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ORIGINAL ARTICLE

Response of rhizosphere soil physicochemical properties and microbial community structure to continuous cultivation of tobacco

Bo Gong[1,2], Yi He[3], Zhenbao Luo[3], Huawei Peng[3], Heqing Cai[3], Yuening Zhu[1,2], Jun Bin[1,2]* and Mengjiao Ding[1,2]*

Abstract

Purpose The health of rhizosphere soil microorganisms is an important indicator to evaluate soil quality. Therefore, understanding the response of rhizosphere soil microorganisms to tobacco crop succession is crucial for promoting the sustainable development of agriculture.

Methods The microbial diversity and community structure of rhizosphere soil in continuous cropping and non-cropped tobacco for 7 years were analyzed by the Illumina platform.

Result (1) Continuous cropping tobacco cause rhizosphere soil acidification and reduction in alkaline nitrogen (AN) and soil organic matter (SOM). (2) Continuous cropping tobacco reduces the diversity of rhizosphere soil microbial communities, increasing harmful functional microorganisms and declining beneficial ones. (3) The abundance of bacteria that perform nitrification and saprophytic fungi in the rhizosphere soil of continuous cropping areas decreases, inhibiting carbon and nitrogen cycling processes. (4) The composition and diversity of the soil rhizosphere microbial community are affected by the imbalance in the physicochemical property of the rhizosphere.

Conclusion Continuous cropping tobacco cause rhizosphere soil acidification and nutrient imbalance, and the carbon and nitrogen cycles involved in microorganisms were damaged. Furthermore, the decreased diversity of rhizosphere soil microorganisms and the increased abundance of pathogenic fungi contribute to the continuous cropping obstacles of tobacco.

Keywords Tobacco, Continuous cropping obstacle, Rhizosphere microorganisms, Microbial communities and diversity, Soil physicochemical properties, Nutrient cycling

*Correspondence:
Jun Bin
jbin@gzu.edu.cn
Mengjiao Ding
952156929@qq.com
Full list of author information is available at the end of the article

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Introduction

Tobacco plays a crucial role in the economic industry of Guizhou Province and is a major contributor to tobacco production in China. Due to the limitations of arable land resources and the market economy, as well as a lack of sustainable planting concepts (Wang et al., 2020), continuous tobacco cropping has become prevalent (He et al. 2011). Several issues have arisen due to long-term continuous cropping, such as decreased soil quality, increased disease-causing pathogens (Rao et al., 2022; Li et al. 2022), and decreased tobacco yields (Liu et al. 2012; Guo et al. 2017; You et al. 2015). These problems negatively impact the sustainability of the tobacco growing industry.

Rhizosphere soil microorganisms are important components of soil ecosystems and play crucial roles in soil nutrient transformation and cycling (Jacoby et al. 2017), plant growth and development, and pathogen defense (Shao et al. 2021; Liu et al. 2021; Wu et al. 2022; Yang et al. 2019; Jacoby et al. 2017). They are also significant indicators of soil health. Continuous cropping cause many issues, for instance, the decline in the diversity of soil microbial populations and the rise of the dominant pathogenic species (Liu et al., 2023; Liu et al. 2022; Zheng et al. 2022). Those conditions are not conducive to the sustainable development of soil ecosystems. Few studies have investigated the alterations in the structure and physicochemical characteristics of rhizosphere soil microbial communities and their interrelationships in the "honey-sweet and fragrant type" of tobacco continuous cropping in Guizhou Province. Thus, comprehending the alterations in the microbial community structure and physicochemical properties of soil in tobacco rhizosphere due to continuous cropping obstacles is crucial to alleviate the problems related to such cropping in tobacco plantation soil. This understanding will contribute to the sustainable utilization of the soil and ensure the superior quality of tobacco production.

In this study, high-throughput sequencing technology were used to investigate the structure and diversity of rhizosphere soil microorganisms in continuous cropping tobacco fields, and Pearson correlation and mantel correlation analysis was adopted to analyze the relationship between rhizosphere soil microorganisms and physicochemical properties. This study aims to provide a reference for mitigating continuous crop barriers.

