore, we think that these 2 parameters are major physicochemical factors significantly influencing the bacterial community of cultivated land soil in this study.

Fig. 3. Phylogenetic molecular ecology networks (pMENs) of bacterial communities in cultivated land soils based on the RMT analysis of OTU profiles; (*a*) and (*b*) are networks of soils under DVRT and CT, respectively. Modules larger than 5 nodes are labeled with different colors in the respective networks. The links with red color represent negative interactions and those with blue represent positive interactions. OTUs that acted as generalists were labeled in the networks. The networks were constructed using an RMT-based model and visualized by Cytoscape 3.5.1. Nodes represent OTUs, and lines represent connecting nodes (links). DVRT, deep vertical rotary tillage; CT, conventional rotary tillage. [Color online.]



Molecular ecological network analysis of soil bacterial communities under DVRT and CT treatments

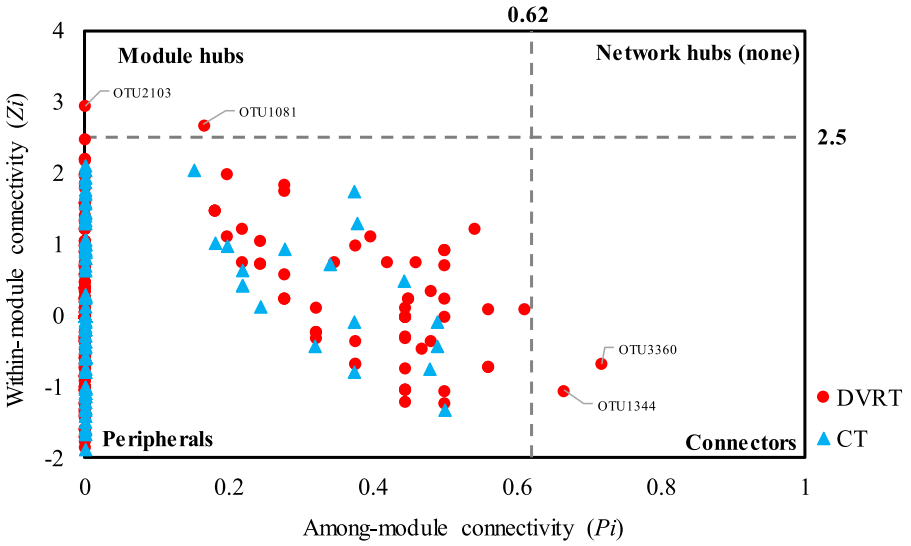
To reveal the difference in cultivated land soil bacterial community under DVRT and CT treatments, phylogenetic molecular ecology network analysis using 16S rRNA gene data was carried out (Fig. 3). The topology properties of the network were summarized in Table S2. The visualized networks of DVRT (Fig. 3*a*) and CT (Fig. 3*b*) treatments processed 13 and 8 modules, respectively. The heatmap of correlations between module eigengenes and soil properties for the DVRT and CT networks based on the Mantel test is shown in Fig. S2. There is a greater number of significant correlations between the module and soil characteristics in the DVRT network ($P < 0.05$) (11 correlations, with 3 being negative) compared with the CT network (5 correlations, with 1 being negative). In the DVRT network, the pH was significantly associated with 5 modules, the concentrations of TOC, AN, TP, and AP were significantly correlated with 1 module, and the TK concentration was significantly associated with 1 module. In the CT network, the pH was significantly correlated with 2 modules, and the EC was significantly associated with 3 modules. Overall, these results indicate that network structure and interactions are related to different soil characteristics under each treatment regime.

A Zi–Pi plot was drawn to show the distribution of OTUs in soils under DVRT and CT treatments based on their module-based topological roles (Fig. 4). In the plot, depending on the simplified classification, all nodes in the network are divided into 4 quadrants under the threshold of Zi = 2.5 and Pi = 0.62. The DVRT processing network is attributed to *norank_OLB14* (OTU2103; in Chloroflexi) and *norank_Microscillaceae* (OTU1081; in Bacteroidota) as 2 nodes classified as module hubs (generalists), which are highly connected to many nodes in their modules. Two nodes of the DVRT processing network, *norank_JG30-KF-CM45* (OTU3360; in Chloroflexi) and *norank_SC-I-84* (OTU1344; in Proteobacteria), are classified as connectors that are highly associated with multiple modules. No nodes belong to the network hub (supergeneralists). Therefore, generalists are key microorganisms in the soil bacterial community. The above results indicate that DVRT treatment increased the characteristic OTUs and key bacteria in the soil and had a great impact on the topology.

