



Low-disturbance farming regenerates healthy deep soil toward sustainable agriculture - Evidence from long-term no-tillage with stover mulching in Mollisols



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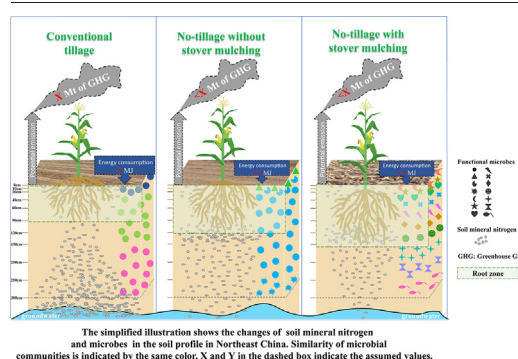
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HIGHLIGHTS

- Low-disturbance farming practices regenerated healthy soils as deep as three meters.
- Low-disturbance farming practices improved soil water and nutrient conditions.
- Low-disturbance farming practices restored bacterial diversity, richness and potential metabolic function.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, global agricultural development is in a critical period, as it contends with a growing population, degraded farmland, and serious environmental issues. Although low-disturbance practices are recommended to improve soil health, it is unclear whether such practices benefit critical deep soil functioning. Here, we compared the soil bacterial communities and physicochemical parameters across 3-m deep soil profiles in a Mollisol of Northeast China at the end of the dormant season after 10 years of farming under conventional tillage without stover mulching (CT), no-tillage without stover mulching (NTNS), and no-tillage with stover mulching (NTSM). We found that low-disturbance practices (NTNS and NTSM), compared with CT, evidently promoted soil bacterial species richness and diversity and enriched potential metabolic diversity. When compared to the bacterial communities in CT, the vertical dissimilarity of bacterial communities in NTNS decreased, while that in NTSM increased, indicating that no-tillage alone homogenized the composition of the bacterial community through soil depth profiles, but straw mulching enhanced the uniqueness of community composition at each layer. In comparison to CT, no-tillage with stover mulching significantly increased the soil water content and root-associated organic carbon (SEOC), and decreased soil pH. Mineral nitrogen declined with depth to 60 cm and then increased to its maximum at 250–300 cm under CT and at 120–150 cm under NTNS and NTSM. More mineral nitrogen at 0–150 cm under low-disturbance practices would provide more available nitrogen for crops in the coming growing season, while the accumulated nitrogen at 150–300 cm under CT may leach into the groundwater. Taken together, our results show that low-disturbance practices can regenerate whole-soil bacterial diversity and potential function, and promote water retention and nitrogen holding capacity within the root

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zone, thus reducing the dose of nitrogen fertilizer and mitigating nitrogen contamination to deep groundwater, ultimately contributing to agricultural sustainability in Mollisol regions.

1. Introduction

Since the industrial revolution, intensive conventional farming methods with high energy inputs (fertilizers and other chemicals) and disturbance (e.g., tillage/compaction, burn/remove stover) have caused a series of environmental issues, such as soil carbon (C) loss, biodiversity loss, freshwater depletion, pollution and climate change (Foley et al., 2005; Tsiafouli et al., 2015; Sanderman et al., 2017). To sustainably feed growing populations (United Nations, World Population Prospects 2019), options to expand farmland area horizontally at the expense of nature and biodiversity, which are already under pressure, are limited (Springmann et al., 2018). Then, could we expand the topsoil toward deeper zones, thus maintaining production with minimal environmental impact?

In contrast to conventional tillage, the conservative low-disturbance practices (e.g., reduced tillage, no-tillage and stover mulching) could promote SOC (soil organic carbon) storage (Li et al., 2020a), improve nutrients and water use efficiency (Jat et al., 2020; Qin et al., 2021), and reduce nutrient losses caused by heavy storms and winds (Gao et al., 2015; Seitz et al., 2018; Prasuhn, 2020). Therefore, since the 1970s, low-disturbance practices have gradually been applied to restore soil health (Phillips et al., 1980). As a living system, healthy soil has the capacity to sustain food production, maintain or enhance water and air quality, and restore or preserve soil biodiversity to meet human needs (Doran and Zeiss, 2000; Kibblewhite et al., 2008). Because soil microorganisms contribute to soil structure formation, modulate the biogeochemical cycling of nutrients, drive soil carbon transformation and stabilization (Schimel and Schaeffer, 2012; Bardgett and Van Der Putten, 2014; Liang et al., 2017), and respond quickly to changes in soil environmental conditions (Nielsen et al., 2002), the effect of farming practices on soil microbial communities is a research hotspot (Helgason et al., 2010; Sun et al., 2018; Li et al., 2020b; Rincon-Florez et al., 2020). However, most studies to date have mainly focused on farmland topsoil or soils within a 1-m depth (Hartman et al., 2018; Nevins et al., 2018; Alahmad et al., 2019). Soil deeper than 1 m, however, contains more than 50% topsoil soil C (Jobbágy and Jackson, 2000) but frequently is not studied.

As a part of Earth's critical zone, deep soils (> 1 m) play important roles in bridging aboveground vegetation with parent soils and even act as essential buffers in protecting underground water (Chorover et al., 2007). Microbial communities residing in deeper soil control mineral weathering, soil formation and long-term carbon sequestration (Richter and Markewitz, 1995; Rumpel and Kögel-Knabner, 2010; Schulz et al., 2013). Therefore, ignoring the impact of farming practices on deep soil microbial communities would constrain our knowledge of the long-term impacts. Studies have shown that microorganisms in deep soils are limited by fresh C supply, which is likely an important factor for long-term SOC stabilization (Fontaine et al., 2007). The roots of many crops can grow in excess of 1 m depending on the species and management, and the uptake/exude functions of these deep-rooting crops may impact the deep-soil microbial community (Canadell et al., 1996; Fan et al., 2016; Thorup-Kristensen et al., 2020). Moreover, the decomposition of stover mulching on the soil surface may redistribute dissolved organic matter into the deep soil profile, thus changing microorganisms and long-term carbon storage (Uselman et al., 2007; Kaiser and Kalbitz, 2012; Kirkby et al., 2016). We thus hypothesized that different tillage practices would affect bacterial community structure, even in deep soil (>1 m), and these changes are closely related to the vertical distribution of soil physicochemical properties.

In addition, low-disturbance practices, which leave the surface soil undisturbed and mulch with crop residues, are conducive to topsoil microbial communities that could enhance microbial biomass, community diversity and stability, and promote ecosystem multifunctionality (Wang et al.,

2017; Li et al., 2020b; Wittwer et al., 2021). Besides, the study showed that no-tillage could promote root growth into deeper soil (Kemper et al., 2011), thus critically maximizing the exploitation of nutrients and water deeper in the soil profile (Billings et al., 2018). Bacterial diversity, activity and abundance in the rhizosphere are generally higher than those in the bulk soil (Li et al., 2014; Prashar et al., 2014; Wang et al., 2020). We then further hypothesized that low-disturbance practices mimicking natural ecosystems would exert a beneficial influence on the deep soil bacterial community.

Recent research shows that, globally, the corn belts in the U.S.A., Western Europe, and China have experienced the most soil carbon loss (Sanderman et al., 2017). The corn belt in Northeast China is considered the “breadbasket” of the country, as it has the largest grain production and overlaps with China's most fertile black soil (Mollisol) region, which sustains 3% of the global population (Liu et al., 2010) and accounts for more than 30% of corn production in the country (Liu et al., 2012). Here, a 10-year manipulative experiment was conducted in a temperate continuous corn production system in Northeast China, investigating farming practices with three levels of disturbance: high disturbance—conventional tillage without stover mulching (CT), low disturbance—no-tillage without stover mulching (NTNS) and the lowest disturbance—no-tillage with 100% stover mulching (NTSM) (described in the Methods and Fig. S1). We compared the soil physicochemical properties, root-associated organic carbon, and bacterial communities of the 3-m soil profiles at the end of the dormant season (legacy effects of practices) after the 10-year manipulation. We aimed to explore the long-term impact of low-disturbance practices on ecosystem function and develop effective strategies for sustainable agriculture.

