

Study on the influence of *Amorphophallus muelleri* and flue-cured tobacco rotation on the soil microbial community in Southwest China

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Abstract

The continuous cropping of tobacco soil is an obstacle in current flue-cured tobacco production. To explore the effects of the different planting patterns on soil nutrients and microbial community structure, soil samples from the *Amorphophallus muelleri* continuous cropping (MMT1), *A. muelleri* + flue-cured tobacco rotation (MKT2) and flue-cured tobacco continuous cropping (KKT3) systems were collected at the same experimental site. The characteristics and relationships between the soil microbial community and soil nutrients under the different planting patterns were analyzed via Illumina MiSeq high-throughput sequencing. The results showed that the soil organic matter content, hydrolyzable nitrogen content and pH value in the *A. muelleri* + flue-cured tobacco rotation treatment were significantly higher than those in the other two treatments. The composition of the soil microbial communities under the different planting patterns was similar at the phylum level, but the relative abundances of Proteobacteria, Mortierellomycota and Basidiomycota in the *A. muelleri* + flue-cured tobacco rotation treatment were 0.31, 0.56 and 0.39 times higher than those in *A. muelleri* continuous cropping and flue-cured tobacco continuous cropping, respectively. The abundance of Ascomycota in the flue-cured tobacco continuous cropping treatment was 0.02 times higher than that in the *A. muelleri* continuous cropping of *A. muelleri* + flue-cured tobacco ($P < 0.05$). At the genus level, the abundances of *Sphingomonas*, *Arthrobacter*, *Bacillus* and *Mortierella* in the *A. muelleri* + flue-cured tobacco rotation treatment were 0.84, 0.006, 0.36 and 0.07 times higher than those in the *A. muelleri* continuous cropping, and 0.78, 1.41 and 0.24 times higher than those in the flue-cured tobacco continuous cropping treatment. The dominant bacteria and fungi were significantly correlated with the soil nutrients. From the perspective of microbial function, the bacterial KEGG enrichment pathway of the tested soil samples was mainly the metabolism pathway. The energy metabolism, carbohydrate and amino acid gene abundances of the soil bacterial community after *A. muelleri* + flue-cured tobacco rotation were higher than those after *A. muelleri* continuous cropping and flue-cured tobacco continuous cropping. The rotation planting pattern plays a positive role in regulating soil nutrients, improving the microbial community structure and increasing the functional metabolism of beneficial bacteria. The results of this study can provide a reference for the production of high-quality flue-cured tobacco.

Introduction

The continuous cropping obstacle refers to the problems of slow plant growth, frequent pests and diseases, and decreased yield and quality after continuous planting of the same crop or near-source crop on the same land; these problems are frequent in Solanaceae plants, melons, beans and roots¹. Flue-cured tobacco is an important economic crop in Southwest China and is constantly being developed and utilized in food, medicine and other industries². Due to the mountainous areas and limited arable land in Southwest China, the duration of continuous cropping of flue-cured tobacco has been increasing in recent years, and the expansion of continuous cropping areas has led to the worsening of continuous cropping obstacles in cultivated land and the decline of flue-cured tobacco yield and quality, which seriously restricts the development of local sustainable agriculture³. Rotation is a method for alleviating continuous cropping obstacles and improving economic benefits⁴. Studies have shown that the rotation of legumes and potatoes can increase soil enzyme activity; increase soil total N, P, K and organic matter; and improve soil quality⁵. After the rotation of *Polygonatum sibiricum* and *Panax notoginseng*, the abundance of pathogenic fungi in the soil decreased, and the proportion of pathogenic bacteria decreased significantly, which alleviated the continuous cropping obstacle of *Panax notoginseng* to a certain extent⁶. The total nitrogen and available nitrogen contents in soil increase significantly after rice and milk vetch rotation, which significantly improves the comprehensive fertility and soil microecological environment of paddy soil⁷.

Flue-cured tobacco is a kind of Solanaceae plant that is not resistant to continuous cropping. Its roots secrete a large number of secondary metabolites⁸, which accumulate in the soil for a long time and cause toxic effects on plants⁹. Allelopathic autotoxic substances are secreted into the soil mainly through the root system. These secretions contain a large number of allelopathic active substances that have a greater impact on plants¹⁰. *A. muelleri* is a general term for *Amorphophallus* with bulbils on the leaf surface and has the characteristics of strong disease resistance and high yield¹¹. Konjac bulbs contain a large amount of alkaloids. Alkaloids have strong antibacterial and bactericidal effects on a variety of plant pathogens and can also act on harmful insects^{12,13}; moreover, the Konjac bulb is rich in nutrients and is a good nutrient source for various microorganisms in the soil¹⁴. Crop growth is closely related to soil microorganisms and nutrients. Reasonable rotation of different crops can fully leverage soil fertility, reduce the accumulation of allelochemicals, and improve soil yield and quality. There are many studies on the continuous cropping of flue-cured tobacco¹⁵, cultivation management¹⁶ and key technologies for improving the quality of flue-cured tobacco¹⁷. However, there are few reports on the mechanism of tobacco and *A. muelleri* rotation on soil nutrients and microbial community composition. In this paper, flue-cured tobacco and *A. muelleri* were used as research materials to study the soil microbial composition and soil nutrient changes in flue-cured tobacco/Konjac root systems under different rotation modes to provide new ideas and methods for flue-cured tobacco + *A. muelleri* rotation cultivation.

Materials and methods

Experimental location

The test site is located in Daxichong village, Hongxi town, Mile city, Honghe Prefecture, 103°N, 24°E. The soil pH at the test site is approximately 6.0, and the soil texture is loamy. The tested flue-cured tobacco variety was the local main variety 'Yunyan 87', and *A. muelleri* variety was 'Zhuyajin No.1'.

Experiment setting

For each planting pattern, 3 replicates were established, for a total of 6 treatments, a plot area of 333.3 m², and a random block arrangement. The detailed rotation methods are shown in Table 1-1.

Table 1-1 Different rotation mode processing

Dispose	First year	Ensuing year
MMT1	<i>A. muelleri</i>	<i>A. muelleri</i>
MKT2	<i>A. muelleri</i>	Flue-cured tobacco
KKT3	Flue-cured tobacco	Flue-cured tobacco

Determination of soil physical and chemical properties

The contents of soil organic matter, available nitrogen, available phosphorus and available potassium, and pH were determined according to the methods of Cheng¹⁸.

DNA extraction and PCR amplification of soil fungi and bacteria

Soil fungal DNA was extracted with a PowerSoil DNA DNA Soil Genomic DNA Kit, soil bacterial DNA was extracted with a Fast DNA SPIN Kit (MP, USA), and DNA quality was detected via 1% agarose gel electrophoresis. The ITS1 PCR amplification primers used were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-TGCCTTCTTCATCGATGC-3')¹⁹. The amplification mixture was 20 µL, and the amplification procedure was predenaturation at 95 °C for 3 min, 95 °C for 3 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and extension at 72 °C for 10 min for a total of 36 cycles. The primers used for PCR amplification of the 16rDNA (V3-V4 region) gene were 338F (5'-ACTCCTACGGGAGGAGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3')²⁰; the amplification mixture was 20 µL, and the amplification procedure involved predenaturation at 95 °C for 5 min and 95 °C for 30 s; annealing at 58 °C for 30 s; and 35 cycles of extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The amplification products were subsequently sent to a sequencing company for Illumina MiSeq high-throughput sequencing (Majorbio).

