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Research Article

Effects of chemical fertilizer nitrogen substitution with rapeseed cake on microbial community in tobacco rhizosphere soil

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Abstract

Rapeseed cake is an important agricultural waste and it has the potential to improve soil fertility, crop productivity and microbial activities. Nonetheless, the effects of rapeseed cake on microbes of tobacco rhizosphere have not studied yet. Therefore, this study was conducted to investigate the effects of rapeseed cake fertilizer on the inter-root soil microbial community of roasted tobacco. The present study was comprised of different proportions of nitrogen replacement fertilizers such as rapeseed cake fertilizer (N30:30%, N60:60%, N100:100%), and recommended nitrogen application as control. The results of diversity analysis showed that cake fertilizer significantly increased the Ace and Chao indices of soil microbial populations, and Shannon and *Invsimpson* indices were significantly higher. Furthermore, cake fertilizer also significantly increased the relative abundance of *Actinobacteria, Ascomycetes, Intrasporangium, Chaetomium* and *Mortierella* and decreased the relative abundance of *Firmicutes, Basidiomycota* and *Fusicolla*. Moreover, cake fertilizer also significantly increased the abundance of stress-tolerant bacteria and animal pathogen-dung saprotroph-endophyte-epiphyte-plant saprotroph-wood saprotroph fungi, and decreased the abundance of their potentially pathogenic and anaerobic bacteria and unknown saprophytic types and plant pathogenic fungi. Further, available phosphorus (AP), soil organic carbon (SOC), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were identified as the main factors affecting the structure of the inter-root soil microbial community of baked tobacco. In conclusion, rapeseed -cake based fertility could be an important strategy to modulate the composition of bacterial and fungal communities in tobacco rhizosphere by improving soil properties, and decreasing the abundance of pathogenic bacteria.

Keywords: microbial community; nitrogen; rapeseed cake; rhizosphere soil; tobacco

Introduction

The application of chemical fertilizers is important to improve crop productivity and quality (He *et al.,* 2023). However, excessive use of chemical fertilizers and neglected use of organic fertilizers cause can decrease soil fertility, crop productivity, environment quality and microbial activities in soil rhizosphere (He *et al.,*

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2023). The application of organic fertilizers is considered is effective strategy to improve soil health, growth and yield of crops (Ji *et al.,* 2020; Zhang *et al.,* 2020). However, it is difficult to meet the nutrient demand of crops due to the slow release of nutrients from organic fertilizers. Furthermore, the application of organic fertilizers is also costly due to their handling and application (Song *et al.,* 2023). Thus, the use of raw materials has become an effective way to improve crop productivity, soil fertility and microbial activities (Song *et al.,* 2023).

Replacing chemical fertilizer with organic fertilizer is one of the important measures for China to implement zero growth of chemical fertilizer in 2020 (Zhang *et al.,* 2024). Rapeseed cake is an imperative organic fertilizer that contains an appreciable amount of nutrients and it showed to promising results in improving crop productivity (Song *et al.,* 2023). Cake fertilizer show positive effects on soil nutrient supply and sustainability, improving soil fertility thereby leading to improve growth and yield of crops (Yu *et al.,* 2020). The increasing application of organic fertilizer while reducing the input of chemical fertilizer has become an important measure to improve the soil fertility and yield and quality of flue-cured tobacco. For instance, Zhu *et al.* (2016) showed that the application of cake fertilizer significantly increased soil nutrients, nitrogen use efficiency and soil carbon, nitrogen mineralization and crop productivity. The application of cake fertilizers also increases the supply of nitrogen, phosphorus and potassium and soil organic matter content which in turn increases the yield and quality of tobacco (Manzoor *et al.,* 2022; Wang *et al.,* 2023). The application of rapeseed cake-based fertilizers could also maintain soil organic matter, nutrient status and microbial activity and enhance root proliferation and subsequent plant growth and biomass production (Fu *et al.,* 2021; Manzoor *et al.,* 2022). Cake fertilizers also increase the activity of soil enzymes and soil microbial activities which in turn improve the plant performance (Li *et al.,* 2019). The combined rapeseed and green manure application significantly enhanced the bacterial (*Acidobacteria*, *Proteobacteria* and *Actinobacteria*) and fungal abundance (*Basidiomycota*, *Ascomycota* and *Zygomycota*) by increasing soil nutrient availability (Fu *et al.*, 2021). In another study, Chen *et al.* (2023) found that combined rapeseed cake and organic manures significantly increased *Spingomonas* bacteria and *Chaetomium* and *Penicillium* fungi abundance in tobacco field. Further, the study findings of Jiang *et al.* (2019) showed that nitrogen substitution with cake fertilizer significantly increased the relative abundance of *Proteobacteria* and changed the bacterial community structure in the rhizosphere soil of flue-cured tobacco. Rhizosphere soil microorganisms affect the soil fertility, nutrient transformation and quality formation of crops (Dlamini *et al.,* 2022; Chaudhary *et al.,* 2023). However, the effect of nitrogen substitution fertilizer such as cake fertilizer on the rhizosphere microbial community of flue-cured tobacco remains to be clarified. We hypothesized that the substitution of nitrogen fertilizers with rapeseed cake-based fertility will modulate soil microbial diversity by changing soil properties. Therefore, this study was conducted with the following objectives: i) to determine the effect of cake fertilizers on soil physical and chemical properties, and enzyme ii) to determine the effects of cake fertilizer on the diversity, and structure composition of microbial communities in the rhizosphere of flue-cured tobacco.

