Tillage Practice Impacts on the Carbon Sequestration Potential of Topsoil Microbial Communities in an Agricultural Field

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Abstract: Soil microorganisms are the core force driving the conversion of plant residues into soil organic carbon (SOC). Identifying the changes in soil microorganism responses to tillage practices is a key step in understanding the SOC sequestration potential. The aim of this study is to assess the impacts of different tillage practices on microbial communities and functions in agricultural soils. A field experiment involving no tillage (NT), rotary tillage (RT), and deep tillage (DT) in winter wheat-summer maize double cropping was performed to determine the structure of the microbial community and its functions using metagenomics. We found that tillage practices changed the composition of soil microbial communities and their functions related to the C cycle. The relative abundance of fungi in DT was significantly higher than that of the NT and RT treatments and primarily facilitated the growth of the fungi community. Moreover, DT treatment increased the relative abundance of genes involved in carbohydrate transport and metabolism genes and carbohydrate metabolism pathway genes, in addition to those encoding carbohydrate-binding modules. Therefore, we concluded that DT increases the transformation potential of straw-C to SOC in the North China Plain where large amounts of wheat and maize straw are returned to the field every year.

Keywords: tillage; microbial community; metagenomics; KEGG; CAZy

1. Introduction

Soil degradation as a result of intensified cropping systems is the main factor restricting agricultural sustainability [1]. The loss of soil organic carbon (SOC) leads to severe degradation, diminishing soil biodiversity, and poor productivity [2]. Improvement in SOC content occurs when the C input is higher than the C output, both of which are influenced by various factors such as land use, field management, temperatures, and nutrient availability [3,4]. Therefore, management practices that increase C inputs, such as straw incorporation, which is known to restore SOC content, are applied as integrated approaches. The optimization of tillage management with the return of straw is being promoted for ameliorating soil conditions, which will facilitate soil quality and sustainable crop productivity [5–12]. Moreover, it is of great significance to clarify the response characteristics of soil quality to different tillage methods.

Tillage is the primary agricultural practice that impacts soil microbes, especially coupled with the return of straw. Physical disturbance leads to alterations and the mechanical
distribution of soil particles in addition to various degrees of mixing of crop residues within the soil matrix [13]. Microbial communities are affected by the multiple physicochemical and biological characteristics of the soil and, in return, are responsible for numerous soil ecosystem functions [14]. Microbes, which mediate approximately 80–90% of the processes in soils, contribute to a wide variety of soil functions involving the key biogeochemical cycles of organic matter and nutrients along with maintain soil structure and soil ecosystem stability [15]. These functions are closely associated with essential soil ecosystem goods (e.g., food and wood) and services (e.g., sequestrating SOC and suppressing pathogens) [16,17]. Thus, soil microbial properties are considered a sensitive indicator of dynamic changes in SOC [18]. Recently, studies have raised concern about the impact on SOC sequestration of microbial communities resulting from different tillage methods. For example, reduced tillage and crop residue retention strengthen the presence of beneficial bacterial groups [19], improve the diversity of bacterial communities [20], and support the richness and diversity of active soil bacteria [21]. A meta-analysis based on 232 data pairs on a global scale reported that NT can improve soil microbial biomass C [22]. The tillage effects on both species richness and composition were more evident for bacterial communities than for fungal communities [23]. However, detailed studies on microbial communities considering functional genes, carbohydrate metabolism pathways, and carbohydrate-active enzymes under different tillage methods is lacking. It was also argued that the information about the microbial taxonomic composition itself is usually insufficient to predict the various functions [24].

To obtain information about the diversity of functional genes and the functional potential of soil microbiology, microbial community profiles have been characterized by metagenomic sequencing [25]. Metagenomics is used to directly detect and quantify DNA sequences, thus avoiding the trouble of PCR amplification bias and providing annotation of functional capabilities via gene enrichment analysis [26,27]. Metagenomics allows researchers to answer questions about what the microbial communities do, how soil functions change, and how soil functions respond to the microenvironment through quantifying the functional composition of those communities [24,28]. The metagenomics technique is based on using the available gene sequences to reveal taxonomic structures according to the NR database [29], providing functional annotation according to the unsupervised orthologous groups database [30], elucidating the metabolic pathway using the KEGG database [31], and examining the carbohydrate-active enzymes and carbohydrate metabolism pathway using the CAZy database [32]. Therefore, based on knowledge of the soil microbial community composition, we can generalize about the functional gene composition and carbohydrate metabolism pathways related to SOC cycling [15,24,33]. Metagenomic sequencing analysis provides an opportunity to explore the functional genes related to SOC cycling, where crop residues and root exudates are the main C sources. This method helps to more deeply understand how tillage methods affect the functional capabilities of microbial communities to degrade straw residues.