Functional prediction of bacterial communities in cultivated land soils under DVRT and CT treatments

Tax4Fun was used to predict the microecological functions of OTUs of cultivated land soil microbes under DVRT and CT treatments. The main soil functional groups detected were related to “Carbohydrate metabolism”, “Metabolism of cofactors and vitamins”, “Energy metabolism”, “Amino acid metabolism”, “Xenobiotics biodegradation and metabolism”, “Nucleotide metabolism”, “Membrane transport”, and “Signal transduction” (relative abundance ≥ 5%) (Fig. 5*a*). In addition, the abundance of functional groups in DVRT treatment soil “Xenobiotics biodegradation, metabolism” and “Signal transduction” and “Metabolism of cofactors and vitamins” was significantly higher than that of CT treatment ($P < 0.05$). The heatmap analysis between bacterial phyla and functional groups showed that most bacteria

Fig. 4. Zi–Pi plot showing the distribution of OTUs based on their topological roles. Each symbol represents an OTU in the DVRT (red circle) or CT (blue triangle) network. The threshold values of Zi and Pi for categorizing OTUs are 2.5 and 0.62, respectively. DVRT, deep vertical rotary tillage; CT, conventional rotary tillage. [Color online.]



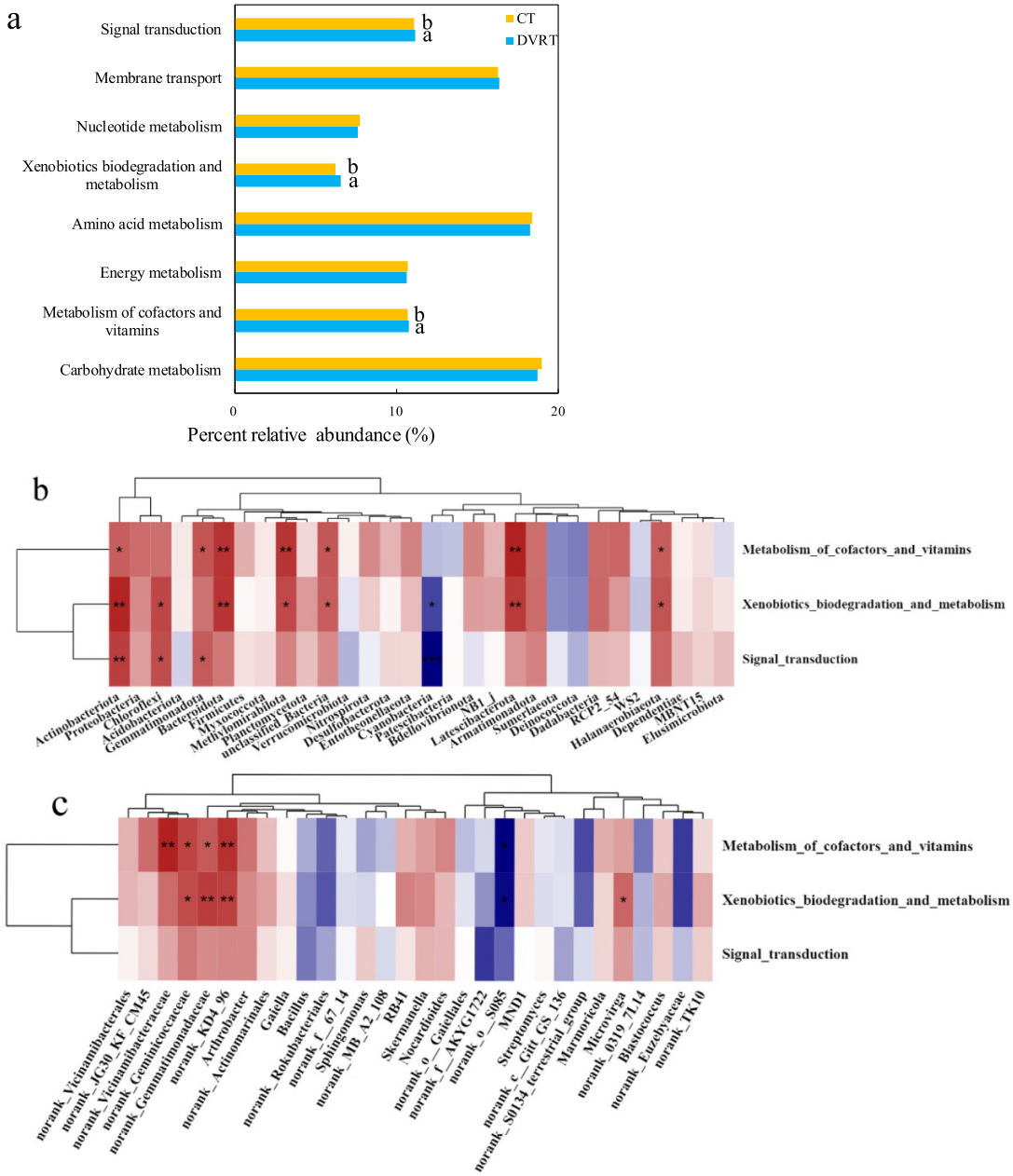
exhibited "Xenobiotics biodegradation and metabolism", "Metabolism of cofactors and vitamins", and "Signal transduction" (Fig. 5*b*). The DVRT and CT processing functions predicted significant differences in the secondary metabolic paths of several bacterial phyla, for example, "Metabolism of cofactors and vitamins" (Actinobacteriota, Gemmatimonadota, Bacteroidota, Methylomirabilota, unclassified_Bacteria, Latescibacterota, Halanaerobiaeota), "Xenobiotics biodegradation metabolism" (Actinobacteriota, Chloroflexi, Bacteroidota, Methylomirabilota, unclassified_Bacteria, Cyanobacteria, Latescibacterota, Halanaerobiaeota), and "Signal transduction" (Actinobacteriota, Chloroflexi, Gemmatimonadota, Cyanobacteria). The heatmap analysis between bacterial genus and functional groups showed that most bacteria exhibited "Xenobiotics biodegradation and metabolism" and "Metabolism of cofactors and vitamins" (Fig. 5*c*). The DVRT and CT processing functions predicted significant differences in the secondary metabolic paths of several bacterial genera, for example, "Metabolism of cofactors and vitamins" (*norank_Geminicoccaceae*, *norank_Gemmatimonadaceae*, *norank_KD4-96*, *norank_S085*) and "Xenobiotics biodegradation metabolism" (*norank_Vicinamibacteraceae*, *norank_Geminicoccaceae*, *norank_Gemmatimonadaceae*, *norank_KD4-96*, *norank_S085*, *Microvirga*).