2. Materials and methods

2.1. Site description

The field experiment was established in 2007 at the Lishu Conservation Tillage Research and Development Station of the Chinese Academy of Sciences in Jilin Province, Northeast China (43.19° N, 124.14° E). The region has a humid continental climate with a mean annual temperature of 6.9 °C and a mean annual precipitation of 614 mm. The region has warm summers and cold winters; the growing season is from early May until mid-October, and the dormant season is from mid-October to early May of the following year. The soils are classified in the Mollisol order (black soil in Chinese Soil Classification) with a clay loam texture (IUSS Working Group, 2007). The initial properties of the 0–20 cm soil layer were 11.3 g/kg of SOC, 1.2 g/kg of total N, and pH 7.1 in 2007. The site has been continuously planted with maize for more than 30 years using conventional plowing practices.

In 2007, we set up an experiment using a randomized complete block design with four blocks, and each block arranged five treatments (Fig. S1). Each plot area was 261 m² (8.7 m × 30 m). The five treatments included 1) conventional tillage without stover mulching: the plots were moldboard plowed annually in spring to a depth of 25–30 cm shortly before planting, and the stover was removed from the field after harvest; 2) no-tillage without stover mulching: the plots were undisturbed, and the stover was removed from the field after harvest; and 3) no-tillage with three levels of stover mulching (33%, 67% and 100% of newly produced maize stover): all stover was harvested completely using sickles (15–25 cm above ground level) and evenly spread over the soil surface as mulch. For each treatment, 240 kg/ha N, 110 kg/ha P₂O₅, and 110 kg/ha K₂O were applied by no-till planters (DEBONT2405) when planting. The no-tillage planter contains two angled disc openers, one for seeding at a depth of 3–5 cm and another

for fertilizing at a depth of 8–12 cm, separating seeds from fertilizer to an offset distance higher than 5 cm (Fig. S2). In this experiment, three treatments, including conventional tillage without stover mulching (CT), no-tillage without stover mulching (NTNS), and no-tillage with 100% stover mulching (NTSM), were selected for the comparison of tillage practices.

2.2. Soil sampling

On April 27, 2017, triplicate soil cores (0–3 m) were collected from each treatment at the end of the dormant season. Taking samples at that time could minimize the influence of crops (especially root turnover) to the greatest degree and determine what the soil could provide (e.g., available nitrogen) at the beginning of the growing season, which may reflect the long-term legacy impacts of practices. After removing the surface stover, we took soil cores (diameter: 4.18 cm) using a stainless-steel hand auger and sliced each of them into ten layers: 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–90 cm, 90–120 cm, 120–150 cm, 150–200 cm, 200–250 cm, and 250–300 cm. In total, 90 soil samples were collected and transported to the laboratory within 3 h and then passed through a 2-mm sieve. All visible roots, crop residues and stones were removed. Each soil sample was divided into three subsamples: one subsample for DNA extraction and soil salt-extractable organic carbon (SEOC) measurement, which was immediately placed into a polyethylene plastic bag and stored at -80°C ; one for chemical measurements including ammonium nitrogen ($\text{NH}_4^+\text{-N}$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) (within one day); and one for determination of other soil physicochemical properties, which was air-dried.

2.3. Soil properties

The soil total nitrogen (TN) content was measured by a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). As an elemental analyzer, Vario EL III does not differentiate between organic and inorganic C, and significant amounts of inorganic C may be present in these alkaline soils. SOC was converted from soil organic matter that was measured by potassium dichromate oxidation (Nelson and Sommers, 1996), and the conversion factor was calibrated with an alkaline reference soil (GBW07461). Soil pH was measured in deionized CO_2 -free water (1:2.5 w/v). The gravimetric soil water content (SWC) was determined by oven-drying fresh soil to a constant weight at 105°C . Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were extracted from fresh soil using 2 M KCl and measured by a continuous flow analytical system (AA3, SEAL, Germany). To reflect soil soluble, exchangeable, mineral-bound and root-associated OC, SEOC was extracted from the frozen soil samples with 0.5 M K_2SO_4 (1:5 w/v) for 30 min and filtered at $0.45\ \mu\text{m}$ (Jones and Willett, 2006; Toosi et al., 2012). Soil mineral N stocks of 0–150 cm increments were calculated based on the equivalent soil mass method (ESM) using cubic spline interpolating functions by using the R script provided by von Haden et al., 2020. It is important to note that while we refer to soils by fixed depth increment, we are still reporting mineral N stock at “ESM depths” rather than fixed depths. The soil bulk density (BD) of the 0–90 cm soil layer (0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–90 cm) was measured in 2015 using the core method (Abdel-Magid et al., 1987). Although it is not ideal to apply these bulk density data to 2017 soils, the treatments were established in 2007, and it is likely that the 2015 measurements captured any soil density differences between the CT and NT treatments.

2.4. DNA extraction, PCR amplification and pyrosequencing

Soil DNA was extracted from the frozen soil samples (0.5 g wet weight) using a MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the instructions of the manufacturer. The quality of DNA was determined by 1% agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified by PCR using the primers 338F and 806R with barcodes for Illumina MiSeq sequencing. PCR was performed in a total volume of 50 μl containing 30 ng DNA as a template, 20 mol of each primer, 10 mM dNTPs, 5 μl Pyrobest buffer ($10\times$) and

0.3 U of Pyrobest polymerase (Takara Code: DR005A). Each sample was amplified for three replicates. The PCR products from the same sample were pooled, checked by 2% agarose gel electrophoresis and then purified using an AxyPrepDNA agarose purification kit (AXYGENT). Finally, purified PCR products were sequenced on an Illumina MiSeq platform PE300 sequencer (Illumina, USA).

The raw sequence data were further analyzed by the following protocol. Low-quality sequences with an average quality score less than 20 were filtered by employing Trimmomatic (Bolger et al., 2014). FLASH software was used to merge overlapping ends and treat them as single-end reads (Derakhshani et al., 2016). The nonamplified region sequences, chimeras and shorter tags were also removed using Usearch and Mothur (Mysara et al., 2016). The resulting high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using Usearch (Version 8.1.1861 <http://www.drive5.com/usearch/>). OTUs were then classified against the Silva database (Release-0119 <http://www.arb-silva.de>), and the taxonomic information of each representative OTU sequence was annotated using the RDP Classifier (Wang et al., 2007). A total of 3,255,693 high-quality reads were obtained from all soil samples, which were clustered into 9573 unique OTUs at 97% sequence similarity. The Good's coverage of all the samples ranged from 0.93 to 0.98, which indicates an adequate level of sequencing to identify the majority of diversity in the samples.

2.5. Statistical analyses

Soil properties were analyzed and plotted using Sigmaplot 12.5 software. Alpha diversity indices were calculated in QIIME (version v.1.8) and used to reflect the diversity and richness of the bacterial community in different samples. The relative abundances of individual phyla in different samples were computed by R packages. Two-way ANOVA was performed to test the effects of tillage disturbance practices, depth and their interaction on soil properties, alpha diversity indices and the relative abundances of individual phyla, which were implemented in IBM SPSS 25. Percentage data were transformed using the arcsine square root function prior to ANOVA. One-way ANOVA was performed in IBM SPSS 25 to test the effects of tillage disturbance practices on the SOC stock and mineral N stock across different depth increments. All statistical tests were significant at $p \leq 0.05$ (multiple comparisons determined using Duncan's test). The indicator analysis based on genera specific to each soil depth was conducted using the indicspecies package of R with 9999 permutations, and the P values were corrected for multiple testing using the qvalue package of R (Cáceres and Legendre, 2009). Functional profiles of the bacterial community were predicted by Tax4fun (an open-source package in R) (Aßhauer et al., 2015), and further statistical analysis was conducted by STAMP (v2.1.3) using a two-sided Welch t -test. The 95% confidence interval was based on Welch's inverted test (Parks et al., 2014). Although this function prediction approach has its uncertainty, Tax4fun is a powerful and widely used tool for broader studies to infer the functional capabilities of bacterial communities based on 16S rRNA sequencing datasets. Nonmetric multidimensional scaling (MDS) was performed by the “vegan” package of R to describe differences in bacterial community structure among samples. Permutational multivariate analysis of variance (PERMANOVA) was employed for Bray–Curtis distances to test the differences in soil bacterial communities among the three tillage practices and among soil depths under each tillage practice. Redundancy analysis (RDA, Canoco 5 software) was conducted to identify the correlations between bacterial community composition and environmental variables. All correlation (Pearson and Spearman) analyses between soil properties and bacterial variables were performed in R.

