TILLAGE ROTATION SHORT-TERM ALTERS THE FUNGAL COMMUNITY DISTRIBUTION AND SOIL CHARACTERISTICS FOR WHEAT IN THE DRYLANDS OF NORTHERN CHINA

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Abstract. Tillage rotation is extensively used for wheat-fallow cropping in the drylands of northern China, altering soil compaction, and water availability. However, the roles of tillage rotation in the composition and structure of rhizosphere soil fungal communities remain unclear. High-throughput sequencing technology was used to investigate effects of different tillage rotations, including the control with no-tillage-no-tillage (NT-NT), subsoil-deep tillage (SS-DT), subsoil-subsoil (SS-SS), as well as deep tillage-deep tillage (DT-DT), during the wheat-fallow period on fungal diversity and community structure in the rhizosphere soil. The soil fungal diversity and composition structures under SS-SS showed similarities with NT-NT indicating that SS-SS had disturbed the soil fungal community less than other technologies. The fungal structures were similar in DT treatments (SS-DT and DT-DT) and caused higher soil, with Basidiomycota, Zygomycota, as well as Ascomycota as the most abundant phyla. The relative abundance of dominant genera was 73.1% in NT-NT, SS-SS increased the abundance to 86.9%, while SS-DT and DT-DT decreased the abundance of dominant genera. SS-SS significantly reduced the abundance of Pathotroph (*Aspergillus and Bipolaris*) and Pathotroph-Saprotroph-Symbiotroph fungi (*Alternaria, Acremonium, Fusarium, Entoloma, and Pyrenochaetopsis*). These findings suggested that tillage rotation can affected soil fungal community by altering available nitrogen (AN), soil organic matter (SOM), nitrate-nitrogen (NO_3-N), as well as ammonium-nitrogen (NH_4-N). This approach could have significant implications for sustainable dryland agriculture and farming practices.
Keywords: *no-tillage, chemical properties, diversity, abundance, fallow period*

Introduction

Soil fungi are important parts of the ecosystem, and participate in a series of important ecological functions, such as soil organic matter decomposition, plant growth, disease development, the elevation of the water holding capacity, and the regulation of carbon and nutrient balance (Bhattacharyya et al., 2022; Sommermann et al., 2018; Devi et al., 2020). Soil fungi can produce cellulose, hemicellulose, lignin, and penicillin, etc. The fungal community structure in the soil is influenced by its physical and chemical properties (Schappe et al., 2017). Tillage conservations are important agricultural practices including decreased tillage, no-tillage, deep tillage, as well as subsoil techniques that can change soil structure, improve soil water storage, enhance crop yield, as well as reduce soil erosion (Li et al., 2020; Zhang et al., 2022). Different

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tillage techniques, each of which has its own advantages and disadvantages, can have diverse influences on the soil structure and microbial community, as well as tillage rotation is warranted in order to equilibrate these influences. For example, these soil tillage measures were combined in a previous study to address the negative effects of long-term reduced tillage and no-tillage and were based not only on saving costs and increasing efficiency, but also on the comprehensive improvement of farmland soil quality (Xia et al., 2020; Liang et al., 2019).

Dryland wheat is a widely used wheat production practice in North China. Chen et al. (2016) reported that the wheat planting area in Shanxi Province reaches up to 700,000 ha, with dryland wheat accounting for 70%. Precipitation is the only source of water in the Loess Plateau, and the annual precipitation is mainly concentrated in summer (July to September), which is the fallow period of winter wheat (Chen et al., 2016; Ren et al., 2022). Therefore, drought is the biggest limiting factor of wheat production in this area. Numerous researches have concentrated on the impacts of single conservation tillage for a long period on wheat yield, soil physicochemical properties, water use efficiency, as well as soil microbial communities (Badagliacca et al., 2021; Xue et al., 2019; Wang et al., 2016a, b). Long-range implementation of less tillage and no-tillage may lead to the increase of soil bulk density, make it difficult for the nutrients to permeate and enrich in the surface layer soil result in the lack of soil fertilizer, and finally limit crop growth. While consecutive deep ploughing leads to higher water loss from soil affecting crop growth and microbial flora in the soil (Sun et al., 2018; Abidela et al., 2019). Hence, it is urgent to address deficiencies of single tillage through the combination of no-tillage, subsoil, and deep tillage in the fallow period in order to restore soil moisture, improve soil quality, increase crop yield, and precisely depict the discrepancies of the rhizosphere fungal communities after different tillage rotations.

It was found that various tillage rotations of no-tillage (NT), subsoil (SS), as well as deep-tillage (DT) could have crucial functions in the wheat yield, and water use efficiency (Sun et al., 2018). Among them, Xia et al. (2020) demonstrated that SS-SS could enhance soil water, keep a balance of aerobic and anaerobic bacteria, as well as improve the metabolic abilities of rhizosphere soil bacteria. Nonetheless, little studies have focused on the roles of different tillage rotations in fungal community structure and diversity in the dryland wheat producing areas. Therefore, rhizosphere soil samples were harvested from four types of two-year tillage rotation treatments, namely, SS-SS, NT-NT (control), SS-DT, and DT-DT, as well as compared the fungal communities. We hypothesized the various tillage rotations could lead to different soil chemical properties as well as soil fungi. The purposes of our research were (1) to study the influence of different tillage rotation treatments on soil chemical properties; (2) to explore the roles of diverse tillage rotations in abundance, diversity, as well as composition of rhizosphere soil fungi; (3) to identify the relationships between the soil chemical properties and the differential soil fungi under the treatments of various tillage rotation.

