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Effects of Tillage Depth on Nutrients and Microbial Communities in Tobacco-Planting Soil

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Abstract

The implementation of appropriate tillage practices is of great significance for agricultural production. However, the effects of different tillage depths on soil nutrients content and microbial communities in tobacco-planting soils are still lacking systematic research. In this study, three different tillage depths of 15 cm (T1), 20 cm (T2), and 30 cm (T3) were set up for field experiments in Liupanshui, Guizhou Province, to explore the effects of tillage depth on tobacco-planting soil nutrients and bacterial and fungal communities based on 16S rRNA and ITS sequencing and figure out the key factors affecting soil microbial communities. The results showed that T2 and T3 increased the contents of organic matter, total nitrogen, total phosphorus, available phosphorus, and available potassium in tobacco-planting soil, and increased the diversity of bacterial communities compared with T1. There was no significant difference in the structure of bacterial and fungal communities in different tillage depth treatments, but some dominant genera were significantly enriched in T2 and T3. *Desulfobacter*, *Setophoma*, *Humicola*, and *Acremonium* were significantly enriched in T2. *Chthonomonas* and *Fusarium* were significantly enriched in T3. These genera favor the decomposition of organic matter and the cycling of nutrients, and control soil pests and diseases. Redundancy analysis indicated that TP and AK were the key factors influencing the dominant genera of bacteria and fungi. This study provides a scientific basis for the selection of soil tillage depth for tobacco production in this region.

Keywords

Tillage Depth, Tobacco, Soil Nutrients, Bacterial Community, Fungal Community

1. Introduction

Tobacco is a crucial cash crop that prioritizes both quality and yield [1]. As the foundation for plant growth, soil plays a vital role in determining the yield and quality of tobacco production. The soil chemical properties and fertility level are closely related to microbial activity, tobacco growth metabolism, and product quality [2]. As one of the crucial tobacco-producing regions in China, Guizhou Province faces various challenges in tobacco cultivation, including heavier-textured soil, shallow cultivation layer, and poor air and water permeability [3] [4] [5]. Notably, the long-term single shallow tillage pattern in Guizhou has led to increasingly prominent problems in the soil tillage structure of tobacco-planting fields, such as a significantly shallower tillage layer, soil compaction, and the subsoil thickening and upward movement of the plow layer [6] [7]. The problem of tillage structure seriously restricts the water storage and fertilizer retention capabilities of tobacco soil. As tobacco is a ridge culture crop, the depth of the tillage layer directly influences the growth of the root system, which in turn affects the yield and quality of tobacco [6] [8]. It is reported that shallow tillage is one of the important reasons for the gap between the quality of tobacco in the southwest region of China and that of high-quality tobacco abroad [8]. Therefore, the implementation of appropriate tillage practices for tobacco-planting soil is conducive to the creation of a favorable soil environment, which is of great significance for agricultural production.

Deep tillage measure increases soil porosity and reduces bulk density by breaking up the compacted soil layer and loosening the soil [9] [10]. It facilitates the entry of air and water infiltration and improves the effectiveness of soil nutrients and soil fertility [11]. Soil microorganisms, as an important part of the soil ecosystem, play an important role in the transformation and cycling of soil nutrients, as well as an important basis for the formation and sustainable development of fertility [12] [13] [14]. On the one hand, deep tillage measure makes oxygen and water enter the deep soil, which enhances the life activities of aerobic microorganisms in the soil and accelerates the oxidation and decomposition of organic matter. On the other hand, it can distribute crop residues into the deep soil, which affects microbial metabolism and the structure of microbial communities in the soil [15].

Currently, research on tillage depth focuses on tobacco yield, quality, root growth, and soil physical properties. However, there is limited information available regarding comparative studies on the soil microbial community structure under different tillage depths. Few systematic field trials have been reported. Both bacterial and fungal communities play important roles in soil nutrient cycling. Therefore, our study focuses on the effects of different tillage depths (15 cm, 20 cm, and 30 cm) on the soil nutrients and the bacterial and fungal community structure and identify the main drivers of microbial community response to tillage depths. It is of great significance to explore the influence me-

chanism of tillage depth on soil fertility and microbial community. It provides a reference basis for promoting the production of high-quality tobacco in this region and is crucial to achieve sustainable agricultural development.