Materials and methods

Study site and soil sampling

The sampling location for soil was in Longping Village (106° 3′ E, 27° 23′ N), Bijie City, Guizhou Province. The region sits between 1390 and 1420 m above sea level. The local region has an average annual temperature of around 15 °C. This study was set two tobacco cropping systems, including non-continuous tobacco soil, and soil continuous tobacco for 7 years. Both cropping patterns had the same soil characteristics and management practices. Soil samples were collected from the rhizosphere of five tobacco plants in each field during the peak period of tobacco growth (60 days after transplanting) using the 5-point sampling method. A total of six mixed samples (two treatments with three replicates each) were collected. The tobacco plants were carefully extracted and shaken to remove any excess soil, and the rhizosphere soil within 0–4 mm of the roots was collected. The soil samples were immediately transported to the laboratory with dry ice. After removing visible residues, part of the samples was dried naturally to measure soil physical and chemical indices, and the remaining was stored at -80 °C for measuring microbial indicators.

Soil physicochemical analysis

The soil's physical and chemical properties were determined according to the "Soil Agricultural Chemical Analysis" (Bao et al., 2000). Soil pH was measured using a pH meter with a suspension ratio (w/v) of 1:2.5 using the potentiometric method. The soil organic matter (SOM) was oxidized using the potassium dichromate heating method in an oil bath under heating conditions. The soil organic carbon was oxidized with potassium dichromate-sulfuric acid and titrated using a 0.2 mol/L ferrous ammonium sulfate standard solution. The alkaline nitrogen (AN) was measured by the alkaline diffusion method, in which nitrate nitrogen ($NO-3$-N) in the soil was converted to ammonium nitrogen ($NH+4$-N) under alkaline conditions. The absorption was done using 20 g/L boric acid, and titration was performed using a 0.01 mol/L standard sulfuric acid solution. The soil's available phosphorus (AP) was extracted using a 0.5 mol/L sodium bicarbonate solution and measured by the molybdenum blue colorimetric method. Color development was achieved using an aqueous solution of molybdenum-antimony (a combination of molybdate and antimony potassium tartrate), followed by detection using an ultraviolet spectrophotometer at a wavelength of 700 nm. The soil's available potassium (AK) was measured via flame photometry, with a neutral 1 mol/L solution of ammonium acetate as the leaching agent. Ammonium ions within the solution were substituted with potassium ions on the soil colloid surface, and potassium ions in the leachate were detected using flame photometry.

DNA extraction and sequencing

The DNA was extracted with the TGuide S96 Magnetic Soil /Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.) according to manufacturer instructions. The

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DNA concentration of the samples was measured with the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Oregon, USA). The 338F: 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′ universal primer set was used to amplify the V3-V4 region of 16S rRNA gene from the genomic DNA extracted from each sample. The internal transcribed spacer 1 between 18S and 5.8S rRNA genes (ITS1 rRNA) was amplified by PCR using primers 5′-CTTGGTCATTTAGAGGAA GTAA-3′ and 5′-GCTGCGTTCTTCATCGATGC-3′. The PCR was performed in a total reaction volume of 10 µl: DNA template 5–50 ng, Vn F (10 µM) 0.3 µl, Vn R (10 µM) 0.3 µl, KOD FX Neo Buffer 5 µl, dNTP (2 mM each) 2 µl, KOD FX Neo 0.2 µl, ddH$_2$O up to 10 µl. Vn F and Vn R were selected according to the amplification area. After with initial denaturation at 95 ℃ for 5 min, followed by 25 cycles of denaturation at 95 ℃ for 30 s, annealing at 50 ℃ for 30 s, extension at 72 ℃ for 40 s, and a final step at 72 ℃ for 7 min. The PCR amplicons were purified with Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN) and quantified using the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Oregon, USA). After the individual quantification step, amplicons were pooled in equal amounts. Illumina NovaSeq 6000 (Illumina, Santiago CA, USA) was used for sequencing for the constructed library.