Materials and methods

Test protocol and tillage rotations

Field experiment was carried out in the arid areas at Qiujialing village (35°09’ N, 110°59’ E), Wenxi County, Shanxi province, China (*Fig. 1A*). The test sites were on a hill in the heavy-arid areas in Shanxi (*Fig. 1B*). The average annual precipitation was 450–630 mm in this area, as well as no irrigation was performed during the

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experimental period. In addition, during the fallow season, from July to September, over 60% of the rainfall occurred.

The soil samples were harvested during a two-year tillage rotation trail that initiated from 2013. The experimental processing integrated three tillage rotations with wheat continuous cropping. The three tillage patterns were designed during the fallow season as follows: no tillage during two years (NT), shallow tillage 30–40 cm (SS), as well as deep tillage 25–30 cm (DT), and four tillage combinations, namely, NT-NT (control), SS-DT, SS-SS, as well as DT-DT (*Fig. 1C*). On June 8, 2013 and June 10, 2014, we harvested the previous wheat, leaving 30-40 cm of stubble in the field to retain soil moisture. After that, the three tillage patterns were conducted with two different plows on the 10th to 15th day after the previous collection. Our study employed a randomized block design with 3 replicates, as well as the total area of the tested field was 666.7 m × 12 m.

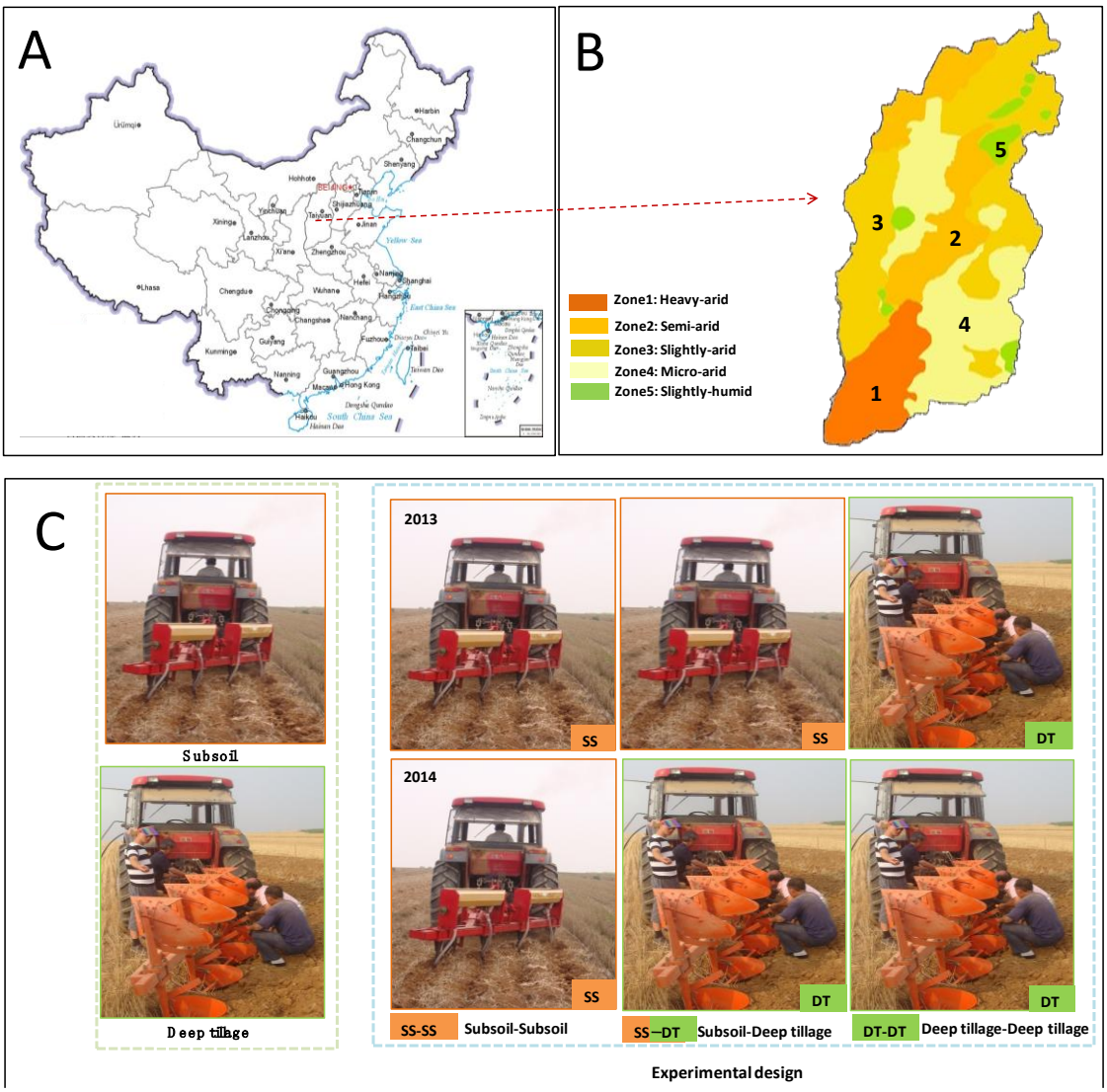

Figure 1. *Map of Shanxi province in China (A). Sampling point in the heavy-arid areas in Shanxi (B). Different tillage rotation treatments applied to the experimental field (C) are labeled as follows: SS-SS: subsoil- subsoil; SS-DT: subsoil- deep tillage; DT-DT: deep tillage - deep tillage; NT-NT: no tillage - no tillage*

