

# Residue Return Effects Outweigh Tillage Effects on Soil Microbial Communities and Functional Genes in Black Soil Region of Northeast China

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**Abstract:** Conservation tillage as an effective alternative to mitigate soil degradation has attracted worldwide attention, but the influences of conservation tillage on soil microbial community and especially function remain unclear. Shotgun metagenomics sequencing was performed to examine the taxonomic and functional community variations of black soils under three tillage regimes, namely no-tillage with residue (maize straw) return (NTS), moldboard plow with residue return (MPS), and moldboard plow without residue return (MPN) in Northeast China. The results revealed: 1) Soil bacterial and archaeal communities differed significantly under different tillage regimes in contrast to soil fungal community. 2) The overlay of less tillage and residues return under NTS led to unique soil microbial community composition and functional composition. Specifically, in contrast to other treatments, NTS increased the relative abundances of some taxa such as *Bradyrhizobium*, *Candidatus Solibacter*, and *Reyranelia*, along with the relative abundances of some taxa such as *Sphingomonas*, Unclassified Chloroflexi and *Nitrososphaera* decreased; NTS had a unique advantage of increasing the relative abundances of genes involved in ‘ATP-binding cassette (ABC) transporters’ and ‘quorum sensing (QS)’ pathways, while MPN favored the genes involved in ‘flagellar assembly’ pathway and some metabolic pathways such as ‘carbon’ and ‘glyoxylate and dicarboxylate’ and ‘selenocompound’ metabolisms. 3) Significantly different soil bacterial phyla (Acidobacteria, Gemmatimonadetes, and Chloroflexi) and metabolic pathways existed between MPN and another two treatments (NTS and MPS), while did not exist between NTS and MPS. 4) Dissolved organic carbon (DOC) and soil bulk density were significantly affected ( $P < 0.05$ ) by tillage and accounted for the variance both in microbial (bacterial) community structure and functional composition. These results indicated that a change in tillage regime from conventional to conservation tillage results in a shift of microbial community and functional genes, and we inferred that residue return played a more prominent role than less tillage in functional shifts in the microbial community of black soils.

**Keywords:** no-tillage; microbial community composition; Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways; soil properties

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## 1 Introduction

Black soils (Mollisols) in Northeast China are one of the four major Mollisol regions in the world, supporting sustainable crop production (Kravchenko et al., 2011). However, soil degradation caused by intensive conventional tillage has been the main factor in the decline of soil health, for example, conventional tillage disrupted the natural structure of soils, resulting in a higher erosion rate and a lower soil organic carbon (SOC) content (Zhao et al., 2018). Conservation tillage encompassing no-tillage (NT) and residue return has attracted extensive attention worldwide in recent years and has been increasingly adopted to improve soil health (Harper et al. 2018). It was well-documented that conservation tillage was beneficial to improve soil physical structure, enhance soil water content, and increase SOC content (Li et al., 2019; Zhang et al., 2019a). Improved soil physical and chemical characteristics in conservation tillage could create a better habitat for soil microbes, leading to higher microbial biomass and activity (Sekaran et al., 2020) and shifting soil microbial communities (Wang et al., 2017; Hao et al., 2019). Soil microorganisms regulate crucial soil processes such as SOC decomposition and nutrient availability and consequently may exert effects on soil ecosystem function (O'Donnell et al., 2001; Smith et al., 2015). Therefore, a comprehensive understanding of soil microbial communities under different soil management practices is critical for the development of sustainable agricultural ecosystems.

In recent years, studies on the effects of conservation tillage on soil microbial communities have benefited from advances in high-throughput sequencing approaches such as 16S rRNA (Yang et al., 2020; Bu et al., 2020). Results about the effects of tillage on soil microbial alpha diversity have sometimes been contradictory; some studies have shown that NT increased soil microbial (bacterial and fungal) alpha diversity relative to conventional tillage systems (Li et al., 2020a), while other studies showed no significant effect (Rincon-Florez et al., 2020) or even a decrease (Degruno et al., 2016). Shifts in microbial community composition under different tillage regimes have been widely reported (Dong et al., 2017; Romero-Salas et al., 2021). Conservation tillage was reported to favor the copiotrophic groups (Wang et al., 2020a) and symbiotic fungal

groups in contrast to conventional tillage (Schmidt et al., 2019; Wang et al., 2021). Despite the breadth of research exploring the impacts of conservation tillage on soil microbial community composition, it has provided limited information about their function; it is still unclear how to relate these microbial community changes to their functions.

Compared with amplicon sequencing, the metagenomic approach provides higher taxonomic resolution and possesses a unique advantage in aiding the identification of metabolic pathways and functional gene sequences of the soil environment (Liu et al., 2021a). Recently, it has been demonstrated that some metabolism-related sequences were significantly affected by tillage treatments using comparative metagenomics, NT with more sequences related to the metabolism of the aromatic compound might favor these higher efficiency C-metabolic pathways, whereas conventional tillage has more sequences associated with carbohydrate metabolism because a greater diversity of metabolic pathways were required to adapt to the lower content of organic matter condition (Souza et al., 2015; Miura et al., 2016). The combined effect of crop residue retention and minimum tillage, the two cores of conservation tillage, on soil microbial community has often been studied (Pankhurst et al., 2002; Dhaliwal et al., 2020), moreover, organic input appeared to be more important than tillage for soil microbial functional indices based on the results of enzyme assays (Chen et al., 2020). Therefore, it is essential to distinguish the effect of residue return and disturbance induced by the tillage system to better understand the benefits of conservation tillage to soil microbial community function. In this study, metagenomic sequencing was employed to elucidate the influence of tillage treatment and residue return on soil microbial composition and function in black soil. We hypothesized that residue return was the dominant factor that influenced soil microbial community and function under the tillage system instead of a reduction in soil disturbance induced by the conservation tillage treatment (less tillage). The objectives of this study were to 1) reveal the differences in soil microbial taxonomic and functional composition under no-tillage (residue return and less disturbance) and conventional tillage both with and without residue return systems; 2) explore the relationship between soil environmental factors and soil microbial community and function in

Northeast China.