Materials and Methods

Experimental site

The present field experiment was conducted at Yanzhong Village, Mashui Town, Leiyang City, Hunan Province (26°40'N, 112°58'E). The experiment site has a monsoon climate with a wet summer and cool winter. The soil samples were collected from 0-20 cm to determine different soil properties. The soil had pH 7.43, alkali-hydrolyzable nitrogen 79.88 mg kg^{-1}, available phosphorus 23.55 mg kg^{-1}, available potassium 154.36 mg kg^{-1}, and organic matter 34.93 g kg^{-1}.

Experimental design and crop management

The field experiment was carried out from 2021 to 2022 and total 4 treatments were set-up: N1 (recommended fertilization); N30 (rapeseed cake fertilizer nitrogen replaced 30% chemical fertilizer nitrogen); N60 (rapeseed cake fertilizer nitrogen instead of 60% chemical fertilizer nitrogen); N100 (rapeseed cake fertilizer nitrogen replaced 100 % chemical fertilizer nitrogen). The total plot area was 6×10 m^2 and each treatment had three replications and experiment was conducted in randomize complete block design. The fermented rapeseed cake fertilizer contained 4.98% N, 2.65% P_2O_5 and 0.97% K_2O, C/N 9:30. The nitrogen fertilizer was replaced by the same amount of cake fertilizer, and the insufficient phosphorus and potassium were supplemented by calcium magnesium phosphate fertilizer (16% P_2O_5) and potassium sulfate (52% K_2O). The flue-cured tobacco variety ('Yunyan 87') was used in experiment and all the cultivation and management measures were kept same as suggested for local flue-cured tobacco production.

Data collection

The soil samples were collected with the help of a soil auger from different locations of the experiment field (0-20 cm). The soil samples were temporarily stored in a container with liquid nitrogen, and then completely homogenized through a 2 mm soil sieve in a low temperature environment in the laboratory. Then soil was divided into three parts; one part was stored in a refrigerator at -80 °C for high-throughput sequencing analysis of rhizosphere soil microbial communities. Another part was stored in a refrigerator at 4 °C to determine microbial biomass and other indicators. The remaining soil samples were dried naturally and sieved for the determination of soil properties.

Determination of soil physical and chemical properties

The soil microbial biomass carbon (MBC) was determined by chloroform fumigation extraction-potassium dichromate volumetric method. Furthermore, microbial biomass nitrogen (MBN) was determined by fumigation extraction method, and microbial biomass phosphorus (MBP) was determined by the chloroform fumigation-sodium bicarbonate extraction method. Urease activity was determined by indophenol blue colorimetric method, the activity of sucrase (SC) was determined by the 3,5-dinitrosalicylic acid colorimetric method, and the activity of phosphatase (AKP) was determined by disodium phenyl phosphate colorimetric method.

Soil microbial sequencing

The soil DNA was extracted by taking 0.5 g soil sample with a DNA extraction kit (DNeasy ® PowerSoil ® Pro Kit, QIAGEN, USA). DNA purity and concentration were detected by spectrophotometer. An appropriate amount of samples was taken for DNA integrity detection according to the conventional method (1% agarose gel electrophoresis, voltage 5 V cm^{-1}, time 20 min). Finally, the qualified DNA was stored in liquid nitrogen. Later, primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) were used were used to amplify the 16S rRNA of bacteria. Moreover, primers iTS1F (GGAAGTAAAAGTCGTAACAAGG) and ITS2R (GCTGCGTTCTT CATCGATGC) were used for PCR amplification of fungal 18S rRNA ITS region. The amplified products were detected by 2% agarose gel electrophoresis, and the qualified PCR products were purified and quantified. NEXTFLEX ® Rapid DNA-Seq Kit was used to construct the library and sequencing was performed using the Illumina Miseq PE250 platform (commissioned by Shanghai Meiji Biotechnology Co., Ltd.).

Data analysis

The data on different traits were analyzed by one-way ANOVA using SPSS 22.0 software (Steel *et al.,* 1996). The significance among means were separated by the least significant test (LSD) and Pearson correlation analysis was used to determine the relations between different traits. The analysis of soil microbial community structure was carried out by using the cloud platform of Shanghai Meiji Biological Company and figures were created by using Orign2023 b and R language.

Results

Soil physicochemical and biochemical properties

The results indicate that the application of different rates of cake-based fertilizers significantly increased soil NPK and organic carbon (SOC) concentration (Table 1). The contents of MBC, MBN and MBP were significantly increased with the application of cake fertilizers and each index increased with the increase of the proportion of cake fertilizer. The urease and phosphatase activities were significant higher in N60, however, other treatments showed a non-significant impact on urease and phosphatase activities (Table 1). There was no significant difference in AN, MBC and MBN between N60 and N100. The content of available potassium (AK) increased significantly with the increasing proportion cake fertilizers. The cake fertilizer also significantly increased the contents of available nutrients, SOC, MBC, MBN and MBP in the rhizosphere soil of flue-cured tobacco. The AN, AK MBC and MBN contents in the rhizosphere soil of flue-cured tobacco were significantly increased by 60% and 100% nitrogen substitution of cake fertilizer compared with 30 % nitrogen substitution (Table 1).

