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Deep tillage reduces the dependence of tobacco on AMF and promotes the growth of tobacco(*Nicotiana tabacum* L.) in dryland

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Abstract

The traditional shallow tillage method makes the soil quality declining, and affects the efficiency of agricultural production. Taking the conventional rotary tillage(12 cm) as the control, Yunyan 87 as the test variety, and the soil type of the test site is paddy soil, we studied the effects of deep tillage(Subsoiling 30 cm) on soil nutrients, arbuscular mycorrhizal fungi (AMF) and tobacco (*Nicotiana tabacum* L.) growth. The results showed that deep tillage increased the contents of organic carbon, available phosphorus(AP) and available potassium(AK) in 20 ~ 40 cm soil layer. The community of AMF was changed by deep tillage. Glomus, the dominant genus in both group, increased significantly in the soil after deep tillage. The colonization rate of AMF was lower than that of conventional rotary tillage. Deep tillage was beneficial to the growth of tobacco in the middle and late stages. Root growth and nutrient content of tobacco increased. Deep tillage significantly improved the output value of tobacco. It can be seen that deep tillage is conducive to improving soil fertility, promoting the vigorous growth of root, reducing the dependence of tobacco on AMF, and promoting the high quality and yield of tobacco in drylands of Hunan.

Keywords: Deep tillage; Arbuscular mycorrhizal fungus; Tobacco; Soil nutrients;

Output value

Introduction

The direct effect of tillage on soil can change soil physical and chemical properties and affect crop yield. Some results (Zhai et al. 2017) showed that deep tillage could reduce soil bulk density, promote corn growth and obtain higher yield. Compared with conventional rotary tillage, deep tillage (Xie et al. 2015) could significantly increase the organic carbon and ammonium nitrogen(N) contents in the 20~40 cm soil layer of the wheat field. Researches also showed that deep tillage could reduce the bulk density and enable the crops to take root deeper and more densely so as to improve the crop yield (Gajri et al. 2010). Different soil tillage measures disturb the soil with machine, also affect the activity of soil microorganisms (Rosner et al. 2020).

Soil microorganisms, water, nutrients and other factors constitute the soil environment. The microorganisms in the soil are very active parts, and AMF, belonging to the phylum Glomeromycota, can form arbuscular mycorrhiza (AM) with the plant root. This mycorrhizal symbiosis is the most important and common type of endophytic mycorrhiza, and also a very important part in the whole ecosystem, playing an important role in farmland (Kadowaki et al. 2018). AMF form material transportation channels between soil and plant roots. Some studies (Smith et al. 2011) have indicated that AMF improve the absorption of mineral nutrients by plants by forming a huge hyphal network. The hyphae outside the roots absorb the mineral nutrients in the soil and transport them to the roots and then to all parts of the plant. AMF can also obtain organic carbon from plants through the arbuscular structure symbiotic with the plants.

Tobacco-maize rotation is a common dryland farming mode in tobacco-growing

areas (Chen et al. 2020). However, the long-term traditional shallow tillage mode made the soil fertility of tobacco-growing soil in Hunan decreased (Liu et al. 2020), and hence affected the yield and quality of tobacco, which showed that the soil urgently needed to improve. Due to the transportation and transmission of material, energy and information among soil, microorganisms and tobacco (Hartmann et al. 2008), the changes of microorganisms in soil can also reflect the effects of tillage methods on soil fertility and crop growth. In this study, the subsoiling, a method of deep tillage, was used on the dry land soil with tobacco-maize rotation. After the maize was harvested, the deep tillage mechanical spiral drill was used to subsoil the soil at one time, which promoted the expansion of soil nutrients, water and oxygen without disturbing the soil layer. This technology has been proved having a good effects on the yield increase, quality and soil improvement (Ji et al. 2014) of crops such as sugarcane (Naseri et al. 2007), cotton (Wesley et al. 2001), wheat (Farooq et al. 2014) and rice (Yoichiro et al. 2007), but its application in flue-cured tobacco is less, and its effect on microorganism in tobacco-growing soil is not clear. Therefore, we assumed that deep tillage could help to increase the content of available nutrients in the soil, promote tobacco growth, improve tobacco yield, and affect the community composition of AMF and mycorrhizal symbiosis. Based on the symbiotic perspective of flue-cured tobacco and AMF, through studying the changes of soil nutrients, AMF population and flue-cured tobacco growth in dry land flue-cured tobacco soil after deep tillage, the effects of deep tillage on the root environment of dry land flue-cured tobacco and the growth of flue-cured tobacco were clarified, which may provide a theoretical support for the application of deep

tillage in flue-cured tobacco planting and promoting the high-quality production of dry land flue-cured tobacco.