2. Material and Methods

2.1. Experimental Design and Sampling

This study was conducted in Shuicheng District, Guizhou Province, China (located at 104˚58'54"E, 26˚25'43"N). This area has a subtropical monsoon climate with an annual average temperature of 12.3˚C and an annual average precipitation of 1300 mm. The soils in this study were classified as yellow-brown earth. This experiment set three tillage depth treatments: 15 cm tillage depth (T1), 20 cm tillage depth (T2), and 30 cm tillage (T3), with three replications for each treatment. 15 cm (T1) is the local conventional tillage depth. The fertilization strategy was as follows: 50 kg of basal fertilizer ($N:P_2O_5:K_2O$ = 10:10:24) per mu, and 15 kg of topdressing ($N:P_2O_5:K_2O$ = 15:0:30) per mu at rosette stage. The experiment was set up in March 2021, and soil samples were collected in July 2023 after two years of experimentation. The samples were collected during the top pruning stage. Five sampling points were selected within each plot using a five-point sampling method, and soil samples were collected from the 0 - 30 cm soil layer. The collected samples were divided into two portions. One portion was stored at 4˚C in a refrigerator for the determination of soil physicochemical properties, while the other portion was stored at −80˚C in a freezer for microbial community analysis.

2.2. Soil Chemical Properties Analysis

Soil chemical properties were analyzed with the reference to Handbook of Soil Analysis [16]. Soil and ultra-pure water were mixed at a ratio of 1:10 to determine soil pH. Soil organic matter was determined by the sulfuric acid-potassium dichromate volumetric method. Soil total phosphorus (TP) and available phosphorus (AP) were determined by the alkaline dissolution-molybdenum antimony anti-colorimetric method. The Kjeldahl method was used to determine the soil total nitrogen (TN) and the atomic absorption spectrophotometric method was used to determine soil available potassium (AK).

2.3. DNA Extraction and High-Throughput Sequencing

Total community DNA was extracted from 0.5 g fresh soil by using the Fast DNA Spin Kit for soil (MP Biomedicals, Santa Ana, CA) following the manufacturer's instructions. After purification, the extracted DNA was dissolved in 80 μL of DES buffer and stored at −20˚C before use. The concentration and quality of the DNA were evaluated using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The bacterial and fungal communities were analyzed by 16S rRNA and ITS1 amplification and sequencing. The amplified products were sequenced using the Illumina Novaseq platform at

Novagene Bioinformatics Technology Co. Ltd. (Beijing, China). To cluster the obtained sequences into operational taxonomic units (OTUs), the Uparse software (version 7.0.1001) was utilized, with a similarity level of 97%. The resulting OTU clustering provided information on corresponding species annotations and their distribution in terms of abundance.

2.4. Statistical Analysis

Statistical analysis of soil chemical properties and diversity indices was performed using Origin 2021 software, and differences between treatments were compared using one-way analysis of variance (ANOVA). Fisher-LSD method was used to test the significance of differences between treatments. Bacterial and fungal community structures were analyzed by PCoA analysis based on the Bray-Curtis distance. Differences for significance were tested by using Adonis test in the "Vegan" package in R. The relative abundance of bacterial and fungal dominant genera was analyzed using the "pheatmap" package in R, and T-test was performed to identify species with significant differences in abundance ($P < 0.05$). Redundancy analysis (RDA) was performed using the "vegan" package in R to clarify the relationship between dominant genera and soil nutrients. Variance Partitioning Analysis (VPA) was performed using the "rdacca.hp" package in R to reveal the importance of a single property [17].

3. Results and Discussion

3.1. Effects of Tillage Depth on Tobacco-Planting Soil Chemical Properties

The effects of tillage depth on soil chemical properties are shown in **Figure 1**. Tillage depth had a minimal impact on soil pH while having a significant influence on soil nutrients. Soil organic matter is an important nutrient source for tobacco-growing soil [18]. The deep-tilled soils T2 and T3 had a significant increase in organic matter compared to T1, with increments of 28% and 32.26% respectively ($P < 0.05$) (**Figure 1(b)**).

Nitrogen, phosphorus, and potassium are crucial indicators for evaluating soil fertility, as their content directly affects tobacco growth, yield and quality [19]. The nitrogen level in tobacco-planting soil is closely related to the synthesis of nitrogen-containing compounds, such as nicotine [20]. As a potassium-loving crop, tobacco quality depends on the available potassium content in the soil [21]. It was shown that the total nitrogen and total phosphorus were significantly affected by tillage depth ($P < 0.05$). Compared to T1, there was an increase of 7.70% and 11.49% in total nitrogen content in T2 and T3 respectively (**Figure 1(c)**). Additionally, the total phosphorus content showed increments of 24.30% and 13.20% in T2 and T3 respectively (**Figure 1(d)**). The available phosphorus and potassium content in T2 and T3 increased slightly but the difference was not significant (**Figure 1(e)** & **Figure 1(f)**).