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Collection of soil sampling

On June 14, 2015, we acquired winter wheat, as well as the rhizosphere soil samples of winter wheat were obtained at a depth of 0-20 cm of the four diverse tillage rotations, with three subsamples of each sampling point. The entire plant containing extra soil within a 15-cm radium around the plant base was gathered, as well as the soil samples were put in a sterile petri dish without the roots. Non-rhizosphere soil is removed by gently shaking the roots. Then, the flame sterilizing tweezers were employed to collect the rhizosphere soil remaining on the roots, as well as a 2-mm mesh was applied for screen to remove the large rocks and roots (Yang et al., 2016). After that, each composite soil sample was homogenized, and used for DNA isolation. A total of twelve soil samples (4 tillage rotations with 3 repeat samples per tillage rotation) were selected for subsequent analysis.

DNA isolation, fungal ITS gene PCR amplification, and Illumina sequencing

Firstly, a DNA extraction kit (MP Biomedicals, Santa Ana, CA, USA) was utilized to isolate the total fungi genomic DNA, and then a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and agarose gel electrophoresis were employed to quantity and quality of the isolated total DNA. The ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3')/ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') primer was employed to amplify the fungal ITS-1 region (Mello et al., 2011), as well as then sample-specific 7-bp barcodes were incorporated into the primers for multiple sequencing.

The PCR constituents were 5 μL of Q5 reaction buffer (5×), 5 μL of Q5 High-Fidelity GC buffer (5×), 0.25 μL of Q5 High-Fidelity DNA Polymerase (5 U/μL), 2 μL of dNTPs (2.5 mM), 1 μL of each forward and reverse primer (10 μM), 2 μL of DNA template, and 8.75 μL of ddH_2O. of the PCR reaction was started at 98°C for 2 min, followed by 25 cycles of at 98°C for 15 s, 55°C for 30 s, and at 72°C for 30 s, and finally extension at 72°C for 5 min. Afterwards, the PCR amplication products were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN), as well as quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After a single quantitative step, the amplified products were aggregated in equal numbers for paired end 2×300 bp sequencing by the Illumina MiSeq platform with a MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd, Shanghai, China).

The sequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline. Simply, the original sequencing readings that exactly match the barcode were assigned to the corresponding samples, as well as identified as the valid sequences. The low-quality sequences with a length of < 150 bp, ambiguous bases, average Phred scores of < 20, as well as mononucleotide repeats of > 8 bp were filtered (Gill et al., 2006; Chen and Jiang, 2014). FLASH software was applied for assembling of paired-end reads (Magoč and Salzberg, 2011). After chimera determination, the rest high-quality sequences were treated by UCLUST with 97% sequence-consistent clustering as operational taxonomic units (OTUs) (Edgar, 2010).

Analyses of soil chemical properties

A series of soil chemical properties with 0–20 cm layer were further measured. The contents of soil organic matter (SOM) was measured by the improved Walkley-Black method (Wang et al., 2016). The level of available nitrogen (AN) was tested by the

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alkaline hydrolysis multiplication method. Additionally, the contents of available P (AP) (Olsen-P), available K (AK), and inorganic N (NH_4^+ and NO_3^-) were determined by extraction with 0.5 mol/L $NaHCO_3$ followed by colorimetric measurement of P using the molybdate-ascorbic acid method, by extraction with 1 mol/L ammonium acetate followed by a flame photometer, and based on the methods as previously described by Berthrong et al. (2013), respectively.

Statistical and bioinformatic analyses

Bioinformatics analyses were carried out by the Qiime software (R package, version 3.2.0). On the basis of the species abundance for each soil sample in the OTU list, the community diversity indices (Shannon and Simpson indexes) and richness indices (Chao1 and ACE) were calculated using Mothur software. The total number of species was estimated using Chao1 and ACE (Pitta et al., 2010). Then, in order to visualize the shared and unique OTUs among groups, a Venn diagram (R package) was plotted (Zaura et al., 2009). According to the OTU results, the species proportions were obtained at phylum, class, and order levels using Qiime (DeSantis et al., 2006); as well as the principal component analysis (PCA) was then performed from the genus aspect (Ramette, 2007). The relationship between soil traits and fungi was evaluated by redundancy analysis (RDA). Spearman's rank correlations between the richness or diversity estimators, and soil chemical characteristics were performed using the R packages. Additionally, all statistical analyses at $P < 0.05$ (analysis of variance and Tukey's test) was used to determine differences between treatment means were calculated by the SAS software (SAS Institute Inc., North Carolina, USA).

Results

Rhizosphere soil chemical properties

Comparison with the NT-NT treatment, various tillage rotations influenced the rhizosphere soil chemical properties. The contents of AP, AK, and NO_3-N were significantly increased in response to SS-SS by 25.0%, 19.7%, and 14.2% ($P < 0.05$), respectively than under NT-NT. In contrast, all the chemical characteristics of the soil under SS-DT and DT-DT treatments were lower than those of the NT-NT treatment. The SS-SS treatment showed an advantage in improving soil traits (*Table 1*).