Knowledge of the abundance of genes coding for specific enzymes in the soil metagenomes can help us understand the functional potential of microorganisms in SOC sequestration [24]. Compared to 16S rRNA-based measurements, examining the gene characteristics of microorganisms based on their functional capabilities is a better tool for deducing changes in microbial functions’ responses to environmental changes [25]. To deeply study the effects of tillage on the C sequestration potential of microbial communities, we conducted a site-specific field experiment of tillage methods (e.g., no tillage (NT), deep tillage (DT), and rotary tillage (RT)) under a maize–wheat double cropping system in the North China Plain. We hypothesized that the tillage practices would lead to dynamic changes in the microbial community structure and functions related to the SOC cycle. To test the above hypothesis, we analyzed the shotgun soil metagenomes and compared various treatments for tillage. The specific objectives were (a) to test whether tillage practices affect the functional genetic diversity of microbial communities; and (b) how tillage methods affect the functional potential of microbial communities and if
these functions are related to the taxonomic composition. This study will help to systematically understand the SOC sequestration mechanisms under different tillage practices.

2. Materials and Methods

2.1. Study Site and Soil Characteristics

This study was conducted in 2012 at the Longshan Experimental Station of Maize Research Institute, Shandong Academy of Agricultural Sciences, Jinan City, Shandong Province (117°32' E, 36°43' N). Before 2012, the experimental plots were all under winter wheat–summer maize rotations, which is the most widely employed cropping system in the region, and were managed by local farmers via conventional tillage. Straws of both wheat and maize were returned to the fields following the addition of 50 and 60 kg ha\(^{-1}\) compound fertilizer to the wheat and maize straw, respectively. Maize was sown annually in early June and harvested in early October. Following maize harvesting, wheat was sown in October and harvested in early June of the following year. This area is characterized by a temperate monsoon climate with mean annual precipitation of approximately 600.8 mm, which is mainly experienced from June to August. The mean annual temperature is 12.8 °C. The hottest month is July, with an average monthly temperature of 26.5 °C, with 2647.6 h of sunshine and 209 frost-free days per year. The antecedent soil contained 14.81 g kg\(^{-1}\) organic matter, 0.85 g kg\(^{-1}\) total nitrogen, 19.26 g kg\(^{-1}\) Olsen-extractable phosphorus, and 46.37 g kg\(^{-1}\) ammonium acetate-exchangeable potassium, at pH 7.64, in a 1:5 soil to water ratio.

2.2. Experiment Design

In October 2012, treatments were implemented in different plots subjected to various soil tillage methods (no tillage, NT; deep tillage (35 cm), DT; rotation tillage (20 cm), RT), with NT treatment used as the control. Each treatment was replicated three times using a split plot design. The area of each plot was 6 m \(\times\) 45 m = 270 m\(^2\). The tested cultivars of maize (Zea mays L.) and wheat (Triticum aestivum L. em. Thell.) were “Ludan 9066” and “Jimai 22”, respectively. Maize was directly planted using no tillage with a row spacing of 60 cm at a sowing rate equivalent to 67,500 plants per hectare. Plowing treatment was performed prior to wheat sowing. Wheat was planted using a wide-precision planting pattern at a sowing rate of 172.5 kg seeds per hectare. Both maize and wheat straw returned to the soil (local practice) were obtained from field harvesting of the aboveground biomass, which is the most widely used cultivation method in this region. The field was fertilized with 600 kg ha\(^{-1}\) compound fertilizer (N/P\(_2\)O\(_5\)/K\(_2\)O = 17:17:17) prior to crop planting, and urea was applied at a rate of 225 kg ha\(^{-1}\) as a topdressing during the jointing stage of the wheat and the bell bottom period of the maize.