3. Results

3.1. Soil properties

Two-way ANOVA results showed a distinct vertical pattern of the measured soil properties and identified the significant effects of tillage practices

on the soil pH, soil water content, mineral N, SEOC and SEOC/SOC, but not on SOC, TN and C/N (Table 1). In addition, we found a significant interactive effect between tillage practices and depth on soil NH_4^+ -N, NO_3^- -N, mineral N and SEOC/SOC (Table 1). More specifically, the SOC, TN and C/N ratio substantially decreased from the soil surface to a depth of approximately 150 cm and then remained unchanged within the 150–300 cm layer (Fig. 1a, f and g). NTSM reduced the soil pH (Fig. 1d) while increased the soil water content (Fig. 1e). In the CT plots, the soil NO_3^- -N concentration first decreased and then increased markedly, ranging from 4.19 to 23.32 mg kg^{-1} (Fig. 1i). However, under the NTNS and NTSM treatments, soil NO_3^- -N decreased significantly in the 0–40 cm layer and then increased to a maximum at 120–150 cm. Interestingly, above the 120–150 cm layer, soil mineral N was significantly higher under low-disturbance practices than under conventional tillage, while the soil below 150 cm under low-disturbance practices had much less mineral N than under conventional tillage (Fig. 1j). The NTNS plots contained much higher amounts of ammonium than the CT and NTSM plots (Fig. 1h). Soil salt-extractable organic carbon (SEOC), a proxy for biotically derived organic acids, declined from the surface to 40–60 cm and then increased to its peak at 60–90 cm under CT, at 90–120 cm under NTNS and at 120–150 cm under NTSM (Fig. 1b). NTSM increased the SEOC concentration compared with CT and NTNS (Fig. 1b); at the surface and 120–150 cm depth, the content of SEOC in NTSM was twice as high as that in CT. SEOC/SOC in NTSM was also higher than that in CT and NTNS, particularly at the 0–10 and 120–150 cm soil depths (Fig. 1c).

3.2. Bacterial diversity, composition, and structure

As shown in Table 1, both tillage disturbance practices and soil depth significantly affected the bacterial diversity indices, while their interaction effect was not significant. Low-disturbance practices significantly increased bacterial richness (Chao1), observed number of species (observed species) and diversity (Shannon index) (Fig. 2 and Table S2). The vertical pattern

of the alpha diversity indices was similar under each disturbance practice, first increasing within 0–20 cm, decreasing from 20 to 90 cm (or 120 cm), and then increasing thereafter with a fluctuating pattern (Fig. 2 and Table S2).

There were 54 bacterial phyla across all soil samples. The dominant phyla (relative abundance >1% across all soil samples) were Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Nitrospirae, Gemmatimonadetes, and Planctomycetes, and these phyla accounted for 60–91% of the total bacterial abundance of all soil samples (Fig. S3a). Bacteroidetes, Verrucomicrobia, Latescibacteria, Parcubacteria, Firmicutes, Microgenomates and Saccharibacteria were less dominant (relative abundance >0.1% across all soil samples) but were still found in all soil samples (Fig. S3a). These phyla were significantly affected by soil depth, and a few of them (Actinobacteria, Planctomycetes, Bacteroidetes, Microgenomates and Verrucomicrobia) were also significantly affected by tillage disturbance (Table 1). In addition, we found more nondominant phyla with higher relative abundance under the low-disturbance practices than under conventional tillage (Fig. S3b and Table S4).

Indicator analysis identified 16 and 51 clearly classified genera (relative abundances >0.005%) in the NTNS and NTSM plots, respectively, while no indicator genera were found in the conventional tillage plots (Fig. 3 and Table S5). The indicator genera in the NTNS plots belonged to Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes and Planctomycetes, and most of them appeared in the surface soil (0–20 cm), with only one genus below 150 cm. Importantly, additional indicator genera — belonging to Bacteroidetes, Acidobacteria, Deferritobacteres, Firmicutes, Verrucomicrobia, Chlorobi and Spirochaetae existed in the NTSM plots, and we observed seven genera at a depth below 150 cm (Fig. 3 and Table S5).

Bacterial community structures were visualized by nonmetric multidimensional scaling (MDS) and were tested by PERMANOVA based on Bray–Curtis distance. A clear separation of the bacterial community structure by soil depth was observed, while the separation by treatment was

Table 1

Two-way ANOVA results of the effects of tillage disturbance practices and depth on soil properties and bacterial variables. Bold values indicate statistical significance ($p < 0.05$).

Variables		Tillage disturbance practices (T)		Depth (D)		T*D	
		F	p	F	p	F	p
Soil properties	SOC	0.519	0.598	116.569	<0.001	0.847	0.640
	TN	0.855	0.430	89.501	<0.001	1.228	0.270
	CN	1.727	0.187	25.778	<0.001	1.161	0.322
	pH	5.975	0.004	5.128	<0.001	1.026	0.446
	SWC	3.930	0.025	23.980	<0.001	1.280	0.234
	NH_4^+ -N	38.114	<0.001	5.542	<0.001	2.369	0.007
	NO_3^- -N	1.155	0.322	7.911	<0.001	2.691	0.002
	Mineral N	4.179	0.020	8.798	<0.001	2.686	0.002
	SEOC	10.707	<0.001	2.895	0.007	1.161	0.322
	SEOC/SOC	10.178	<0.001	14.973	<0.001	1.920	0.031
Alpha diversity indices	Chao1	7.091	0.002	3.385	0.002	0.460	0.965
	Observed_species	8.850	<0.001	5.435	<0.001	0.667	0.829
	Shannon	7.555	0.001	14.016	<0.001	1.294	0.225
Dominant phyla	Acidobacteria	1.779	0.178	6.656	<0.001	1.543	0.107
	Actinobacteria	6.074	0.004	23.243	<0.001	0.870	0.615
	Chloroflexi	1.633	0.204	18.128	<0.001	0.903	0.577
	Gemmatimonadetes	0.938	0.397	7.150	<0.001	0.362	0.990
	Nitrospirae	2.932	0.061	23.810	<0.001	0.916	0.563
	Planctomycetes	5.789	0.005	13.044	<0.001	1.387	0.172
	Proteobacteria	1.541	0.223	12.258	<0.001	1.782	0.049
Less dominant phyla	Bacteroidetes	5.665	0.006	2.850	0.007	0.787	0.706
	Firmicutes	2.511	0.090	1.933	0.064	0.562	0.913
	Latescibacteria	2.086	0.133	10.140	<0.001	1.330	0.203
	Microgenomates	4.299	0.018	18.941	<0.001	0.692	0.805
	Parcubacteria	0.064	0.938	29.463	<0.001	0.920	0.558
	Saccharibacteria	2.536	0.088	5.791	<0.001	1.329	0.204
	Verrucomicrobia	6.251	0.003	19.120	<0.001	0.962	0.512

SOC = soil organic carbon; TN = total nitrogen; C/N = ratio of SOC to TN; SWC = soil water content; NH_4^+ -N = ammonium nitrogen; NO_3^- -N = nitrate nitrogen; Mineral N = NH_4^+ -N + NO_3^- -N; SEOC = salt-extractable organic carbon; SEOC/SOC = ratio of SEOC to SOC.