Materials and methods

Experimental Site

The field experiment was conducted in the Yunyuan Experimental Base of Hunan Agricultural University (28°11'18" N, 113°04'56" E), and the tobacco variety was Yunyan 87. The soil type is paddy soil, and soil texture is loam, with tobacco-maize multiple cropping and continuous cropping. The land is relatively flat and well drained. The soil samples were taken before tillage in late November 2018 to detect the basic nutrient status of tobacco fields : pH=6.04, organic matter 22.03 g kg⁻¹, total nitrogen (TN) 1.21 g kg⁻¹, total phosphorus (TP) 0.74 g kg⁻¹, total potassium (TK) 28.57 g kg⁻¹, alkali-hydrolyzable nitrogen (AN) 116.36 mg kg⁻¹, available phosphorus (AP) 48.45 mg kg⁻¹, and available potassium (AK) 245.02 mg kg⁻¹.

Experiment design

The experimental farmland was divided into two different tillage measures, deep tillage and conventional rotary tillage. Conventional rotary tillage was used as the control group and deep tillage as the treatment group. There were three repetitions for each tillage measure, and three plots with the area of each plot about 92m² were set. The deep tillage was recorded as DT, and the conventional rotary tillage CT. The conventional rotary tillage method was carried out as following: artificial ridging after rotary tillage

before tobacco transplanting in March 2019 using a LS804 tractor as rotary tiller, and the tillage depth being 12cm. The deep tillage method was : subsoiling the field in late November 2018 after maize harvesting in the autumn of 2018 using a SGL-160 as deep tillage machine, and the tillage depth being 30cm, and artificial ridging before tobacco transplanting in March 2019.

Other tobacco cultivation management measures were the same for both tillage methods. The Yunyan 87 tobacco seedlings were transplanted in all cultivated lands on April 8, 2019. All fertilizers were purchased at Yong 'an Station of Liuyang Tobacco Company. Before the tobacco was transplanted, special base fertilizer for tobacco, biological organic fertilizer (cake fertilizer), calcium fertilizer, magnesium fertilizer and phosphate(P) fertilizer were applied in the field. The seedling raising fertilizer was applied twice on the day of transplantation and 7 d after transplantation, respectively. Potassium nitrate and potassium sulfate were applied 14 d and 28 d after transplanting, respectively. The total fertilizer application amount before and during the growth period of tobacco was 120 kg N ha⁻¹, 130 kg P₂O₅ ha⁻¹, and 320 kg K₂O ha⁻¹.

Determination of AMF in soil

Sample collection

75 d after transplantation (the mature stage of tobacco), a soil sample around the roots was taken. Three sampling points were randomly selected from each plot, and the slope with a depth of about 30cm was dug at the ridge surface 5cm away from the main stem of the tobacco, and the soil near the root was collected. Three soil samples from the

same plot were mixed evenly, with three replicates in each group, and the obtained soil samples were stored in liquid N. After being taken back to the laboratory, the samples were stored at -80 °C refrigerator for further examination. The complete roots of different groups of tobacco (9 for DT and 9 for CT) were dug out from the corresponding sampling points. The lateral roots of tobacco plants were collected and packaged in sealed bags according to different groups, stored in an ice box at 4 °C, brought back to the laboratory, and stored in a refrigerator at -20 °C for the determination of AMF colonization rate.

Soil AMF community composition and diversity detection

AMF community composition and diversity were analyzed for three replicates in each group of DT and CT. Soil samples were taken from the -80 °C refrigerator, and 5g soil samples were taken from each group and placed in dry ice for transportation. AMF in soil was tested by Biomarker Technologies (<http://www.biomarker.com.cn/>), raw sequence reads obtained from Illumina Hiseq sequencing. Primer sets AMV4.5NF/AMDGR were used to amplify an 18S rRNA gene fragment.