## 2 Materials and Methods

### 2.1 Site description and experimental design

The study was conducted on an ongoing tillage and crop rotation experiment at the Changchun Observation Station (44°00'N, 125°24'E) (Fig. 1), Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. The field experiment was established in 2012 in Changchun, Jilin Province, China. The climate is continental temperate monsoon. This site has an average 4.4°C annual temperature (1982–2009) and receives 515 mm annual precipitation with most of the precipitation occurring from June to August (Zhang et al., 2022). The soil is classified as Mollisols (USDA Soil Taxonomy), and before the experiments, soil pH, total C, and total N are 7.3, 19.1 g/kg, and 1.6 g/kg,

For the present study, three tillage treatments with three replicates (7.8 m × 25 m each plot) under a continuous maize cropping system were studied: a) NTS: no-tillage with residue return; b) MPS: moldboard plowing with residue return; c) MPN: moldboard plowing without residue return. In the NTS plots, no soil disturbance occurred except for the planting with a no-till planter (KINZE-3000NT, Williamsburg, IA, USA). After harvesting, the maize residue was cut into about 30 cm pieces and then laid on the soil surface. MPN treatment included post-harvest removal of residue, moldboard ploughing (20 cm) after harvest in fall, and secondary seedbed preparation in the spring by disking (7.5–10 cm). In the MPS plots, the residue was remo-

ved prior to fall moldboard plowing and then manually put back. The residue was incorporated into the soil mechanically during spring cultivation of the next year and then mixed to a deeper depth with moldboard ploughing in the autumn. The average aboveground biomass maize residues were 11.0 Mg/ha. Residue return in MPS and residue removal in MPN was the only difference between MPS and MPN. For fertilizer management, the application rates of N, P, and K were the same in each tillage treatment. Base fertilizers in the form of urea, ammonium phosphate, and potassium sulphate (100.0 kg N/ha, 45.5 kg P/ha, and 78.0 kg K/ha) were applied, and an additional 45.0 kg N/ha was applied as top dressing at the V-6 stage. For all tillage treatments, maize weeds and pests were controlled by herbicides and insecticides applied before and after seeding following conventional practices.

### 2.2 Soil sampling and analysis

Soil samples (about 15 cm distance from the maize rows) were collected at a depth of 0–10 cm in April 2019. In each plot, seven soil cores were taken in a zig-zag pattern using a hand auger (2.5 cm in diameter) and then pooled to form a composite sample. Soil samples were stored in a box with ice and brought back to the laboratory promptly after collection. Visible roots, leaves, and stones were removed and the soils were sieved through a 2-mm mesh. A portion of each soil sample was stored at –20°C for subsequent total DNA extractions, and the other part of the fresh soil sample was used for the determination of soil water content (SWC), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ), soil dissolved organic carbon (DOC) and dissolved total nitrogen (DTN). In addition, cutting rings (100 cm<sup>3</sup>) were used in each plot to collect soil samples for the determination of soil bulk density (SBD). Soil water content and bulk density were determined by the over-drying method; soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were extracted with 2 mol/L KCL solution and measured by a continuous flow analyzer (SKALAR SAN ++, Holland). Soil DOC and DTN were extracted with 0.5 mol/L  $\text{K}_2\text{SO}_4$  solution and measured by a total organic carbon analyzer (TOC-L CPH, SHIMADZU, Japan).

### 2.3 DNA extraction and sequencing

DNA was extracted using the MoBIO PowerSoil<sup>®</sup> DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA,

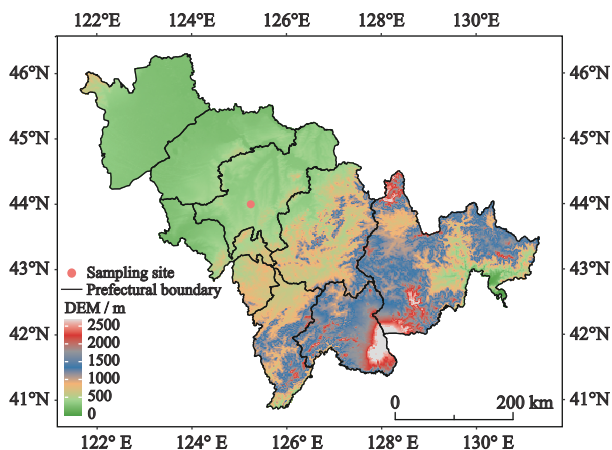


Fig. 1 Location of study area

USA), following the manufacturer's instructions. The concentration and purity of genomic DNA were checked with a TBS-380 mini-fluorometer (Turner BioSystems, Inc., Sunnyvale, CA, USA) and NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. The DNA quality was examined by 1% agarose gel electrophoresis. Covaris M220 was used to shear DNA into about 400 bp fragments. A paired-end library was constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA) and then sequenced on Illumina HiSeq 4000 sequencing platform (paired-end 150 bp reads) (Illumina Inc., San Diego, CA, USA) at Majorbio BioPharm Technology Co., Ltd. (Shanghai, China). All of the raw metagenomic datasets in this study are publicly available in the NCBI Sequence Read Archive (SRA) database with an accession number PRJNA692995.

#### 2.4 Metagenome sequencing data analysis

The raw sequence reads were trimmed using FASTP v0.20.0 software (<https://github.com/OpenGene/fastp>) to remove adaptor and low-quality sequences. After quality filtering, the clean reads were assembled by MEGAHIT v1.1.2 (Li et al., 2015) to acquire contigs of over 300 bp. MetaGene (<http://metagene.cb.k.u-tokyo.ac.jp/>) (Noguchi et al., 2006) was used to predict open reading frames (ORFs). All predicted genes were clustered by an identity of 95% and coverage of 90% using CD-HIT (Li and Godzik, 2006), and the longest sequences were chosen as representative sequences, resulting in a non-redundant gene catalog. The cleaned data for each sample were mapped to the non-redundant gene with SOAPaligner v2.21 software (Li et al., 2008) with a criterion of 95% identity to determine the number of reads that mapped to the genes in each sample.

Taxonomic and functional annotations were carried out by aligning the non-redundant gene catalog to the NCBI-NR database and KEGG (Kyoto Encyclopedia of Genes and Genomes) database, respectively by DIAMOND v 0.8.35 (Buchfink et al., 2015) with optimum E-value of  $10^{-5}$ .

#### 2.5 Statistical analysis

The effect of tillage regimes on soil physiochemical properties and abundant microbial phyla or genera were determined by analysis of variance (ANOVA) with the post-hoc LSD test by SPSS 23.0 (SPSS Inc., Chicago,

IL, USA). Principal co-ordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were performed using the Adonis function based on Bray-Curtis distance within the R package vegan (Oksanen et al., 2013). Based on a statistical analysis of metagenomics profiles (STAMP) (Parks and Beiko, 2010), using Welch's *t*-test for pairwise comparisons, significantly different metabolic pathways and KO functional categories between different tillage regimes were identified. The relationships between microbial community and environmental variables as shaped by soil tillage practices were visualized using distance-based redundancy analysis (db-RDA) and tested statistically using the envfit analysis. Contribution and regression analysis between species and function were performed to explore the relationships between soil microbial species and function. The db-RDA, envfit, and contribution and regression analyses were performed on the online platform of Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)).