Discussion

The choice of tillage method shapes the soil microbes and other soil properties (Wei et al. 2017; X.F., Zhang et al. 2018; M. Chen et al. 2020; Liu et al. 2022). However, there has been less concern about how vertical rotary tillage affects the soil bacterial community and ecosystem functions in corn farmlands. Studies have shown that DVRT changes the soil properties of cultivated land (Chen et al. 2017; Zhai et al. 2017; X.F. Zhang et al. 2018). Previous studies have demonstrated that DVRT treatment could improve the pH value and TK concentration in soils (Wei et al. 2017; M. Chen et al. 2020). These findings are consistent with our results, i.e., the application of DVRT could lower the soil pH and increase the TK concentration (Table 1). Yin et al. (2015) found that the soil microbial community structure and abundance are related to changes in soil pH value and TK concentration. The distribution and activity of microorganisms are principally affected by soil pH, and TK has also been proven to be a key factor in determining the composition and diversity of the soil bacterial community (Zi et al. 2018). In addition, the PCoA analysis results revealed that there were significant differences in soil microbial community structure between different tillage methods, which is also in good agreement with previous studies(Zhou et al. 2020). The soil bacterial communities were altered by the change of tillage method through the mediation of soil properties, and some of these changes, especially for pH and TK concentration, were significantly correlated with bacterial diversity and community structure. Therefore, the soil samples under DVRT exhibited higher bacterial diversity, and the corn yield significantly increased year by year.

In our study, the dominant phyla in soil samples in both tillage treatments (Fig. 1*c*) were generally Actinobacteriota (23.52%), followed by Proteobacteria (23.60%). Two consecutive years of DVRT treatment increased Acidobacteria and Bacteroidota in the soil. In a study by Yang (2021), the phylum-level analysis of the community revealed that Fenlong tillage (DVRT) significantly increased the abundance of dominant phyla in soil compared with CT, despite the different proportions of the top 10 bacterial phyla, which is consistent with our results. In our experiment, the bacterial genera whose relative abundance changed significantly were the key factors that led to the variation in the diversity of the bacterial commu-

Fig. 5. Tax4Fun prediction of soil bacterial community function under DVRT and CT. (*a*) Relative major functional groups; different letters indicate statistically significant differences at $P < 0.05$ as determined by the Student's *t* test for independent samples. (*b*) Correlation between bacterial phylum level and predictive function group. (*c*) Correlation between bacterial genus level and predictive function group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. DVRT, deep vertical rotary tillage; CT, conventional rotary tillage. [Color online.]



nity. Genus-level studies also showed that DVRT treatment significantly increased the abundance of *norank_ Vicinamibacterales*, *norank_Geminicoccaceae*, *norank_Gemmatimonadaceae*, *norank_KD4-96*, *norank_Actinomanarales*, and *Gaiella*, and that *norank_JG30-KF-CM45* and *norank_Vicinamibacteraceae* were significantly increased in 2020 compared with 2019. *Norank_Vicinamibacterales* and *norank_Vicinamibacteraceae* belong to Acidobacteriota; these microorganisms are decomposers of organic matter and can improve soil quality (Zi et al. 2018). *Norank_Geminicoccaceae* belongs to Proteobacteria and may be related to the decomposition and transformation of organic matter in soil (Noah 1839; Li et al. 2016). Previous studies have shown that DVRT increased soil organic matter content (Nie et al. 2017; Wei 2017). On the one hand, weeds deplete soil nutrients and reduce organic matter content (Chen and Xue 2004). DVRT will reduce weed abundance in the field, decrease the competition between crops and weed roots, and alleviate the consumption of organic matter (Wei et al. 2011). On the other hand, DVRT enhances the stability of soil aggregates in cultivated land, decreases the mineralization and oxidation rates of organic matter in the aggregates, and increases the content of organic matter (S.L. Chen et

al. 2020). This might partly explain why the abundance of Proteobacteria and Acidobacteriota was significantly higher under DVRT treatment than CT treatment. In addition, the *norank_Vicinamibacterales* and *norank_Vicinamibacteraceae* belong to Acidobacteriota, which may degrade cellulose, and are involved in the metabolism of single-carbon compounds (Radajewski et al. 2002; Pankratov et al. 2011). *Arthrobacter*, *norank_Actinomarinales*, and *Gaiella* belong to Actinobacteriota. *Arthrobacter* could conduct nitrification and denitrification effectively (He et al. 2016). We conclude that DVRT has a potential to significantly increase bacteria involved in nutrient cycling, and 2 years of continuous tillage can further enhance this effect.