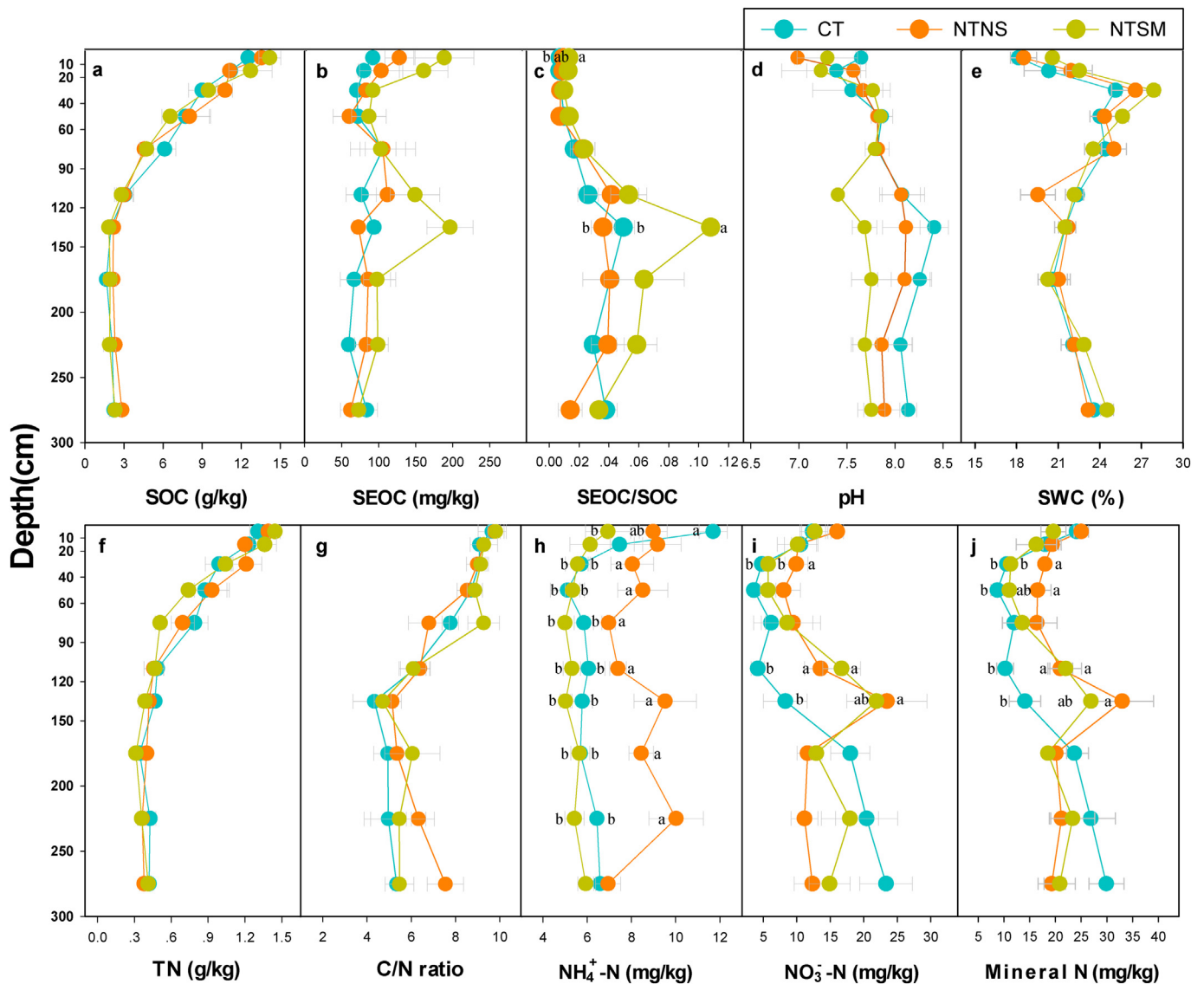


Fig. 1. Soil properties (mean \pm SE, $n = 3$) with soil depth under different practices. a, SOC = soil organic carbon; b, SEOC = salt-extractable organic carbon; c, SEOC/SOC = ratio of SEOC to SOC; d, soil pH; e, SWC = soil water content; f, TN = total nitrogen; g, C/N = ratio of SOC to TN; h, $\text{NH}_4^+\text{-N}$ = ammonium nitrogen; i, $\text{NO}_3^-\text{-N}$ = nitrate nitrogen; j, mineral N = $\text{NH}_4^+\text{-N}$ + $\text{NO}_3^-\text{-N}$. Error bars indicate standard errors ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among disturbance practices.

less obvious (Fig. 4a) and showed only marginal separation at the 10–20 cm and 250–300 cm soil depths (PERMANOVA $p < 0.1$). The disturbance practices influenced the vertical distribution dissimilarity in the bacterial community structure (Fig. 4b–d). Three clusters — 0–10 cm and 10–20 cm, 20–150 cm and 150–300 cm — were observed in the CT plots (PERMANOVA- $F = 9.57$, $p = 0.0001$) (Fig. 4b). In the NTNS plots, an independent cluster formed at 0–10 cm, while other soil depths showed some separation (e.g., 20–120 cm was separated from the 150–300 cm soil depth by axis 1); however, Bray–Curtis distances between adjacent depths were too close to be separated (PERMANOVA- $F = 8.18$, $p = 0.0001$) (Fig. 4c). The NTSM treatment clustered 0–10 cm and 10–20 cm together and 120–150 cm, 150–200 cm, 200–250 cm and 250–300 cm separately, and the other depths also showed some separation (PERMANOVA- $F = 11.32$, $p = 0.0001$) (Fig. 4d).

3.3. Predicted ecological functions of bacterial communities

According to the bacterial diversity, composition and structure, the metabolic capabilities of the bacterial community in the whole 3-m soil profile were evaluated using Tax4Fun (Fig. 5). The low-disturbance practices

significantly increased the abundance of predicted functions related to carbohydrate metabolism, nucleotide metabolism, glycan biosynthesis and metabolism, lipid metabolism and metabolism related to cofactors and vitamins (Fig. 5a). Moreover, the relative abundances of genes encoding assimilatory nitrate reduction under low-disturbance practices were higher than those under conventional tillage practices (Fig. S4 and Table S6). The results suggested that under low disturbance practices, the bacterial community prefers to convert nitrate to ammonia, which can reduce nitrogen loss. We then further assessed the impact of stover mulching on functional profiles (Fig. 5b). The extended error bar plot shows that NTNS enriched the abundance of amino acid metabolism and lipid metabolism, while NTSM enriched the functions associated with energy metabolism, carbohydrate metabolism, biosynthesis of secondary metabolites, glycan biosynthesis and metabolism as well as the metabolism of cofactors and vitamins (Fig. 5b).