Data evaluation and analysis

The obtained sequence was spliced by FLASH software to obtain the original sequence number, and Usearch (version 7.0) software was used to filter the original sequence number to obtain the effective sequence. Mothur (version v.1.30.1) software was used to plot the sample dilution curve to determine the presence of microbial communities in the sample. The coverage, Shannon, Simpson and Chao1 indices of the library were calculated to determine the richness and diversity of microorganisms in the soil samples from the different planting patterns. Source tracker software was used to visualize the dominant species in the samples. PcoA principal coordinate analysis was performed using QIIME software (version 1.7.0). PICRUST2 (2.2.0) software was used to predict bacterial function. All data were analyzed and processed using the IBM SPSS statistical software (2016) and Microsoft Office Excel (2019).

Results and analysis

Differences in soil nutrient content under different treatments

The soil nutrient contents in the different treatment groups are shown in Fig. 1-1. There were significant differences in soil pH, organic matter, Hydrolyzable nitrogen and available potassium among the three treatments. The soil pH and organic matter content in the MMT1 and MKT2 treatment groups were 0.17 and 0.15 times higher, respectively, than those in the KKT3 treatment group. 0.25 times, 0.25 times. The content of Hydrolyzable nitrogen was the highest in the MKT2 treatment group, at 155.74 mg/kg, which was 0.03 times and 0.06 times higher than that in the MMT1 and KKT3 treatment groups, respectively. The available potassium content in the KKT3 treatment group was the highest, at 264.25 mg/kg, which was 0.41 and 0.42 times higher than that in the MMT1 and MKT2 treatment groups, respectively.

Analysis of the OTU abundance and alpha diversity of soil bacteria and fungi under different treatments

The dilution curves of bacteria and fungi based on the Shannon index under different treatments are shown in Fig. 1-2. With increasing sequencing number, the dilution curves of bacteria and fungi tended to be gentler, indicating that the sequencing reads were long enough to support subsequent data analysis. The differences in the soil bacterial and fungal community structures under the three planting patterns were explored. PCoA (Adonis, R2=0.8335, P=0.0050) and (Adonis, R2=0.8425, P=0.0050) revealed significant differences in the soil bacterial and fungal communities among the three treatments, indicating that the different planting patterns had significant effects on the soil microbial community composition (Fig. 1-3).

The analysis of the alpha diversity of the soil bacterial and fungal communities in the different treatment groups is shown in Table 1-2. The coverage of the bacterial and fungal communities exceeded 99%, which can effectively reflect the diversity of bacteria and fungi in the samples. The higher the Shannon index and the lower the Simpson index were, the higher the community richness was. The Chao1 index represents the predicted OTU number, and the Ace index represents the actual observed OTU number. The bacteria exhibited significant differences in Simpson and Ace indices among the three treatments. The Simpson index was the lowest in the KKT3 treatment (0.00), and the Ace index was the highest in the MMT1 treatment (4032.85) (P<0.05). Overall, these findings indicated that the richness of the bacterial community in the soil was higher in the flue-cured tobacco continuous cropping treatment (KKT3).

The Shannon, Simpson and Ace indices of the three treatments were significantly different. The Shannon index (4.55) and Ace index (618.71) were the highest in the MKT2 treatment group, and the Simpson index in the MMT1 treatment was the lowest (0.03), indicating that the soil fungal community richness was the highest in the *A. muelleri*+flue-cured tobacco rotation treatment (MKT2).

Table 1-2 Differences in Alpha diversity of soil bacterial and fungal community composition under different treatments

Bacterial	/		MMT1	MKT2	KKT3
		Number of original sequences	81038.00±7720.51a	77805.67±5858.34a	74430.33±2762.45a
Alpha diversity index	Shannon		6.49±0.02a	6.52±0.07a	6.49±0.01a
	Simpson		0.01±0.00a	0.01±0.01a	0.00±0.00b
Evenness index	Ace		4032.85±216.68a	3754.64±13.93b	3406.22±29.36c
	Chao1		3925.47±173.27a	3715.52±12.87b	3381.92±39.09c
		Coverage(%)	0.99±0.00a	0.99±0.00a	0.99±0.00a
Fungi		Number of original sequences	104866.00±8336.65a	88355.3369±2752.61b	87269.00±2831.37b
	Alpha diversity index	Shannon	4.46±0.07a	4.55±0.18a	3.96±0.07b
		Simpson	0.03±0.00b	0.03±0.01b	0.05±0.01a
	Evenness index	Ace	537.96±12.87b	618.71±23.06a	308.87±3.84c
		Chao1	540.43±14.18b	621.30±19.36a	309.29±4.39c
		Coverage(%)	1.00±0.00a	1.00±0.00a	1.00±0.00a

Note: The data in the table are mean±standard deviation, and different lowercase letters indicate P<0.05.

The number of OTUs in the different treatment groups is shown in Figs. 1-4. A total of 14360 bacterial OTUs were detected in the three treatment groups, and the total number of bacterial OTUs was 2538. There were 692 bacterial OTUs shared by the MMT1 and MKT2 treatments, 462 bacterial OTUs shared by the MMT1 and KKT3 treatments, and 694 bacterial OTUs shared by the MKT2 and KKT3 treatments. The number of OTUs of specific bacteria in the three treatments was the highest in the MMT1 treatment (1404) and the lowest in the KKT3 treatment (741). Overall, these findings indicated that the bacterial species compositions of the *Amorphophallus muelleri*+flue-cured tobacco rotation treatment (MKT2) and flue-cured tobacco continuous cropping treatment (KKT3) were similar. Although the bacterial community richness in the flue-cured tobacco continuous cropping treatment (KKT3) was high, the abundance of endemic bacterial species was low.

A total of 2469 fungal OTUs were detected in the three treatment groups, and the total number of fungal OTUs was 199. There were 197 fungal OTUs shared by the MMT1 and MKT2 treatments, 58 fungal OTUs shared by the MMT1 and KKT3 treatments, and 100 fungal OTUs shared by the MKT2 and KKT3 treatments. The number of unique fungal OTUs in the three treatments was the highest in the MKT2 treatment (497) and the lowest in the KKT3 treatment (213). The results showed that the fungal species compositions of the *A. muelleri* continuous cropping treatment (MMT1) and *A. muelleri*+flue-cured tobacco rotation treatment (MKT2) were similar, and the fungal community richness and endemic species richness were higher in the *A. muelleri*+flue-cured tobacco cropping treatment (MKT2).

Differences in the soil bacterial and fungal species community structures under the different treatments

The composition of the soil bacterial community under the different planting patterns is shown in Fig. 2-1. Among the bacterial phyla with relative abundances in the top 10, Actinobacteria, Proteobacteria, Chloroflexi and Acidobacteriota were the dominant bacterial phyla. The abundances of Actinobacteriota and Chloroflexi were the highest in the MMT1 treatment group (27.12% and 16.36%, respectively), which were 0.08 times and 0.26 times higher than those in the MKT2 treatment. These values were 0.18 and 0.16 times higher than those in the KKT3 treatment, respectively. The abundance of Proteobacteria in the MKT2 treatment was the highest (28.88%), which was 0.31 times higher than that in the MMT1 treatment. This value was 0.22 times higher than that in the KKT3 treatment group. The abundance of Acidobacteriota in the KKT3 treatment was the highest (16.51%), which was 0.19 times higher than that in the MMT1 treatment. This value was 0.38 times higher than that of the MKT2 treatment (P<0.05).