Bioinformatics

The bioinformatics analysis of this study was performed with the aid of the BMK Cloud (Biomarker Technologies Co., Ltd., Beijing, China). According to the quality of a single nucleotide, raw data was primarily filtered by Trimmomatic v0.33 (Edgar et al., 2013). Identification and removal of primer sequences were achieved by Cutadapt v1.9.1 (Callahan et al. 2016). PE reads previously obtained were assembled by USEARCH v10 (Segata et al., 2011), followed by chimera removal using UCHIME v8.1 (Quast et al. 2013). The obtained high-quality reads were used in the bioinformatics statistical analysis.

Statistical analysis

Sequences with similarity ≥ 97% were clustered into the same operational taxonomic unit (OTU) by USEARCH

v10.0 (Edgar et al., 2013), and the OTUs with abundance < 0.005% were filtered. Taxonomy annotation of the OTUs was performed based on the naive Bayes classifier in QIIME2 (Bolyen et al. 2019) using the SILVA database (Quast et al. 2013) (release 132) with a confidence threshold of 70%. Sequences with similarity ≥ 97% were clustered into the same operational taxonomic unit (OTU) by USEARCH v10.0 (Edgar et al., 2013), and the OTUs with abundance < 0.005% were filtered. Taxonomy annotation of the OTUs was performed based on the Naive Bayes classifier in QIIME2 (Bolyen et al. 2019) using the SILVA database (Quast et al. 2013) (release 132) with a confidence threshold of 70%. The Alpha diversity was calculated using the QIIME2 and R software. Beta diversity was calculated to measure the similarity of microbial communities from different samples using QIIME. Principal component analysis (PCA) was used to analyze the beta diversity. Linear discriminant analysis (LDA) effect size (LEfSe) was adopted to test the significant taxonomic difference among groups. A logarithmic LDA score 4.0 was set as the discriminative feature threshold to identify significant variation. To explore the dissimilarities of the microbiome among different factors, the correlation between microbial communities and soil properties was determined using the Mantel test and the Pearson test. The fungal functions were analyzed using FUNGuild v1.0 (Nguyen et al. 2016). The bacterial functions were analyzed using FAPROTA v1.2.6 (Louca et al. 2016). Soil physicochemical parameters, microbial alpha diversity indices, and functional prediction data were compared, and a normal distribution was ensured. Statistical analyses were conducted using SPSS Statistics v26. The statistical significance of the two treatments was determined using an analysis of variance (ANOVA) with post hoc multiple comparisons of variances (Amenu et al., 2023).

Results

Physicochemical properties of rhizosphere soils

The physical and chemical properties of continuous and non-continuous rhizosphere tobacco soils are shown in Table 1. There has a significant difference in physicochemical properties between the CK and CM groups. All physicochemical properties (pH, SOM, AP, AK, and AN) of CM are lower than those of the CK group, indicating that the nutrient status of the soil with continuous

Table 1 Analysis of variance of soil physicochemical properties

Sample	pH	SOM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
CK	6.80 ± 0.00a	39.21 ± 0.71a	102.63 ± 1.42a	60.00 ± 1.08a	178.47 ± 0.31a
CM	4.83 ± 0.01b	20.81 ± 0.48b	69.99 ± 0.90b	35.83 ± 0.90b	169.70 ± 0.34b

CK soil from non-continuous crop farmland, *CM* soil from planting tobacco for 7 years. Data are presented as the mean ± standard error (*n* = 3), and different letters in the same column indicate significant differences between different treatments (*p* < 0.01)

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cropping diseases is lower than that of the soil with normal non-cropped conditions. According to the classification of soil nutrient levels for tobacco cultivation (Wu et al. 2014; Yang et al. 2022), the rhizosphere soil in the CM group presents a nutrient imbalance with acidic pH and AN deficiency. However, the effective nutrients in the rhizosphere soils of the CK group were generally harmonized and showed partial enrichment levels, and the soil pH was neutral. It can be inferred that continuous tobacco cultivation over multiple years has led to soil acidification, resulting in an imbalance and deficiency of soil nutrients.