2.3. Microbial Analysis

Soil samples were collected at the mature stage of the wheat and maize in 2017. Five sites were randomly selected from each plot where soil samples were taken from a depth of 0–20 cm. Soil samples from each plot were then uniformly mixed to form a composite sample. Visible roots and residues were removed, and DNA was extracted from 0.4 g of a homogenized subsample with three replicates using a Quick Soil Isolation Kit (OMEGA, Norcross, GA, USA) according to the manufacturer’s protocols. The yield and purity of the DNA were measured using a spectrophotometer (NanoDrop® ND-1000, Thermo Scientific, Wilmington, NC, USA). The quality of the soil DNA was examined using a 1% agarose gels electrophoresis system. The total DNA was fragmented to around 300 bp in size using a Covaris M220 system (Gene Company Limited, Shanghai, China) for paired-end (PE) library construction. The PE library was prepared using a TruSeqTM DNA Sample Prep Kit (Illumina, San Diego, CA, USA). The sequencing was accomplished using an Illumina HiSeq4000 platform (Illumina Inc., San Diego, CA, USA) from Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). During this process, the HiSeq 3000/4000 PE Cluster and HiSeq 3000/4000 SBS kits were applied according to the manufacturer’s
instructions. The sequences were submitted to the NCBI Sequence Read Archive (SRA) with accession number PRJNA673669.

Raw sequence reads were stripped from the adapter in 3’ and 5’ ends for quality control using SeqPrep (https://github.com/jstjohn(SeqPrep). Subsequently, low-quality reads were removed by Sickle (https://github.com/najoshi/sickle) using the parameters of quality value < 20, length < 50 bp, or the presence of N bases so that high-quality paired-end reads and single-end reads could be obtained. Multiple megahits were employed to assemble the short reads via Megahit (https://github.com/voutcn/megahit) and Newbler (https://ngs.csr.uky.edu/Newbler) [34]. Contigs with lengths over 300 bp were retained. We evaluated the quality and quantity of the contigs generated using Quast 4.3. Open reading frames (ORFs) from the contigs of samples were predicted by MetaGene (http://metagene.cb.k.u-tokyo.ac.jp/) [35]. The predicted ORFs with a length ≥100 bp were blasted and translated using the NCBI translation table (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/index.cgi?chapter=tcodes#SG1). To evaluate the gene abundance, high-quality reads were mapped to the non-redundant gene catalog with 95% identity using SOAPaligner (http://soap.genomics.org.cn/) [37]. Using the basic local alignment search tool (BLASTP 2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi), the non-redundant gene catalog was aligned to the NR database [38], eggNOG database [39], KEGG database [40], and CAZy database [32] with an optimized e-value cutoff of 1e−5. The abundance of a particular taxonomic group/module/KEGG orthology/enzyme was calculated by summing the abundance of genes annotated with the same feature(s).

2.4. Calculation and Statistics

Circus figures were developed in Circos-0.67-7 to show the relative abundance of dominant phyla. The figures provide a visual circle diagram that describes the corresponding relationship between the treatment and the dominant phyla as well as the distribution ratio of dominant phyla among different samples. A comparison of the relative abundance of soil bacteria, archaea, and fungi in different treatments was conducted using one-way analysis of variance (ANOVA) and multiple comparisons with Duncan’s test (SPSS 22.0, SPSS Inc., Chicago, USA). Data for the principal co-ordinate analysis (PCoA) were obtained from the NR database, eggNOG database, KEGG database, and CAZy database using Qlime 2. Bray–Curtis distances were used to estimate the beta diversity among different treatments. Variance analysis of the function-related differences of COG, KEGG, and CAZy was conducted using one-way ANOVA with procedures for controlling the false discovery rates (FDR), and post-hoc analysis was conducted using Tukey–Kramer testing. The PCoAs of the microbial community contributing to COG, KEGG, and CAZy were performed using the vegan package in R software (Version 3.6.3).