The dominant bacterial genera with relative abundances in the top 10 at the genus level were *norank_f__norank_o__Vicinamibacterales*, *Sphingomonas*, *norank_f__Gemmatimonadaceae*, *norank_f__norank_o__Gaiellales*, *Intrasporangium*, *norank_f__Roseiflexaceae*, *Arthrobacter*, *norank_f__Vicinamibacteraceae*, and *Bacillus*, *norank_f__JG30-KF-CM45*. The abundances of *Intrasporangium*, *norank_f__Vicinamibacteraceae* and *norank_f__JG30-KF-CM45* treated with MMT1 were the highest, at 4.83%, 3.34% and 2.96%, respectively. These values were 1.17, 0.84 and 0.48 times higher than those of the MKT2 treatment group. Compared with those in the KKT3 treatment group, these values increased 2.16-fold and 0.21-fold, respectively. The abundances of *Sphingomonas*, *Arthrobacter* and *Bacillus* were highest in the MKT2 treatment (6.15%, 3.38% and 2.94%, respectively). Compared with those in the MMT1 treatment group, these values increased by 0.84 times, 0.006 times and 0.36 times, respectively. Compared with those in the KKT3 treatment, the abundances of *Sphingomonas*, *Arthrobacter* and *Bacillus* increased by 0.78 times, 1.41 times and 0.24 times, respectively. The abundances of *norank_f__norank_o__Vicinamibacterales*, *norank_f__Gemmatimonadaceae*, *norank_f__norank_o__Gaiellales* and *norank_f__Roseiflexaceae* were the highest in the KKT3 treatment, at 4.96%, 4.32%, 4.19% and 3.14%, respectively. Compared with those in the MMT1 treatment, the values were 0.12, 0.69, 0.84, and 0.08 times higher; compared with those in the MKT2 treatment, the values were 0.32, 0.35, 0.24 and 0.42 times higher respectively (P<0.05).

The dominant bacteria with a relative abundance in the top 10 at the species level were *uncultured_Sphingomonadaceae_bacterium_g__Sphingomonas*, *unclassified_g__norank_f__norank_o__Vicinamibacterales*, *uncultured_bacterium_g__Intrasporangium*, *unclassified_g__Arthrobacter*, *uncultured_bacterium_g__norank_f__Roseiflexaceae*, *unclassified_g__norank_f__norank_o__Gaiellales*, *unclassified_g__norank_f__Vicinamibacter*, *Bacillus_sp._X20141*, and *unclassified_Chuiibacter_unclassified*.

The abundances of *Intrasporangium*_ sp. and *Vicinamibacteraceae*_ sp. were the highest in the MMT1 treatment, at 4.83% and 1.86%, respectively. These values were 1.17 and 1.19 times higher than those in the MKT2 treatment group. Compared with those in the KKT3 treatment, these values increased 2.16-fold and 0.46-fold, respectively. The *Sphingomonas*_ *Sphingomonadaceae*, *Arthrobacter*_ sp., *Gaiellales*_ sp. and *Bacillus*_sp._X12014 treated with MKT2 had the highest abundances, 5.71%, 3.38%, 1.93% and 1.73%, respectively. Compared with those in the MMT1 treatment, the values were 0.94, 0.006, 1.72, and 0.70 times higher; compared with those in the KKT3 treatment, the values were 0.74, 1.41, 0.18 and 0.48 times higher, respectively. The highest abundances of *Vicinamibacterales*_ sp., *Roseiflexaceae*_ sp., *Chujaibacter*_ sp., and *Gemmatimonadaceae*_ *Gemmatimonadales* were observed in the KKT3 treatment group at 3.72%, 2.40%, 2.43% and 1.57%, respectively. Compared with those in the MMT1 treatment, the abundance values were 0.06, 0.38, 12.5, and 0.54 times higher; compared with those in the MKT2 treatment, the values were 0.49, 0.62, 0.90 and 0.35 times higher, respectively ($P < 0.05$).

The composition of the soil fungal community under the different planting patterns is shown in Fig. 2-2. Among the fungal phyla with relative abundances in the Top 10, Ascomycota, Mortierellomycota, Basidiomycota, Chytridiomycota, unclassified_k_Fungi and Rozellomycota were the dominant phyla. The abundance of Chytridiomycota was the highest in the MMT1 treatment group (5.22%). This value was 0.97 times higher than that of the MKT2 treatment. This value was 0.24 times higher than that of the KKT3 treatment. Similarly, the abundances of Mortierellomycota, Basidiomycota, unclassified_k_Fungi and Rozellomycota were the highest in the MKT2 treatment group, at 14.03%, 7.13%, 4.76% and 3.56%, respectively. Compared with those in the MMT1 treatment group, the values were 0.56, 0.39, 1.25, and 355 times higher; compared with those in the KKT3 treatment, the values were 0.66, 0.77, 2.38 and 4.39 times greater, respectively. The abundance of Ascomycota was highest in the KKT3 treatment group (80.12%). This value was 0.02 times higher than that in the MMT1 treatment group. This value was 0.2 times greater than that of the MKT2 treatment ($P < 0.05$).

The dominant fungal genera with relative abundances in the Top 10 at the genus level were *Penicillium*, *Mortierella*, *Fusarium*, *Aspergillus*, *Purpureocillium*, *Chaetomium*, *unclassified_k_Fungi*, *Acremonium*, and *unclassified_f_Chaetomiaceae*. The abundances of *Aspergillus*, *Chaetomium* and *Acremonium* were highest in the MMT1 treatment group, at 12.01%, 6.10% and 7.14%, respectively. Compared with those in the MKT2 treatment group, these values increased by 18.69 times, 2.72 times and 17.31 times, respectively. Compared with those in the KKT3 treatment, the abundances of *Aspergillus*, *Chaetomium* and *Acremonium* increased by 7.52 times, 0.44 times and 53.92 times, respectively. The abundances of *Mortierella*, *Fusarium*, *Purpureocillium* and *unclassified_k_Fungi* were the highest in the MKT2 treatment group (9.61%, 6.54%, 10.47% and 4.76%, respectively). Compared with those in the MMT1 treatment, the values in the MKT2 treatment increased by 0.07 times, 0.69%, 129.88 times, and 1.25 times; 0.17 times, 0.11 times, 6.32 times and 2.38 times, respectively, compared with those in the KKT3 treatment group. The abundances of *Penicillium* and *unclassified_f_Chaetomiaceae* were highest in the KKT3 treatment group, at 29.49% and 3.39%, respectively. Compared with those in the MMT1 treatment group, these parameters increased 4.21-fold and 0.04-fold; these values were 2.37-fold and 4.65-fold higher, respectively, than those in the MKT2 treatment group ($P < 0.05$).

The dominant fungi with relative abundances in the top 10 at the species level were *unclassified_g_Penicillium*, *Mortierella_elongata*, *unclassified_g_Fusarium*, *unclassified_g_AspERGILLUS*, *Penicillium_simplicissimum*, *unclassified_k_Fungi*, *Mortierella_alpina*, *unclassified_f_Chaetomiaceae*, *Purpureocillium_lilacinum*, and *Neocosmospora_rubicola*. The abundances of *Aspergillus* sp. and *Mortierella_alpina* were highest in the MMT1 treatment group, at 10.95% and 4.68%, respectively. Compared with those in the MKT2 treatment group, these values increased 59.83-fold and 2.66-fold, respectively. The abundances of *Aspergillus* sp. and *Mortierella_alpina* were 13.22 and 1.26 times higher than those in the KKT3 treatment group. The abundances of *Mortierella_elongata*, *Fusarium* sp., *Fungi* sp. and *Purpureocillium_lilacinum* were the highest in the MKT2 treatment group (7.07%, 5.65%, 4.76% and 6.03%, respectively). Compared with those in the MMT1 treatment, the values were 0.69, 0.67, 1.25, and 75.38 times higher; compared with those in the KKT3 treatment group, the values were 0.69, 0.07, 2.38 and 5.41 times higher, respectively. The abundances of *Penicillium* sp. and *Penicillium_simplicissimum* were the highest in the KKT3 treatment group, at 19.83% and 9.06%, respectively. Compared with those in the MMT1 treatment group, these values increased 6.72-fold and 225.5-fold; compared with those in the MKT2 treatment group, these values increased 1.62-fold and 8.15-fold ($P < 0.05$).