Diversity of bacterial and fungal communities in rhizosphere soil

Table 2 presents the results of the Alpha diversity indices ANOVA for bacteria and fungi obtained through amplicon sequencing. The diversity and richness indices (Simpson and Shannon indices, ACE, and Chao1 indices) of soil bacterial communities in the CM group were significantly lower than those of the CK group. This finding suggests that successive years of cultivating tobacco have significantly reduced the diversity of soil bacterial communities. Within the fungi group, the Simpson and Shannon indices of the CM community were considerably lower than those of the CK group. The ACE and Chao1 indices were higher in the CM group than in the CK group, but the differences were not statistically significant. These findings reveal that the continuous cultivation of tobacco increases the richness of rhizosphere soil fungi. However, a significant decrease is observed in both the abundance and evenness of these fungi.

The structure and composition of bacterial and fungal communities in the rhizosphere soil

OTU clustering was performed on each set of soil samples to conduct a comparative study of the bacterial and fungal community composition in rhizosphere soils of continuous cropping areas and non-continuous normal soils. This analysis has yielded 25 phyla, 77 classes,

170 orders, 282 families, 493 genera, and 554 species of bacteria. Additionally, 8 phyla, 27 classes, 65 orders, 135 families, 262 genera, and 307 species were analyzed for fungi. In the bacterial phylum-level analysis (Fig. 1a), the top 10 bacteria in relative abundance are Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Patescibacteria, Nitrospirae, Firmicutes, and Proteobacteria is the most dominant bacterium in this group. The relative abundance shares of Proteobacteria, Actinobacteria, Verrucomicrobia, and Firmicutes increased in the CM group compared with the CK group. Conversely, the relative abundance percentage of Acidobacteria, Gemmatimonadetes, Chloroflexi, Bacteroidetes, and Nitrospirae decreases. At the genus level of bacteria (Fig. 1c), the ten most abundant bacteria in terms of relative abundance are *Sphingobium*, *Rhodanobacter*, *Bradyrhizobium*, and *Gemmatimonas*, plus one additional "Candidatus Udaeobacter" and five "uncultured bacterium" bacteria. *Sphingobium* is the dominant bacterium. Compared with the CK group, the relative abundance percentage of *Sphingobium* decreases in the CM group, while the relative abundance ratio of *Rhodanobacter* and *Bradyrhizobium* increases. These findings suggest that continuous cropping alters the community structure of rhizosphere soil bacteria.

Regarding the fungal phylum level (Fig. 1b), the most abundant fungi, in descending order, are Ascomycota, Basidiomycota, Mortierellomycota, Chytridiomycota, Rozellomycota, Olpidiomycota, Glomeromycota, and Kickxellomycota. Ascomycota is the most prevalent fungus. Compared with the CK group, the relative abundance of Ascomycota and Mortierellomycota decreases in the CM group, whereas the proportion of Basidiomycota and Chytridiomycota increases. At the genus level of fungi (Fig. 1d), the most abundant genera among the top 10 are *Purpureocillium*, *Fusarium*, *Mortierella*, *Penicillium*, *Aspergillus*, *Gliocladiopsis*, *Cladosporium*, *Ilyonectria*, *Epicoccum*, and *Conocybe*, with *Purpureocillium* being the most dominant. Compared with the CK group,

Table 2 Analysis of variance of bacterial and fungi alpha diversity index

Sample ID	ACE	Chao1	Simpson	Shannon	Coverage
Bacteria					
CK	1994.04 ± 9.12a	2005.19 ± 6.61a	0.9941 ± 0.00a	9.34 ± 0.12a	0.9974
CM	1790.59 ± 37.72b	1834.71 ± 72.46b	0.9914 ± 0.00b	8.59 ± 0.06b	0.9964
Fungi					
CK	658.79 ± 8.41a	676.29 ± 16.54a	0.9810 ± 0.00a	7.05 ± 0.04a	0.9997
CM	678.37 ± 29.36a	681.26 ± 31.16a	0.9721 ± 0.00b	6.50 ± 0.16b	0.9996