The specific method of tillage is considered to be one of the key factors controlling the function of soil ecosystems in agricultural production. Drijber et al. (2000) found that under different tillage methods, no tillage made the distribution of soil microbial community structure tend to be distributed on the surface. Zhang et al. (2020) concluded that no-tillage treatment provided suitable conditions for the reconstruction of soil microbial genotypes and improved bacterial structure. However, to better reveal the interactions of soil bacteria in the network, it is not enough to study bacterial diversity alone. Our study is the first to apply the functional molecular ecological network method to the investigation of DVRT soil bacterial community structure and function. Herein, the use of network analysis to assess the differences in soil ecosystem functions in cultivated land between DVRT and CT treatments illuminated new aspects of the underlying mechanisms associated with different tillage methods. The constructed network graphs revealed different structures for the bacterial communities of cultivated land soils under DVRT and CT treatments (Figs. 5*a* and 5*b*); the bacterial community in soils with DVRT treatment was more organized and diverse, with a greater number of functionally interrelated modules (13 modules) than those in soils under CT treatment (8 modules). Because highly connected microbes that co-occur within a module may share similar ecological characteristics within communities (Kladivko 2001), our results indicate that soils under DVRT treatment could possess a more diverse array of ecologically functional groups. Chen et al. (2017) reported that the cultivation of 2 energy crops (corn and soybean) alters the co-occurrence patterns of bacterial communities and yields more ecological function modules. Different tillage methods lead to different community structures and abundances, potentially indicating that these methods could affect the functional communities of soil ecosystems (Kladivko 2001; Spedding et al. 2004; Zhou et al. 2020). Thus, DVRT treatment could enhance the ability of soil microorganisms in nutrient utilization, and optimize the long-term health of the agricultural farmland ecosystem, which became more organized and diversified than CT treatment soil.

The generalists of Zi–Pi plot (Fig. 4) are the keystone nodes in the soil microbial network (Gu et al. 2019). Herein, generalists with different OTUs in DVRT treatment were assigned to different module hubs and connectors, which indicated that different key bacterial populations could shape the soil communities of cultivated land under DVRT. The 4 OTUs in DVRT treatment soils belonged to the genera *norank_OLB14*, *norank_Microscillaceae*, *norank_JG30-KF-CM45*, and *norank_SC-I-84* as generalists. The *norank_JG30-KF-CM45* is the most abundant keystone node, and the higher its abundance, the higher the productive function of the soil bacterial community (Shen et al. 2022). The *norank_OLB14* and *norank_JG30-KF-CM45* belong to Chloroflexi, and both seem to feed on the debris of lysed bacterial cells to ferment carbohydrates and degrade other complex polymeric organic compounds into low molecular weight substrates that support their growth and that of other bacterial populations, and participate in the carbon cycle (Hug et al. 2013; Speirs et al. 2019). The *norank_Microscillaceae* belongs to Bacteroidota, and might degrade polysaccharides, proteins, and complex organic matter (Wolińska et al. 2017). The *norank_SC-I-84* belongs to Proteobacteria, and might be the dominant microorganism involved in the cycling and recycling of carbon, nitrogen, and sulfur compounds, and their abundance is significantly related to soil pH value (Campbell et al. 2006; He et al. 2020). In addition, members of generalists have been proven to indicate the physicochemical properties of soil (Zi et al. 2018; Wu et al. 2021),which might be an important observation with respect to improving the soil bacterial diversity and community structure of cultivated land by DVRT treatment. Zheng et al. (2021) concluded that green manure returning under DVRT can improve the cumulative mineralization of paddy soil compared with CT. On the one hand, DVRT enhances soil potential mineralizable organic carbon and mineralization rate, which is beneficial to the sequestration of organic carbon in soil. On the other hand, DVRT increased the rate of organic carbon utilization by bacteria in the soil, promoted the transformation of soil nutrients, and improved the structure and quantity of related key microorganisms.