Table 1. *The chemical characteristics of rhizosphere soil under different tillage rotation treatments*

Tillage	SOM (g/kg)	AP (mg/kg)	AN (mg/kg)	AK (mg/kg)	NO_3-N (mg/kg)	NH_4-N (mg/kg)
SS-SS	9.57 a	7.74 a	51.67 a	250.88 a	11.32 a	4.06 a
SS-DT	8.88 bc	5.99 b	46.29 ab	156.54 c	6.89 c	2.29 b
DT-DT	8.60 c	5.45 c	44.02 b	145.07 c	5.31 d	1.33 c
NT-NT	9.35 ab	6.19 b	49.79 ab	209.57 b	9.91 b	3.79 a

Values are the mean of three soil samples. SOM: soil organic matter; AP: available phosphorus; AN: available nitrogen; AK: available potassium. NO_3-N: nitrate nitrogen; NH_4-N: ammonium nitrogen. The different tillage rotations are labeled as follows: SS-SS: subsoil-subsoil; SS-DT: subsoil-deep tillage; DT-DT: deep tillage-deep tillage; NT-NT: no tillage-no tillage. Different letters in a column indicate significant differences (ANOVA, $P < 0.05$, Tukey's HSD post-hoc analysis) among tillage rotation treatments

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The composition of soil fungal communities

The OTU-based method indicated that the tillage rotation altered the soil fungal richness (Chao1 and ACE) as well as diversity (Simpson and Shannon) indices compared to NT-NT treatment (*Table 2*). A total of 511,611 quality sequences and 15,162–68,101 sequences of each sample (average = 42,638) were acquired in all the soil samples by high-throughput sequencing (*Fig. 2A*). A total of 279, 344, 327, as well as 279 fungal OTUs were obtained in the NT-NT, SS-SS, SS-DT, as well as DT-DT groups respectively. The largest amount of unique OTUs was observed in the SS-SS treatment (92), followed by SS-DT (48), NT-NT (42), and DT-DT (29). The results showed that SS-SS was beneficial to the growth of rhizosphere soil fungi.

Table 2. *Alpha diversity indices of rhizosphere soil fungi under different tillage rotation treatments*

Tillage	Richness index		Diversity index	
	Chao1	ACE	Simpson	Shannon
SS-SS	440.24 a	429.80 a	0.96 a	5.55 a
SS-DT	393.74 b	395.52 b	0.87 b	4.80 ab
DT-DT	312.36 c	325.63 c	0.82 b	3.95 b
NT-NT	415.04 ab	418.44 ab	0.89 ab	5.20 ab

Values are the mean of three soil samples. The different tillage rotations are labeled as follows: SS-SS = subsoil-subsoil; SS-DT = subsoil-deep tillage; DT-DT = deep tillage-deep tillage; NT-NT = no tillage-no tillage. Different letters in a column indicate significant differences (ANOVA, $P < 0.05$, Tukey's HSD post-hoc analysis) among tillage rotation treatments

The Ascomycota, Basidiomycota, as well as Zygomycota were the dominant fungal phyla among all the soil samples with relative abundances of 1.0% to 6.2%, 73.2% to 75.6%, as well as 12.8% to 22.9%, respectively (*Fig. 2B*). Also, Chytridiomycota, Glomeromycota, Rozellomycota, and the other three infrequent phyla were shown at low abundance in all the samples. Ascomycota (relative abundance > 73%) contributed the most in all tillage rotation treatments. Ascomycota was increased in the SS-SS treatment, whereas Zygomycota and Basidiomycota were decreased.

From the aspect of class, taxonomic classification revealed a high abundance of fungi belonging to Eurotiomycetes, Sordariomycetes, Dothideomycetes, as well as Tremellomycetes (*Fig. 2C*). These classes accounted for more than 96.5% of the total sequences obtained across all soil samples. Pairwise tests of dissimilarities under the class aspect showed the smallest differences between the SS-SS and NT-NT, as well as SS-DT and DT-DT. Furthermore, the differences between the two groups were mainly because of the differences in the relative abundance of Sordariomycetes (50.4%) as well as Dothideomycetes (16.5%).

To further analyze the actions of tillage rotations in the structure of rhizosphere soil fungi, the fungal classes with relative abundance > 1% were analyzed at the level of order and family. The orders Sordariales, Hypocreales, Mortierellales, and Pleosporales were dominant with mean relative abundances of 17.8%, 15.8%, 14.2%, and 11.1%, respectively (*Fig. 2D*). These orders could account for the observed differences between each pair of tillage rotations. From the aspect of the order, the smallest dissimilarity was found in the comparison of SS-SS vs. NT-NT. Twelve strains of fungi with relative abundances greater than 1% were isolated from soil samples at the family level, nine

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families of which, including Lasiosphaeriaceae, Nectriaceae, Pleosporaceae, Chaetomiaceae, Hypocreaceae, Trichocomaceae, Sporormiaceae, Cucurbitariaceae, and Myxotrichaceae, belong to Ascomycota (accounting for 75%). While families Mortierellaceae and Entolomataceae belong to Zygomycota and Basidiomycota, respectively (*Fig. 2E*).