This study showed that deep tillage of 20 cm (T2) and 30 cm (T3) was effective

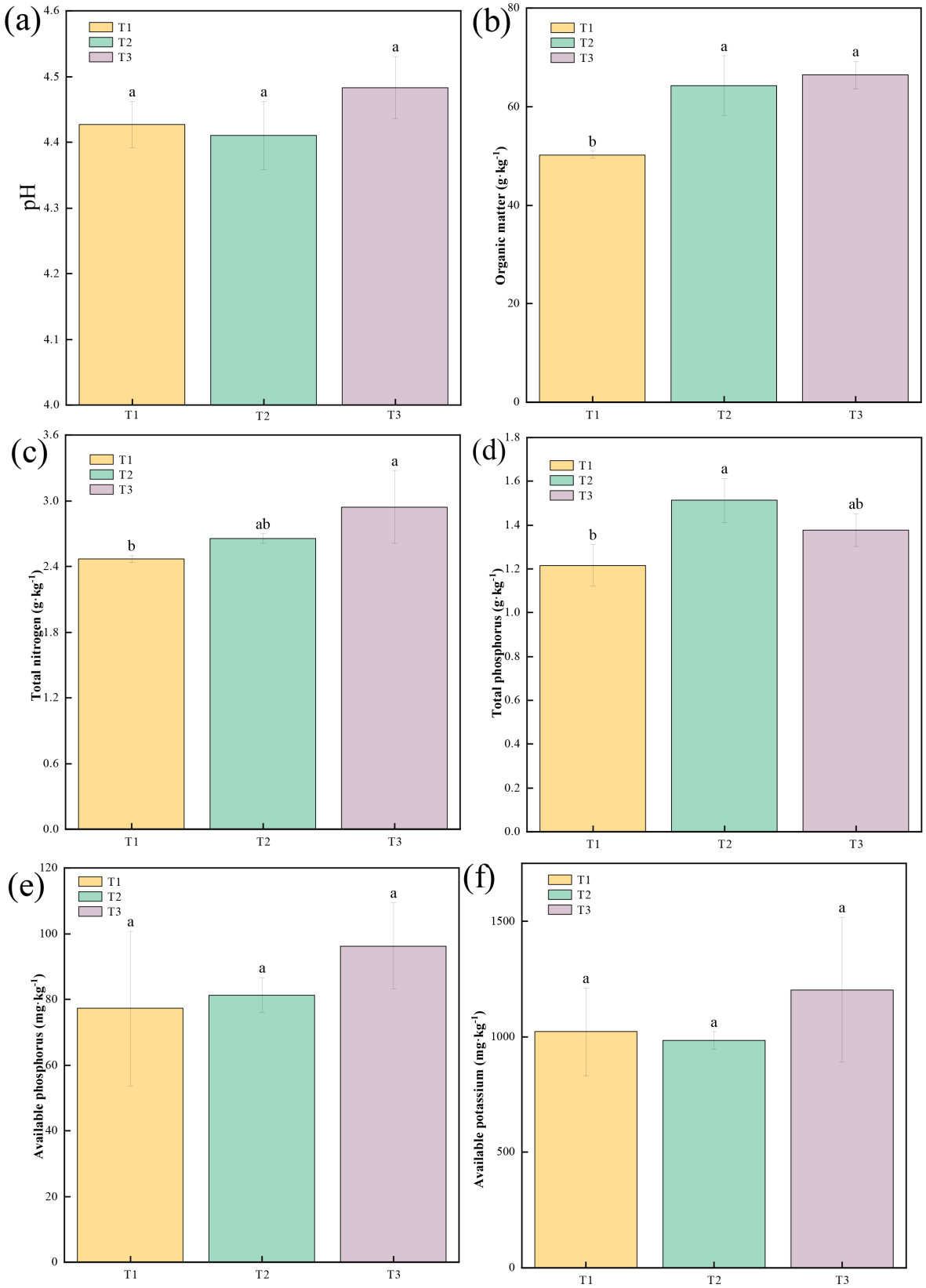

Figure 1. Soil physiochemical properties with different tillage depths. Different letters represent the significant effects of deep tillage on soil chemical properties at $P < 0.05$.

in increasing the content of soil organic matter, total nitrogen, total phosphorus, available phosphorus, and available potassium compared to local conventional tillage depths (T1). Among them, deep tillage significantly affected the contents of organic matter and total nitrogen and total phosphorus in soil ($P < 0.05$). It could be explained that deep tillage can bring the straw stubble on the soil surface into the deep soil and promote the entry of oxygen and water into the deep soil [22] [23]. It is beneficial to stimulate the activity of soil microorganisms and promote the humification and mineralization of soil organic matter [24].