Correlation analysis of soil bacteria, fungi and soil nutrients under different treatments

The results of Spearman correlation analysis between bacteria, fungi (genus level) and soil nutrients and the relative abundance in the top 20 bacteria under the different planting patterns are shown in Figs. 2-3. Soil pH was significantly positively correlated with *norank_f_norank_o_norank_c_KD4-96*, *norank_f_JG30-KF-CM45*, *Arthrobacter* and *Intrasporangium*. It was significantly negatively correlated with *norank_f_Xanthobacteraceae*, *norank_f_67-14*, *RB41*, *norank_f_norank_o_norank_c_TK10*, and *norank_f_norank_o_Gaiellales*. Soil organic matter was significantly positively correlated with *norank_f_norank_o_norank_c_KD4-96*, *norank_f_JG30-KF-CM45*, *Arthrobacter* and *Intrasporangium*. It was significantly negatively correlated with *Nitrospira*, *norank_f_Xanthobacteraceae*, *RB41*, and *norank_f_norank_o_norank_c_TK10*. The hydrolysable nitrogen content was significantly positively correlated with the *Sphingomonas* abundance. It was significantly negatively correlated with *norank_f_norank_o_norank_c_MB-A2-108*, *Gemmatimonas*, *Gaiella*, and *norank_f_Vicinamibacteraceae*. Effective phosphorus was significantly positively correlated with *Nitrospira*. It was significantly negatively correlated with *Bacillus*. Available potassium was positively correlated with *Nitrospira*, *norank_f_Xanthobacteraceae*, *RB41*, *norank_f_norank_o_norank_c_TK10*, *norank_f_Roseiflexaceae*, and *norank_f_Gemmatimonadaceae*. There was a significant negative correlation between *norank_f_norank_o_norank_c_KD4-96* and *Intrasporangium* ($P < 0.05$).

The fungal community showed that pH was significantly positively correlated with *Pseudeurotium*, *Microascus*, *Acremonium* and *unclassified_k_Fungi*. pH was significantly negatively correlated with *Penicillium*. Organic matter was significantly positively correlated with *Pseudeurotium*, *unclassified_f_Nectriaceae*, *Microascus* and *unclassified_k_Fungi*. *Fusicolla* and *Penicillium* were significantly negatively correlated. Hydrolyzable nitrogen was significantly positively correlated with *unclassified_f_Didymellaceae*, *Plectosphaerella*, *unclassified_p_Rozellomycota*, *unclassified_p_Mortierellomycota*, *Penicillium*, and *Fusarium*. Hydrolyzable nitrogen was significantly negatively correlated with *Byssoschlamys*, *unclassified_p_Chytridiomycota*, *Chaetomium* and *Aspergillus*. Effective phosphorus was significantly positively correlated with *unclassified_f_Chaetomiaceae*. There was a significant positive correlation between available potassium and *Fusicolla*, was significantly negatively correlated with *Pseudeurotium*, *unclassified_f_Nectriaceae* and *Microascus* ($P < 0.05$).

KEGG pathway enrichment analysis of soil bacteria in different treatment groups

In the prediction of soil bacterial function under the different planting patterns, the enrichment of KEGG pathways in the top 20 pathways is shown in Figs. 2-4. PICRUST2 software was used to predict the KEGG functions of the potential microorganisms. A total of 19791 KOs were detected in all soil samples, and the detected KOs were assigned at a higher classification level (level 1-level 3). As shown in Figs. 2-4, metabolism accounted for most of the predicted KOs, and the enrichment of the six metabolic pathways at Level 1 was the highest in the MKT2 treatment group. At Level 2, the global and overview maps pathways accounted for most of the total protein levels. Among the top 20 pathways, 16 pathways had the highest enrichment in the MKT2 treatment group, of which 9 pathways were related to metabolism. The top three pathways were global and overview maps; carbohydrate metabolism; amino acid metabolism; and signal transduction and substance transport pathways. The translation and folding, sorting and degradation pathways were the most enriched pathways in the MMT1 treatment group, and the glycan biosynthesis and metabolism and cell motility pathways were the most enriched pathways in the KKT3 treatment group. The metabolic pathway at Level 3 accounted for most of the pathways at this level. Among the top 20 pathways, 14 pathways were the most enriched in the MKT2 treatment group, of which 11 pathways were related to metabolism. There were 5 pathways with the highest enrichment in the MMT1 treatment group. Only one pathway (ribosome) was the most enriched pathway in the KKT3 treatment group. The metabolic function of bacteria treated with MKT2 was higher than that of bacteria treated with the other two agents.

Discussion

Crop rotation from different sources can change the soil microenvironment, reduce the frequency of continuous cropping obstacles, fully utilize soil nutrients, promote crop production and regulate soil fertility²¹. In this study, the soil nutrient index pH, hydrolyzable nitrogen content, effective phosphorus content and organic matter content of *A. muelleri* + flue-cured tobacco (MKT2) were higher than those of *A. muelleri* continuous cropping (MMT1) and flue-cured tobacco continuous cropping (KKT3). This finding is consistent with the findings of Wei²², who reported that the available potassium content in flue-cured tobacco from continuous cropping was the highest, possibly because the roots of flue-cured tobacco absorb less nutrients under continuous cropping conditions. Flue-cured tobacco plants have a high demand for potassium, and long-term potassium deficiency leads to plant growth retardation and yield reduction²³.