3. Results

3.1. Diversity and Abundance between Treatments

In the present study, 96.50–118.27 million (average: 108.50 million) quality-filtered reads were obtained from each sample. Among these sequences, abundant phyla, i.e., relative abundance >1%, for the wheat and maize seasons, respectively, were Proteobacteria (31.25% and 31.34%), Actinobacteria (26.30% and 22.60%), Acidobacteria (11.06% and 9.26%), Chloroflexi (5.03% and 7.01%), Gemmatimonadetes (4.02% and 3.00%), Firmicutes (2.54% and 5.98%), Thaumarchaeota (2.46% and 2.43%), Bacteroidetes (2.07% and 3.37%), Planctomycetes (1.86% and 1.88%), Cyanobacteria (1.86% and 2.00%), Nitrospirae (1.35% and 1.11%), and Verrucomicrobia (1.02% and 1.68%) (Figure 1). Bacteria accounted for more than 94% of the sequences in all the treatments (Table 1). In the wheat season, the relative abundance of Bacteria in DT was significantly lower than that of the NT treatment (p < 0.001). Interestingly, the relative abundance of fungi in the DT treatment was significantly increased by 21.49% (p = 0.029) compared to that of the NT treatment.
However, according to the relative abundance of microorganisms in the maize season, there was no significant difference in the relative abundance of bacteria, archaea, and fungi among treatments (Table 1).

Table 1. The relative abundance of soil bacteria, archaea, and fungi in the different tillage treatments.

| Index | Wheat Season | Maize Season |
|-------|--------------|--------------|
|       | NT           | DT           | NT           | RT           | DT           |
|       | Bacteria     | Archaea      | Fungi        | Bacteria     | Archaea      | Fungi        |
|       | 95.96 ± 0.12 a | 2.83 ± 0.09 b | 1.21 ± 0.06 b | 95.31 ± 0.29 b | 3.46 ± 0.19 a | 1.23 ± 0.11 b |
|       | 94.79 ± 0.01 c | 3.73 ± 0.14 a | 1.47 ± 0.15 a | 96.07 ± 0.31  | 3.43 ± 0.33  | 0.50 ± 0.02   |
|       | 96.06 ± 0.24  | 3.42 ± 0.22   | 0.52 ± 0.05   | 95.94 ± 0.23  | 3.56 ± 0.26   | 0.50 ± 0.03   |

Values (mean ± standard deviation) indicate the absolute amount of each characteristic; different letters in columns indicate significant differences ($p < 0.05$) across treatments according to Duncan’s multiple comparison. There was no significant difference in the relative abundance of bacteria, archaea, and fungi among treatments in the maize season.

3.2. Microbial Community and Function Structure

The taxonomic, gene, and metabolic diversity of the microbiomes differed according to the type of tillage method (Figure 2). In the wheat season, the PCoA ordination plot indicated that 61.68% and 19.33% (NR), 50.48% and 18.76% (COG), 53.18% and 17.05% (KEGG) and 68.21% and 18.83% (CAZy) of the variation in the community composition could be explained, respectively, by the first two axes. In the maize season, the PCoA ordination plot indicated that 52.70% and 27.00% (NR), 29.13% and 21.01% (COG), 31.38% and 19.02% (KEGG) and 55.71% and 15.41% (CAZy) of the variation in the microbial community and functional composition could be explained by the first two axes. This result indicates that samples within the same treatment were clustered together, which suggests strong similarities among samples from the same treatment but variations among samples from different treatments (Figure 2). Clusters between NT, RT, and DT treatments...
were situated further apart, indicating that the microbial taxonomic, gene and C metabolic communities between the different treatments were dissimilar.

### Figure 2

Principal co-ordinate analyses of variation among the (a) NR database; (b) eggNOG database; (c) KEGG database, and (d) CAZy database gene profiles based on Bray–Curtis distances. The relative abundance of dominant phyla in the 0–20 cm layer with no tillage (NT), rotary tillage (RT), and deep tillage (DT). The left charts represent the wheat season, and the right charts represent the maize season.