3.2. Effect of Tillage Depth on Tobacco-Planting Soil Bacterial and Fungal Diversity and Structure

The alpha diversity of soil bacterial and fungal community under different tillage depths are shown in **Figure 2**. The results indicated that deep tillage (T2 and T3) significantly increased the richness and diversity of the bacterial community but had no significant effect on the fungal community. It showed that bacterial communities were more sensitive to tillage depth than fungal communities, which may be due to the dominance of bacteria in soils driving material decomposition and nutrient cycling [15] [25]. To figure out the effect of tillage depth on the structure of bacterial and fungal communities, PCoA analysis of bacterial and fungal communities was performed based on the Bray-Curtis distance. Adonis test was used to assess the significance of the differences in community structure. It was shown that there were no significant differences in the structure of soil bacterial and fungal communities among different tillage depths ($P > 0.05$).

3.3. Effect of Tillage Depth on Tobacco-Planting Soil Bacterial and Fungal Communities Composition

The composition of soil bacterial community under different tillage depths is shown in **Figure 3**. The dominant bacterial phyla are Chloroflexi (29.46% - 31.65%), Actinobacteriota (21.51% - 24.57%), Proteobacteria (14.64% - 16.00%), and Acidobacteriota (12.07% - 12.99%). At the genus level, *Acidothermus* (3.78% - 3.92%), *Bryobacter* (2.22% - 2.63%), and *JG30a-KF-32* (2.04% - 2.79%) were the dominant bacterial genera with relative abundances (RA) > 0.02.

To further explore the difference at the genus level, clustering analysis was performed on the relative abundance of the top 35 ranked genera. The results indicated that there were differences in the abundance of dominant bacterial genera in the soil under different tillage depths. *Acidothermus* and *Bryobacter* had higher abundance in T3 compared to T1 and T2. *Acidothermus* is a genus that is related to the decomposition of organic matter [26]. Previous studies have reported that *Bryobacter* can effectively promote soil carbon cycling and is closely related to soil enzyme activity [26]. By performing T-test on the differing species, the results showed that there were no significant differences observed among the three treatments at the phylum level. At the genus level, *Desulfobacter*