Table 1. Basic physical, chemical and biochemical indexes of soil

Soil properties	N_I	N_{30}	N_{60}	N_{100}
pH	7.64±0.23a	7.68±0.25a	7.62±0.12a	7.58±0.25a
AN (mg/kg)	95.37±8.55c	106.06±5.69b	128.85±6.51a	129.81±6.45a
AP (mg/kg)	51.02±4.7b	72.02±4.99a	79.23±6.47a	80.09±6.71a
AK (mg/kg)	218.81±7.98d	254.50±10.01c	285.97±13.88b	296.18±11.29a
SOC ($g \cdot kg^{-1}$)	22.14±3.00b	29.27±1.41a	30.73±1.54a	30.82±2.01a
MBC ($mg \cdot kg^{-1}$)	121.06±18.77c	195.26±11.23b	246.83±22.56a	256.79±23.92a
MBN ($mg \cdot kg^{-1}$)	8.90±1.91c	21.60±2.27b	33.20±2.29a	37.15±5.47a
MBP ($mg \cdot kg^{-1}$)	19.92±2.76c	24.24±3.11ab	32.53±6.57a	32.84±11.72a
SC ($mg \cdot g^{-1} \cdot d^{-1}$)	11.86±0.84a	12.23±0.91a	12.33±0.48a	12.17±0.86a
UE ($mg \cdot g^{-1} \cdot d^{-1}$)	48.82±7.66b	48.14±7.64b	55.93±1.07a	48.95±7.05b
AKP ($mg \cdot g^{-1} \cdot d^{-1}$)	0.65±0.09b	0.67±0.09b	1.05±0.02a	0.82±0.13ab

Note: different lowercase letter indicates significant difference at $p < 0.05$. AN: available nitrogen, AP: available phosphorus, AP: available potassium, SOC: soil organic carbon, MBC: microbial biomass carbon, MBN: microbial biomass nitrogen, MBP: microbial biomass phosphorus, SC: sucrose, UE: urease, AKP: alkaline phosphatase.

α-diversity of soil microbial community

The results indicate Ace and Chao indexes of soil microbial communities in N30, N60 and N100 were significantly increased as compared with NI (Table 2). The Ace indexes of bacteria increased by 6.85%, 10.43% and 8.42%, respectively, and the Chao indexes increased by 7.16%, 10.12% and 8.1%, respectively in N30, N60 and N100. The Ace indexes of fungi increased by 17.1%, 26.27% and 15.86%, respectively, and the Chao indexes increased by 15.69%, 22.44% and 15.78%, respectively N30, N60 and N100 (Table 2).

Composition of soil microbial bacterial community

The dominant phyla of soil bacterial community in each treatment included *Actinobacteria* (23.05%-35.88%), *Proteobacteria* (17.03%-25.25%), *Chloroflexi* (14.02%-21.94%), *Acidobacteria* (7.66%-15.09%) and *Firmicutes* (1.24%-2.45%). Compared with NI, the relative abundance of *Actinobacteria* in N30, N60 and N100 was increased by 44.99%-55.66%, and the relative abundance of *Proteobacteria* increased by 6.28%-

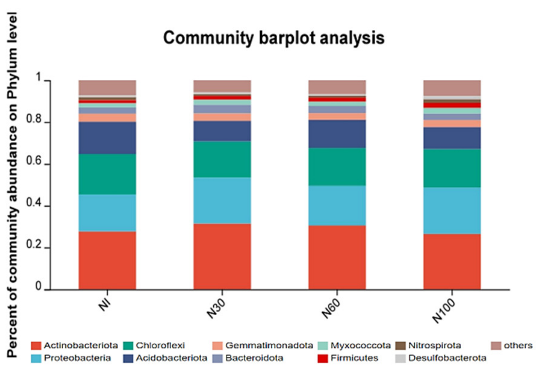
48.67%, among which the increment of N100 reached a significant level. The relative abundance of *Chloroflexi, Acidobacteria* and *Firmicutes* decreased. Among them, the relative abundance of *Chloroflexi* and *Acidobacteria* in N100 decreased significantly by 36.10% and 49.24 %, respectively, and the relative abundance of *Firmicutes* in N60 and N100 decreased significantly by 49.39% and 40%, respectively.

Table 2. Bacterial and fungal community richness index and community diversity index

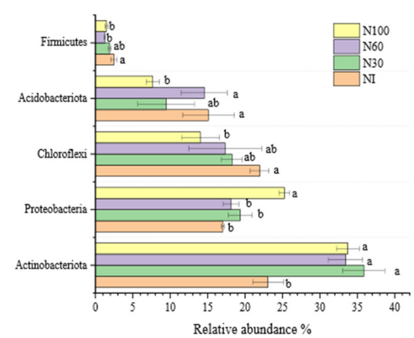
Treatment	Bacteria				Fungi			
	Ace	Chao	Shannon	Invsimpson	Ace	Chao	Shannon	Invsimpson
NI	2991.95±59.06b	2974.9±69.75b	6.13±0.12b	73.42±4.63b	578.40±37.41b	579.29±31.91b	3.48±0.11b	17.32±2.03b
N30	3196.57±70.68a	3187.52±82.53a	6.45±0.18a	81.46±3.29a	678.39±49.28a	681.87±48.23a	3.78±0.12a	21.45±2.37a
N60	3303.79±69.22a	3294.12±79.84a	6.52±0.21a	85.47±6.73a	746.88±63.59a	751.18±57.85a	3.86±0.26a	22.21±3.77a
N100	3243.93±38.25a	3216.04±63.45a	6.47±0.17a	82.29±3.46a	669.07±31.37a	669.90±32.13a	3.76±0.16a	19.36±3.56ab

Note: different lowercase letter indicates significant difference at $p < 0.05$.