3.4. Relationships between bacterial communities and soil properties

Forward selection in redundancy analysis (RDA) revealed that soil depth (pseudo- $F = 48$, $p = 0.002$), SOC (pseudo- $F = 11.5$, $p = 0.002$),

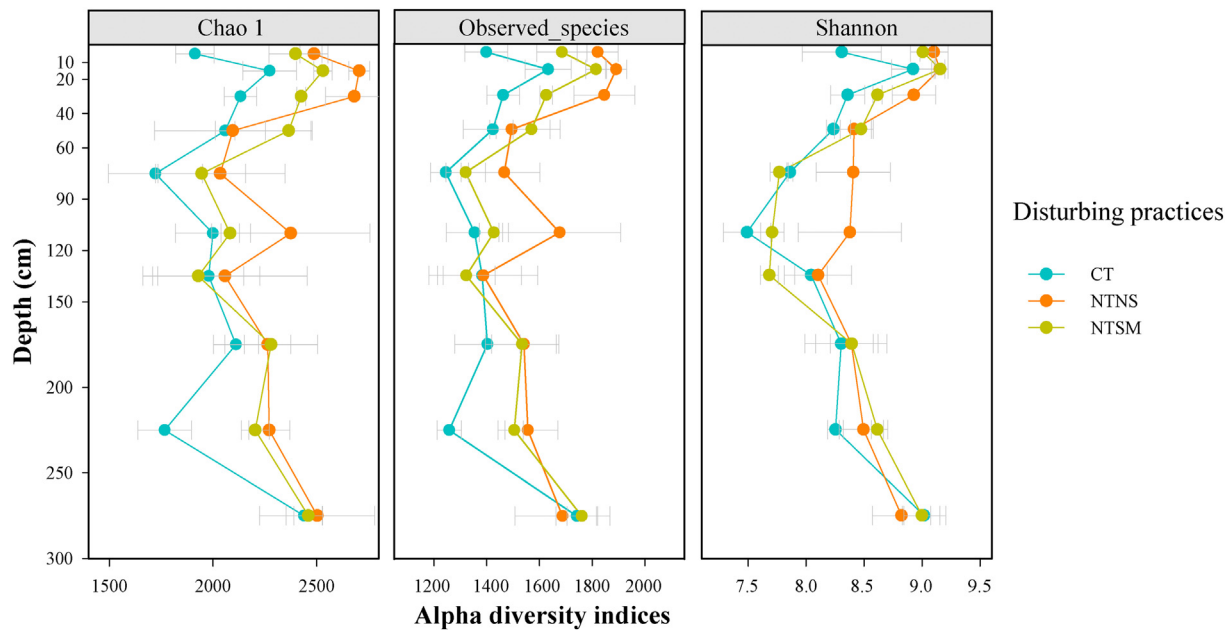


Fig. 2. Bacterial richness (Chao1), observed number of species (observed species) and diversity (Shannon index) in the CT, NTNS and NTSM plots. Error bars indicate standard errors ($n = 3$). Different letters indicate significant differences ($P < 0.05$) among disturbing practices.

SWC (pseudo- $F = 3.4$, $p = 0.012$), soil pH (pseudo- $F = 2.3$, $p = 0.018$) and soil NH_4^+ -N (pseudo- $F = 2.7$, $p = 0.026$) were significantly related to the vertical distribution of the bacterial communities (Fig. 6a). Furthermore, the soil properties that regulated the distribution of soil bacteria were different under different disturbance practices. Under the CT treatment, the soil bacterial community was mainly related to soil NH_4^+ -N (pseudo- $F = 4$, $p = 0.002$) and soil NO_3^- -N (pseudo- $F = 2.3$, $p = 0.012$), which mainly came from applied fertilizer (Fig. 6b). The bacterial community was positively correlated with soil NH_4^+ -N in the 0–20 cm layer, negatively correlated with soil NO_3^- -N in the 20–150 cm layer, and positively correlated with soil NO_3^- -N at a depth below 150 cm (Fig. 6b). Under the NTNS treatment, soil pH (pseudo- $F = 3.7$, $p = 0.004$) constrained the distribution of the bacterial community; strong negative correlations were observed in the 0–10 cm soil layer, and a positive correlation was observed in the 90–150 cm soil layer (Fig. 6c). Under the NTSM treatment, the soil TN (pseudo- $F = 11$, $p = 0.002$), SWC (pseudo- $F = 2.6$, $p = 0.004$) and C/N ratio (pseudo- $F = 1.8$, $p = 0.016$) significantly influenced the separation of the soil bacterial community (Fig. 6d). In general, the bacteria positively correlated with the soil TN and C/N ratio in the surface soil (0–40 cm) and with SWC in the middle layers (40–150 cm), while they were mainly influenced by depth in the deeper soil (150–300 cm) (Fig. 6d).

4. Discussion

4.1. Effect of low-disturbance practices on soil properties

The effects of tillage practices on the vertical distributions of the soil properties (Fig. 1) demonstrated that low disturbance (no-till) plus stover mulching enhanced the stratification of the soil. Specifically, soil salt-extractable organic carbon (SEOC), of which a relatively high proportion of compounds are labile, was significantly higher under NTSM than under CT (Fig. 1b), which may have resulted from stover decomposition and root exudates. During the dormant season, stover decomposition may be promoted due to the destructive effect of freeze–thaw cycles (Wu et al., 2010). Additionally, we found that NTSM decreased soil pH compared with CT (Fig. 1d). No-tillage tends to decrease soil pH, as reported in previous studies (Sithole et al., 2016; Li et al., 2019), which may be attributed to

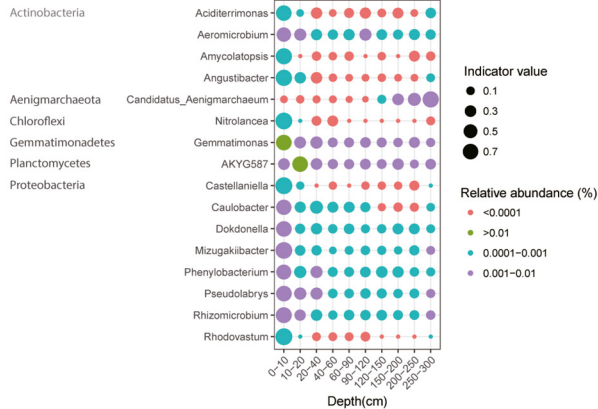
the higher amount of organic hydrogen ions released by the decomposition of organic matter; a lack of soil mixing that prevents nitrogenous fertilizers from leaching into the deeper soil layer, thus enhancing the acidification of the surface soils; and no-tillage that promotes root growth and increases root exudates, which contain various organic acid ions, likely contributed to the lower soil pH (Dakora and Phillips, 2002). However, SEOC, as a proxy for biotically derived organic acids (Billings et al., 2018), showed only a significant negative correlation with soil pH in the NTSM treatment, not in the NTNS and CT treatments (Table S8). Combined with the SEOC data, these results jointly suggested that no-tillage was not the sole factor for the lower soil pH in the NTSM treatment, which was attributed to the combination of no-tillage plus stover mulching.

As expected, low-disturbance practices promoted soil nutrient and water holding capacities. The accumulated NO_3^- -N at 120–150 cm depth under low-disturbance practices could potentially be used during the growing season, while corn roots cannot reach 250–300 cm deep under the CT treatment (Fig. 1i), which indicates that nitrate will leach to the ground water later. The results indicated that low-disturbance practices could sustain NO_3^- -N, possibly by promoting root growth into deep soil (Thorup-Kristensen, 2006; Kemper et al., 2011). Additionally, some leached nitrate likely had been removed by denitrification in deep soil, as a higher relative abundance of denitrification bacteria (*Caldithrix* and *Pseudomonas*) was detected in NTSM (Fig. 3); alternatively, no-tillage improved the soil physical structure as a result of soil disturbance decreases, such as increased soil penetration resistance, which resulted in decreased drainage and leaching (Celik et al., 2017). Soil NH_4^+ -N, another form of soil mineral N, was significantly higher in NTNS than in CT and NTSM, which can be explained by the changes in genes involved in N cycling (Fig. S4) and the result of the ^{15}N -labeled fertilizer experiment from our station (Yuan et al., 2021). First, the increased abundance of dissimilatory and assimilatory nitrate reduction genes suggested that low-disturbance practices tended to convert nitrate to ammonium (Fig. S4). However, under the NTSM treatment, ammonium may subsequently transform into soil organic N, as the isotopic tracer indicated that maize stover mulching significantly enhanced the transformation of fertilizer N into organic N pools compared with NTNS (Yuan et al., 2021). In addition, from October 10, 2016 (after harvest) to April 27, 2017 (our sampling date), the total precipitation was only 66.4 mm (data are obtained from a local meteorological administration),