The effective sequences were clustered into different operational taxonomic units (OTUs) at 97% sequence similarity using the UPARSE. Taxonomic assignment was performed by blasting the representative sequences against the Silva 132. All samples were normalized to the lowest sample size (97794 sequences) for further analysis. α diversity analysis of each sample were calculated. The difference of AMF community among groups was studied by β diversity analysis. Through significant difference analysis between groups, the genera with significant difference between groups were

found. All raw sequence reads have been deposited in the NCBI-Sequence Read Archive with the submission Accession Number: PRJNA744658.

Arbuscular mycorrhizal fungal colonization

The experiment was performed by staining and microscopic examination with reference to the method of Lu Shanshan et al. (2019). The roots of tobacco were divided into two groups, DT and CT. A plurality of lateral roots, with the length of about 1cm, were respectively taken and placed in two conical flasks. 5mL of formalin (38% formaldehyde), 5 mL of glacial acetic acid and 90 mL of 70% alcohol were taken to prepare Formalin-Aceto-Alcohol (FAA), 20 mL of FAA was taken and placed in the conical flasks, the root segments were immersed and fixed. Then the root segments were taken out and rinsed for 3 to 4 times with distilled water. 10mL of 10% KOH solution was added into the root segments of different groups, and the segments were decolorized in a water bath at 92 °C. The segments were taken out after water bath for 1h and then acidified in 2% lactic acid at room temperature for 3min. The acidified root segments were then stained with 0.05% acid fuchsin staining solution for 45 min at 92 °C. The stained root samples were pressed into tablets and made into temporary slides, which were then successively examined under the microscope to observe the colonization of AMF therein and to calculate the colonization of AMF in the roots. Under the microscope, the part with AMF colonization showed deep color.

$$\text{Colonization rate (\%)} = (\text{root area with AM}/\text{observed root area}) \times 100\%$$

Determination of soil nutrients

Before cultivation, the soil were collected by sampling in five locations from each plot, and a 0~30 cm soil column was taken. After the soil column was fully and evenly mixed, pH of the soil and the main nutrients, including soil organic matter, soil TN , TP , TK, AN, AP, and AK, were detected. In the vigorous growth period of tobacco (55 d after transplantation), three soil columns with the size of 0~50 cm were selected on the ridge surface 5cm away from the main stem of the tobacco plant in each group as replicates and stored in a layered and mixed manner at the size of 0~10cm, 10~20cm, 20~30cm, 30~40cm and 40~50 cm. The content of soil organic carbon(SOC), soil TN,TP, TK, AN, AP and AK were detected: Soil TN determination method with reference to the Kjeldahl method(Morikawa et al. 2004), Soil TP and TK in soil were first boiled by H₂SO₄-H₂O₂ (Mala J and Lagova M, 2014), and then determined by molybdenum-antimony colorimetric method (Sun et al. 2019) and flame photometric method(Vaiciulyte et al. 2017) respectively. The determination method of AP in soil refers to the above-mentioned molybdenum-antimony colorimetric method, The determination method of soil AK refers to the flame photometry mentioned above. Soil AN determination method reference diffusion absorption method, (Lu,2000), and the SOC determination reference to the method of hydrated hot complex acid potassium oxidation–colorimetric method (Wu et al. 2011).

Determination of main nutrients and dry matter accumulation in tobacco

The plants with complete roots were collected four times every 15 days (35 d, 50 d, 65 d and 80 d) from the beginning of topping stage (35 d after transplantation). Three sites

were randomly selected from each group for each sampling, and two representative tobacco were selected from each site to detect the root characteristic indexes of each sample, including root diameter, root volume, lateral root number and root depth. First, classified and weighed the fresh root, stems and leave of each plant, secondly, dry and weighed each part of the two plants, then analyzed the accumulation condition of dry substances in each part of each group of tobacco plants. The Kjeldahl method of N determination, molybdenum-antimony colorimetric method and flame photometry (Li, 2003) were used to detect the N, P and K contents in each part of the tobacco, and to analyze the nutrient absorption and distribution characteristics of tobacco under different tillage practices.