### 3 Results

#### 3.1 Soil physiochemical properties

ANOVA revealed that the effects of tillage on soil water content, DOC, and soil bulk density were significant ( $P < 0.05$ ), and soil water content and soil bulk density were higher in no-tillage with residue (NTS) than in moldboard plow with residue return (MPS) and moldboard plow without residue return (MPN) ( $P < 0.05$ ); DOC was higher in NTS and MPS than in MPN ( $P < 0.05$ ). The highest soil  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and DTN were found in NTS, although the effects were not significant (Table 1).

#### 3.2 Soil bacterial and archaeal community compositions

All filtered reads were aligned against the NCBI-NR database. Soil bacterial community composition (Adonis,  $P = 0.008$ ,  $R^2 = 0.55$ ) and archaeal composition (Adonis,  $P = 0.016$ ,  $R^2 = 0.62$ ) differed significantly among tillage treatments, and PCoA plot showed that soil bacterial community composition (genus level) under NTS was obviously apart from MPN, and soil archaeal composition under NTS was apart from both MPN and MPS (Fig. 2). While soil fungal composition was not significantly different among tillage treatments



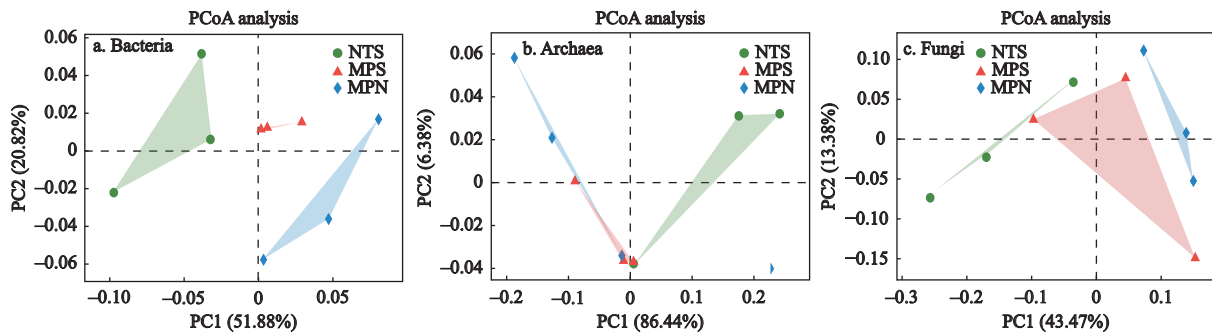
**Table 1** Soil physicochemical properties under different tillage treatments (means  $\pm$  SE)

Samples	NH <sub>4</sub> <sup>+</sup> -N / (mg/kg)	NO <sub>3</sub> <sup>-</sup> -N / (mg/kg)	SWC / %	DOC / (mg/kg)	DTN / (mg/kg)	SBD / (g/cm <sup>3</sup> )
NTS	2.08 $\pm$ 0.10	1.99 $\pm$ 0.43	17.34 $\pm$ 0.58a	75.22 $\pm$ 3.22a	54.41 $\pm$ 1.92	1.36 $\pm$ 0.03a
MPS	1.86 $\pm$ 0.07	1.88 $\pm$ 0.19	13.01 $\pm$ 1.19b	73.19 $\pm$ 1.59a	45.02 $\pm$ 5.44	1.18 $\pm$ 0.02b
MPN	1.90 $\pm$ 0.02	1.84 $\pm$ 0.28	12.17 $\pm$ 0.12b	56.54 $\pm$ 6.93b	48.58 $\pm$ 5.93	1.21 $\pm$ 0.01b

*F* values of ANOVA

Tillage	2.81	0.06	9.93*	5.18*	0.98	22.15**
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Notes: NTS represents no-tillage with residue (maize straw) return; MPS represents moldboard plow with residue return (MPS); MPN represents moldboard plow without residue return; SWC and SBD are abbreviations of soil water content and soil bulk density, respectively; NO<sub>3</sub><sup>-</sup>-N, soil nitrate (NO<sub>3</sub><sup>-</sup>) contents; NH<sub>4</sub><sup>+</sup>-N, soil ammonium (NH<sub>4</sub><sup>+</sup>) contents; DOC, soil dissolved organic carbon; DTN, soil dissolved total nitrogen; different letters in each tillage treatment indicate significant differences \*  $P < 0.05$ ; \*\*  $P < 0.01$



**Fig. 2** Principal coordinate analysis (PCoA) plots based on Bray-Curtis distances between samples presenting bacterial, archaeal, and fungal communities (genus level) in soils of different tillage regimes encompassing NTS, MPS, and MPN. NTS represents no-tillage with residue (maize straw) return; MPS represents moldboard plow with residue return (MPS); MPN represents moldboard plow without residue return

(Adonis,  $P = 0.052$ ).