Soil microorganisms play a positive role in maintaining soil fertility and promoting nutrient cycling²⁴. Planting patterns directly or indirectly affect the composition of soil microbial communities²⁵. According to the study of Jia Jian²⁶, the higher fungal abundance in the continuous cropping soil of flue-cured tobacco was inconsistent with the findings of the highest bacterial microbial abundance in the continuous cropping of flue-cured tobacco and the high fungal microbial abundance in the *A. muelleri* + flue-cured tobacco (MKT2) treatment group in this study, which may be due to the different rotation crops. From the perspective of soil bacterial and fungal community structure, the soil microbial community associated with *Amorphophallus muelleri* continuous cropping was mainly composed of Actinobacteriota, Chloroflexi and Chytridiomycota. The *A. muelleri* + flue-cured tobacco cropping system was composed mainly of Proteobacteria, Mortierellomycota and Basidiomycota, and the flue-cured tobacco continuous cropping system was composed mainly of Acidobacteriota and Ascomycota. The abundances of Proteobacteria, Ascomycota, Mortierellomycota and Actinobacteriota in these phyla are significantly correlated with soil nutrients, indicating that microbial communities can affect soil nutrient content²⁷. The bacteria in the phylum Proteobacteria decompose and utilize organic acids as secreted metabolites in plant roots, thereby reducing the accumulation of soil organic acids and nitrogen^{28,29}. Zhao³⁰ reported that the application of *Pseudomonas acidophilus* CLP-7 could increase the soil nutrient content and microbial community richness in continuous cropping tobacco fields and effectively control tobacco bacterial wilt. *Arthrobacter* has a strong ability to degrade pesticide residues³¹ and can remediate contaminated soil³². *Sphingomonas* has very rich degradation-related genes and has obvious advantages in degrading aromatic compounds such as pesticides, dioxins, polycyclic aromatic hydrocarbons and heterocyclic aromatic hydrocarbons³³. In addition, *Sphingomonas* also has a variety of mechanisms for assisting in phytoremediation, promoting biosorption, promoting active efflux transport and reducing toxicity³⁴. Xiao³⁵ isolated a strain of *Bacillus* from garlic endophytic bacteria that could use phthalate (PAE) as a carbon source to grow and remediate PAE-contaminated soil. *Mortierella* fungi can dissolve phosphorus in soil, use carbohydrates in soil for growth and metabolism, and decompose organic substances to promote plant roots to absorb mineral elements and secrete antibiotics³⁶. Ascomycota, which has a negative effect on plant growth, can cause fungal diseases such as root rot, stem rot, and shoot blight³⁷. In this study, the abundances of *Sphingomonas*, *Arthrobacter*, *Bacillus* and *Mortierella* in *A. muelleri* + flue-cured tobacco cropping soil were 0.84, 0.006, 0.36 and 0.07 times higher than those in *A. muelleri* continuous cropping and flue-cured tobacco continuous cropping (0.78, 1.41, 0.24 and 0.17 times, respectively). The abundance of Ascomycota in the flue-cured tobacco continuous cropping system was 0.02 times higher than that in the *A. muelleri* continuous cropping and *A. muelleri* + flue-cured tobacco cropping systems ($P < 0.05$). We speculate that a large amount of glucomannan (KGM) in the bulb of *A. muelleri* may provide a high-quality carbon source for soil microorganisms and affect the community composition of soil bacteria through the combined action of other enzymes and plant secretory metabolites. A large number of alkaloid metabolites in Konjac corms can regulate the competition between soil bacteria and enrich the microbial community structure. The synthesis of plant alkaloids may lead to increased microbial abundance via the expression of alkaloid synthesis-related genes^{38,39}. However, there are few studies on the metabolomics and proteomics of *A. muelleri*, and the mechanism of action of its metabolites on soil microecology is still unclear.

From the perspective of microbial ecological function, the KEGG pathway enrichment of the soil bacteria in the three treatment groups was mainly related to the metabolism pathway. After *A. muelleri* + flue-cured tobacco cropping, the abundance of genes related to energy metabolism, carbohydrates and amino acids in the soil bacterial community increased. Active bacterial metabolism in soil is highly important for plant growth and development. For example, energy metabolism is closely related to energy intake⁴⁰. Carbohydrates are important ways for bacteria to uptake carbon sources. Amino acid metabolites may be beneficial to soil fertility^{41,42}.

Conclusion

The results of high-throughput sequencing analysis showed that *A. muelleri*+ flue-cured tobacco could regulate soil nutrients and increase the pH of acidified soil. The relative abundance of soil functional microorganisms increased. The abundance of beneficial bacteria and fungi, such as Proteobacteria, Mortierellomycota and Basidiomycota, increased. The abundance of soil bacterial sugars, amino acids and substance conversion genes increased. Correlation analysis of microorganisms and environmental factors was used to determine that Proteobacteria, Ascomycota, Mortierellomycota and Actinobacteriota were significantly correlated with soil nutrients, which indicated that microbial communities can affect soil nutrient content. The rotation planting pattern plays a positive role in regulating soil nutrients, improving the microbial community structure and increasing the functional metabolism of beneficial bacteria. The results of this study can provide a reference for high-quality flue-cured tobacco production.

Declarations

Author Contribution

Nong was mainly responsible for manuscript writing and picture production, and Yu and Huang were responsible for revision; The remaining authors were responsible for sample and data collection.

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Figures

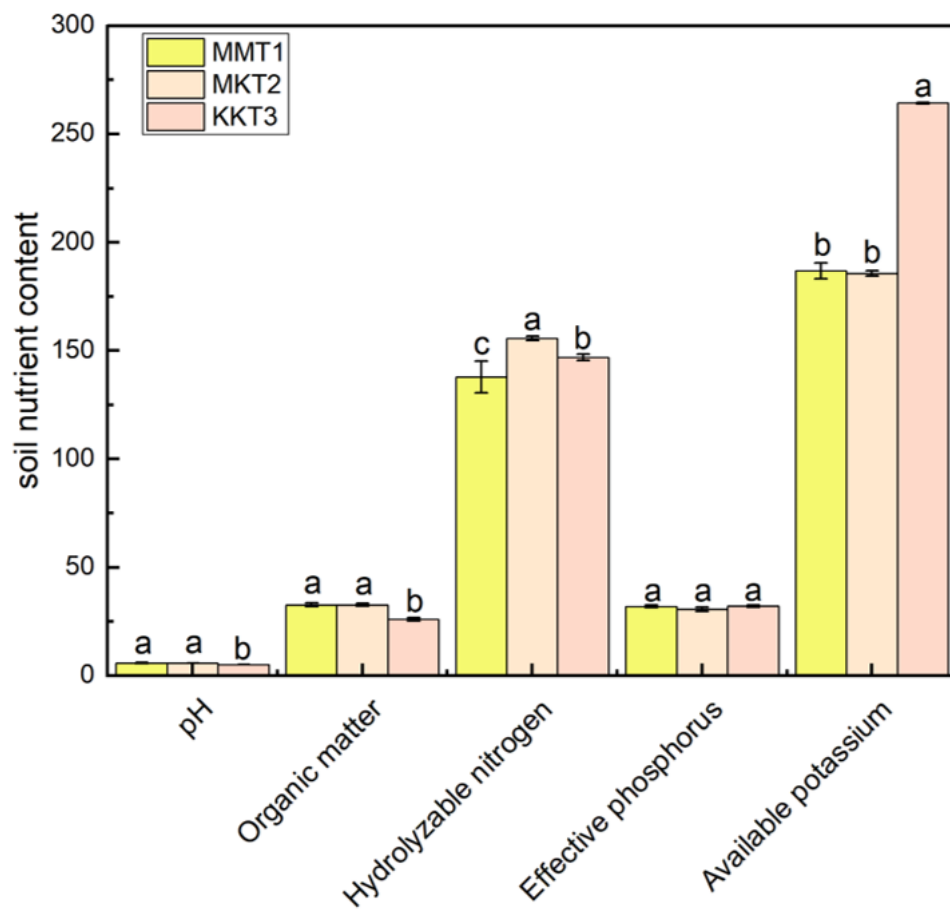
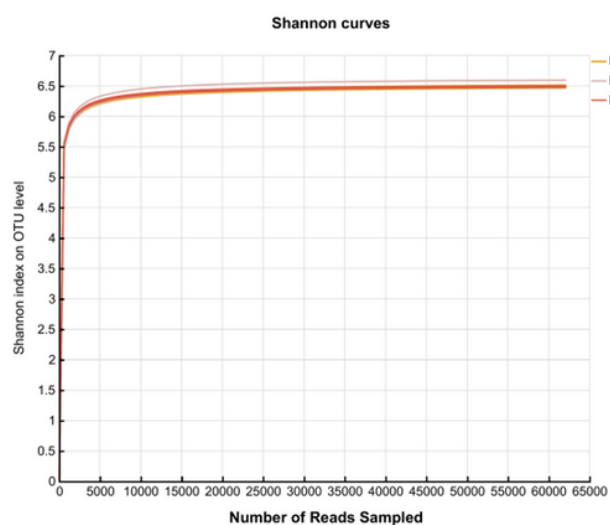
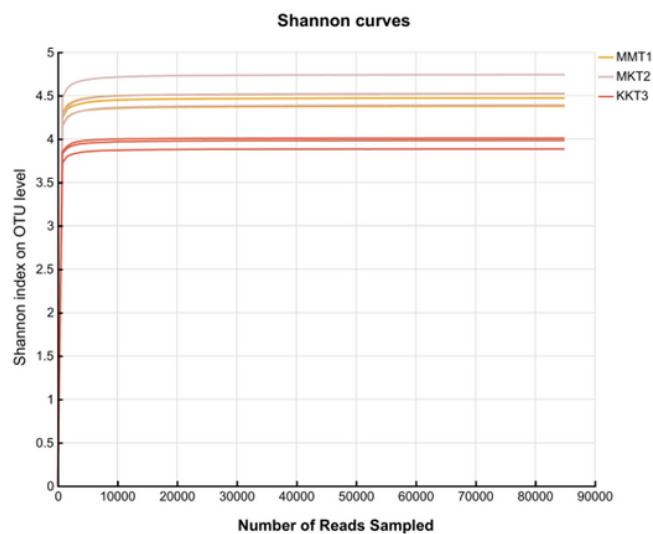