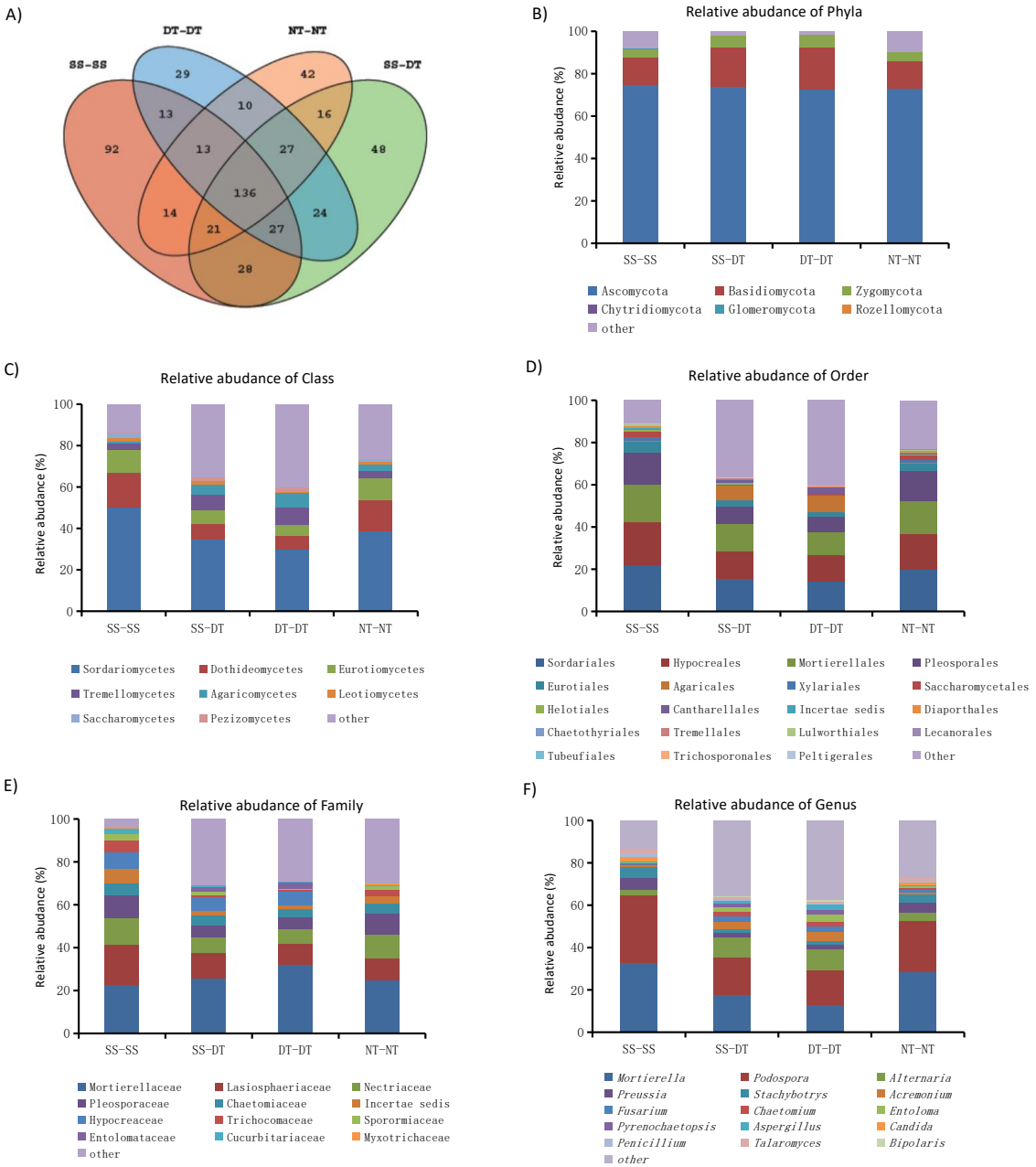

Figure 2. *The Venn diagram (A) and relative abundance of the dominant fungal phyla (B), class (C), order (D), family (E), and genus (F) for the rhizosphere soil samples under different tillage rotation treatments. SS-SS: subsoil-subsoil; SS-DT: subsoil-deep tillage; DT-DT: deep tillage-deep tillage; NT-NT: no tillage-no tillage*

In addition, a total of 15 strains of fungi with relative abundances greater than 1% were isolated and identified at the genus level (*Fig. 2F*). The 15 identified genera

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belong to Ascomycota (accounting for 86.7%), including *Podospora, Alternaria, Preussia, Stachybotrys, Acremonium, Fusarium, Chaetomium, Pyrenochaetopsis, Aspergillus, Candida, Penicillium, Talaromyces, and Bipolaris*. *Mortierella* belongs to Zygomycota. *Entoloma* belongs to Basidiomycota. *Mortierella, Podospora,* and *Alternaria* were the preponderant genera, with their average abundances of 22.9%, 22.5%, and 6.5%, respectively. Moreover, the relative proportion of these ascendant genera was about 73.1% in the control (NT-NT), SS-SS increased the abundance to 86.9%, while SS-DT and DT-DT decreased the abundance of dominant genera.

Relationship between soil chemical properties and fungal community

The PCA based on the genus-level fungus community abundance was studied under different tillage rotation modes (*Fig. 3*). The contribution rates of PC1 and PC2 were 43.3% and 35.9%, respectively. Compared with the NT-NT treatment, the SS-SS treatment was concentrated in the positive region of PC1 and PC2, followed by the SS-DT treatment. Therefore, tillage rotation had a marked implication on increasing the abundance of the fungal community, among which SS-SS had the best effect.