Among different substitution ratios, the abundance of *Proteobacteria* in N100 was significantly higher than that in N30 and N60, while the abundance of *Acidobacteria* was significantly lower than that in N60 (Figure 1). This indicates that the substitution of nitrogen fertilizer such as cake fertilizer significantly increased the relative abundance of *Actinobacteria* in the rhizosphere soil of flue-cured tobacco, while the relative abundance of *Firmicutes* decreased significantly.



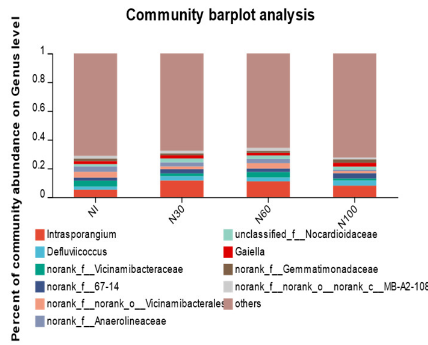
(A)



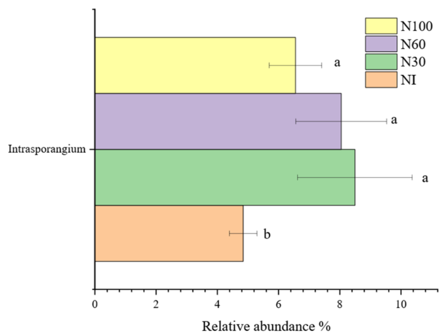
(B)

Figure 1. Bacterial community composition and its relative abundance at phylum level

The relative abundance of soil microbial community bacteria in each treatment ranked the top 10 from high to low; *Intrasporangium* (4.84%- 8.50%), *Defluviicoccus* (2.26%-3.21%), *norank _f_ Vicinamibacteraceae* (2.28%- 3.08%), *norank _f_* (2.27%-3.01%) and other 10 species, accounting for about 30 % of the total abundance (Figure 2A). Compared with NI, the relative abundances in N30, N60 and N100 were significantly increased by 75.62%, 66.32% and 35.33 %, respectively (Figure 2B). There was no significant difference between different proportions of cake fertilizer and other nitrogen substitution fertilizer treatments (Figure 2).



(A)

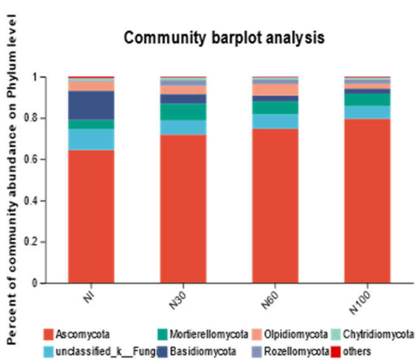


(B)

Figure 2. Bacterial community composition and its relative abundance at genus levels

Composition of soil microbial fungal community

The dominant phylum of soil fungal community in each treatment was composed of Ascomycota (62.49%-83.32%), *Mortierellomycota* (5.46%- 11.46%) and *Basidiomycota* (2.07-14.90%), accounting for more than 90% of the total abundance (Figure 3A). The relative abundance of Ascomycota in N30, N60 and N100 increased by 18.40% -31.73%. Further, rape cake fertilizer decreased the abundance of *Basidiomycota* 86.11% and 79.26%, respectively. Moreover, there was no significant difference between different proportions of cake fertilizer and other nitrogen substitution fertilizer treatments. Different proportions of nitrogen fertilizer such as cake fertilizer had no obvious effect on the abundance of fungi at the phylum level.



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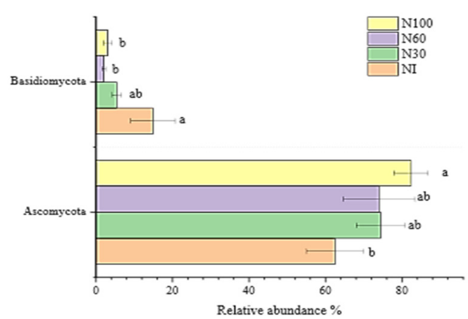
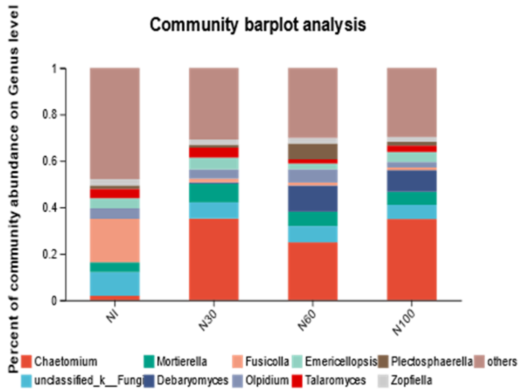


(B)

Figure 3. Fungal community composition and its relative abundance at phylum level

The relative abundance of fungal genera in the soil microbial community of each treatment ranked the top 10 from high to low, followed by: *Chaetomium* (1.92%- 32.94%), *Mortierella* (4.12%-7.88%), *Debaryomyces* (0.93%-11.56%), *Fusicolla* (1.30%-18.47%) and other genera (Figure 4A), accounting for more than 50%. The main genera of N30, N60 and N100 were *Chaetomium.* Compared with NI, the relative abundance of *Chaetomium* and *Mortierella* in N30, N60 and N100 (Figure 4B) increased significantly by 1174.32%-1615.63% and 50.59%-91.26%, respectively. The relative abundance of *Debaryomyces* in N60 and N100 increased significantly by 1147.31% and 940.32%, while the relative abundance of *Fusicolla* decreased significantly by 1054.56% and 1321.04%. The relative abundance of *Debaryomyces* in N60 and N100 was significantly higher than that in N30.