Determination of tobacco yield and quality

After the tobacco was mature(80 d sfter transplanting), each group and each plot were separately harvested and roasted. The leaves were classified according to the national standard formulated by State Tobacco Monopoly Administration of China and weighed. The average price was obtained by dividing the total price acquired by the local tobacco department at different prices of classified tobacco by the total weight acquired. The yield and output value of tobacco as well as the proportion of medium and high grade tobacco were counted.

Data processing

Statistical significances between groups were evaluated by one-way ANOVA using SPSS 21.0. Raw sequence data was analyzed on the BMKCloud

(<http://www.biocloud.net/>) for finding Alpha diversity and Beta diversity. A Venn diagram, rarefaction curves and histogram of relative abundance were formed employing Origin 2021. R 4.0.5 and R studio (21.0.0.0.184) were used to evaluate the colonization rate and STAMP was used to find AMF with significant differences.

Result

Impacts on structural diversity and colonization of soil AMF communities

Sequencing data and OUT analysis

After sequencing of each sample, CT and DT obtained Raw Reads 397673 and 362298, respectively; there were 380527 effective sequence in CT and 347388 in DT respectively, with the effective rates of 93% and 93%, and the average sequence lengths of 219 bp and 218bp. A total of 417 OUTs were obtained from the tested soil samples (Table 2), including 332 OTUS for CT, 314 OTUs for DT and 229 OUTs for both groups (Figure 1a). With the increase in the number of sequenced samples, the dilutability curve (Figure 1b). was tended to be stable, indicating that the amount of each sample sequenced was sufficient for subsequent analysis of microbial diversity.

Analysis of AMF community composition

In both groups, four genera, Acaulospora, Claroideoglomus, Glomus, and Paraglomus, were detected at the genus level, except for Unassigned AMF (Figure 2). Glomus was most abundant in both the DT and CTs, accounting for 48% and 40%, respectively, and increased significantly by 19% in the DT; the second important group was

Claroideoglossum, which was more in CT (37%) and only 32% in DT. Acaulospora and Paraglossum accounted for minimal amounts, only 2 % in each group.

Analysis of α diversity of AMF

The Shannon index in the CT was higher than that in the DT, and the Simpson index was lower than that in the DT, suggesting that the species diversity in the CT was higher than that in the DT (Table 3). But the ACE index in the CT was higher than that in the DT, and the Chao1 index was lower than that in the DT. The analysis of variance of α diversity index of each sample showed that the p -values of ACE, Chao1, Shannon and Simpson were all greater than 0.05. Therefore, the differences of ACE index, Chao1 index, Shannon index and Simpson index between the two groups were not significant. This showed that there is no significant difference in species abundance and species diversity of AMF between DT and CT, and the coverage of the statistical samples is 0.999, so this result could represent the AMF in the samples.

Analysis of β diversity of AMF

Based on the Bray-Curtis dissimilarity, β diversity of AMF in different groups was analyzed (Figure 3). Non-metric Multi-dimensional Scaling analysis and unweighted pair-group method with mathematical mean both showed differences in microbial communities among different groups and similarities in microbial species composition within the same group. As shown in Figure 3a and b, the community compositions of CT and DT were significantly different, and the species composition of AMF in the same group was more similar. It could be seen that different tillage practices had significant

effects on the community structure of AMF in soil.

Significant analysis of difference between groups

The significant differences of AMF communities in CT and DT were analyzed (Figure 4), and the species with statistical difference among different groups were found. At the genus level, the abundance of *Glomus* was significantly different between the CT and the DT, and significantly increased in the DT.

Comparison of colonization rates of AMF among different groups

The significant difference in AMF colonization rates between the two groups of CT and DT was analyzed, and the results are shown in Figure 5. The results showed that the average colonization rate of CT was 5%, the minimum colonization rate was 3%, and the maximum colonization rate was 7%, while those of the DT was 3%, 1% and 5% respectively, which indicated that the colonization rate of DT was lower than that of CT, and *P* value was 0.008, less than 0.01. Therefore, the difference of AMF colonization rate between CT and DT was very significant, and the colonization rate of AMF was extremely significantly reduced.