#### 3.3. COG

All of the ORFs from the metagenome and metaproteomes were searched against the clusters of orthologous groups of proteins (COGs) using the non-supervised orthologous
groups (eggNOG) database. One of the most notable results was that the genes responsible for carbohydrate transport and metabolism decreased under NT treatment in the maize season \((p < 0.05, \text{Figure } 3)\). Analyses of the function-related differences between treatments focused on the phyla represented by >1% of all annotated genes contributing to the carbohydrate transport and metabolism function. The carbohydrate transport and metabolism pathway was dominated by Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Bacteroidetes, Gemmatimonadetes, Cyanobacteria, Thaumarchaeota, Planctomycetes, Verrucomicrobia, and Nitrospirae (Figure 4). In the maize season, PCoA analysis showed that the clusters among DT, RT, and NT treatments were situated further apart, indicating that the microbial communities between the three treatments were dissimilar (Figure 4). The abundance of Proteobacteria, Actinobacteria, Acidobacteria, and Chloroflexi was higher than the abundance in other groups (Figure 4). The relative abundance of Actinobacteria, Acidobacteria, and Chloroflexi in the DT treatment increased significantly compared to NT treatment in the maize season (Figure 4). However, there were no differences in Proteobacteria abundance across the treatments (Figure 4, \(p > 0.05\)).

**Figure 3.** Analyses of function-related differences focused on clusters of orthologous groups of proteins (COG) using the evolutionary genealogy of genes: non-supervised orthologous groups (eggNOG) represented the top 15 of all COGs under different treatments in the \((a)\) wheat and \((b)\) maize seasons. E, Amino acid transport and metabolism; C, Energy production and conversion; G, Carbohydrate transport and metabolism; T, Signal transduction mechanisms; L, Replication, recombination and repair; P, Inorganic ion transport and metabolism; M, Cell wall/membrane/envelope biogenesis; J, Translation, ribosomal structure, and biogenesis; K, Transcription; O, Post-translational modification, protein turnover, and chaperones; I, Lipid transport and metabolism; V, Defense mechanisms; Q, Secondary metabolite biosynthesis, transport and catabolism; H, Coenzyme transport and metabolism; F, Nucleotide transport and metabolism. *, ** mean significant difference among treatments at \(p = 0.05, 0.01\), respectively.
Figure 4. Top: Principal co-ordinate analysis (PCoA) of the microbial community composition relevant to carbohydrate transport and metabolism against clusters of orthologous groups of proteins (COG) genes at the phyla level based on Bray–Curtis distances. Bottom: The relative abundance of dominant bacterial phyla relevant to carbohydrate transport and metabolism genes (focused on the metabolic pathways represented by >1% of all annotated genes); the data are the means (n = 3) for each treatment. * Indicates a significant difference between treatments at p < 0.05. ** Indicates a significant difference between treatments at p < 0.01. *** Indicates a significant difference between treatments at p < 0.001. Red * and black * indicate the wheat season and the maize season, respectively. Left: wheat season, right: maize season. The same as below.

3.4. KEGG

To confirm the functional profiles, individual genes annotated by KEGG2 were summarized, as shown in Figure 5. Compared with NT treatment, the relative abundance of carbohydrate metabolism pathway genes was increased under DT and RT treatments in the maize season (p < 0.05, Figure 5). In both the wheat season and the maize season, the carbohydrate metabolism pathway was dominated by Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Bacteroidetes, Gemmatimonadetes, Thaumarchaeota, Cyanobacteria, Planctomycetes, Verrucomicrobia, Nitrospirae, Candidatus_Rokubacteria, and unclassified_d_Bacteria (Figure 6). These taxa accounted for more than 93% of the metabolism pathway genes in all treatments. PCoA analysis showed that samples within each treatment in the maize season clustered tightly together but were differentiated between different treatments (Figure 6). Compared with NT treatment, in the maize season, the relative abundances of Actinobacteria, Acidobacteria, and Chloroflexi in DT treatment increased by 7.26%, 6.87%, and 8.24% respectively (p < 0.05, Figure 6). There were no differences in Proteobacteria abundance across all treatments (Figure 6, p > 0.05).
Figure 5. Analyses of the function-related differences focused on pathway level 2 using KEGG provided the top 15 represented pathways under different treatments in the wheat (a) and maize (b) seasons. *, ** indicate significant difference among treatments at $p = 0.05, 0.01$, respectively.
3.5. CAZy