CT Treatment

0 indicator genera

NTNS Treatment



NTSM Treatment

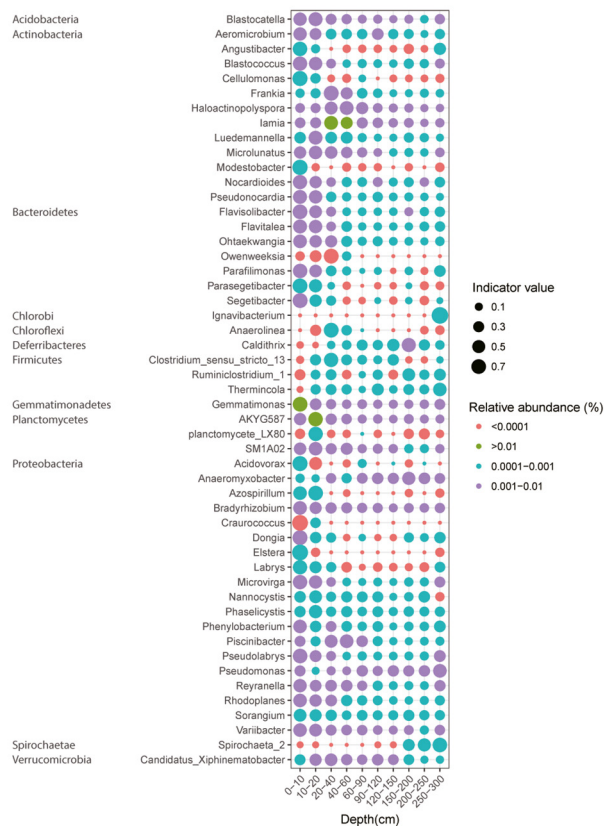


Fig. 3. Indicator genera that were significantly ($q < 0.1$) associated with tillage practices. The size of each circle represents the indicator value of a specific genus at different soil depths. The color indicates the relative abundance of each indicator genus. Taxonomic information, indicator values, P values, and q -values of all indicator genera are given in Table S5. No indicator genera were identified in the CT treatment. More indicator genera indicate more diverse bacterial functions.

yet we found that the soil water content was significantly higher in the NTSM plots than in the CT plots (Fig. 1e). We therefore speculated that mulching stover helped mitigate potential negative (e.g., drought) impacts on crop establishment, which then increased corn yields because stover

mulching could sustain the soil water content by reducing water evaporation and surface runoff (De Vita et al., 2007; Prosdocimi et al., 2016).

4.2. No-tillage with stover mulching promotes bacterial diversity, richness, and potential function

Under the CT treatment, tillage heavily disturbed the topsoil and liberated occluded organic materials. Microbes tend to rapidly use available nutrients in the plowed layer (e.g., NH_4^+ -N) (Ramirez-Villanueva et al., 2015), thereby causing a reduction in bacterial community diversity (Fig. 2) and metabolic diversity (Fig. 5a). Then, the resistance of the soil to stress or disturbance may decrease (Kremen, 2005). In deeper soil layers, due to shallower roots, NO_3^- -N could quickly move downward and intensely accumulate in deeper soils (Fig. 1i), which may be the main factor influencing the shaping of bacterial community (Fig. 6b). Because NH_4^+ -N and NO_3^- -N are the predominant forms of soil mineral nitrogen, the bacteria under CT were mainly influenced by added chemical fertilizer (Wood et al., 2015). Since chemical fertilizer was applied in the same manner and at the same rate for all treatments, the reason why the bacterial communities in CT were strongly influenced by fertilizer may be attributed to soil loosening from tilling; on the one hand, tillage likely allows the applied fertilizer to be better distributed - this would mean that a greater volume of soil (and the bacteria within it) was impacted by fertilizer; on the other hand, tillage adds oxygen to the soil that allows bacteria to react. For low-disturbance practices, more stratification effects (both lateral and vertical) occur around the fertilizer band, so a relatively low volume of soil is directly impacted by the fertilizer (Lupwayi et al., 2001). The presence of stover mulch is also likely to immobilize some of the fertilizer N in near-surface soils as the stover decomposes (Grageda-Cabrera et al., 2011). In addition, no indicator genera were identified for each soil depth in the CT treatment, while a large number of indicator genera were observed in NTNS and NTSM (Fig. 3 and Table S5). Indicator genera for soil depths might play diverse putative functions (Zhang et al., 2017), and when combined with both the increase in genes related to metabolic pathways from functional prediction analyses under low-disturbance practices (Fig. 5) and the initial soil properties in 2007, yield results suggesting that low-disturbance practices result in the diversification of bacterial diversity, structure, and potential function, although the changes are attributed to treatments.

Under the NTNS treatment, soil pH was the major edaphic factor strongly correlated with the bacterial community, and it changed in a similar direction and with a similar magnitude with depth (Fig. 6c). This is reasonable because bacteria often show a narrow tolerance to soil pH (Rousk et al., 2010). Under the NTNS treatment, the soil pH increased with depth and was highly variable (ranging from 6.99 to 8.11) (Fig. 1d and Table S1). Moreover, because of the surface application of chemical fertilizer and the absence of disturbance, the surface soil pH was significantly lower than that under CT (Table S1). This stratification of soil pH could further influence nutrient availability, ion toxicity, and rooting patterns (Lauber et al., 2009; Rousk et al., 2010; Zhalnina et al., 2015). These results might explain why a greater number of indicator genera belonged to shallower soils (Table S5) and were negatively correlated with soil pH (Table S7).

In contrast to NTNS, soil pH showed no significant changes with depth under the NTSM treatment, and TN and C/N were significantly correlated with the soil bacterial community (Fig. 6d). Since soil C/N and TN in bulk soil did not differ with treatment at almost all depths, changes in substrate availability (or quality) may play a key role (Cookson et al., 2008). Studies have reported that following the application of maize stover mulching, more organic N, amino acid N, and amino sugar N were observed in soil (Liu et al., 2016; Lu et al., 2018), which, when combined with a higher abundance of genes related to amino acid metabolism and carbohydrate metabolism from functional prediction analyses (Fig. 5), jointly suggesting that NTSM shifts the acquisition of soil nitrogen.

The increased available nitrogen and labile carbon in deep soil under NTSM may increase the resilience and resistance of maize to disturbances. Resistance to disturbance or stresses is the nature of healthy soil and is