Figure 1

Fig.1-1 Differences in soil nutrient content under different treatments



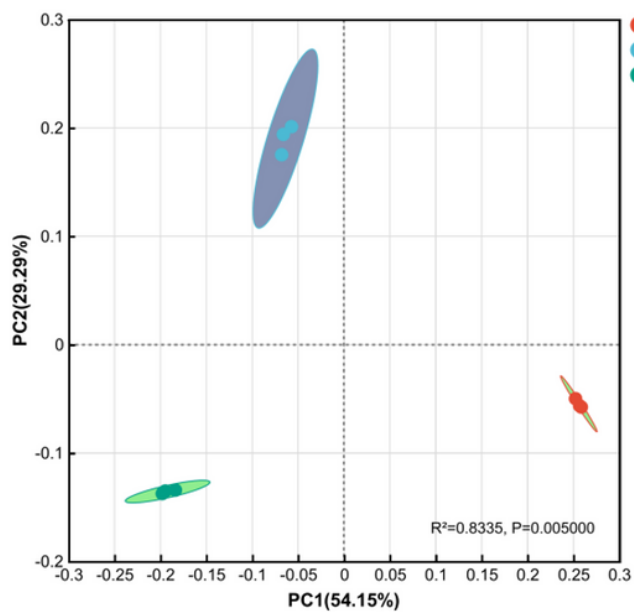
Bacteria



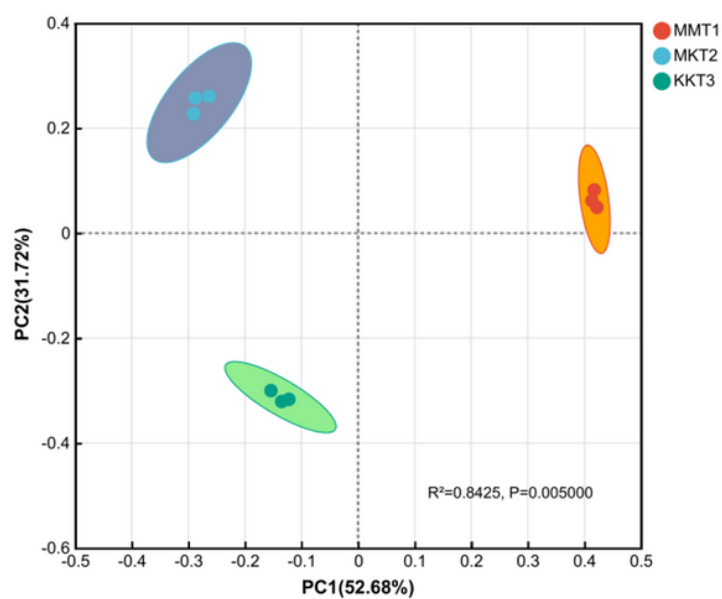
Fungi

Figure 2

Fig.1-2 Dilution curves of soil bacteria and fungi under different treatments



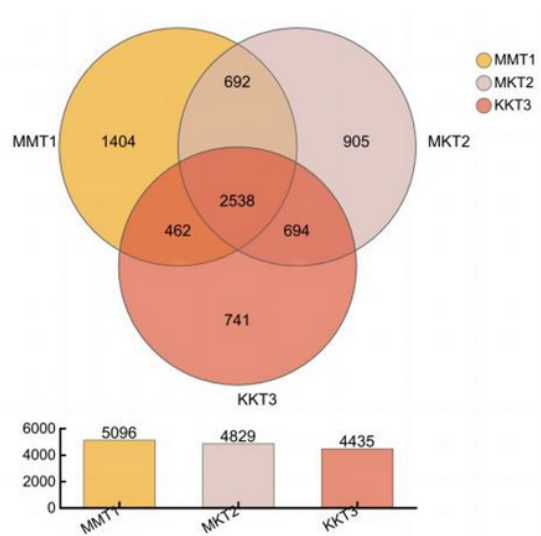
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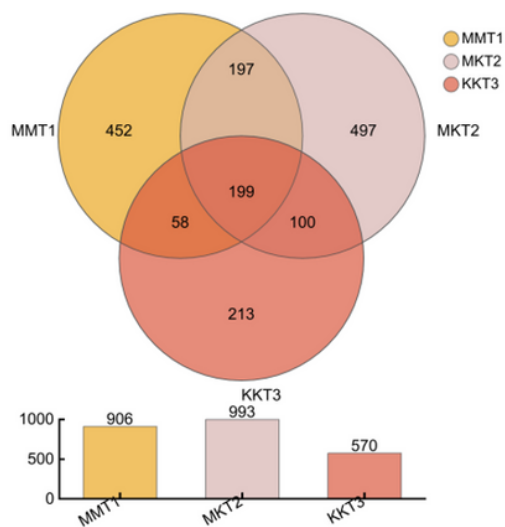
Fungi

Figure 3

Fig.1-3 PCoA scatter plots of soil bacterial and fungal community composition based on different OTU levels.