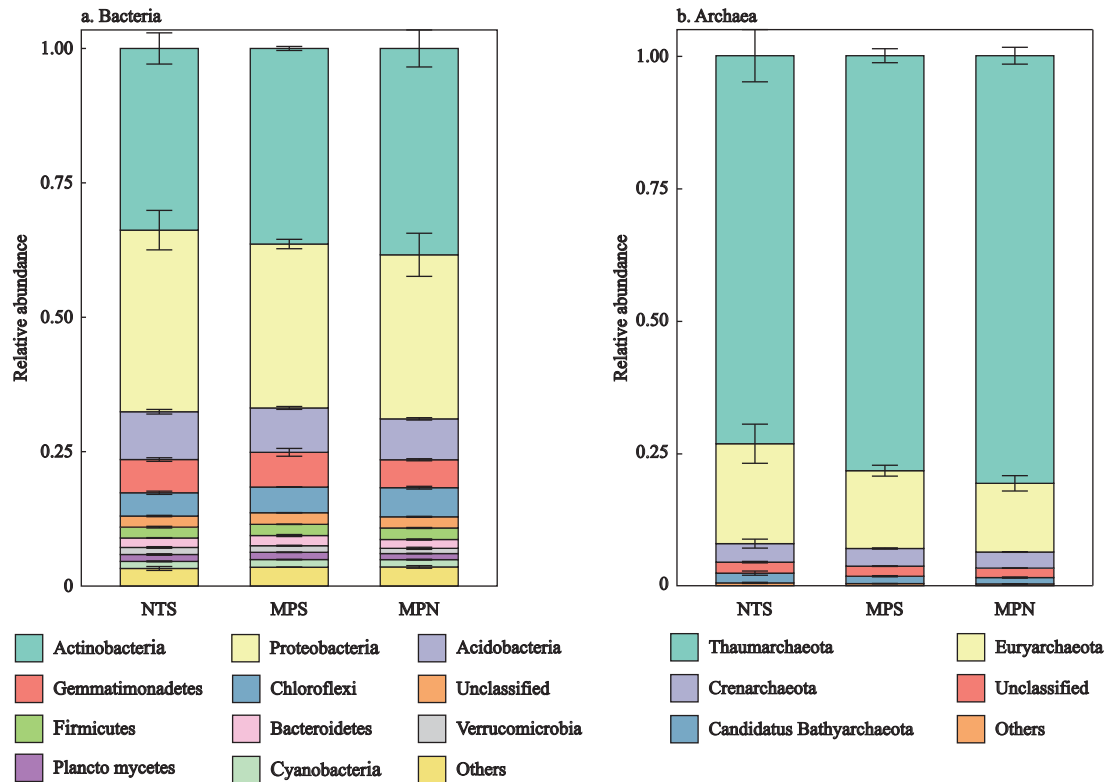
Members of soil bacterial and archaeal communities were further analyzed in this study. Abundant phyla and genera with a relative abundance of over 1% were shown (Fig. 3, Table 2), among these bacterial phyla, Actinobacteria was the most abundant taxa with an abundance percentage of 36.20% trailed by Proteobacteria (30.27%), Acidobacteria (8.25%), Gemmatimonadetes (5.94%) and Chloroflexi (5.02%) (Fig. 3). For archaeal phyla, Thaumarchaeota (77.39%) and Euryarchaeota (15.50%) predominated in all treatments (Fig. 3). Several abundant bacterial and archaeal phyla were significantly affected by tillage practice (Fig. 3, Table 2), for example, soil bacteria phyla including Acidobacteria, Gemmatimonadetes, and Chloroflexi significantly differed between MPN and another two treatments (NTS and MPS), while did not change between NTS and MPS (Fig. 3, Table 2). Specifically, Chloroflexi was significantly less abundant under NTS and MPS than MPN, while Gemmatimonadetes and Acidobacteria were more abundant under NTS and MPS than MPN (Table 2). Soil archaeal phyla including unclassified Ar-

chaea and Candidatus Bathyarchaeota showed higher relative abundance under NTS than MPN (Fig. 3, Table 2).

For the bacterial genera (Table 2), a total of 21 abundant genera accounted for about 42.70% of all the reads (data not shown). *Bradyrhizobium* (Rhizobiales) and Candidatus *Solibacter* significantly differed among three tillage treatments with a relative abundance of NTS > MPS > MPN. *Reyranella* exhibited significantly higher relative abundance under NTS than MPS and MPN (Table 2), while *Sphingomonas* and unclassified Geodermatophilaceae exhibited significantly lower relative abundance under NTS than MPS and MPN (Table 2). For the archaeal genera, unclassified Candidatus Bathyarchaeota showed higher relative abundance under NTS than MPN. *Candidatus Nitrosocosmicus* showed significantly lower relative abundance under NTS than MPN, and *Nitrososphaera* showed significantly lower relative abundance under NTS than MPS and MPN (Table 2).

### 3.3 Soil microbial community functions

A total of 407 level-3 KEGG pathways were obtained across all sampling sites, and KEGG pathways with a



**Fig. 3** Compositions of the soil bacterial and archaeal communities at phylum levels under NTS, MPS, and MPN treatments. Groups accounting for < 1% are integrated into others. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return

relative abundance > 1% were analyzed (Fig. 4). The dominant pathways were carbon metabolism, biosynthesis of amino acids, ABC transporters, pyrimidine metabolism, and quorum sensing with 5.1%, 4.5%, 3.0%, 2.9%, and 2.7% of the total annotated genes, respectively. Compared with MPN, ABC transporters and quorum sensing were more abundant in soils under NTS, and alanine, aspartate, and glutamate metabolism, pyrimidine metabolism, and carbon metabolism were less abundant in soils under NTS (Fig. 4); starch and sucrose metabolism was more abundant in soils under MPS; citrate cycle, pyrimidine metabolism, alanine, aspartate, and glutamate metabolism and purine metabolism were less abundant in soils under MPS (Fig. 4). There were no significantly different pathways between MPS and NTS (Fig. 4). Further, at KO (KEGG Orthology) level, there was statistically divergence (Adonis,  $P = 0.019$ ,  $R^2 = 0.40$ ) among different tillage regimes, and PCoA plot showed that MPN was apart from NTS and MPS (Fig. 5), while NTS and MPS were relatively closer. The top 50 abundant KO functional categories were further analyzed to show the different categories among tillage regimes (Fig. 6a). Specifically, compared

with MPN, the four KO functional categories (K01997, K01998, K01999, and K02051) were more abundant in soils under NTS (Fig. 6a) representing ABC transporters and quorum sensing pathways. A total of six KO functional categories (including K03657, K01992, K03086, K02027, K00384, and K03798) were less abundant in soils under NTS representing selenocompound metabolism, nucleotide excision repair, mismatch repair, flagellar assembly, and ABC transporters pathways. The two KO functional categories (K03088 and K06147) representing transcription and ABC transporters were overrepresented in soils under MPS compared to MPN; a total of seven KO functional categories including amino acid and nucleotide metabolism (K01955, K00548, K00525), fatty acid degradation (K00626), carbon and carbohydrate metabolism (K00626 and K01681) and others (K03086 and K02355) were underrepresented in soils under MPS than MPN (Fig. 6a).

### 3.4 The relationships and driving factors of taxonomic and functional composition

Regression analysis revealed that changes in microbial functional composition (KO level) were highly in line

**Table 2** The results of the post-hoc LSD test on the abundant soil microbial taxa (phylum and genus level) that were significantly affected by tillage regimes