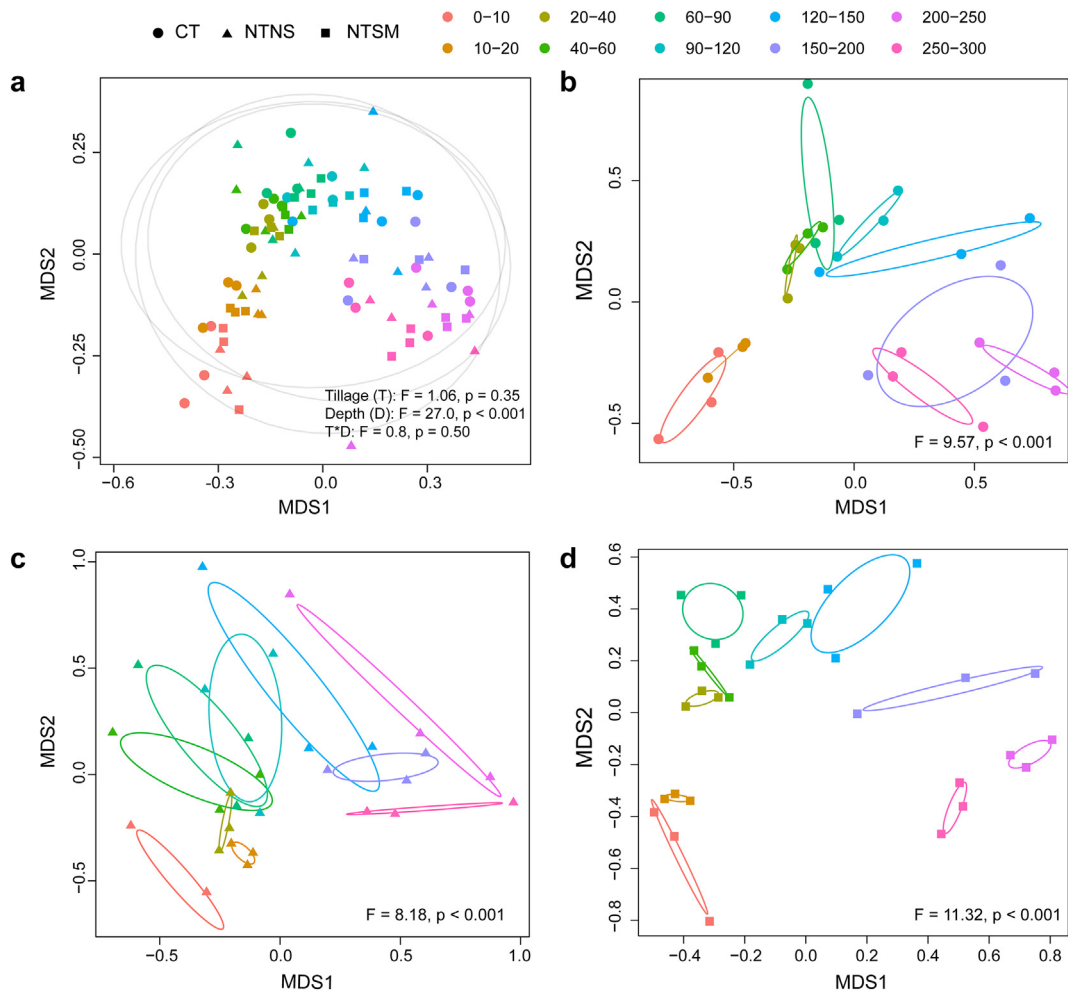


Fig. 4. Nonmetric multidimensional scaling (MDS) ordination of soil bacterial community structures based on Bray–Curtis distances. (a) Ordination of all samples from the three tillage disturbance practices. (b–d) Ordination of samples from CT, NTNS and NTSM. Permutational multivariate analysis of variance (PERMANOVA) results are shown at the bottom right of each figure. Circles, triangles and squares represent CT, NTNS and NTSM, respectively.

essential for maintaining ecosystem functions, such as decomposing organic matter (Kibblewhite et al., 2008; Zhang et al., 2013a). Under the NTSM treatment, the microorganisms associated with the degradation of relatively stable carbon compounds, such as Planctomycetes and Verrucomicrobia (Table S3) (Herlemann et al., 2013; Erbilgin et al., 2014), as well as the indicators *Cellulomonas* and *Azospirillum* (Fig. 3 and Table S5) with the function of cellulose decomposition (Halsall and Goodchild, 1986; Pathma et al., 2019), were increased. The predicted functional profiles related to energy metabolism (carbon fixation pathways in prokaryotes), carbohydrate metabolism (TCA cycle, amino sugar, nucleotide sugar, galactose, fructose), biosynthesis of secondary metabolites (carotenoid and betalain) and glycan biosynthesis were increased, suggesting higher metabolic activity and a change in substrate quality (Fig. 5). In addition, more indicator genera at each soil depth were identified under NTSM than under the NTNS and CT practices (Fig. 3 and Table S5), and these indicators at different soil depths might enhance the ability of NTSM to resist disturbance. For example, the denitrification bacteria *Caldithrix* and *Pseudomonas* (Koike and Hattori, 1975; Miroshnichenko et al., 2003) were the indicator genera at 150–200 cm and 250–300 cm, respectively (Fig. 3 and Table S5), which might explain the low amount of NO_3^- -N in the deep soil in NTSM. *Ignavibacteria* and *Spirochaeta*, the indicator genera of deep soil, have the ability to grow under strictly anaerobic conditions (Iino et al., 2010) or severely limited nutrient conditions (Terracciano and Canale-Parola, 1984), respectively. Surface indicator genera belonging to Bacteroidetes might have the ability to degrade organic matter that is difficult to decompose (Thomas et al., 2011).

4.3. Implications for climate change and food security

To detect what amount of available nitrogen the soil can provide at the beginning of the growing season, we estimated the root zone of each tillage practice. Fine roots (diameter < 2 mm) play a major role in releasing root exudates and absorbing nutrients and water, while measuring fine roots is still technically difficult, especially in situ (Pierret et al., 2016). Instead of detecting fine roots directly, we measured SEOC as the indicator of root depth, as SEOC is a proxy for biotically derived organic acids and is a sensitive signal of root density (Billings et al., 2018). According to the change in SEOC with soil depth under different disturbance practices (Fig. 1b), we estimated that corn roots reached 60–90 cm, 90–120 cm and 120–150 cm under CT, NTNS and NTSM, respectively, which is in line with reported corn root depths (~150 cm) (Canadell et al., 1996; Kemper et al., 2011). Then, we estimated the mineral N retained in the root-zone soil based on the equivalent soil mass approach (von Haden et al., 2020), and it was approximately 170.84, 344.89, and 418.94 kg/ha under CT, NTNS and NTSM, respectively (Table S9).

Generally, corn roots reach their maximum depth at the silking stage (Archontoulis and Licht, 2017), which is also the time when the heaviest rainfall occurs in northeastern China. We therefore expect that the available N retained in the root zone would be utilized by crops in the upcoming growing season before storms could leach nitrogen into the groundwater, meaning that fertilizer N could be reduced to meet crop growth requirements, at least in Northeast China, and prevent reactive N losses. The energy consumption and greenhouse gas emissions during fertilizer