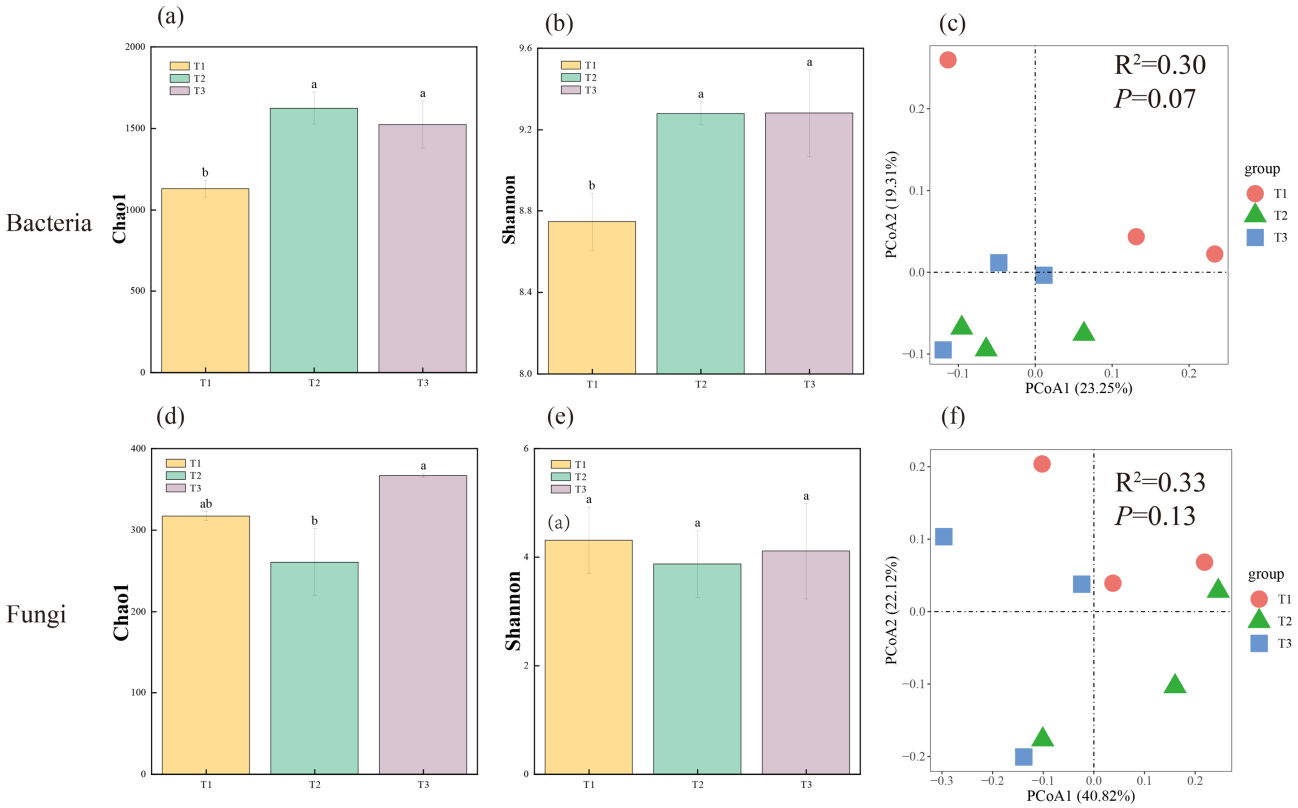

Figure 2. Bacterial and fungal community diversity and structure under different tillage depths. The R^2 in the Adonis analysis indicates the degree of explanation of the sample variance by different groups. The larger R^2 indicates that the grouping explains more of the variance. $P < 0.05$ is statistically significant.

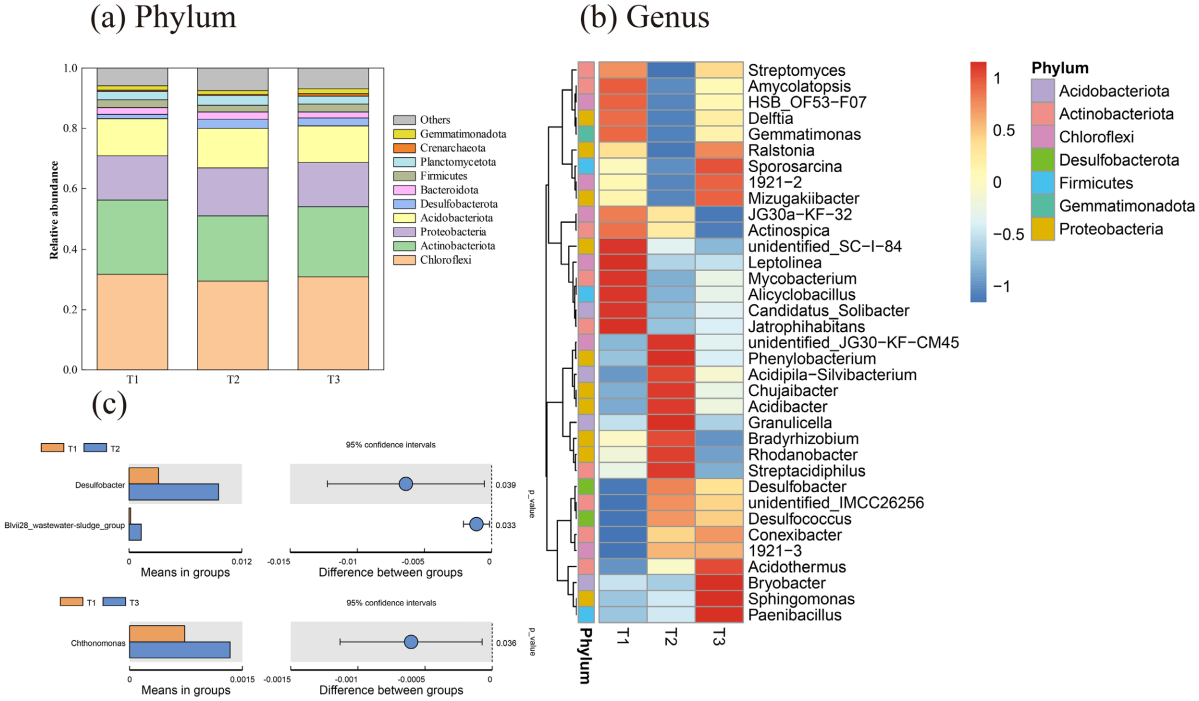

Figure 3. The composition of bacterial community at the phylum (a) and genus level (top35) (b) and the different species between different tillage depths at the genus level (c).

and *Blvii28_wasrewater_sludge_group* were significantly enriched in T2, while the genus *Chthonomonas* was significantly enriched in T3 ($P < 0.05$). *Desulfobacter* was identified as a nitrogen-fixing sulfate-reducing bacterium (SRBs) [27]. Shen [28] found that *Chthonomonas* has a high abundance in the disease-suppressive soil, this may be indicated that this genus could suppress a variety of pathogens. However, there is limited physiological data available and its ecological role in soil remains unclear [29].