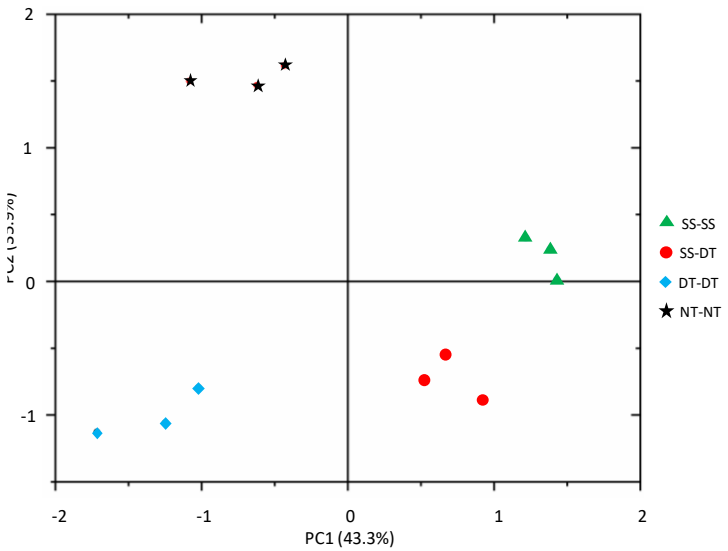

Figure 3. *Principal component analysis (PCA) of the fungal community. PC: principal coordinate. The different tillage rotations are labeled as follows: SS-SS: subsoil-subsoil; SS-DT: subsoil-deep tillage; DT-DT: deep tillage-deep tillage; NT-NT: no tillage-no tillage*

The db-RDA showed that the soil chemical properties, including NO_3-N, SOM, AP, AN, AK, as well as NH_4-N, significantly affected the rhizosphere soil fungal community (*Fig. 4*). The arrows for the soil properties mostly point to the SS-SS, suggesting a strong contribution of SS-SS, which was due to the relatively high content of SOM, AN, NO_3-N, and NH_4-N.

The Spearman's rank correlation coefficients implied a close relationship between the richness or diversity estimators and the soil chemical features (*Fig. 5*). Rhizosphere soil chemical properties, such as soil SOM, AN, NO_3-N, and NH_4-N values, were evidently ($P < 0.05$) linked with fungal community richness (Chao1 and ACE), as well as diversity indexes (Simpson and Shannon).

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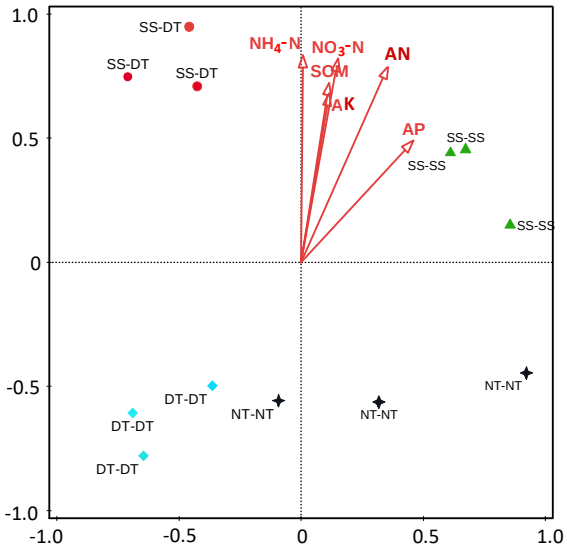

Figure 4. *Distance-based redundancy analysis (db-RDA) showing a significant correlation of rhizosphere soil chemical properties with the rhizosphere fungal community. SOM: soil organic matter; AP: available phosphorus; AN: available nitrogen; AK: available potassium; NO_3-N: nitrate-nitrogen; NH_4-N: ammonium-nitrogen. SS-SS: subsoil-subsoil; SS-DT: subsoil-deep tillage; DT-DT: deep tillage-deep tillage; NT-NT: no tillage-no tillage*

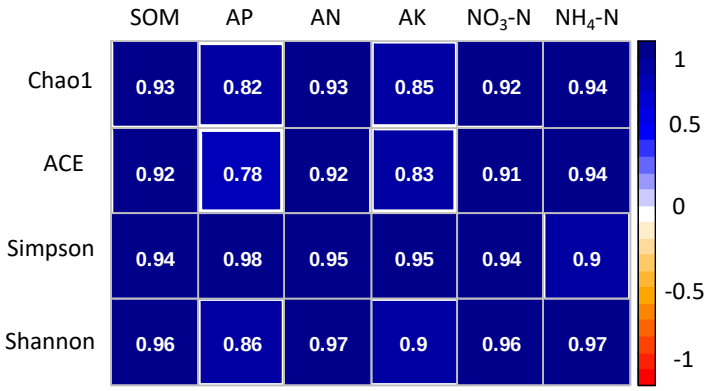

Figure 5. *Spearman's rank correlation coefficients between alpha diversity and rhizosphere soil chemical properties. SOM: soil organic matter; AP: available phosphorus; AN: available nitrogen; AK: available potassium; NO_3-N: nitrate nitrogen; NH_4-N: ammonium nitrogen*

Classification of fungal trophic and functional groups based on FUNGuild database

To further clarify the implications of diverse tillage rotation treatments in the fungal communities of the rhizosphere soil, we classified the fungal species at trophic levels (*Fig. 6*). In addition to Pathotroph, Symbiotroph, and Saprotroph, facultative fungal communities were also divided into two multitrophic modes, i.e., Saprotroph-Symbiotroph as well as Pathotroph-Saprotroph-Symbiotroph. Among them, Saprotroph-Symbiotroph fungi were most abundant accounted for 48.3%-72.8% in all treatments. The relative abundance of Saprotroph-Symbiotroph fungi was 70.6% in NT-NT, which was increased by 3.8% under SS-SS treatment, while decreased by 22.2% and 22.4% in SS-DT as well as DT-DT. The saprotroph was the second most abundant fungal group

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in NT-NT and SS-SS treatments. The DT-DT and SS-DT increased the abundance of Pathotroph-Saprotroph-Symbiotroph fungi where it was the second most abundant, while SS-SS treatment reduced their abundance as compared to NT-NT.