Bacteria



Fungi

Figure 4

Fig.1-4 Venn diagram of soil bacteria and fungi OTUs under different treatments

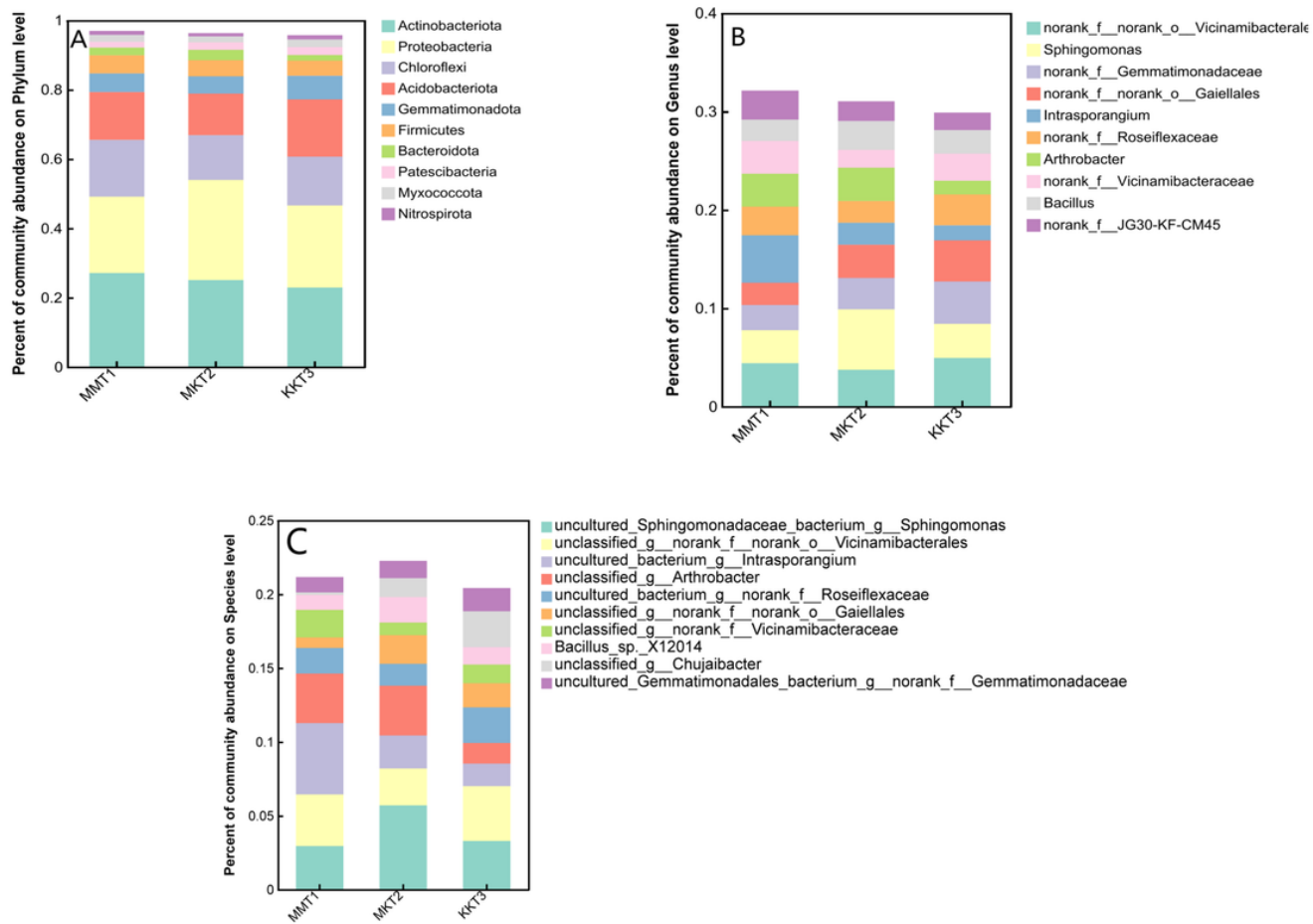


Figure 5

Fig.2-1 The relative abundance of soil bacterial community composition in different treatments (A: phylum level; b: Genus level; c: species level)

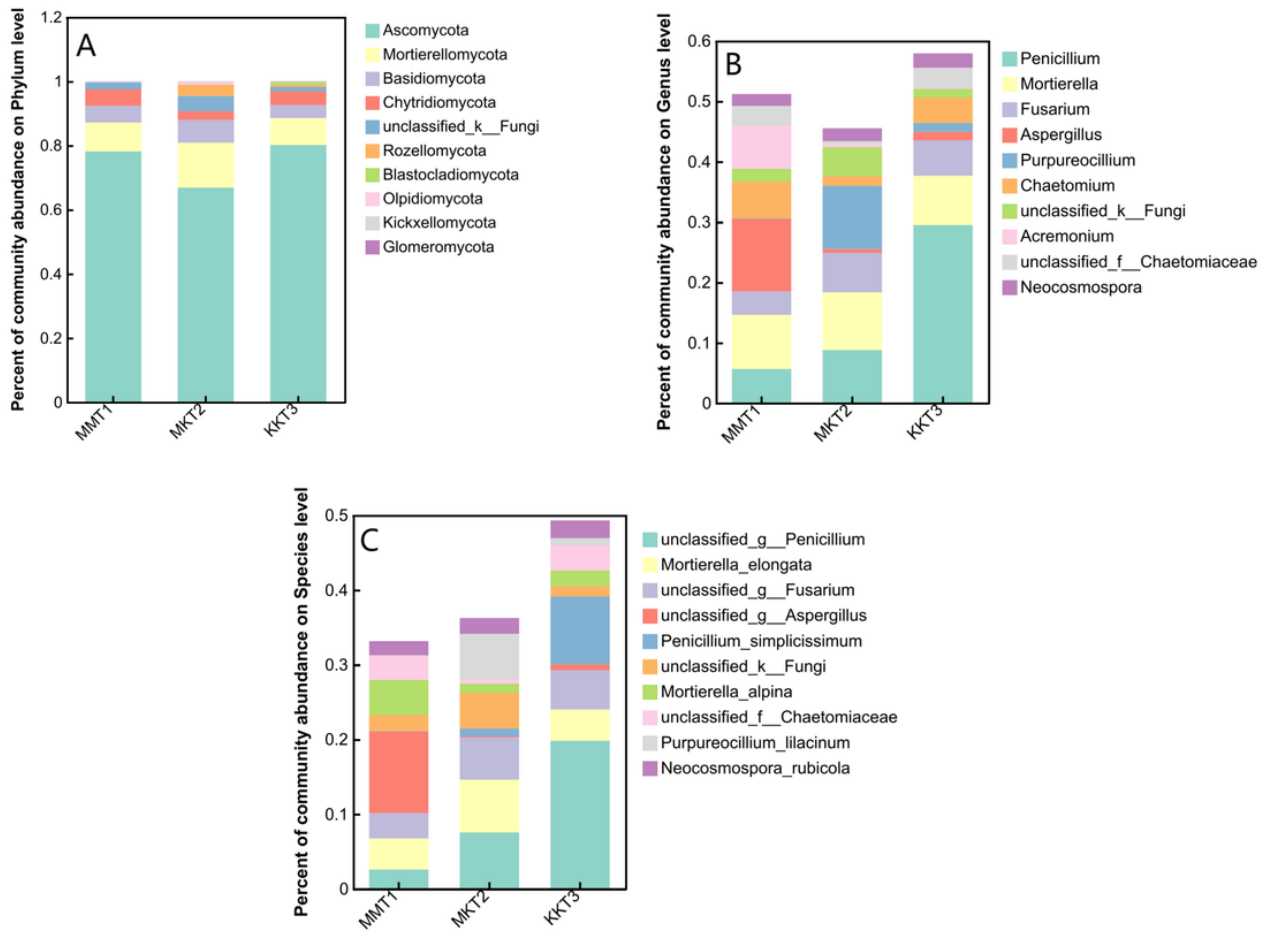


Figure 6

Fig.2-2 The relative abundance of soil fungal community composition in different treatments (A: phylum level; b: Genus level; c: species level)