The composition of soil fungal community is shown in **Figure 4**. Ascomycota (64.63% - 69.86%) was the dominant phylum in all treatments, followed by Basidiomycota (15.16% - 20.49%). There were no significant differences observed among the three treatments at the phylum level. *Saitozyma* (10.08% - 15.04%), *Fusarium* (8.01% - 22.89%), and *Penicillium* (3.02% - 31.14%) were the dominant fungal genera (RA > 0.02). There were significant differences observed in the dominant fungal genera.

T-test revealed that *Setophoma*, *Humicola*, and *Acremonium* were significantly enriched in T2, while *Fusarium* was significantly enriched in T3. *Setophoma* has been reported that have a better cellulase production capacity [30]. *Humicola* was found to possess the ability to break down complex natural substrates and is an important source of enzymes such as cellulase and xylanase. It also produces structurally diverse natural compounds that have significant bioactivities such as antibacterial and insecticidal effects [31]. Meanwhile, it was also observed that

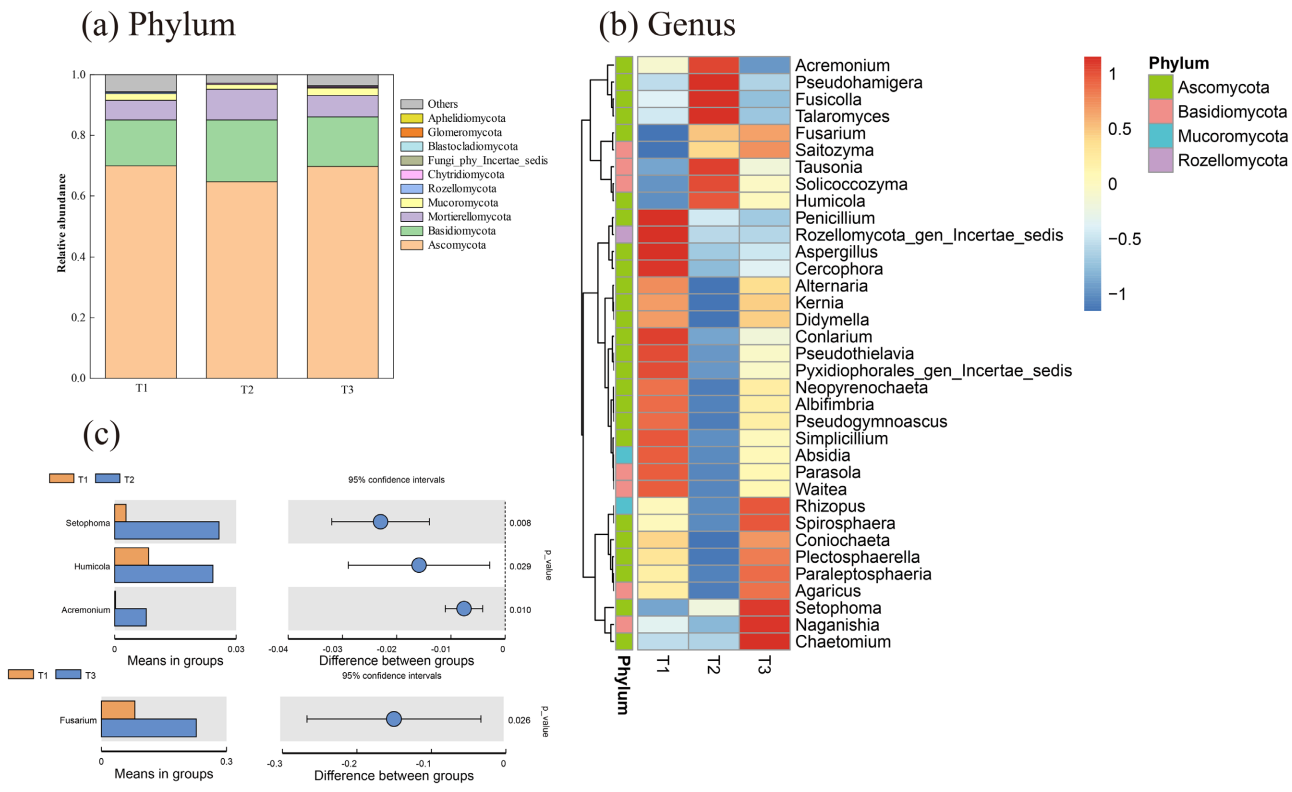

Figure 4. The composition of fungal community at the phylum (a) and genus level (top 35) (b) and the different species between different tillage depths at the genus level (c).