Taxonomy	Name	NTS vs MPS		NTS vs MPN		MPS vs MPN	
		Mean Difference	<i>P</i>	Mean Difference	<i>P</i>	Mean Difference	<i>P</i>
Bacteria (Phylum)	Acidobacteria	0.0063	0.050	0.0129**	0.003	0.0065*	0.046
	Gemmatimonadetes	-0.0028	0.505	0.0098*	0.045	0.0126*	0.018
	Chloroflexi	-0.0045	0.054	-0.0109***	0.001	-0.0063*	0.015
Fungi (Phylum)	Basidiomycota	-0.0695*	0.014	-0.0510*	0.044	0.0185	0.393
	Unclassified Fungi	-0.0180	0.450	-0.0721*	0.018	-0.0541	0.052
Archaea (Phylum)	Unclassified Archaea	0.0017	0.070	0.0029**	0.009	0.0012	0.165
	Candidatus Bathyarchaeota	0.0045	0.065	0.0064*	0.019	0.0019	0.165
Bacteria (Genus)	<i>Bradyrhizobium</i>	0.0075***	0.001	0.0120***	0.000	0.0045**	0.009
	<i>Gemmatirosa</i>	-0.0020	0.425	0.0066*	0.030	0.0086**	0.010
	<i>Sphingomonas</i>	-0.0093*	0.035	-0.0106*	0.021	-0.0014	0.707
	Unclassified Chloroflexi	-0.0031	0.063	-0.0020**	0.003	-0.0036*	0.037
	Candidatus <i>Solibacter</i>	0.0021*	0.050	0.0043**	0.002	0.0023*	0.034
	<i>Reyranella</i>	0.0045**	0.002	0.0056***	0.001	0.0011	0.257
	Unclassified Geodermatophilaceae	-0.0039**	0.005	-0.0050**	0.002	-0.0010	0.303
	<i>Rubrobacter</i>	-0.0054	0.086	-0.0113**	0.005	-0.0060	0.061
	Fungi (Genus)	<i>Rhizopus</i>	-0.0021	0.410	-0.0077*	0.018	-0.0056
<i>Pseudogymnoascus</i>		-0.0098*	0.013	-0.0041	0.194	0.0057	0.087
<i>Fonsecaea</i>		-0.0018	0.466	-0.0111**	0.003	-0.0093**	0.008
<i>Spizellomyces</i>		-0.0076**	0.003	-0.0021	0.224	0.0054*	0.013
<i>Rhodotorula</i>		-0.0133**	0.013	-0.0040	0.338	0.0093	0.051
Archaea (Genus)	<i>Nitrososphaera</i>	-0.0640*	0.042	-0.0784*	0.020	-0.0144	0.583
	Candidatus <i>Nitrosocosmicus</i>	-0.0044	0.287	-0.0129*	0.014	-0.0085	0.066
	Unclassified Archaea	0.0017	0.070	0.0029**	0.009	0.0012	0.165
	Unclassified Candidatus Bathyarchaeota	0.0045	0.065	0.0064*	0.019	0.0019	0.387

Notes: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

with changes in bacterial composition (Fig. 7), and functional contribution analysis showed that *Solirubrobacter*, *Sphingomonas*, and *Bradyrhizobium* contributed most to those different KO functional categories, notably, *Bradyrhizobium* contributed most strongly to K01997, K01998, and K01999 (Fig. 8).

We further established the relationships between soil taxonomic, functional compositions and several soil environmental factors (DOC, DTN,  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, soil water content, and soil bulk density), and db-RDA analysis revealed that environmental factors explained 47.90% and 70.70% of the variability of the bacterial and archaeal composition, respectively. Among them, soil water content ( $R^2 = 0.790$ ,  $P = 0.013$ ), DOC ( $R^2 = 0.669$ ,  $P = 0.026$ ), and soil bulk density ( $R^2 = 0.669$ ,  $P = 0.026$ ) were the main environmental factors that contributed to the soil bacterial composition differences (genus level), whereas DOC ( $R^2 = 0.669$ ,  $P = 0.02$ ) was the key

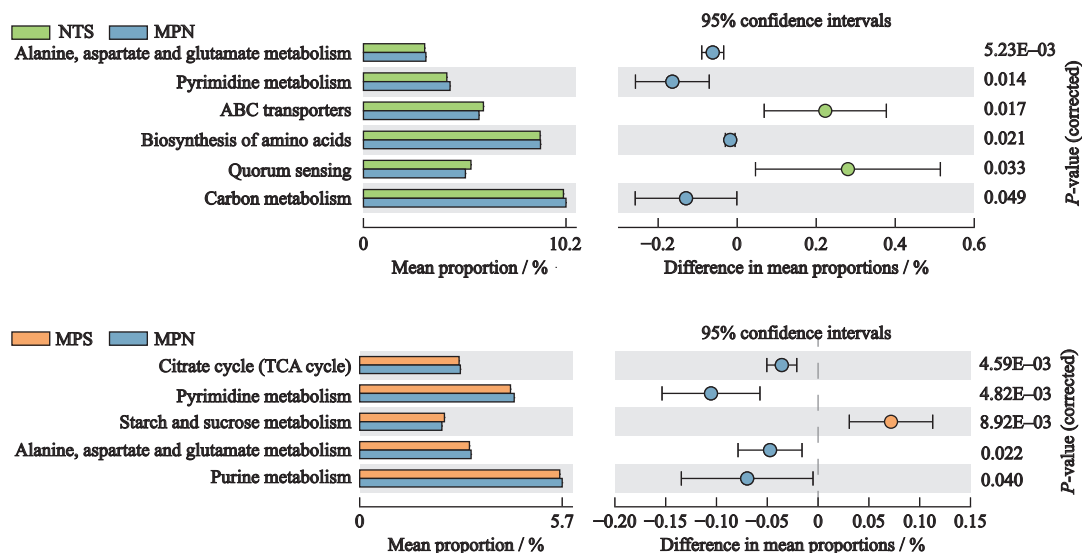
factor that influenced the archaeal community structure. Additionally, soil bulk density ( $R^2 = 0.817$ ,  $P = 0.011$ ) and DOC ( $R^2 = 0.658$ ,  $P = 0.007$ ) were the main influential factors that contributed to differences in the soil microbial function (Fig. 9).

More abundant KO functional genes in NTS soil involved in ABC transporters and quorum sensing such as K01997, K01998, and K01999 were significantly positively correlated with dissolved organic carbon (DOC) (Fig. 6b). Besides, K00384 was significantly negatively correlated with soil DOC (Fig. 6b).

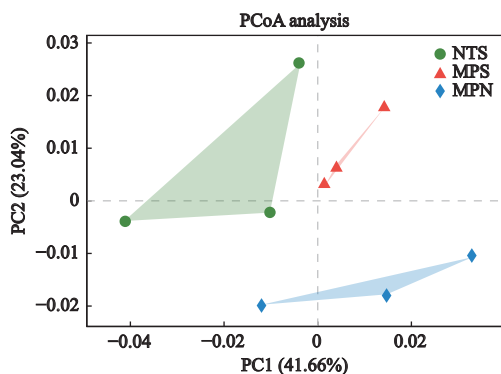
## 4 Discussion

### 4.1 Soil taxonomic composition of soil microbial communities

Soil bacterial and archaeal communities, in contrast to soil fungal community, were more sensitive to soil till-



**Fig. 4** Statistical Analysis of Metagenomic Profiles (STAMP) showing the differentially abundant metabolic pathways among the three tillage regimes. Welch's two-sided *t*-test for each different two tillage treatments with one filter ( $P < 0.05$ ) to generate extended error bar figures. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return