Based on function predictions, the percentage relative abundances of "Metabolism of cofactors and vitamins", "Xenobiotics biodegradation and metabolism", and "Signal transduction functional bacteria" were higher in cultivated land soils under DVRT treatment compared with soils under CT treatment (Fig. 5*a*). "Metabolism of cofactors and vitamins" consists of the primary pathways of anaerobic digestion in anaerobic bacterial communities (Wang et al. 2020). Meanwhile, breaking down large macromolecules to sugars, amino acids, and fatty acids by hydrolytic and fermentative bacteria is a significant ecosystem function (Mamun and Torii 2017). Xenobiotic biodegradation and metabolism was shown as an effective functional pathway for microorganisms to improve the degradation capacity of an ecosystem toward aromatics (Ma et al. 2020). Our findings showed that the DVRT treatment significantly enhanced the functional groups involved in "Metabolism and Environmental Information Processing", which were mainly performed by Actinobacteriota, Chloroflexi, Bacteroidota, Methylomirabilota, etc. (Fig. 5), indicating that under DVRT treatment, soil bacteria decompose relatively more organic matter from the soil, which improves their diversity. The predicted potential functions of these bacteria could indicate the increase of bacterial diversity and improved ecosystem functions in cultivated land soils under DVRT treatment. In conclusion, DVRT increased soil bacterial diversity, altered network structure and the key bacteria, im-

proved potential ecosystem functions, and increased the crop yield, indicating that this tillage application is beneficial to the development of sustainable agriculture.

Conclusions

The results of this paper highlighted the impact of different tillage methods on the soil bacterial diversity and community structure of cultivated land. In comparison to CT treatment, DVRT treatment increases corn yield, reduces the values of soil pH and EC, improves soil nutrient and water contents, and increases the soil bacterial diversity, thereby altering the network structure and key bacterial organisms and eventually improving potential ecosystem functions. Based on the network analysis results, future research efforts should strive for a deeper understanding of the relationship between key bacteria (e.g., generalists) and their potential functions, and explore the possibility of supporting soil bacterial communities in cultivated land to improve soil fertility and crop production.

In addition, this study only focused on the analysis of the bacterial structure of cultivated soil during the harvest period for 2 consecutive years. In the future, long-term positioning research should be pursued on the changes in soil nutrients and soil microecology caused by DVRT. It is also necessary to consider physiological and biochemical identification of unknown bacteria, soil aggregates, labile C, and soil respiration in the future to fully understand the mechanism of DVRT and improve the soil ecosystem functions in cultivated soil.

Acknowledgements

The support provided by the College of Life Science, Ningxia University, is highly appreciated. We also thank all the authors for their endeavors in this study.

Article information

History dates

Received: 21 December 2021
Accepted: 18 May 2022
Accepted manuscript online: 2 June 2022
Version of record online: 14 November 2022

Copyright

Data availability

The datasets generated or analyzed during the current study are not publicly accessible due to the unfinished project but are available from the corresponding author upon reasonable request.

Author information

Author ORCIDs

Wanyu Xia https://orcid.org/0000-0003-1061-1537

Author contributions

WX—conceptualization, validation, formal analysis, investigation, visualization, and writing original draft. XR—investigation, resources, and project. YC—methodology, resources, supervision, and writing—review and editing.

Competing interests

The authors declare that there are no competing interests.

Funding information

This research was funded by the Ningxia Hui Autonomous Region Key R&D Program of China (Grant No. 2019BBF02006).

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/CJSS-2021-0208.

References

Afzalinia, S., Khosravani, A., Javadi, A., Mohammadi, D., and Alavimanesh, S.M. 2012. Effect of tillage and planting methods on the soil properties, grain drill performance, and wheat yield. J. Agric. Sci. Technol. **2**(4): 537–543.

Anand, S.R., Rajkumar, R.H., Vishwanatha, J., and Karegoudar, A.V. 2018. Evaluation of different alternative crops for saline vertisols under different tillage methods in rice fallows in tungabhadra command area of Karnataka. J. Prog. Agric. **9**(2): 18–22.

Asargew, M.F., Atsushi, T., Nigussie, H., Enyew, A., Mitsuru, T., Kindiye, E., et al. 2021. Tillage and crop management impacts on soil loss and crop yields in northwestern Ethiopia. Int. Soil Water Conserv. Res. **10**(1): 75–85.

Aßhauer, K., Bernd, W., Rolf, D., and Peter, M. 2015. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics, **17**: 2882–2884. doi:10.1093/bioinformatics/btv287.

Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A., and Richardson, A.E. 2016. Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol. Biochem. **97**: 188–198.

Campbell, B.J., Engel, A.S., Porter, M.L., and Takai, K. 2006. The versatile epsilon-proteobacteria: key players in sulphidic habitats. Nat. Rev. Microbiol. **4**(6): 458–468. doi:10.1038/nrmicro1414.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods, **7**: 335–336. doi:10.1038/nmeth.f.303.

Chen, B.S., Teh, B.S., Sun, C., Hu, S.R., Lu, X.M., Wilhelm, B., and Shao, Y.Q. 2016. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore Spodoptera littoralis. Sci. Rep. **6**: 29505. doi:10.1038/srep29505.

Chen, M., Xiang, J., Liu, Y., Zhang, L., Wu, J., Yang, H., et al. 2020. Effects of deep vertical rotary tillage combined with organic carbon fertilizer on pH, physical property and nutrients of tobacco-planting soil. Crop Res. **34**(4): 349–353.