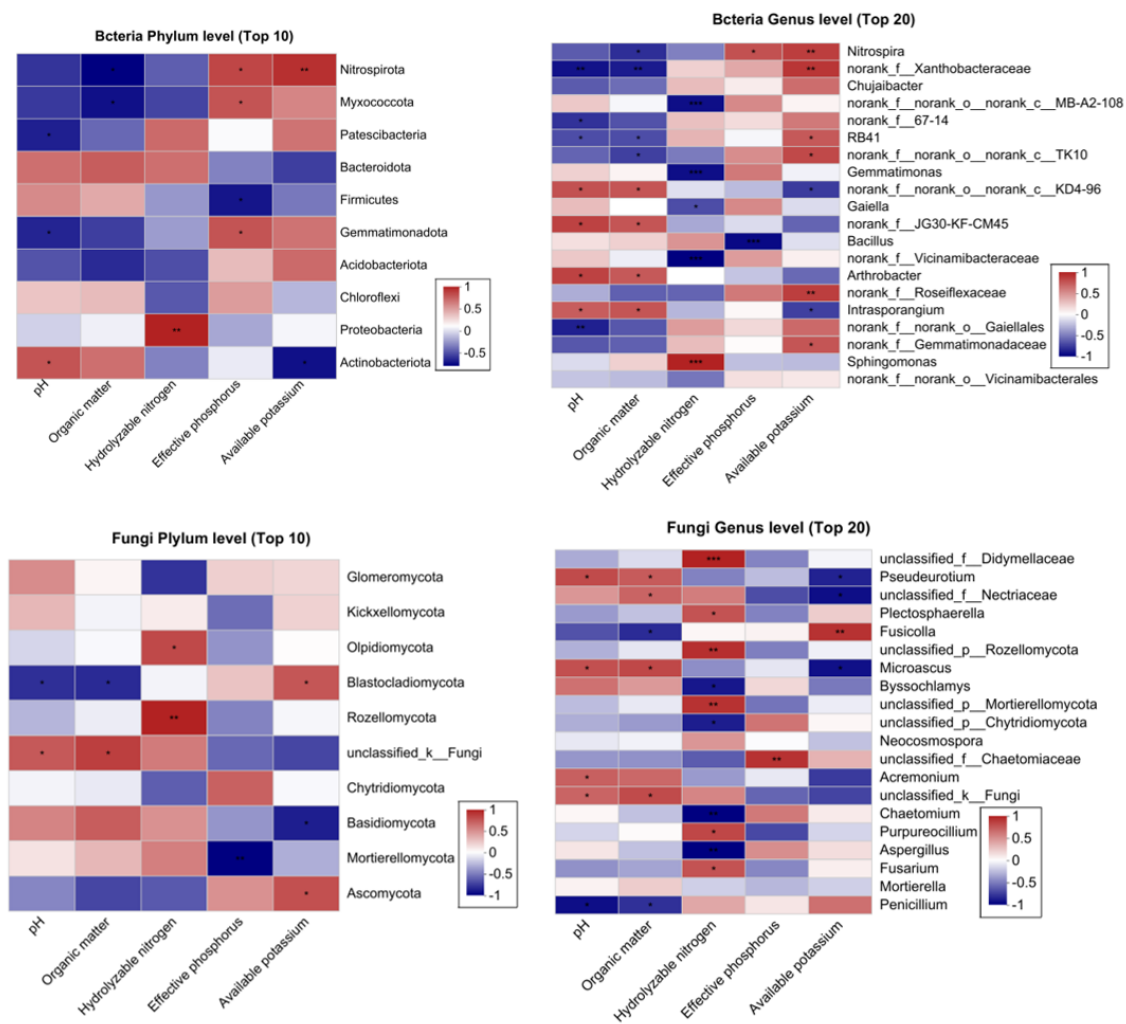


Figure 7

Fig.2-3 Spearman correlation analysis of soil bacteria, fungi and soil nutrients in different treatments (Phylum and Genus level)

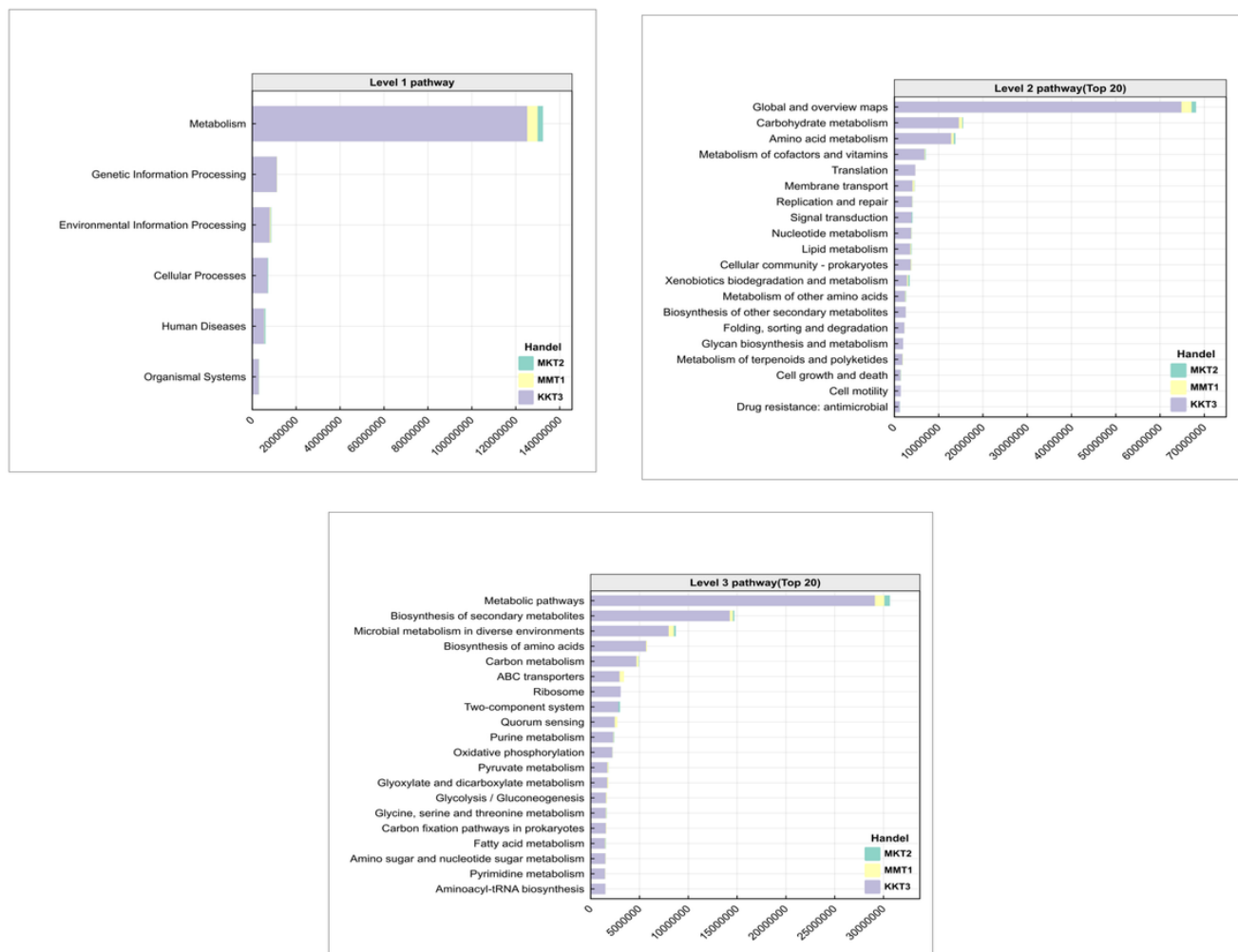


Figure 8

Fig.2-4 KEGG enrichment of soil bacteria under different planting patterns (Level1-3)