CK soil from non-continuous crop farmland, *CM* soil from planting tobacco for 7 years. Data are presented as the mean ± standard error ($n = 3$), with significant differences between alpha diversity index values indicated by different letters in the same column at $p < 0.05$
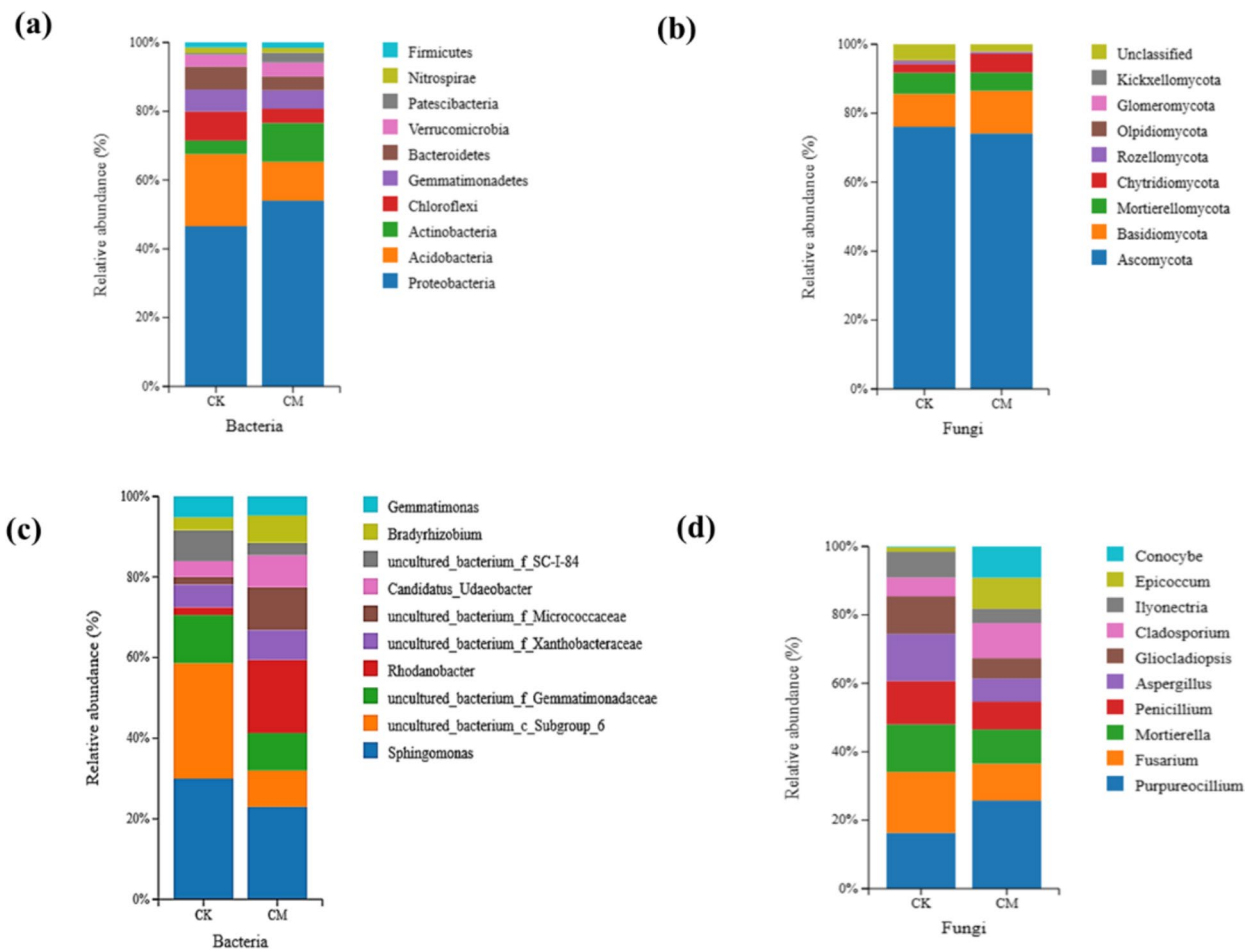
Fig. 1 Relative abundance of bacterial and fungal communities of soil samples. **a** bacterial phyla; **b** fungal phyla; **c** bacterial genera; **d** fungal genera. CK: soil from non-continuous crop farmland. CM: soil from planting tobacco for 7 years