Chen, S.L., Hu, J.M., Huang, Z.H., Li, T.T., Zheng, J.S. Huang, Y.M., et al. 2020. Effects of smash ridging on soil organic carbon mineralization and structure of sugarcane field in flat and slope farmland. Chin. J. Agrometeorol. **41**(5): 299–307.

Chen, W., and Xue, L. 2004. Root interactions: competition and facilitation. Acta Ecol. Sin. **6**: 1243–1251.

Chen, Z., Zheng, Y., Ding, C., Ren, X., Yuan, J., Sun, F., and Li, Y. 2017. Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. Ecotoxicol. Environ. Saf. **145**: 111–118. doi:10.1016/j.ecoenv.2017.07.019.

Choudhury, S.G., Srivastava, S., Singh, R., Chaudhari, S.K., and Sarkar, D. 2014. Tillage and residue management effects on soil aggregation,

organic carbon dynamics and yield attribute in rice–wheat cropping system under reclaimed sodic soil. Soil Tillage Res. **136**: 76–83. doi:10.1016/j.still.2013.10.001.

Coker, O.O., Dai, Z., Nie, Y., Zhao, G., and Yu, J. 2017. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut, **14**(6): 314281.

Deng, Y., Jiang, Y.H., Yang, Y., He, Z., Luo, F., and Zhou, J. 2012. Molecular ecological network analyses. BMC Bioinformatics, **13**: 113. doi:10.1186/1471-2105-13-113.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., and Lyon, D.J. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. **32**(10): 1419–1430.

Edgar, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods, **10**(10): 996–998. doi:10.1038/nmeth.2604.

Essel, E. 2019. Bacterial and fungal diversity in rhizosphere and bulk soil under different long-term tillage and cereal/legume rotation. Soil Tillage Res. **194**: 1–11. doi:10.1016/j.still.2019.104302.

Franzluebbers, A.J., and Arshad, M.A. 1997. Soil microbial biomass and mineralizable carbon of water-stable aggregates. Soil Sci. Soc. Am. J. **61**(4): 1090–1097. doi:10.2136/sssaj1997.03615995006100040015x.

Gu, S., Hu, Q., Cheng, Y., Bai, l., Liu, Z., Xiao, W. et al. 2019. Application of organic fertilizer improves microbial community diversity and alters microbial network structure in tea (*Camellia sinensis*) plantation soils. Soil Tillage Res. **195**: 104356. doi:10.1016/j.still.2019.104356.

He, T.X., Ni, J.P., Li, Z.L., Sun, Q., Zhi, Q., and Xu, Y. 2016. Heterotrophic nitrification and aerobic denitrification of the hypothermia aerobic denitrification bacterium: Arthrobacter arilaitensis. Environ. Sci. **37**(3): 1082–1088.

He, W., Zhang, M., Jin, G., Sui, X., and Song, F. 2020. Effects of nitrogen deposition on nitrogen-mineralizing enzyme activity and soil microbial community structure in a Korean pine plantation. Microb. Ecol. **81**(2): 410–424. doi:10.1007/s00248-020-01595-6.

Hinsinger, P., Bengough, A.G., Vetterlein, D., and Young, I.M. 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil. **321**(1–2): 117–152. doi:10.1007/s11104-008-9885-9.

Hug, L.A., Castelle, C.J., Wrighton, K.C., Thomas, B.C., Sharon, I., Frischkorn, K.R., et al. 2013. Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. Microbiome, **1**(1): 22–22. doi:10.1186/2049-2618-1-22.

Jat, H.S., Choudhary, M., Datta, A., Yadav, A.K., and Sharma, P.C. 2020. Temporal changes in soil microbial properties and nutrient dynamics under climate smart agriculture practices. Soil Tillage Res. **199**: 104595. doi:10.1016/j.still.2020.104595.

Kladivko, E.J. 2001. Tillage systems and soil ecology. Soil Tillage Res. **61**(1–2): 61–76. doi:10.1016/S0167-1987(01)00179-9.

Lao, C.Y., Wei, B.H., Shen, Z.Y., Zhou, L.Z., Li, Y.Y., Zhou, J., et al. 2021. Diversity analysis of fungal in rhizosphere soils of rice under different tillage methods based on high-throughput sequencing technique. Chin. J. Trop. Crops, **42**(9): 2717–2726.

Li, X., Wei, B., Xu, X., and Zhou, J. 2020. Effect of deep vertical rotary tillage on soil properties and sugarcane biomass in rainfed dry-land regions of Southern China. Sustainability, **12**(23): 1–19. doi:10.3390/su122310199.

Li, Y.C., Li, Z., Li, Z.W., Jiang, Y.H., Weng, B.Q., and Lin, W.X. 2016. Variations of rhizosphere bacterial communities in tea (*Camellia sinensis* L.) continuous cropping soil by high-throughput pyrosequencing approach. J. Appl. Microbiol. **121**(3): 787–799. doi:10.1111/jam.13225.