Chaetomium, *Talaromyces*, and *Rhizopus* had higher abundances in T2 and T3. *Fusarium*, *Talaromyces*, and *Rhizopus* have been reported to have the phosphate-solubilizing capacity [32]. Wang [33] reported that healthy soil has higher abundances of beneficial microbes (*Acremonium*, *Chaetomium*), which can improve soil nutrients, promote plant growth, and control soil-borne diseases [34]. *Acremonium* and *Chaetomium* are potential for bio-control of plant disease via the production of lytic enzymes and antimicrobial metabolites to protect plants from pathogens infection [35] [36]. These results showed that deep tillage can increase the abundance of genera that are beneficial in maintaining soil health and mitigating the occurrence of diseases [6].

3.4. Relationship between Soil Nutrients and Dominant Bacterial and Fungal Genera

Relationships between soil nutrients and dominant bacterial and fungal genera (top 10) were obtained by Redundancy analysis. The results showed that soil nutrients (OM, TN, TP, AP, AK) could explain 69.9% and 68.5% of the variation in dominant bacterial and fungal genera, respectively. In bacteria, the first two axes account for 48.1%, while the first two axes 49.8% in fungi (**Figure 5**). TP is the key chemical property influencing the soil bacterial and fungal genera (15.9%, $P = 0.211$; 21.5%, $P = 0.027$), followed by AK (15.1%, $P = 0.261$; 18.4%, $P = 0.113$) (**Figure 6**). *Acidothermus*, *Conexibacter*, and *Bryobacter* were positively correlated with TN and OM, which may indicate that these genera are important players in the metabolism of organic matter in the soil. Some studies have confirmed this conclusion [26] [37].

TP and AK showed an increasing trend with increasing tillage depth (**Figure 1**), and most of the bacterial and fungal dominant genera were positively correlated

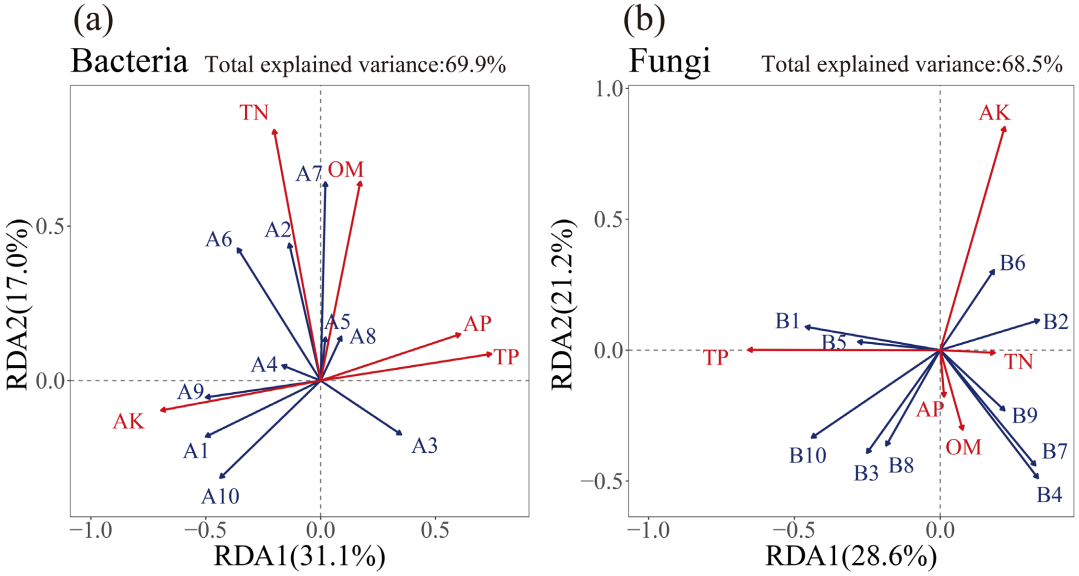

Figure 5. Redundancy analysis (RDA) between soil nutrients and dominant bacterial (a) and fungal (b) genera (top 10).