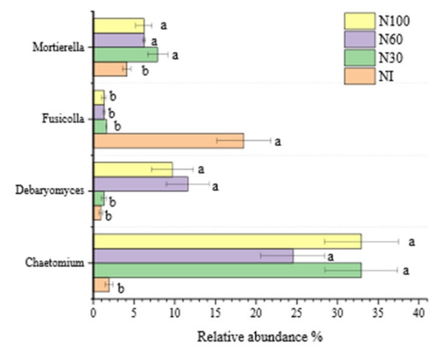
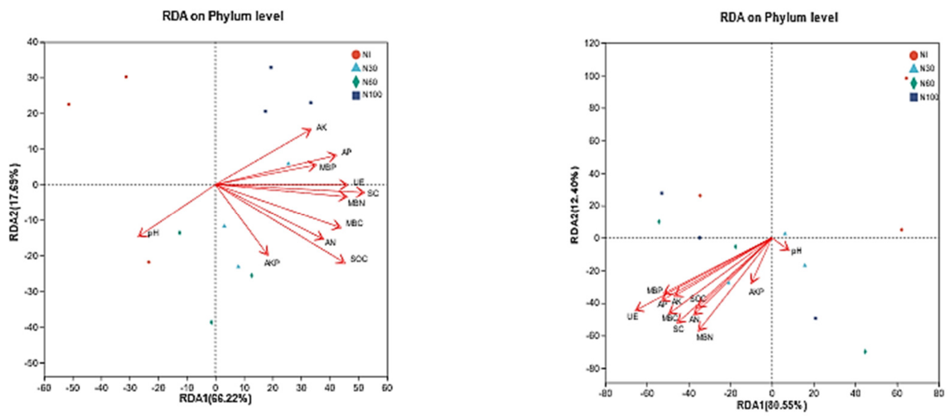


Figure 4. Fungal community composition and its relative abundance at genus levels

Relationship between soil environmental factors and microbial dominant community composition

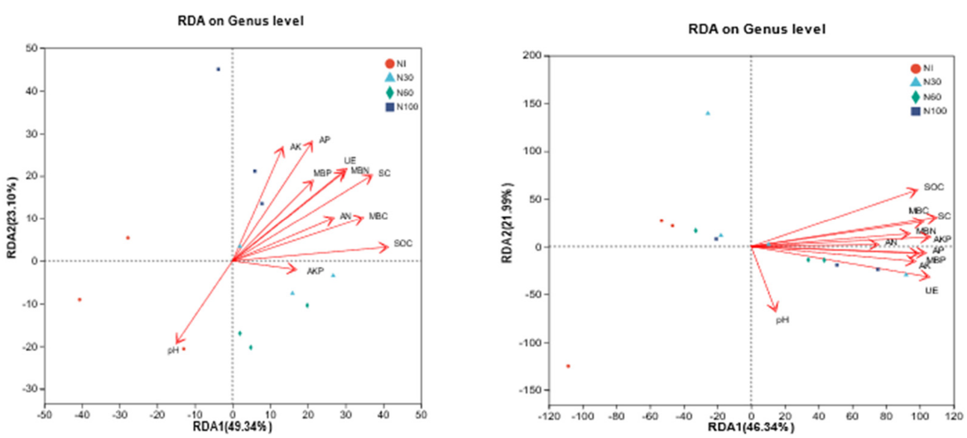
The RDA analysis was carried out on soil physical and chemical and biochemical indexes with soil bacterial and fungal communities at phylum and genus levels (Figure 5). The two axes explained 83.91% of the variation, and the latter explained 92.95% of the variation. SC ($R^2 = 0.7748$, P = 0.004), SOC ($R^2 = 0.7235$, P = 0.017), urease ($R^2 = 0.6075$, P = 0.019), MBN ($R^2 = 0.6044$, P = 0.021), AP ($R^2 = 0.5255$, P = 0.031) and MBC ($R^2 = 0.5862$, P = 0.034) were the main soil environmental factors affecting the phylum composition of bacterial community, while urease ($R^2 = 0.5628$, P = 0.024) was the main soil environmental factor affecting the phylum composition of fungal community (Figure 5). The RDA analysis results of soil physicochemical and biochemical indicators and soil dominant bacterial and fungal communities at the genus level are given in (Figure 6A), RDA1 and RDA2 axes explained 72.44% of the total variation of bacterial communities. SC ($R^2 = 0.7591$, P = 0.003), SOC ($R^2 = 0.7280$, P = 0.006), urease ($R^2 = 0.5876$, P = 0.019), MBC ($R^2 = 0.5522$, P = 0.020), MBN ($R^2 = 0.5584$, P = 0.025) and AP ($R^2 = 0.5244$, P = 0.036) were the main soil environmental factors affecting the bacterial community. The RDA1 and RDA2 axes of the fungal community (Figure 6B) explained 68.33% of the total variation of the fungal community. Among them, SC ($R^2 = 0.5010$, P = 0.016), SOC ($R^2 = 0.5075$, P = 0.03), urease ($R^2 = 0.4675$, P = 0.039) and MBC ($R^2 = 0.4251$, P = 0.044) were the main soil environmental factors affecting the fungal community.



(A) (B)

Figure 5. RDA analysis of different environmental factors and microbial community composition of bacteria (A) and fungi (B) in each treatment

Note: AN: Available nitrogen; AP: available phosphorus; AK: available potassium; SOC: organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus; SC: sucrose activity; UE: urease; AKP: alkaline phosphatase.