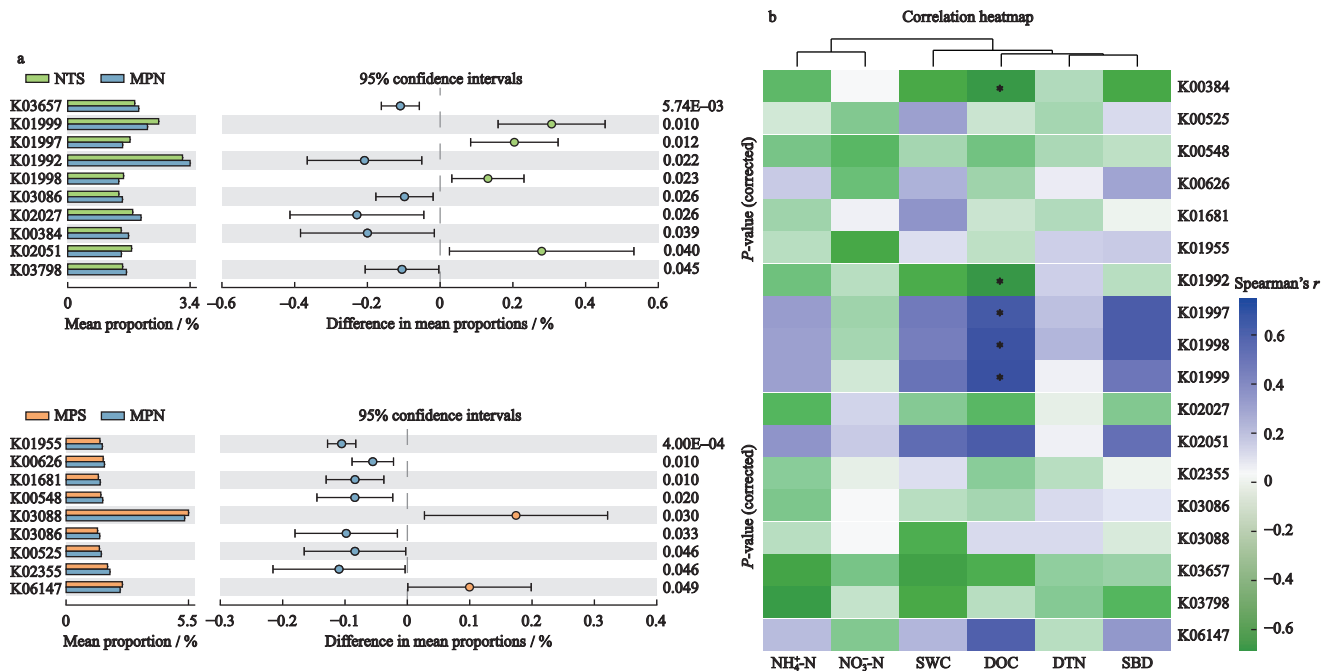
**Fig. 5** Principal coordinate analysis (PCoA) based on Bray-Curtis distances between samples presenting microbial function composition at KO level in soils of different tillage regimes encompassing NTS, MPS, and MPN. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return

age practices and showed a clearer variation under different tillage regimes in our study (Fig. 2), although there was a shift in the relative abundance of several fungal taxa among tillage regimes, but not sufficient to alter the overall fungal community composition (Table 2, Fig. 2). The less sensitivity of soil fungi was not in line with the ITS sequencing results (Sun et al., 2018; Wang et al., 2020a); and the contradictory results are possibly attributed to the relatively higher resilience of soil fungi to environmental changes caused by tillage measures (Legrand et al., 2018; Li et al., 2020b) or different methods used to identify soil fungal community.

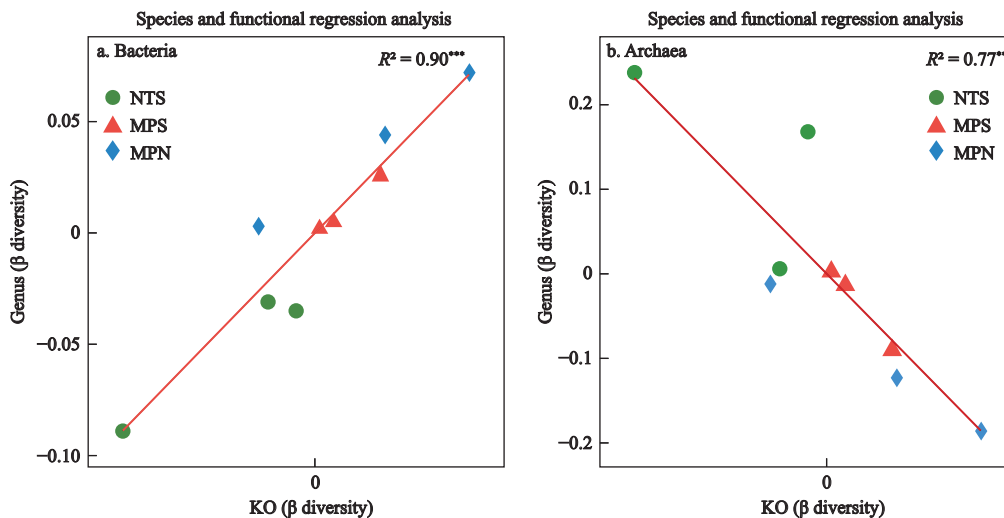
There was less contribution of soil fungi (0.06%–0.08%) to soil microbial communities composition based on the shotgun metagenomics sequencing, which may underestimate the relative abundance compared with the ITS sequencing, therefore, further studies should conduct on using both two methods to better evaluate the response of soil fungal community composition and function to tillage regimes.

Soil bacterial community plays a critical role in regulating soil ecosystem processes (Lin and Lu, 2015). Actinobacteria, Proteobacteria, Acidobacteria, Gemmatimonadetes, and Chloroflexi were the main abundant phyla in all tillage regimes (Fig. 3), which was roughly in congruence with other studies of agricultural soil (Somenahally et al., 2018; Hao et al., 2021). Tillage practice affected three abundant bacterial phyla (Acidobacteria, Gemmatimonadetes, and Chloroflexi) (Table 2). The significantly higher relative abundance of Acidobacteria in soils under NTS and MPS than MPN was related to greater cellulose input with residue cover under these two treatments (Table 2). Acidobacteria has been reported to be capable of degrading cellulose (Kanokratana et al., 2011), such as *Candidatus Solibacter* genus involved in carbon biodegradation (Zhang et al., 2019b). Residue return under NTS and MPS also contributed to the higher relative abundance of Gemmatimonadetes (*Gemmatirosa* genus) which were reported to have a greater ability for using available carbon