Effects on soil nutrients

Deep tillage increased the content of available nutrients in the soil (Table 1). The SOC content in the DT was higher than that in the CT in the 0~40 cm topsoil, especially at 20~40 cm, the organic carbon content in the DT was 43% more than that in the CT. The depth of DT with TP content in the range of 20~30 cm was lower than that of CT, and

the other depths of DT were higher than that of CT. There was no significant difference in TP content in the range of 0~10 cm, and it was significantly higher in the 20~50 cm DT than in the CT. TK was lower in the DT than in the CT at the position of 20~30 cm, and had no significant difference in other soil layers. AP and AK in the soil at the depth of 0~10cm were higher in the CT than in the DT, but with the deepening of the topsoil, between 10~50 cm, the DT was better than the CT. In the 20~40 cm soil layer, the amount of AP in the DT was 495% more than that in the CT, and in the 20~40 cm topsoil, the amount of AK in the DT was 98% more than that in the CT. The TN content in the 0~40 cm topsoil of the DT was significantly lower than that of the CT. The AN content in each group decreased with the deepening of the soil depth. The content in the DT was significantly lower than that in the CT at 10~20 cm, and there was no significant difference in the AN content in other topsoil. After deep tillage, the TN was significantly reduced in the tobacco field soil, while the AN was not significantly changed.

Effects of Deep Tillage on Main Nutrient and Dry Matter Accumulation of Tobacco

3.3.1 Effect on tobacco root

As shown in Table 4, the effects of different tillage practices on the root of tobacco plants suggested that, with the growth of tobacco, the root downbinding depth in the DT was significantly deeper than that in the CT. Especially at the maturity stage, the root downbinding depth in the deep tillage was 35% deeper than that in the conventional rotary tillage. The root diameter of DT in prosperous period, budding period and mature

period was also significantly higher than that of CT. The maximum root diameter of both groups reached 11.7mm and 7.3mm in mature period. The root diameter of DT in prosperous period, budding period and mature period was 12%, 49% and 60% thicker than that of CT. The number of lateral roots in the rosette stage was not significantly different between the DT and the CT. Each lateral root rapidly increased in the prosperous period. The number of lateral roots in the DT was significantly more than that in the CT in the prosperous period, 23% more than that in the CT. The significant difference also existed between the DT and the CT in the mature period, 42% more than that in the CT. The root volumes from prosperous period to mature period were counted. The root volumes of DT were higher than that of CT. And with the growth of tobacco plants, the root volumes became larger and larger. In the mature period, the root volume of DT was 32% larger than that of CT, and there was a significant difference. This indicated that deep tillage was beneficial to the growth of tobacco root.

Effect of deep tillage on dry matter accumulation, and nutrient content of tobacco

The dry matter accumulation and nutrient content of flue-cured tobacco under different tillage practices are shown in Table 5. On the whole, the total contents of dry matter, N, P and K in leaves were higher than those in other parts. From different stage, the dry matter under the two tillage methods was higher in CT than in DT in the rosette stage and the vigorous growth stage in all parts of tobacco, but there was no significant difference. By the budding stage and the mature stage, the dry matter accumulation of the DT was superior to that of the CT. The dry matter weights of roots, stems and leaves in the DT at the budding stage were significantly higher than those of the CT by 23%,

30% and 15%, and those of the roots, stems and leaves at the mature stage were significantly higher than those of the CT by 29%, 30% and 18%. This indicated that in the middle and late growth stage, flue-cured tobacco with deep tillage could accumulate more dry matter. The contents of N, P and K in the rosette stage and vigorous growth stage were mostly higher in the CT than in the DT. However, it was significantly higher in the DT than in the CT at the budding stage and the mature stage. In the budding stage, the N, P and K contents in the leaves of DT were 10%, 11% and 40% higher than those in the CT, respectively. In the mature stage, the N, P and K contents in the leaves of DT were 20%, 18% and 25% higher than those in the CT, respectively. This indicated that the promoting effect of deep tillage on the growth of flue-cured tobacco began to highlight in the middle and late stage of growth and development, and was conducive to the accumulation of N, P and K on the tobacco.

Effect on economic characters of tobacco

The economic traits of cured tobacco were evaluated (Table 6). The yield, output value, average price and the proportion of medium and high grade tobacco in DT were 9%, 11%, 1% and 3% higher than those in CT. As a result, the yield and output value in DT were significantly higher than those in CT, which indicated that deep tillage improved the yield of tobacco and good economic benefits were achieved.