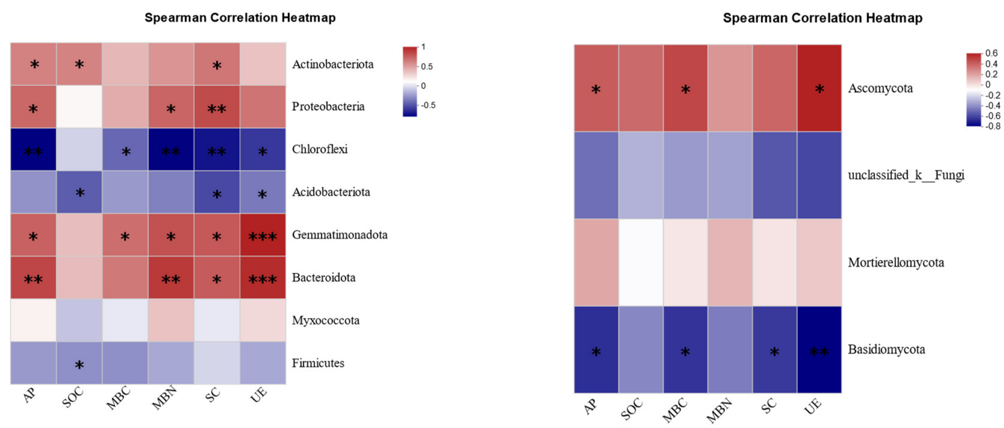


(A) (B)

Figure 6. RDA analysis of different environmental factors and microbial community composition of bacteria (A) and fungi (B) in each treatment

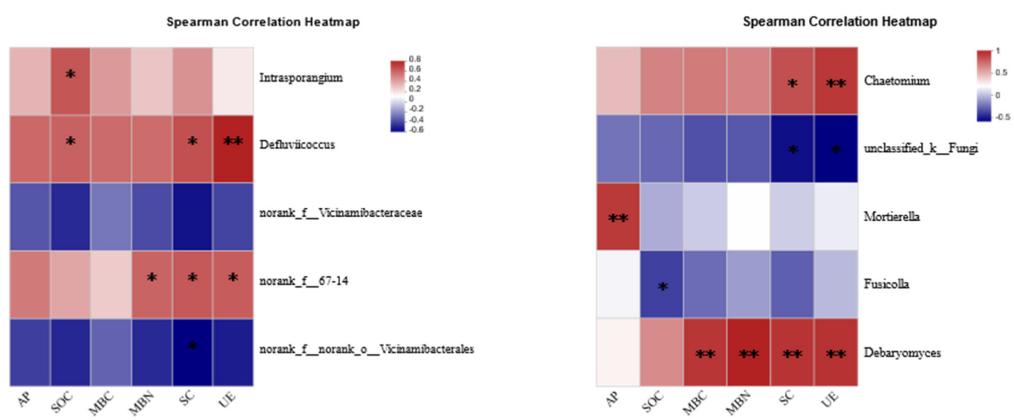
Spearman correlation between soil physicochemical and biochemical indexes and soil bacterial and fungal community structure

Spearman correlation analysis was performed between soil physicochemical and biochemical indicators (Figure 7 and Figure 8). The results of bacterial phylum level showed that *Actinobacteria* of bacteria (Figure 7A) were significantly positively correlated with AP, SOC and SC, respectively. *Proteobacteria* was significantly positively correlated with AP, MBN and SC, respectively. *Chloroflexi* was significantly negatively correlated with AP, MBC, MBN, urease and SC. Moreover, *Acidobacteria* and *Firmicutes* were significantly negatively correlated with SOC, and *Acidobacteria* was also significantly negatively correlated with SC and urease. The results of the genus level of bacteria (Figure 8A) showed that the *Intrasporangium* of *Actinobacteria* was significantly positively correlated with SOC. The fungal community (Figure 8B) showed that *Chaetomium* in *Ascomycota* was significantly positively correlated with SC and urease, while *Debaryomyces* in *Ascomycota* was significantly positively correlated with MBC, MBN, SC and urease. Moreover, *Mortierella* of *Mortierellaceae* was significantly positively correlated with AP and *Fusicolla* genus of *Basidiomycota* was significantly negatively correlated with SOC.



(A) (B)

Figure 7. Bacterial and fungal phylum level correlation analysis



(A) (B)

Figure 8. Bacterial and fungal genus level correlation analysis

BugBase phenotype prediction of bacterial community

BugBase can determine the high-level phenotype in the microbiome samples. The predictive analysis of bacterial phenotype showed that the abundance of stress-tolerant bacteria in N30, N60 and N100 was significantly increased by 11.07%-17.62% compared with NI (Table 3).

The relative abundance of potentially pathogenic and anaerobic bacteria was significantly decreased by 36.05%-40.13% and 54.71%-63.45%. There was no significant difference between different substitution ratios. The substitution for chemical fertilizers such as cake fertilizer can significantly increase the abundance of stress-tolerant bacteria and reduce the abundance of potentially pathogenic and anaerobic bacteria (Table 3).