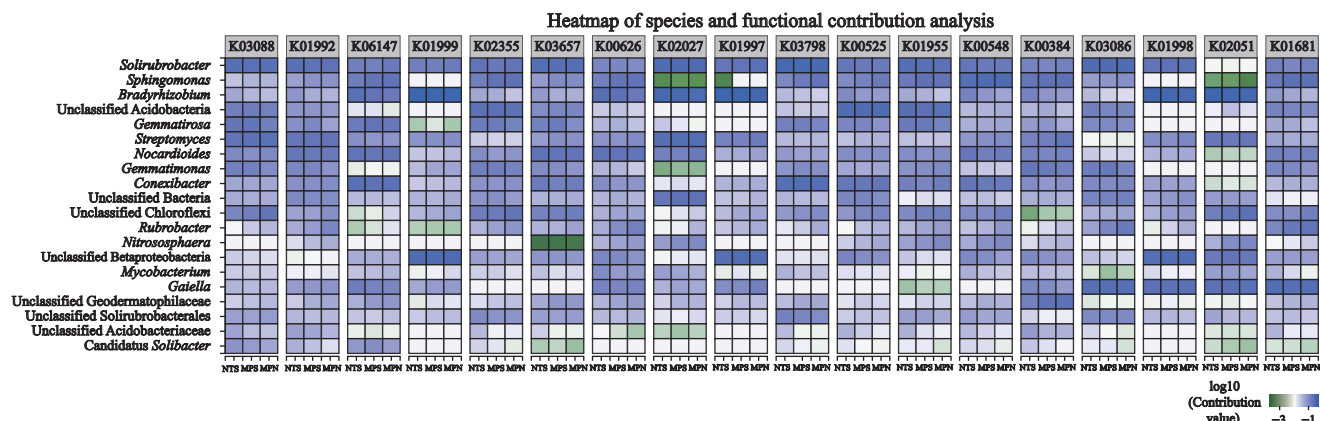
**Fig. 6** (a) Statistical Analysis of Metagenomic Profiles (STAMP) showing the differentially abundant KO functional categories between tillage regimes. Welch's two-sided t-test for each different pair of tillage treatments with one filter ( $P < 0.05$ ) to generate extended error bar figures. (b) Correlation heatmap of different KO functional categories between groups shown in (a) and soil environmental factors. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return. SWC, soil water content; SBD, soil bulk density;  $\text{NH}_4^+\text{-N}$ , soil ammonium ( $\text{NH}_4^+$ ) contents;  $\text{NO}_3^-\text{-N}$ , soil nitrate ( $\text{NO}_3^-$ ) contents; DOC, soil dissolved organic carbon; DTN, soil dissolved total nitrogen



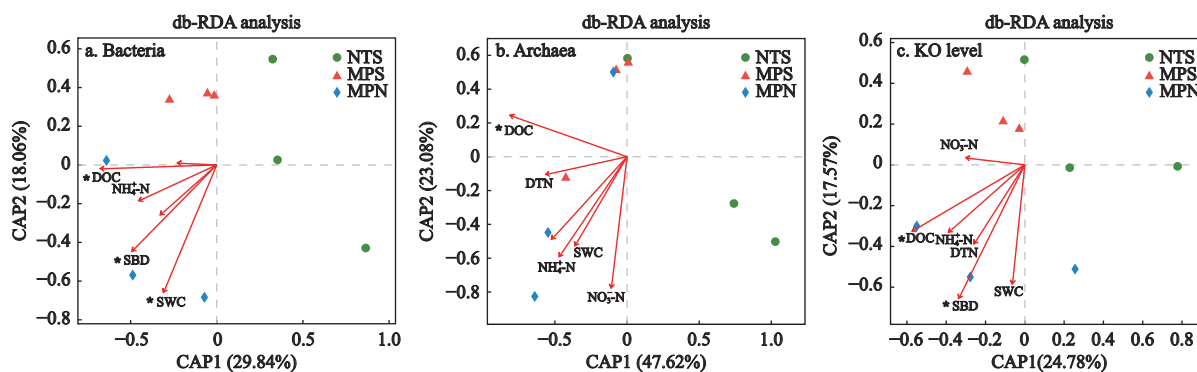
**Fig. 7** Regression analysis of functional composition (KO level) and bacterial, archaeal composition (genus level) based on the Bray-Curtis dissimilarity values for the abundance data. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return

(Yu et al., 2020). Chloroflexi as an oligotrophic population showed higher relative abundance under MPN (Table 2) and has been observed to be better adapted to resource-poor conditions (Wang et al., 2020a; Romero-Salas et al., 2021). These results suggested that a signi-

ficant difference in soil bacterial phyla (Acidobacteria, Gemmatimonadetes, and Chloroflexi) between NTS/MPS and MPN was induced by residue return instead of tillage treatments. Which supported our hypothesis that residue return (NTS and MPS) by increasing substrate



**Fig. 8** Contribution analysis of the top 20 abundant genera to the different KO functional categories among three tillage treatments. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return



**Fig. 9** Distance-based redundancy analysis (db-RDA) between the bacterial (a), archaeal communities (b), KO functional categories (c), and soil environmental factors. Environmental variables which significantly affected bacterial, archaeal communities, and functional compositions are marked with \* ( $P < 0.05$ ) based on the results of envfit analysis. NTS, no-tillage with residue (maize straw) return; MPS, moldboard plow with residue return; MPN, moldboard plow without residue return. SWC, soil water content; SBD, soil bulk density NH<sub>4</sub><sup>+</sup>-N, soil ammonium (NH<sub>4</sub><sup>+</sup>) contents; NO<sub>3</sub><sup>-</sup>-N, soil nitrate (NO<sub>3</sub><sup>-</sup>) contents; DOC, soil dissolved organic carbon; DTN, soil dissolved total nitrogen

availability was the main factor that regulated soil microbial (especially bacterial) community composition.

There were no significant differences between Actinobacteria and Proteobacteria among tillage regimes, but some specific bacterial genera were significantly different (Table 2). For example, *Rubrobacter* (Actinobacteria) exhibited a significantly lower abundance in soils under NTS than that under MPN (Table 2); the unclassified Geodermatophilaceae genus (Actinobacteria) with unique adaptation mechanisms to extreme environments including low nutrient tolerance (Xing et al., 2020) exhibited a significantly lower relative abundance in soils under NTS than MPN (Table 2). *Bradyrhizobium* (Rhizobiales) (Proteobacteria) are frequently associated with plant roots and may participate in enhancing plant growth (Antoun et al., 1998; Schneijder-

berg et al., 2018); they showed higher relative abundance under NTS and MPS than MPN in other studies (Souza et al., 2013; Wang et al., 2020b), our study was consistent with these studies and reconfirmed the preference of *Bradyrhizobium* for tillage treatment with more residues input. *Reyranelia* (Proteobacteria) involved in carbon biodegradation exhibited significantly higher relative abundance in soils under NTS (Table 3; Xing et al., 2020), whereas *Sphingomonas* (Proteobacteria) involved in degrading herbicides (Dong et al., 2017) were reported to dominate in conventional tillage both in our study, and other studies (Degruene et al., 2015; Souza et al., 2013). For archaeal taxa, the relative abundance of Ammonia-oxidizing archaea (AOA) including *Candidatus Nitrosocosmicus* and *Nitrososphaera* was significantly lower in soils under NTS than MPN (Table 2).