As an indicator of the overall genetic potential for carbohydrates, we examined the relative abundances of polysaccharide lyases, auxiliary activities, carbohydrate-binding modules (CBMs), glycoside hydrolases, carbohydrate esterases, and glycosyl transferases. Specifically, DT treatment increased the relative abundance of CBMs in the wheat season and RT treatment increased the relative abundance of CBMs in the maize season when compared to NT treatment ($p < 0.05$, Figure 7). PCoA analysis showed that the clusters among all treatments were situated further apart, indicating that the microbial communities among the three treatments were dissimilar for both wheat and maize seasons. Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Bacteroidetes, Gemmatimonadetes, Thaumarchaeota, and Cyanobacteria were the dominant phyla involved in CBMs at the phylum level (Figure 8). Compared with NT treatment, the relative abundance of Actinobacteria and Chloroflexi was greatly increased (19.86% for Actinobacteria and 26.49% for Chloroflexi) under DT treatment in the wheat season ($p < 0.05$, Figure 8) and increased by 4.12% for Actinobacteria and 12.01% for Chloroflexi in the maize season ($p < 0.05$, Figure 8).
Figure 7. The variation of carbohydrate-active enzymes (CAZy) Database gene class profiles under different tillage methods. Values are the means ± standard deviation (n = 3). (a) represents the wheat season, and (b) represents the maize season. Different letters in the figures indicate significant differences tested by Duncan’s multiple comparison.
4. Discussion

Although there is literature on the effects of tillage practices on microbial communities in croplands based on studies representing various regions around the world, few detailed data exist on the relevant functions of microbial groups and the abundance of enzyme-encoding genes of the C metabolic pathway. In this study, we surveyed soil metagenomic information using a metagenome sequencing approach and analyzed functional information based on the eggNOG, KEGG, and CAZy databases to determine the distinctive effects of tillage methods on the microbial community and its functions related to C cycle. The results indicate that dissimilar tillage practices led to divergences in microbial community structure and functions. This observation can be explained by dissimilarities in the soil structure, moisture, and physicochemical properties under different tillage practices [9,13], which alter the microenvironment for microbial growth and metabolism. These changes in microorganisms likely impacted the straw-C cycle.
Soil microorganisms essentially transfer C between microenvironmental compartments to achieve their fundamental survival [41]. Following straw incorporation, microbes utilize organic compounds of straws as sources of C and energy. Under NT treatment, the surface placement of abundant straws made the microbes less susceptible to microbial breakdown by reducing the contact between straw and soil particles [42]. By contrast, the incorporated straw in deep tillage fully contacts soil particles. The relatively high C/N ratio [43,44] and abundant cellulose and lignin [45,46] in straw created advantageous condition for the proliferation of fungal groups [43,47]. In this way, the DT treatment primarily facilitated the growth of the fungal community (Table 1). The results showed that the ratio of fungi to bacteria in DT was significantly increased by 23.5% compared to that of NT ($p < 0.05$). The ratio of fungi to bacteria has been extensively explored in the context of land management and its effects on SOC sequestration [48]. Firstly, fungi feature more extensive extracellular enzyme types and a stronger ability to degrade organic substances [49]. An increase of fungi in DT treatment promotes the rapid degradation of straw residues, which can more effectively turnover the exogenous straw-C. Secondly, the biomass and growth efficiency were higher for fungi than for bacteria in the soil [49,50], and fungal biomass contained more C per unit N than bacterial biomass [51], which resulted in increased C-use efficiency [48]. Hence, fungi are generally much more efficient at assimilating and storing nutrients than bacteria. The increase of fungi relative abundance in DT is meaningful for agricultural production. Based on the aforementioned arguments, we concluded the stronger and more sustainable transformation potential of straw-C to SOC in DT under the condition of returning significant straw residues to the field (7500 kg/ha/year wheat straw and 9000 kg/ha/year maize straw).