Amongst the top 15 abundant fungi, *Mortierella* and *Podospora* belong to Saprotroph-Symbiotroph, *Preussia*, *Stachybotrys*, *Chaetomium*, and *Talaromyces* belong to Saprotroph, and *Candida* and *Penicillium* belong to Symbiotroph. The abundance of the above fungi was higher in SS-SS treatment. *Alternaria*, *Acremonium*, *Fusarium*, *Entoloma*, and *Pyrenochaetopsis* belong to Pathotroph-Saprotroph-Symbiotroph, and *Aspergillus and Bipolaris* belong to Pathotroph. These two types of fungi were abundant in the more disturbed treatments (such as SS-DT as well as DT-DT).

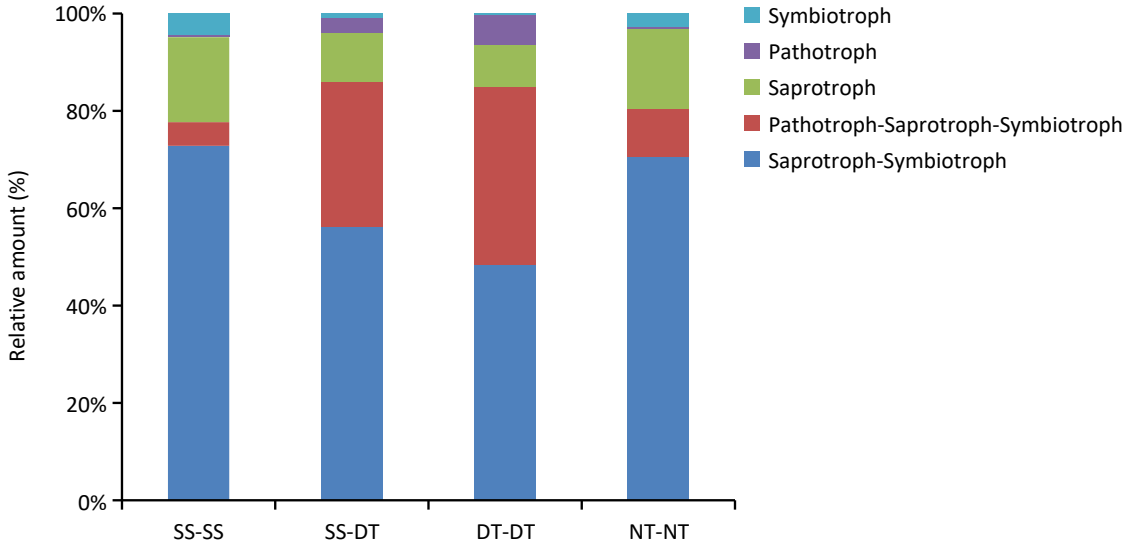

Figure 6. *Relative abundance of fungal trophic mode based on OTU annotation table with distribution frequency level. SS-SS: subsoil-subsoil; SS-DT: subsoil-deep tillage; DT-DT: deep tillage-deep tillage; NT-NT: no tillage-no tillage*

Discussion

Effects of tillage rotation on rhizosphere soil fungal community diversity

Conservation tillage or no-tillage could improve soil microbial biomass and population diversity (Khan et al., 2023). Soil chemical structures (Wang et al., 2017), as major factors affect soil health and ecosystem productivity and are highly correlated with the fungal communities. In the present research, fungal community diversities were observed in the rhizosphere soil of dryland wheat after treated with different tillage rotations at the Loess Plateau. The tillage rotations had significantly influenced the rhizosphere soil fungal richness (Chao1 and ACE indexes) as well as diversity (Simpson and Shannon indexes), which had significantly association with SOM, AN, NO_3-N, and NH_4-N contents. Fungi are known to have a strong link to SOM and are the main driving force for decomposition in the agricultural fields (Liu et al., 2023). The great diversity of soil microbial communities might be through the fungal-driven SOM decomposition (Lu et al., 2023). Additionally, fungal richness was significantly influenced by NO_3-N, AN, as well as NH_4-N. The changes in NO_3-N as well as NH_4-N in rhizosphere soil indicate the contribution of tillage rotations in plant growth by promoting nitrogen utilization and changing fungal diversity (Wang et al., 2020).

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The SS-SS and NT-NT treatments caused relatively less disturbance of soil resulted in a higher fungal diversity. Tillage affects soil by changing the soil aeration and rate of waste decomposition and increases surface area (Angon et al., 2023). Less soil disturbance under NT-NT and SS-SS can increase the use of microbial growth nutrients and protects soil biological components (Essel et al., 2019). While relatively higher disturbance by SS-DT and DT-DT might cause lower fungal diversity and fungal abundances (*Table 2*). One explanation is that the DT treatments (including DT-DT as well as SS-DT) attained the deeper soil below the plow surface layer as well as the mature surface soil to a depth of 25–30 cm, thus preventing the fungi from intercepting soil nutrients such as carbon and nitrogen. This would reduce the nutrients available to the fungi, thus directly affecting own growth and metabolism. In summary, our data showed that the NT-NT as well as SS-SS less disturbed the soil as compared to SS-DT or DT-DT, that were important for the protection of soil biological integrity. But, the NT-NT rotation may contribute to soil compaction, especially topsoil and subsoil layers (Sun et al., 2018). Therefore, SS-SS had been proved as the most effective farming method in the heavy-arid areas of northern China. Nevertheless, the underlying roles of long-term rotation tillage in soil fungal communities need to be further studied.