the proportion of *Purpureocillium, Cladosporium, Epicoccum*, and *Conocybe* increases, while the proportion of *Fusarium, Mortierella, Penicillium, Aspergillus, Gliocladiopsis*, and *Ilyonectria* decreases. These results suggest that the community structure of rhizosphere soil fungi has undergone a significant alteration due to continuous cropping.

Differences in the distribution of bacterial and fungal communities in the rhizosphere soil

The differences in bacterial community structure between non-continuous cropping normal rhizosphere soil and continuous cropping diseased rhizosphere soil were compared using PCA based on the Bary Curtis algorithm. The variation in microbial composition can be explained by the total sum of the first and second principal component axes being over 50%. As shown in Fig. 2, the overall explanatory reliability of bacteria and fungi PC1 and PC2 is high, while the CK and CM groups are distributed independently with a significant

distance between them. The result indicates a significant difference in bacterial and fungal community structure between normal rhizosphere soils with continuous and non-continuous cropping practices. To further determine the differential distribution of species in soil between the CM and CK groups, LEfSe analysis was performed on the results (Fig. 3). There are six species of bacteria at the level of the phylum and three at the level of the cultivable genus of bacteria. These species are classified as Proteobacteria, Patescibacteria, Acidobacteria, Actinobacteria, Chloroflexi, and Bacteroidetes at the phylum level and *Sphingobium*, *Bradyrhizobium*, and *Rhodanobacte*r at the cultivable genus level, as shown in Fig. 3a. There are numerous distinct species of fungi, with two phylum-level and seven genus-level species. The phylum-level species included Ascomycota and Chytridiomycota, while the genus-level species were *Purpureocillium, Fusarium, Aspergillus, Gliocladiopsis, Cladosporium, Epicoccum*, and *Conocybe*, as shown in Fig. 3b.
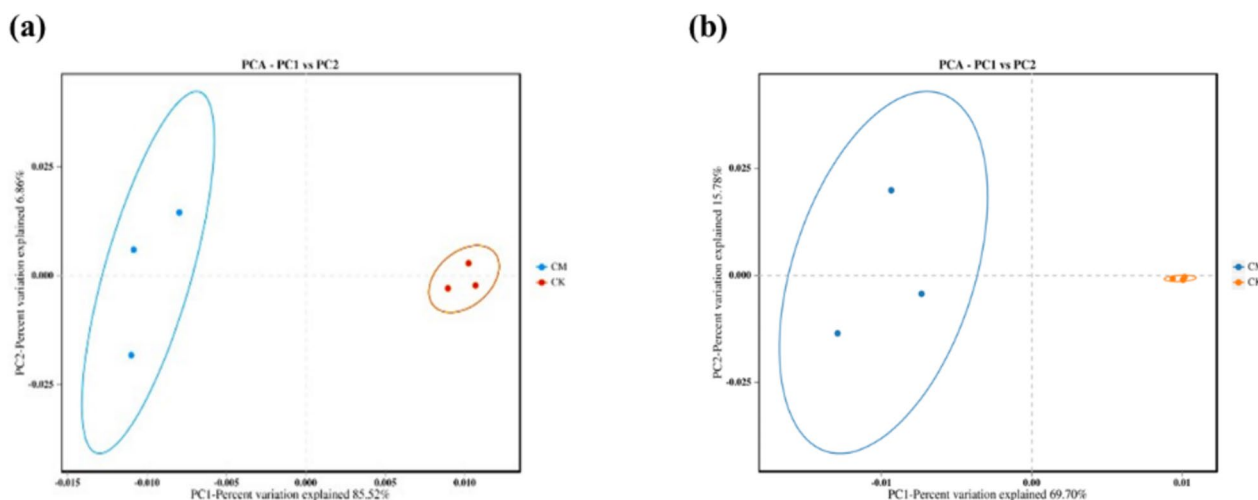
Fig. 2 Major variations in the bacterial and fungal communities in soil samples detected by the PCA of the OTUs. **a** Bacteria. **b** Fungi. CK: soil from non-continuous crop farmland. CM: soil from planting tobacco for 7 years