Correlation analysis between 4.AMF infection rate and root and soil nutrients of flue-cured tobacco

The colonization of AMF is negatively correlated with root growth and soil available nutrients, among which the colonization is negatively correlated with the contents of

Root depth, Root diameter and soil AP (Table 7). Fig. 6 visually shows the negative correlation between Root depth, Root diameter and AP content in soil.

Discussion

AMF are important components of soil microorganisms in farmland ecosystems. Plants can induce hyphal branches and promote the formation of mycorrhizal symbionts by releasing signals to the rhizosphere, which further improve the absorption of phosphorus in soil and the stress resistance of plants. Extraroot hyphae plays an important role in improving the soil by secreting glomalin-related proteins, which stabilizes soil aggregates (Alguacil et al. 2008). In this experiment, the colonization of AMF on the roots of tobacco was low under both deep tillage and conventional rotary tillage, which might be due to fertilization before tobacco planting. A large number of studies (Xie et al. 2014) have shown that under the condition of high nutrient supply, especially P, the colonization rate of AMF reduced, the mycorrhizal structure reduced, and the hypha and development inside and outside the root are inhibited. The availability of P in the soil affected the colonization of AMF on plants, and if the range was out of favor of the formation of mycorrhizae, the series of transformations presented an inverted "U" curve.

Previous studies on AMF have shown that soil tillage could result in decrease fungal biomass and disrupt AMF networks (Mariotte et al. 2018), which might lead to a decrease in P absorption. Interestingly, although the mycorrhizal colonization rate was significantly decreased in deep tillage, the nutrient uptake by tobacco was significantly increased. Mycorrhizal dependency may explain this phenomenon. Plants that grow

faster and have developed roots have less dependence on mycorrhizae (Williams and de, 2020). The effect of deep tillage on the growth of tobacco root is very obvious, no matter from the setting depth, root diameter, lateral root number or volume, the promotion is significantly increased, and this promotion effect is more significant in the late growth and development of tobacco, which is consistent with the research of Yang et al. (2020) on tobacco. The research of Chen et al. (2021) on corn also reflects the promotion effect of deep tillage on root growth. Long-term rotary tillage, will compact 15~30 cm soil layer of soil, make the soil hardening, lead to the unit weight increases or porosity decreases. Raising the tillage depth has obvious effect on the improvement of soil (Tian et al. 2016). Researches show that deep tillage can significantly reduce soil infiltration resistance, increase the porosity of surface or underground soil, reduce the soil bulk density (Tian et al. 2020). As it is well-known that loose soil is beneficial to the growth of plant root. The two tillage methods both cultivate the shallower soil layer. In the early growth stage of tobacco, the root is short, and the promotion effect on the growth of tobacco is not significant. In the late stage, the growth of tobacco root and the deep loosening tillage on the deeper soil make the deeper soil more loose, which is more beneficial to the growth of tobacco root, so the root can absorb more nutrients in the deep soil. And this promotion effect also makes the material accumulation more significant in the middle and late growth stage of tobacco and further improves the yield and quality of tobacco. On the other hand, since plants absorb soil nutrients via two ways (Pan et al. 2020): one is the direct pathway for absorption from the rhizosphere environment through root hair and epidermal cells, and the other is the indirect pathway

for transport through AMF. Although under very low nutrient concentrations, the AMF pathway enabled plants to obtain nutrients by increasing the absorption surface area, when the nutrient utilization rate was high, the host would reduce their dependence on the nutrients obtained by AMF from the soil, and the pathway of direct root absorption was more effective. Subsoiling can increase the content of SOC (Feng et al. 2020), increase the stability of soil aggregates (Hu et al. 2021), and increase the content of AP and AK in the soil (Guo et al. 2016), improve the chemical properties of the soil and improve the fertility of the soil. The results of soil nutrient testing in this study show that the contents of AP and AK in the 10~40cm soil layer are also significantly higher than those under conventional rotary tillage. Therefore, with higher soil available nutrients and more developed tobacco roots in the soil with deep tillage, the tobacco might prefer to absorb the nutrients in the soil through the direct pathway, and reduce the dependence on the indirect pathway, thus reduce the colonization. Therefore, in this experiment, the content of N, P and K and dry matter in the tobacco with deep tillage was also significantly increased. Under the condition of reduced colonization, the absorption of nutrients by the tobacco did not reduce, but significantly increased, which might be explained by the reason mentioned above. When the host reduced their dependence on the nutrients obtained by AMF from the soil, the amount of carbon allocated to the underground would be decreased, which would intensify the competition for C provided by the plants among AMF species (Han et al. 2018), and change the community structure of AMF in soil under conventional rotary tillage and deep tillage.