Table 3. Effects of different fertilization treatments on bacterial community function

Bacterial community function %	Treatment			
	NI	N30	N60	N100
Forms biofilms	20.29a	20.44a	20.66a	19.55a
Mobile functional components	**16.10a**	17.69a	16.69a	18.18a
Stress tolerant	14.81b	17.42a	16.45a	17.24a
Gram negative	16.56a	12.70a	13.44a	13.38a
Aerobic	10.22a	12.25a	11.92a	12.97a
Gram positive	7.98a	10.20a	9.58a	9.21a
Potentially pathogenic	10.79a	6.49b	6.46b	6.9b
Anaerobic	4.46a	2.02b	1.98b	1.63b
Others	0.79a	0.79a	0.82a	0.94a

FUNGuild function prediction of fungal community

FUNGuild (fungi functional guild) is a tool for the classification and analysis of fungal communities through micro-ecological guilds. The guild involves a class of species (whether closely related or not), which can use similar environmental resources in similar ways. According to the results of FUNGuild the functional types of fungal communities mainly included 5 saprophytic types:1 *disease prototype type, 1 parasitic type, 1 endophytic-saprophytic type, and 1 pathogen-saprophytic type* (Table 4). Among them, the abundance of undefined *Saprotroph* fungi accounted for the highest proportion in all samples, reaching 20.96%-38.57%. The abundance of unknown saprophytic and plant pathogen communities in N30, N60 and N100 treatments was significantly lower than that in NI by 35.42%-45.66% and 94.68% 96.51%, respectively. Animal *Pathogen-Dung Saprotroph-Endophyte-Epiphyte-Plant Saprotroph-Wood Saprotroph* community abundance was significantly increased by 1177.08%-1615.63%, but there was no significant difference between different proportions (Table 4).

Table 4. Effects of different fertilization treatments on fungi community function

Functional nutritional type	Functional groups of fungi %	Treatment			
		NI	N30	N60	N100
Saprotroph	Undefined saprotroph	38.57a	20.96b	23.33b	24.91b
	Dung saprotroph-wood saprotroph	4.58a	3.01a	2.29a	1.72a
	Dung saprotroph	3.22a	2.56a	3.29a	1.94a
	Dung saprotroph-plant saprotroph-wood saprotroph	2.89a	3.28a	5.52a	2.97a
	Undefined saprotroph-wood saprotroph	1.10a	1.07a	1.35a	1.75a
Pathogen	Plant pathogen	6.02a	0.21b	0.23b	0.32b
Parasite	Animal parasite-fungal parasite	4.40a	3.98a	5.86a	2.63a
Endophyte-saprotroph	Endophyte-litter saprotroph-soil saprotroph-undefined saprotroph	5.12a	7.88a	6.20a	6.21a
Pathogen-saprotroph	Animal pathogen-dung saprotroph-endophyte-epiphyte-plant saprotroph-wood saprotroph	1.92b	32.94a	27.52a	32.95a
Unknown and others	Unknown	23.10a	17.97a	16.07a	15.08a
	Others	10.08a	7.22a	9.51a	9.79a

Discussion

Soil microorganisms are an important component of soil micro-ecology and they are a key factor driving soil nutrient cycling (Wang *et al.,* 2022; Wu *et al.,* 2024). The microbial Ace and Chao indexes and Shannon and Invsimpson indexes were increased with the application of cake fertilizers. Organic fertilizers improve the physical and chemical properties of soil which provide a good growth environment for soil bacteria resulting in increased microbial abundance (Ye *et al.,* 2022). The rich total carbon, organic acids and phenols in organic fertilizers provide nutrients for the metabolic growth of soil microbial communities which affect the diversity and richness of soil microbes (Wang *et al.,* 2023). Different proportions of cake fertilizer had non-significant effect on the microbial diversity in the rhizosphere soil of flue-cured tobacco. The application of organic source fertilizers does not necessarily lead to an increase in the total category of soil microorganisms (Chernov and Semenov, 2021). Only the appropriate amount of organic fertilizer can help to improve the richness and diversity of soil bacterial and fungal communities. These findings align with the results of Liu *et al.* (19) who also found that no significant difference in the Shannon index of fungi with the application of cow dung and chemical fertilizers.

Soil microorganisms are very sensitive to environmental changes. In this study, *Actinobacteria, Proteobacteria, Chloroflexi and Acidobacteria*, as well as *Ascomycota, Basidiomycota and Mortierella* were the main dominant species in the rhizosphere soil which aligns with earlier studies of Bao *et al*. (2023). The use of cake fertilizer significantly increased the relative abundance of *Actinobacteria* in bacteria and *Ascomycota* in fungi in rhizosphere soil of flue-cured tobacco which could promote the decomposition of soil organic matter (Zhang *et al.,* 2021). At the same time, cake fertilizer significantly increased the relative abundance of bacteria and fungi, and reduced the relative abundance of *Firmicutes, Fungi Basidiomycota* and *Fusicolla* in bacteria. *Chaetomium* is a biological control fungus, and it can produce secondary metabolites with inhibitory activity against plant pathogens (Dwibedi *et al.,* 2023). *Mortierella* can metabolizes fatty acids that inhibit the growth of harmful microorganisms, and *Mesosporangium* can produce antibacterial compounds (Okudoh and Wallis, 2012). Both *Firmicutes and Fusicolla* are potential pathogens (Liu *et al.,* 2022; Ren *et al.,* 2023), while *Basidiomycota* plays an important role in the degradation of organic materials with high lignin content. Therefore, cake fertilizer is beneficial in the prevention and control of soil-borne diseases of flue-cured tobacco, but it is not conducive to the degradation of organic matter containing complex high lignin and cellulose.