The relationship between rhizosphere microorganisms and physicochemical properties

To investigate the relationship among rhizosphere soil microbiota, physicochemical properties, and species diversity, we employed Pearson correlation analysis and the Mantel test to perform a correlation analysis of species diversity, physicochemical properties, and alpha diversity. The results indicate (Fig. 4) positive correlations among the physicochemical properties of the rhizosphere soil each other. Within the bacterial community (Fig. 4a), the bacterial genera showed a significantly positive correlation with soil SOM and AP. Chao1 and ACE, the indicators for bacterial abundance, were also significantly positively correlated with soil pH, SOM, and AK. In addition, the diversity indices (Shannon and Simpson) were significantly positively correlated with soil SOM, AN, and AP. These observations indicate that the species composition and diversity of bacterial rhizosphere soils are affected by soil physicochemical properties. The impacts of soil SOM and AN on the bacterial community were particularly significant. Similarly, the fungal genera showed a significantly positive correlation with soil SOM and AK (Fig. 4b). Shannon and Simpson exhibited a significantly positive correlation with soil SOM, AN, and AK. In contrast, Chao1 and ACE were not significantly correlated with soil physicochemical properties. These findings suggest that soil physicochemical properties primarily affect the rhizosphere soil community composition and species diversity of fungi. Additionally, AN was observed to have a highly significant impact on fungal diversity.

Pearson correlation analyses were conducted to analyze the top 10 rhizosphere soil microorganism communities to explore the effects of soil physicochemical properties on rhizosphere soil species. Concerning the bacterial community (Fig. 5a), the amount of *Bradyrhizobium* was significantly negatively correlated with SOM and AN. Furthermore, the quantity of *Rhodanobacte*r was significantly negatively correlated with pH, SOM, and AN. In the fungal community (Fig. 5b), the abundance of *Purpureocillium* was significantly negatively correlated with SOM and AK. The concentration of *Fusarium* was significantly positively correlated with AK. Additionally, the content of *Penicillium* demonstrated a significantly positive correlation with AK, AN, pH, and SOM. The abundance of *Aspergillus* indicates significant positive correlations with SOM, AP, AN, and pH. A significantly positive correlation was observed between the quantity of *Ilyonectria* in soil and AK, AN, and pH. The abundance of *Epicoccum* was significantly negatively correlated with the pH of SOM, AP, and AN.

Microbial gene function prediction in rhizosphere soil of different groups

The bacterial function was predicted in the two treatment groups using the functional annotation of prokaryotic taxa (FAPROTAX), and bacteria were annotated into 45 functional groups. Ten functional groups with the highest relative abundance were chemoheterotrophy, aerobic chemoheterotrophy, nitrification, ureolysis, nitrate reduction, aerobic ammonia oxidation, nitrogen fixation, fermentation, aerobic nitrite oxidation, and aromatic compound degradation (Fig. 6a).