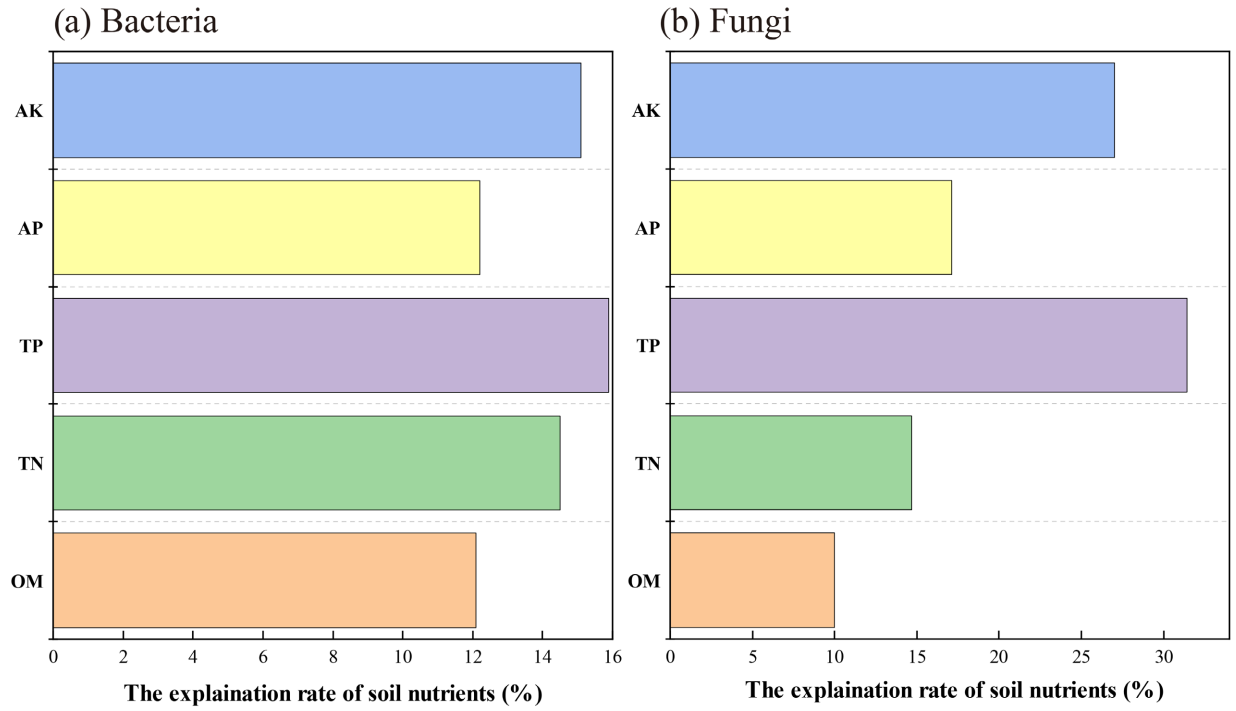

Figure 6. Explanation rates of soil nutrients in dominant bacterial (a) and fungal (b) genera.

with TP and AK. The distribution and operation of phosphorus play an important role in the metabolism of tobacco and the transfer of growth centers, and the level of phosphorus content in the soil directly affects the yield and quality of tobacco [38]. Tobacco is a typical potassium-loving crop with high potassium demand, and the potassium content of tobacco leaves is one of the most important indicators for evaluating the quality of roasted tobacco [21]. These results indicated that deep tillage affects the abundance of dominant bacterial and fungal genera in the tobacco-planting soil through changes in soil nutrients, which play an important role in soil nutrient cycling, setting the stage for the eventual development of higher tobacco yields and quality.

3.5. Effects of Different Tillage Depths on the Quality and Economic Value of Tobacco

According to **Table 1**, 30 cm deep tillage increased the average price of flue-cured tobacco, the proportion of superior tobacco, and the proportion of middle and superior tobacco. This experiment showed that in flue-cured tobacco cultivation, 30 cm tillage depth was the most effective in improving the economic traits of flue-cured tobacco. Compared with T1, the average price of T3 increased by 8.0%, the proportion of superior tobacco increased by 20.9%, and the proportion of middle and superior tobacco increased by 10.0%.

4. Conclusion

Deep tillage (20 - 30 cm) (T2 and T3) significantly increased the content of

Table 1. Effects of different plowing depths on the output value of tobacco.

Treatment	Yield ($kg \cdot hm^{-2}$)	Average price ($RMB \cdot kg^{-1}$)	Economic value ($RMB \cdot hm^{-2}$)	Proportion of superior tobacco (%)	Proportion of middle and superior tobacco (%)
T1	2551.12	28.42	72494.30	56.48	88.90
T2	2544.08	26.39	67136.13	50.15	83.86
T3	2092.20	30.69	64202.25	68.28	97.76

organic matter, total nitrogen, total phosphorus, available phosphorus, and available potassium in the tobacco-planting soil, and increased the diversity of bacterial communities, but had no significant effect on the diversity of fungal communities. Deep tillage treatment did not significantly affect the structure of bacterial and fungal communities, but the abundance of some dominant genera changed. Dominant genera involved in soil nutrient cycling and material metabolism have a higher abundance in deep tillage treatments. *Desulfobacter*, *Setophoma*, *Humicola*, and *Acremonium* were significantly enriched in T2. *Chthonomonas* and *Fusarium* were significantly enriched in T3. RDA analysis showed that TP and AK were the key factors affecting the dominant genera of bacteria and fungi. Therefore, the tillage depth of 20 cm and 30 cm is conducive to improving soil nutrients and influencing soil microbial communities, which lays a good foundation for improving tobacco quality and economic traits.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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