The relative abundance of *Proteobacteria* was significantly increased, but the relative abundance of *Chloroflexi* and *Acidobacteria* was decreased with application of rapeseed cake fertilizer. *Proteobacteria* promotes the decomposition of easily degradable organic carbon components rich in nutrients (Song *et al.,* 2016) while *Chloroflexi* has the ability to degrade macromolecular organic matter to small molecular organic matter (Nie *et al.,* 2023). *Acidobacteria* can effectively degrades complex lignin and cellulose to provide nutrients for the soil (Kalam *et al.,* 2020) therefore, higher proportion of cake fertilizer substitution is beneficial to enhance the bacterial phylum of easily decomposed organic matter. The effect of different proportions of nitrogen substitution fertilizer such as cake fertilizer on the abundance of fungi at the phylum level was not obvious. Therefore, the rhizosphere soil of flue-cured tobacco treated with nitrogen substitution 60% chemical fertilizer such as cake fertilizer has low pathogenicity and can effectively degrade complex lignin and cellulose properties, and has a relatively better micro-ecological environment.

Soil microorganisms are closely related to soil environmental quality, and soil bacteria and fungi are closely related to soil physical and chemical properties. The growth of bacteria and fungi requires sufficient carbon and nitrogen sources. Dou *et al*. (2023) believed that organic carbon and its active organic carbon components are the main factors affecting soil bacterial and fungal communities. The application of organic fertilizer increased the input of SOC, which was an important energy source for heterotrophic microorganisms in soil (Rambaut *et al.,* 2023). The cake fertilizer and other nitrogen substitution fertilizers significantly increased the SOC and MBC contents in the rhizosphere soil of flue-cured tobacco. Therefore, the changes in SOC and MBC content may affect the acquisition of carbon sources required for bacterial and fungal life activities. Organic fertilizer can increase carbon-based substances and soil exogenous nutrients, stimulate enzyme activity and promote microbial nitrogen fixation, thereby increasing soil microbial biomass nitrogen content (Rambaut *et al.,* 2023). Cake fertilizer significantly increased the MBN and MBN content was significantly positively correlated with *Debaryomyces of Proteobacteria and Ascomycota*. Therefore, the change of MBN content in the rhizosphere soil of flue-cured tobacco in this study (Figure 4) may affect the acquisition of nitrogen sources required by bacteria and fungi, which is similar to the results of Guo *et al.* (2024).

The application of cake fertilizer increased the content of active organic carbon components, which was beneficial to the rapid growth of microorganisms in the early stage (Cong *et al*., 2020). There was also a significant negative correlation between MBN content and *Chloroflexi* in this study. AP is one of the nutrient sources for microbial growth, and it can promote crop rooting (Wang *et al.,* 2022). Cake fertilizer and other nitrogen substitution fertilizers significantly increased the AP content. Therefore, AP is an important soil property affecting bacterial and fungal community structure in this study. Cake fertilizer significantly increased the urease activity which is consistent with the results of Song *et al.* (2023). The application of organic fertilizers such as cake fertilizers increases the content of soil organic matter, which prevents soil enzyme activity from denaturation or degradation, and increases the overall urease activity (Zhang *et al.,* 2013). The increase in urease activity increases the content of inorganic nitrogen in the soil, and SC is involved in the decomposition of macromolecular organic matter. Since soil enzymes are mainly derived from microbial cells, changes in the number of soil microorganisms will inevitably lead to changes in soil enzyme activity (Telesiński *et al.,* 2021).

Different fertilization patterns can affect the occurrence of soil-borne diseases by regulating crop rhizosphere communities (Sun *et al.,* 2023). BugBase phenotype prediction showed that nitrogen substitution cake fertilizer significantly increased the abundance of stress-tolerant bacteria and reduced the abundance of potential pathogenic and anaerobic bacteria. This indicates that the combined bio-organic fertilizer had a positive effect on the prevention and control of soil-borne pathogenic microorganisms. The functional prediction of FUNGuild in this study showed that the nutrient type of soil fungal community was higher in saprophytic nutrient type, which may be related to the dominant phylum *Ascomycota,* and most of the *Ascomycota* were saprophytic bacteria (Bai *et al.,* 2022). The cake fertilizer significantly reduced the abundance of unknown saprophytic fungi and plant pathogens, and increased the abundance of animal pathogens-faecal saprophytic fungi-endophytic fungi-plant saprophytic fungi-wood saprophytic fungi. This may be because nitrogen cake fertilizer reduced some potentially pathogenic and saprophytic microbial communities such as *Basidiomycetes*, while animal pathogenic saprophytic fungi inhibited tobacco pests (Semenov *et al.,* 2021; Su *et al.,* 2022).

Conclusions

The rapeseed cake fertilizers increased the relative abundance of *Actinobacteria, Ascomycota, Mesospora, Chaetomium* and *Mortierella* and reduced the relative abundance of *Firmicutes, Basidiomycota* and *Fusicolla*. The rhizosphere soil of flue-cured tobacco with 60% chemical fertilizer replaced by cake fertilizer had a relatively better microbial community structure. Soil-available phosphorus, soil carbon, microbial biomass carbon, and nitrogen were the main physical and chemical factors affecting the microbial community structure of flue-cured tobacco rhizosphere soil. However, there is limited research linking the interaction of soil microbes with nutrient cycling affecting the plant molecular and physiological aspects of plants after rapeseed cake fertilizer.

Authors' Contributions

Writing-original draft, Writing-review and editing: X.W and W.C; Funding acquisition, Supervision: L.C; Visualization: X.P; Resources: X.Z; Software: Z.Y; Data curation: M.M; Validation: X.M

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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