Effects of tillage rotations on rhizosphere soil fungal community composition

Tillage rotation during the fallow period is one of the effective modes of sustainable agricultural production. Compared with conservation tillage, tillage rotation solves the negative effects of long-term no-tillage by reasonably combining soil tillage techniques such as deep tillage, subsoil, and no-tillage, to improve soil drought resistance and fertility and maintain relatively high crop yield. Previous reports also indicated that tillage treatments affected the compositions of soil fungal communities (Sommermann et al., 2018; Wang et al., 2016a). The compositions of the rhizosphere soil fungal communities responded differently to tillage rotation practices (*Fig. 3*). The relative taxonomic composition of soil fungi in the comparisons of SS-SS vs. NT-NT, as well as DT-DT vs. SS-DT treatments was similar at all taxonomic levels. This may be because the most dominant soil fungi at all taxonomic levels belong to Ascomycota, a most abundant fungal phylum (>72.6%) found in the soil samples with various tillage rotations (Maguire et al., 2020). SS-SS can provide a more suitable soil environment for the growth and abundance of Ascomycota. In addition, the results of the pairwise differences at the class level after different ground tillage rotation treatments showed that the differences between the SS-SS and NT-NT treatments, as well as SS-DT and DT-DT treatments. These differences in the two tillage groups were because of the differences in Sordariomycetes (60.4%) and Dothideomycetes (16.5%) proportions. Sordariomycetes belong to the phylum Ascomycota and display a diverse modes types such as endophyte, mycoparasite, and pathogens (Naranjo and Gabaldón, 2019). Dothideomycetes are important for the health and global carbon cycling of the ecosystem as most of the members are saprotrophs (Taylor and Bhatnagar, 2024).

In addition, the genus-level analysis showed fifteen potentially dominant fungi. They belong to the core mycobiome of the investigated field site, which was strongly affected by tillage rotation practice. The proportions of these preponderant genera were 74.1% in NT-NT, and SS-SS increased the abundance to 88.7%, while SS-DT as well as DT-DT decreased their abundance. Further, the results of PCA proved marked divergences of the fungal communities under different tillage rotations. By secreting oxalic acid, *Morphella* stimulates the dissolution of insoluble phosphorus in the soil, promotes the

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absorption of mineral elements by roots, as well as has great ability to decompress cellulose. Meanwhile, it also has the potential to secrete antibiotics and inhibit the growth of some pathogens such as *Fusarium* (Li et al., 2016; Miao et al., 2016). *Chaetomium* as well as *Penicillium* can effectively degrade cellulose and organic matter, and have antagonistic effects with plant pathogens in the growth process (Rashad and Moussa, 2020).

According to the functional comparison of FUNGuild database, the proportion of Saprotroph-Symbiotroph fungi significantly increased after SS-SS treatment, and the Pathotroph-Saprotroph-Symbiotroph and Pathotroph fungi decreased. One explanation is that the conservation tillage had less destructive effects on fungal hyphae, and retain more nutrients in the soil, while suppress the growth of pathogenic microorganisms (Gao et al., 2022). Another explanation is that SS-DT as well as DT-DT treatments destroyed the soil aggregates and disturbed the soil structure, while SS-SS did not break the plow layer but only loosens the soil in the 0–40 cm layer and thus not much alters the microbial community (Sun et al., 2018).

Our findings demonstrated that various tillage rotations including DT (SS-DT and DT-DT) were clustered together, suggesting similar fungal community structure existed in the comparison of SS-DT vs. DT-DT. Additionally, SS-SS had more similar fungal community structure to NT-NT. RDA results suggested that NO_3-N, NH_4-N, and AN were the dominant soil parameters that determined the composition of fungal communities after treatments of diverse tillage rotations. The core rhizosphere soil fungi found in our research are mainly involved in biochemical processes of the soil nitrogen cycle and are significantly related to changes in NO_3-N as well as NH_4-N in soil (Moreau et al., 2019). As plants cannot fix nitrogen directly, NO_3-N as well as NH_4-N are the major inorganic forms of nitrogen absorbed by plants (Rehman et al., 2022). Taken together, the composition of rhizosphere soil fungal communities in the fallow period (pre-sowing) was mainly driven by the levels of SOM, AN, NO_3-N, and NH_4-N. These results are from two years study, and different tillage methods after long-term rotation can better reflect the actual effect of cultivation on the rhizosphere soil fungal community.

Conclusions

In the drylands of northern China, fungal communities in rhizosphere soil of winter wheat subjected to two-years tillage rotations during the fallow period. The tillage rotations had evidently influence in fungal diversity as well as composition by altering contents of SOM, AN, NO_3-N, and NH_4-N. The subsoiling for subsequent years (SS-SS) could be the most effective tillage rotation, and reduced the abundance of Pathotroph (*Aspergillus* and *Bipolaris*) and Pathotroph-Saprotroph-Symbiotroph fungi (*Alternaria, Acremonium, Fusarium, Entoloma*, and *Pyrenochaetopsis*). These findings have practical implications for the rotation of subsoil and subsoil to improve soil quality and for the production of more stable and sustainable soil ecosystems in dryland subsoil rotation in northern China.

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Conflict of interests. We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and company that could be construed as influencing this manuscript.

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