Liu, X., Peng, C., Zhang, W.J., Li, S.Y., An, T.T., Xu, Y.D., et al. 2022. Subsoiling tillage with straw incorporation improves soil microbial community characteristics in the whole cultivated layers: a one-year study. Soil Tillage Res. **215**: 105188. doi:10.1016/j.still.2021.105188.

Lu, L., Yin, S., Liu, X., Zhang, W., Gu, T., Shen, Q., and Qiu, H. 2013. Fungal networks in yield-invigorating and -debilitating soils induced by prolonged potato monoculture. Soil Biol. Biochem. **65**: 186–194. doi:10.1016/j.soilbio.2013.05.025.

Lu, R.S. 2000. Analytical methods of soil agricultural chemistry. China Agricultural Science Press, Beijing, China. pp. 22–36.

Ma, Q., Qu, H., Meng, N., Li, S., and Sun, Y. 2020. Biodegradation of skatole by Burkholderia sp. IDO3 and its successful bioaugmentation in activated sludge systems. Environ. Res. **182**: 109123. doi:10.1016/j.envres.2020.109123.

Magoč, T., and Salzberg, S.L. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics, **27**(21): 2957–2963. doi:10.1093/bioinformatics/btr507.

Mack, I., Cuntz, U., Grämer, C., Niedermaier, S., Pohl, C., Schwiertz, A., et al. 2016. Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints. Sci. Rep. **6**: 26752. doi:10.1038/srep26752.

Mamun, M.A., and Torii, S. 2017. Anaerobic co-digestion technology in solid wastes treatment for biomethane generation. Int. J. Sustain. Energy, **36**: 462–472. doi:10.1080/14786451.2015.1043302.

Montgomery, D.R. 2007. Soil erosion and agricultural sustainability. Proc. Natl. Acad. Sci. USA, **104**(33): 13268–13272. doi:10.1073/pnas.0611508104.

Nie, S.W., Zhang, Y.T., Zhang, Q.P., Guo, Q., Tang, F.S., Wang, H.Q., and He, N. 2017. Effect of smashing ridge tillage on grain yields of winter wheat and summer maize and contents of soil nutrients. Chin. J. Soil Sci. **48**(4): 930–936.

Noah, F. 1839. Toward an ecological classification of soil bacteria. Ecology, **88**(6): 1354–1364.

Pankratov, T.A., Ivanova, A.O., Dedysh, S.N., and Liesack, W. 2011. Bacterial populations and environmental factors controlling cellulose degradation in an acidic sphagnum peat. Environ. Microbiol. **13**(7): 1800–1814. doi:10.1111/j.1462-2920.2011.02491.x.

Quintanilla, E., Ramírez-Portilla, C., Adu-Oppong, B., Walljasper, G., Glaeser, S.P., Wilke, T., et al. 2018. Local confinement of disease-related microbiome facilitates recovery of gorgonian sea fans from necrotic-patch disease. Sci. Rep. **8**: 14636. doi:10.1038/s41598-018-33007-8.

Radajewski, S., Webster, G., Reay, D.S., Morris, S.A., and Murrell., J.C. 2002. Identification of active methylotroph populations in an acidic forest soil by stable-isotope probing. Microbiology, **148**(8): 2331–2342. doi:10.1099/00221287-148-8-2331.

Shannon, P., Markiell, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. **13**(11): 2498–2504. doi:10.1101/gr.1239303.

Shen, M.C., Zhang, Y.Z., Bo, G.D., Yang, B., Wang, P., Ding, Z.Y., et al. 2022. Microbial responses to the reduction of chemical fertilizers in the rhizosphere soil of flue-cured tobacco. Front. Bioeng. Biotechnol. **9**: 812316. doi:10.3389/fbioe.2021.812316.

Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., and Firestone, M.K. 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. Ecol. Lett. **19**(8): 926–936. doi:10.1111/ele.12630.

Spedding, T.A., Hamel, C., Mehuys, G.R., and Madramootoo, C.A. 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem. **36**(3): 499–512.

Speirs, L., Rice, D., Petrovski, S., and Seviour, R.J. 2019. The phylogeny, biodiversity, and ecology of the Chloroflexi in activated sludge. Front. Microbiol. **10**: 2015. doi:10.3389/fmicb.2019.02015.

Stackebrand, E., and Goebel, B.M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44**(4): 846–849.

Tang, H., Xiao, X., Li, C., Shi, L., and Wang, K. 2021. Microbial carbon source utilization in rice rhizosphere soil with different tillage practice in a double cropping rice field. Sci. Rep. **11**: 5048. doi:10.1038/s41598-021-84425-0.

Wang, P., Qiao, Z., Li, X., Su, Y., and Xie, B. 2020. Functional characteristic of microbial communities in large-scale biotreatment systems of food waste. Sci. Total Environ. **746**: 141086. doi:10.1016/j.scitotenv.2020.141086.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. **73**(16): 5261–5267. doi:10.1128/AEM.00062-07.

Wang, Z., Li, Y., Li, T., Zhao, D., and Liao, Y. 2019. Tillage practices with different soil disturbance shape the rhizosphere bacterial community throughout crop growth. Soil Tillage Res. **197**: 104501. doi:10.1016/j.still.2019.104501.