Taken together, these results suggested that increasing substrate input under residue return treatments (NTS and MPS) favored those taxa that can participate in the degradation of complex organic compounds or adapt to the eutrophic environment. Conventional tillage was dominated by microorganisms that adapt to the carbon-limited environment. These positive response of soil microbial communities to residue return would help to understand the effects of conservation tillage under black soils.

#### 4.2 Soil functional composition of soil microbial communities

This information on soil microbial function is important for revealing the roles they take in the soil ecosystem. Our results indicated a relatively closer relationship between soil bacterial community and soil functional community (Fig. 7). And some soil bacterial taxa including *Solirubrobacter*, *Sphingomonas*, and *Bradyrhizobium* contributed most to soil functional shifts (Fig. 8). Farming practices have been reported to shape the soil microbial community structure and function (Babin et al., 2019). In our study, there were more significantly different soil microbial taxa and functional groups between NTS and MPN (Fig. 4, Table 2), caused by the overlay of residue return and less tillage, both common attributes of conservation tillage practices. Meanwhile, there were no significantly different bacteria phyla (Table 2) and metabolic pathways (Fig. 4) between NTS and MPS, and the functional composition between them was relatively similar (Fig. 5), possibly indicating that residue return played a more prominent role than less tillage in the functional shifts of the microbial community. Chen et al. (2020) have indicated that organic substrate input appeared to be more important than tillage interference for soil microbial functional indices using enzyme assays. Functional profiles were less variable than community composition across tillage systems due to functional redundancy (Souza et al., 2013; Miura et al., 2016). In our study, most basic genes related to global and overview maps, carbohydrate metabolism, environmental information processing, and amino acid metabolism were similar among tillage regimes (Fig. 4), which suggested that an abundance of functions was involved in maintaining basic cellular machinery, supporting the growth, and enabling the metabolism of microorganisms (Moran, 2009). However,

some specific genes related to metabolisms, such as carbon, pyrimidine, amino acid, carbohydrate metabolism, and fatty acid degradation were significantly higher in MPN soils (Fig. 6a), indicating an active metabolic strategy for soil microorganisms to adapt to poor nutrition and highly disturbed soil conditions. More sequences related to the metabolism of carbohydrates under residue removal treatment (Souza et al., 2015; Miura et al., 2016) ensures to obtain sufficient resources (Liu et al., 2021b). Meanwhile, we found that the overlays of residues return and less tillage in NTS soil led to a higher relative abundance of some genes involved in ABC transporters such as K01997, K01998, and K01999 (most were contributed by *Bradyrhizobium*) (Fig. 6a), and these genes were significantly positively correlated with DOC (Fig. 6b). ABC transporters are important for the import of available substrates such as ions (inorganic and organic), saccharides (monosaccharide and oligosaccharide), amino acids, and peptides (Nelkner et al., 2019), which may attribute to the more nutritious soil condition under NTS. In addition, these genes (K01997, K01998, and K01999) were also corresponding to quorum sensing pathways in *Agrobacterium tumefaciens* from 4-aminobutanoate (GABA) to succinate semialdehyde (SSA). Quorum sensing is a process where the bacteria population releases small signal molecules (autoinducers) to regulate gene expression (such as the formation of biofilms) (Lang and Faure, 2014). The higher relative abundance of quorum sensing genes under NTS may decrease the virulence of *A. tumefaciens* on plants by GABA regulation (Chevrot et al., 2006). However, it is difficult to draw solid conclusions on the effect of NTS on pathogenic organisms, further studies should focus on these categories under conservation tillage systems.

#### 4.3 The driving factors of taxonomic and functional composition

Changes in soil property caused by various tillage regimes, such as pH, soil aggregates, and organic C content, appeared to exert effects on the soil microbial community (Navarro-Noya et al., 2013; Wang et al., 2019; Li et al., 2021). In particular, our results confirmed the crucial role of active SOC contents on microbial community composition (Yan et al., 2020) with the results that DOC was the key factor in the shaping both bacterial, archaeal communities, and functional communities

of soils under different tillage regimes (Fig. 9).

Overall, NTS and MPS with residue return increased DOC, and other soil physical characteristics such as soil bulk density and soil water content were also altered by tillage regimes (Table 1), and consequently affected soil microbial community and function. Based on an 8-year conservation tillage trial, our study offered empirical evidence on the effect of conservation tillage (especially the effect of residue return) on entire soil microbial compositions and functions, which could provide more supports for the implementation of conservation tillage in black soils of Northeast China and other regions of China. It is unreasonable to draw a conclusion on the response of microbial community structure and functions based on one study, and we must admit that more studies are needed to better understand the effect (Li et al., 2020a).

## 5 Conclusions

We found soil bacterial and archaeal communities showed significant variations with tillage regimes, returning residues (increasing substrate input such as DOC content) favored those taxa that can participate in the degradation of complex organic compounds or adapt to the eutrophic environment, while conventional tillage was dominated by microorganisms that adapt to the carbon-limited environment. Shifts in soil bacterial community composition were highly in line with the changes in soil microbial community function (KO level). There was a divergence in soil microbial function at the KO categories level among tillage regimes. NTS led to a higher relative abundance of some genes involved in ABC transporters and quorum sensing pathways, the increase of these genes may favor the import of available substrates. The higher relative abundance of genes related to metabolisms, such as amino acid metabolism, fatty acid degradation, carbon, and carbohydrate metabolism under MPN indicated that soil microbes may modulate their metabolic strategies to meet resource requirements through altering functional genes. Furthermore, soil microbial functional compositions showed relatively similar between NTS and MPS, and significantly different metabolic pathways existed between MPN and another two treatments (NTS and MPS), but did not exist between NTS and MPS. This suggested that residue return played a more prominent

role than tillage in the functional shifts in soil microbial community. These results may benefit for assessing black soil function from the entire microbial community and function standpoint, and provide insights for the management of conservation tillage. Conservation tillage is an alternative measure to protect black soil and maintain its productivity in Northeast China.

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