CBMs are a class of proteins present in most cellulolytic enzymes, with high specificity and functional diversity. CBMs exist widely in microbial populations such as fungi, bacteria, and actinomycetes [52]. The observed increase in CBM and carbohydrate metabolism (KEGG 2) genes under deep tillage indicated that it was conducive to the growth of cellulolytic microorganisms. The dominant bacterial phyla with significant improvement with respect to increased abundance of genes relevant to CBMs and carbohydrate metabolism (KEGG 2) included Actinobacteria and Chloroflexi in the DT treatment (Figure 7). Actinobacteria are generally associated with the effective degradation of polysaccharides (hemicellulose and cellulose) or phenolic compounds in straw [53,54]. Moreover, Actinobacteria are a copiotrophic group containing abundant functional taxa. Actinobacteria prefer environments with high C availability for rapid growth and to facilitate effective soil nutrient cycling [55]. Therefore, deep tillage promotes the degradation of straw and provides more C sources for microbial growth.

The chosen tillage method affects the soil ecology, including soil respiration, the number of microorganisms, and microbial community structure [56]. Our results further underlined both taxonomic and functional changes in the soil metagenomes and elucidated their ecological implications for SOC storage as a result of tillage methods applied to cropland. The spatial distribution of straw in soil is an important factor for controlling the microbial community and functional gene composition under different tillage types. Residue incorporation promotes the growth of microbial communities, but the type of tillage triggers dissimilar responses, mainly due to changes in C sources exposed to microbial communities via tillage. This corroborates with the results of our previous study, which showed that organic C is only enriched in the topsoil under NT (14.04 g/kg at 0–10 cm and 6.70 g/kg at 10–20 cm) [57]. We speculate that NT restricts microbial access to straw-C (by leaving many residues on the surface). As a result, the C sequestration in the topsoil of NT is mainly due to the accumulation of large amounts of straw residue that has not been used by microorganisms, with great quantities of undecomposed straw left on the surface of the soil. This may be the reason why most research results show that no tillage increases SOC in the soil. Although more straw-C is catabolized by microorganisms in the DT treatment due to the better contact between the soil and straw, more straw is used by microorganisms. Some of the straw-C then turns into microbial necromass, some of which
is released into the air by microbial respiration in the form of CO$_2$. Microbial necromass, exudates, and metabolites are precursors for the stabilization of SOC, which is mainly derived from the progressive decomposition of straw residues in the soil [40]. Microbial debris sustainably contributes to the transformation of soil organic matter and the provision of nutrients to crops in arable land [41,58,59]. These nutrients greatly contribute to the soil’s easy-to-use nutrient pool [60]. In cropland, the roots of wheat and maize, which absorb soil nutrients, are not located only on the soil surface, especially in the later stages of growth. Therefore, we speculate that DT has greater sustainable potential for SOC sequestration than NT when a large quantity of straw is applied to the field under intensive production conditions.

The shift in microbiology itself is a driver of changes in nutrient cycling; thus, the observed changes in soil microbiology are coupled with shifts of nutrients dynamics in the soil system. However, the related biological processes were not considered in our study. Moreover, metagenomic approaches are biased towards the abundance of coded genes; however, the abundance of genes is not reliably related to gene expression. Thus, future studies are needed to combine meta-transcriptomic and metaproteomic approaches to better integrate and more precisely target specific processes of SOC sequestration. This process will provide a better understanding of SOC sequestration mechanisms under different tillage methods.

5. Conclusions

In this work, a metagenomics approach was applied in the analysis of agricultural soils to elucidate the effects of tillage practices on the functional genetic characteristics of microbial communities. A greater abundance of fungus and functional genes related to the C cycle was observed in the deep tillage compared to no tillage. These results provide evidence that tillage practices influence the SOC sequestration potential of the microbial community. Deep tillage is an effective practice to improve the capacity of straw degradation and SOC sequestration potential because it increases the relative abundance of the fungi community and carbohydrate metabolism and carbohydrate-binding module genes. By contrast, no tillage treatment decreases the potential capacity of straw degradation as it lowers the relative abundance of fungi and carbohydrate-binding module genes. We suggest that the C sequestration mechanism of deep tillage is more sustainable under the condition of intensive agricultural production than the alternatives. The C sequestration potential of microorganisms in farmland is of great significance for guiding farmland management, more experiments are required to elucidate the C sequestration mechanisms of different tillage methods in the North China Plain.

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