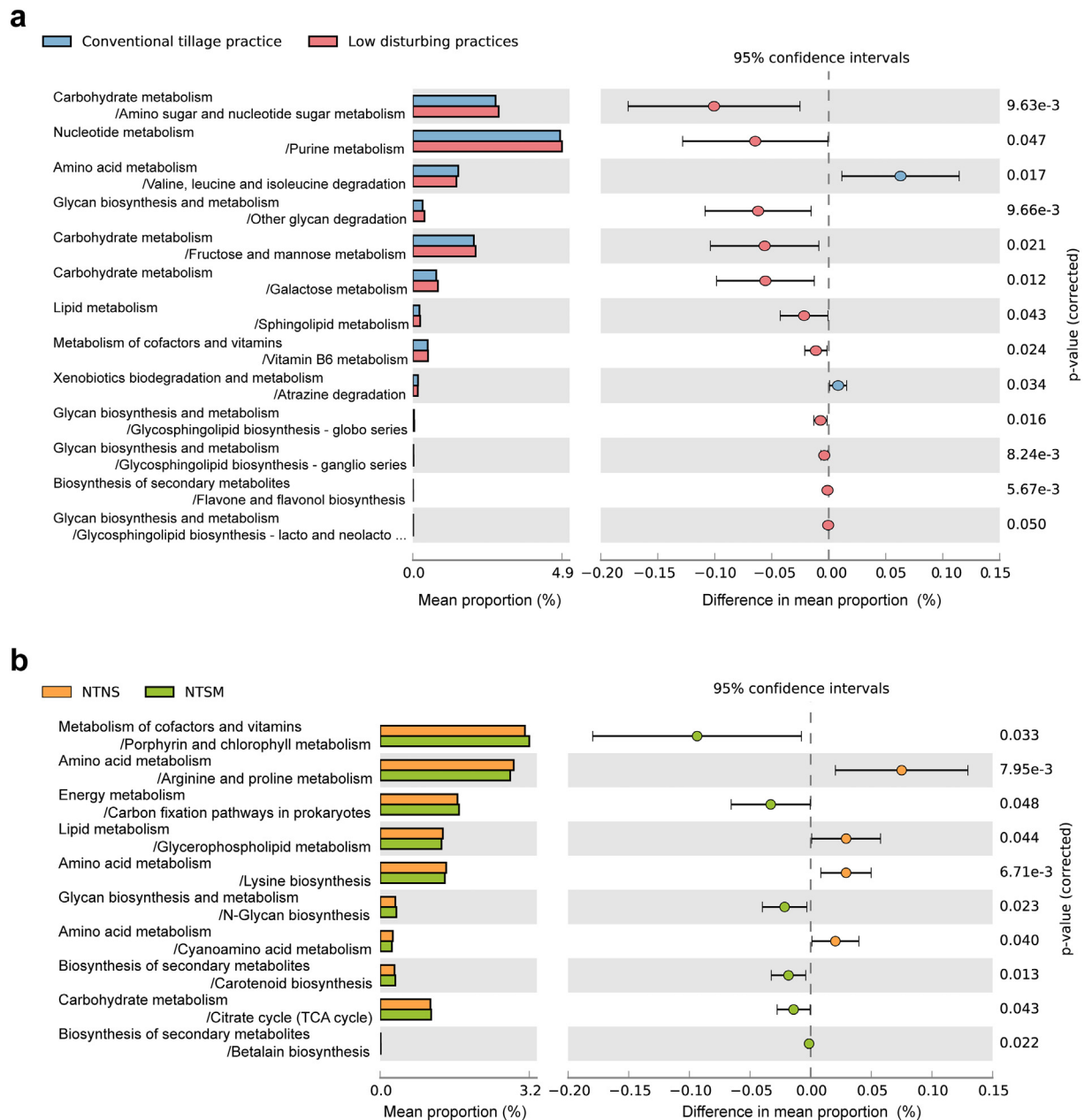


Fig. 5. Extended error bar plots showing significant differences in the mean relative abundance of 16S rRNA gene-predicted functional profiles obtained with Tax4Fun. (a) Difference between mean proportions of conventional practice and low-disturbance practices; (b) Differences between mean proportions of NTNS (no-tillage without stover mulching) and NTSM (no-tillage with 100% stover mulching). “Mean proportion” reflects “mean relative abundance”, as the data were normalized. The graphic shows only the pathways (L2/L3) with statistical differences between treatments with a confidence interval of 95 (Welch’s *t*-test).

production then could be reduced. This is a large amount for the following reasons: 1) for every kilogram of fertilizer-N produced and used on cropland, up to 87.9 MJ of energy is consumed (Kennedy, 2000), and 13.5 kg of CO₂ equivalent (eq) (CO₂-eq) is emitted (Zhang et al., 2013b); 2) maize farmland in Northeast China is approximately 13,000,000 ha (Source: China Statistics Yearbook 2018); 3) low-disturbance practices usually show higher crop yield in this region, which means much more carbon can be sequestered (carbon content ≈ 0.5*biomass (Ma et al., 2018)).

5. Conclusion

Our results clearly showed that long-term low-disturbance practices could save agricultural resources and diversify the bacterial communities in the whole 3-m soil profile. In agroecosystems, more abundant and

diverse bacterial communities generally indicate an increase in soil quality. These findings revealed that agricultural soil under appropriate management may ultimately allow access to nutrients and water from deeper soil without reclaiming more natural land areas, reduce nutrient loss to groundwater, and improve the self-sustaining ability of farmland in the face of climate change.

Data availability

All sequencing data that support the findings of this study have been deposited in the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>) in the Sequence Read Archive (SRA) database (BioProject number: PRJNA488172). Other data supporting the results can be accessed from the corresponding author upon request.

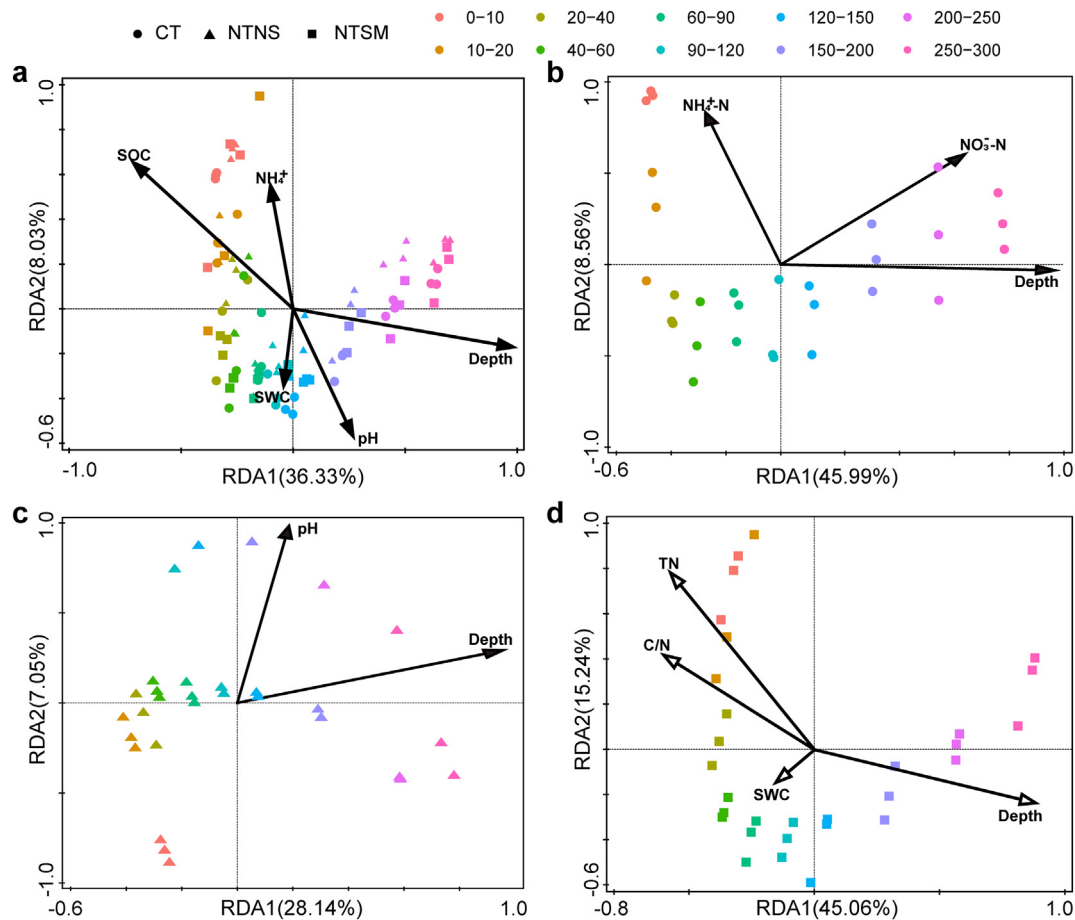


Fig. 6. Redundancy analysis (RDA) of the soil bacterial community originating from bacterial phyla constrained by soil properties among samples. (a) All samples from the three tillage disturbance practices. (b-d) Samples from CT, NTNS and NTSM, respectively. Only soil variables that significantly explained the variability in the bacterial community structure in the forward selection procedure were selected for the ordination (arrows). TN = total nitrogen content; C/N = ratio of SOC to TN; $\text{NH}_4^+\text{-N}$ = ammonium nitrogen; $\text{NO}_3^-\text{-N}$ = nitrate nitrogen; SWC = soil water content. Circles, triangles and squares represent CT, NTNS and NTSM, respectively.

CRediT authorship contribution statement

Hongtu Xie, Xudong Zhang and Chao Liang designed the experiment, Fangbo Deng performed field and laboratory measurements, Fangbo Deng, Hongjun Wang and Chao Liang analyzed the data and wrote the manuscript, and all the authors discussed the results and commented on the manuscript.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.153929>.

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