Conclusion

Deep tillage promoted the nutrients of paddy soil where tobacco is planted, significantly increased SOC, AP and AK in deep soil, activated soil nutrients and promoted soil fertility. The changes of tillage practices also changed the community composition of soil AMF. After deep tillage, the colonization of AMF on tobacco root was reduced, and the mycorrhizal symbiotic relationship was weakened. The root system structure of tobacco is optimized, and the root depth, the number of lateral roots, the root diameter and the root volume are obviously increased, which was beneficial to the growth of tobacco root and the accumulation of N, P and K and dry matter in each part of tobacco, and significantly increased the output value and obtained better economic benefits. In conclusion, deep tillage could promote the growth of tobacco and improve the plant-availability of nutrients in the soil, which would provide a theoretical support for the application of deep tillage in tobacco planting and the promotion of high-quality production of tobacco.

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Conflict of Interest The authors declare no conflict of interest exists.

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Figure legends

Figure 1. OUT Venn (a) and Rarefaction curves (b).

Figure 2. Histogram of relative abundance under genus level in CT and DT.

Figure 3. Non-Metric Multi-Dimensional Scaling(a) And Unweighted Pair-group Method with Arithmetic Cluster tree(b).

Figure 4. Significant analysis of AMF abundance among groups at genus level.

Figure 5. The colonization rate of AMF in the roots of tobacco in different groups.

Figure 6. Regression model of colonization with root growth and soil available nutrients.

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Table 1. Nutrient content of different soil layers in vigorous growing stage of tobacco

Depth (cm)	Tillage	SOC (g·kg ⁻¹)	AP (mg·kg ⁻¹)	AK (mg·kg ⁻¹)	AN (mg·kg ⁻¹)	TP (g·kg ⁻¹)	TN (g·kg ⁻¹)	TK (g·kg ⁻¹)
0~10cm	DT	12.27a	56.68b	245.91b	167.75a	0.96a	1.55b	31.91a
	CT	11.87a	63.88a	323.91a	171.76a	0.93a	1.85a	31.81a
10~20cm	DT	12.16a	87.61a	328.19a	148.49b	1.04a	1.67b	32.24a
	CT	12.02a	48.11b	242.87b	151.10a	0.83b	2.15a	31.97a
20~30cm	DT	11.57a	83.33a	252.19a	138.10a	1.12b	1.44b	30.72b
	CT	8.87b	12.47b	165.33b	144.83a	0.69a	1.77a	31.36a
30~40cm	DT	11.02a	50.69a	234.27a	131.83a	0.88a	1.43b	29.04a
	CT	6.93b	10.06b	80.84b	126.53a	0.53b	1.71a	28.94a
40~50cm	DT	5.17a	8.76a	82.08a	127.54a	0.75a	1.11a	30.20a
	CT	5.85a	3.66b	81.17a	123.90a	0.51b	1.10a	30.18a

Note: Different small letter in the same column means significant difference at $P<0.05$

Table2. Sample sequencing data and OTU results statistics

Tillage	Raw Reads	Clean Reads	Effective Reads	AvgLen (bp)	Effective (%)	OTU
CT	397673	375201	371051	219	93	332
DT	362298	341745	336700	218	93	314

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Table 3. Analysis of α diversity

Alpha_index	Shannon	Simpson	ACE	Chao1	Coverage
CT	4.0297	0.8620	222.0188	224.7842	0.9998
DT	3.9599	0.8758	219.3214	232.4459	0.9997
p_value	0.6862	0.3551	0.8905	0.7172	

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Table 4. Effect of different tillage on root of tobacco in different stage

Stage	Tillage	Root depth (cm)	Root diameter (mm)	Lateral root	Root volume (cm ³)
Rosette stage	DT	13.0a	3.3a	8.3a	
	CT	13.0a	3.6a	7.7a	
Vigorous growth stage	DT	26.0a	6.6a	31.0a	83.7a
	CT	20.8b	5.9b	25.3b	64.1b
Budding stage	DT	36.9a	10.4a	30.3a	129.5a
	CT	27.7b	7.0b	26.7a	123.7a
Mature stage	DT	37.5a	11.7a	31.3a	175.6a
	CT	27.8b	7.3b	22.0b	133.3b