Wei, B. 2017. Fenlong cultivation: the fourth set of farming methods invented in China. Agric. Sci. Technol. **18**(11): 2045–2048+2052.

Wei, B., Shen, Z., Zhou, J., Gan, X., Lao, C., Zhou, L., et al. 2017. Initial exploration on effect of saline-alkali land rebuilding and utilization by Fenlong cultivation. Agric. Sci. Technol. **18**(12): 2396–2400.

Wei, B.H., Gan, X.Q., Chen, B.S., Shen, Z.Y., Yu, J., Ning, X.C., et al. 2011. Comparison of the cultivation of maize and peanuts by smash-riding and traditional land preparation methods. J. Anhui Agric. Sci. **39**(6): 3216–3219.

Wolińska, A., Kuźniar, A., Zielenkiewicz, U., Zizak, D., Szafranek-Nakonieczna, A., Banach, A., and Błaszczyk, M. 2017. Bacteroidetes as a sensitive biological indicator of agricultural soil usage revealed by culture-independent approach. Appl. Soil Ecol. **119**: 128–137. doi:10.1016/j.apsoil.2017.06.009.

Wu, F., Zhai, L., Xu, P., Zhang, Z., Baillo, E.H., Tolosa, L.N., et al. 2021. Effects of deep vertical rotary tillage on the grain yield and resource use efficiency of winter wheat in the Huang-Huai-Hai Plain of China. J. Integr. Agric. **20**(2): 593–605. doi:10.1016/S2095-3119(20)63405-0.

Wu, H., Lin, M., Rensing, C., Qin, X., and Lin, W. 2020. Plant-mediated rhizospheric interactions in intraspecific intercropping alleviate the replanting disease of Radix pseudostellariae. Plant Soil, **454**(1–2): 1–20. doi:10.1007/s11104-020-04659-1.

Yang, W.X. 2021. Effects of Fenlong tillage combined with nitrogen reduction fertilization on microbial community and greenhouse gas emission in cassava soil. Master dissertation, Guangxi University, Nanning, China.

Yin, C., Fan, F., Song, A., Cui, P., and Li, T. 2015. Denitrification potential under different fertilization regimes is closely coupled with changes in the denitrifying community in a black soil. Appl. Microbiol. Biotechnol. **99**(13): 5719–5729.

Zaneveld, J.R., Burkepile, D.E., Shantz, A.A., Pritchard, C.E., McMinds, R., Payet, J.P., et al. 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat. Commun. **7**: 11833. doi:10.1038/ncomms11833.

Zhai, L., Xu, P., Zhang, Z., Li, S., Xie, R., Zhai, L., and Wei, B. 2017. Effects of deep vertical rotary tillage on dry matter accumulation and grain yield of summer maize in the Huang-Huai-Hai Plain of China. Soil Tillage Res. **170**: 167–174. doi:10.1016/j.still.2017.03.013.

Zhang, D., Yang, X., Wang, Y., Zong, J., Ma, J., and Li, C. 2020. Changes in soil organic carbon fractions and bacterial community composition under different tillage and organic fertiliser application in a maize-wheat rotation system. Acta Agric. Scand. B Soil Plant Sci. **70**(6): 457–466.

Zhang, X.F., Xin, X.L., Zhu, A.N., Yang, W.L., Zhang, J.B., Ding, S.J., et al. 2018. Linking macroaggregation to soil microbial community and organic carbon accumulation under different tillage and residue managements. Soil Tillage Res. **178**: 99–107. doi:10.1016/j.still.2017.12.020.

Zhang, Y., Wang, S., Hao, W., Fang, N., Zhang, Y., Dong, Z., et al. 2018. The effects of rotating conservation tillage with conventional tillage on soil properties and grain yields in winter wheat-spring maize rotations. Agric. For. Meteorol. **263**: 107–117. doi:10.1016/j.agrformet.2018.08.012.

Zhang, Y.R., Li, K., Zhu, H.R., and Zhang, H.X. 2017. Community structure of microorganisms and its seasonal variation in Beihai Lake. Environ. Sci. **38**(8): 3319–3329.

Zheng, J.S., Hu, J.M., Wei, X.H., Wei, Y.Y., Su, S.M., Li, T.T., et al. 2021. Effects of green manure returning on soil microbial biomass carbon and mineralization of organic carbon in smash ridging paddy field. Chin. J. Eco-Agric. **29**(4): 691–703.

Zhou, J., Zhou, L., Lao, C., Shen, Z., Li, Y., Hu, P., et al. 2020. Effects of short-term different tillage methods on the diversity of bacterial community in rice rhizosphere soils. J. South. Agric. **51**(10): 2401–2411.

Zi, H.B., Hu, L., Wang, C.T., Wang, G.X., Wu, P.F., Lerdau, M., and Ade, L.J. 2018. Responses of soil bacterial community and enzyme activity to experimental warming of an alpine meadow. Eur. J. Soil Sci. **69**(3): 429–438. doi:10.1111/ejss.12547.