Note: Different small letter in the same column means significant difference at $P<0.05$

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Table 5. Total nitrogen, total phosphorus, total potassium and dry matter accumulation in each part of tobacco

Nutrient content	Stage	Tillage	Root	Stem	Leaf
TN (mg·plant ⁻¹)	Rosette stage	DT	54.40a	85.14b	582.01b
		CT	62.31a	118.56a	625.68a
	Vigorous growth stage	DT	362.87b	736.12a	1363.09b
		CT	471.78a	711.68a	1603.97a
	Budding stage	DT	599.92a	1204.50a	2323.05a
		CT	598.08a	1040.16b	2107.68b
	Mature stage	DT	720.62a	1264.49a	3092.96a
		CT	674.73b	1091.41b	2576.07b
TP (mg·plant ⁻¹)	Rosette stage	DT	7.60a	16.83a	83.37b
		CT	6.83a	17.55a	102.70a
	Vigorous growth stage	DT	89.08b	155.35b	397.81b
		CT	102.23a	222.40a	442.01a
	Budding stage	DT	189.37a	452.65a	534.90a
		CT	130.83b	313.23b	479.87b
	Mature stage	DT	212.21a	490.31a	593.92a
		CT	226.05a	363.17b	501.49b
TK (mg·plant ⁻¹)	Rosette stage	DT	39.80a	99.99a	407.41a
		CT	35.57a	102.57a	467.68a
	Vigorous growth stage	DT	348.46a	717.00a	2201.33a
		CT	338.20a	692.22a	1235.69b
	Budding stage	DT	524.03a	1396.30a	2435.89a
		CT	436.42b	1052.45b	1738.82b
	Mature stage	DT	716.20a	1505.35a	2599.79a
		CT	482.93b	1189.50b	2072.18b
Dry matter (g·plant ⁻¹)	Rosette stage	DT	2.00a	3.30a	15.73a
		CT	2.07a	3.90a	15.80a
	Vigorous growth stage	DT	13.94a	23.90a	70.33a
		CT	16.67a	27.80a	75.53a
	Budding stage	DT	37.14a	76.72a	99.02a
		CT	30.15b	59.10b	86.08b
	Mature stage	DT	44.21a	86.02a	112.06a
		CT	34.25b	66.03b	94.62b

Note: Different small letter in the same column means significant difference at $P < 0.05$; TN, Total nitrogen; TP, Total phosphorus; TK, Total potassium.

Table 6. Economic characteristics of tobacco in different tillage

Tillage	Yield (kg ha ⁻¹)	Output value (yuan ha ⁻¹)	Average price (yuan kg ⁻¹)	Middle and high quality tobacco ratio (%)
DT	2358.45 a	50931.90 a	22.14 a	94 a
CT	2154.15 b	47071.80 b	22.08 a	91 a

Note:Different small letter in the same column means significant difference at *P*<0.05.

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Table7 Pearson correlation between colonization and root growth of tobacco and soil available nutrients

Index	Root depth	Root diameter	Lateral root	Root volume	AP	AK	AN
Colonization	-0.96**	-0.91*	-0.71	-0.65	-0.93**	-0.61	-0.15

Note:*and** indicate significant levels of 0.05 and 0.01, respectively.

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Figure 1. OUT Venn (a) and Rarefaction curves (b)

523x200mm (300 x 300 DPI)

Figure 2. Histogram of relative abundance under genus level in CT and DT

363x263mm (300 x 300 DPI)

Figure 3. Non-MetricMulti-Dimensional Scaling(a) And Unweighted Pair-group Method with Arithmetic Cluster tree(b)

302x123mm (300 x 300 DPI)

Figure 4. Significant analysis of AMF abundance among groups at genus level
198x49mm (300 x 300 DPI)

Figure 5. The colonization rate of AMF in the roots of tobacco in different groups.

399x299mm (300 x 300 DPI)

Figure 6. Regression model of colonization with root growth and soil available nutrients

72x56mm (300 x 300 DPI)