FUNGuild was employed to predict the trophic functions of fungal communities subjected to different treatments. They are dominated by Pathogens and
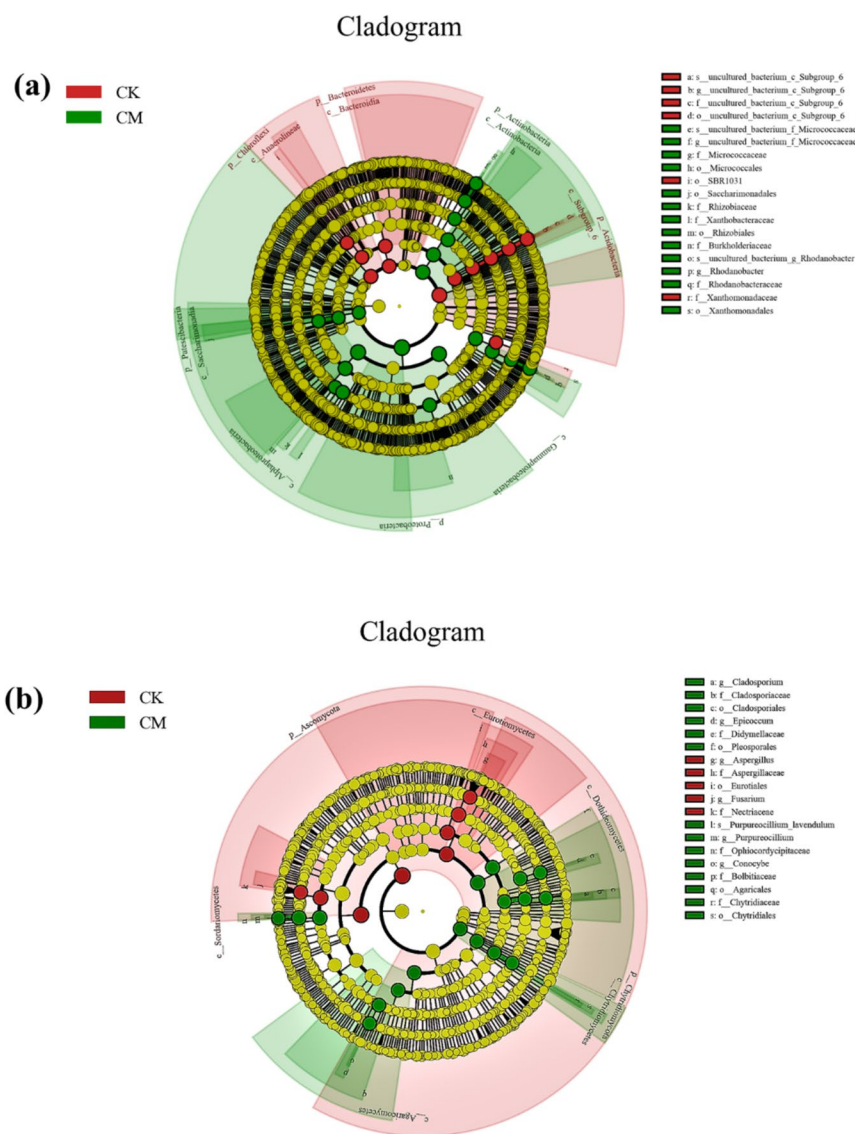
Cladogram

Cladogram

Fig. 3 The Cladogram showing that LEfSe analysis of soil bacteria and fungi. **a** Bacteria. **b** Fungi. CK: soil from non-continuous crop farmland. CM: soil from planting tobacco for 7 years. Taxa with significant differences in abundance between groups are indicated by colored dots, yellow indicates no differences, and the branching map circles represent phylogenetic taxa from phylum to genus

Saprotrophs and were further categorized into 23 functional groups. According to Fig. 6b, the top ten fungal functional groups were ranked by relative abundance, including undefined saprotroph, fungal parasite, plant pathogen, animal pathogen, wood saprotroph, algal parasite, dung saprotroph, plant saprotroph, endophyte, and ectomycorrhizal.

We conducted a variance analysis on the predicted functions in bacteria and fungi to examine the variations in functional genes within the rhizosphere soil microbiome under different treatments. The results show that the bacterial functional genes involved in the nitrogen cycle change significantly in the continuous cropping soils (Fig. 7a), with a significant decrease in the relative abundance of nitrification-related functional genes and an increase in the relative abundance of nitrogen-fixing functional and ureolytic genes. The study suggests a decline in the population of nitrification-related bacteria in soils undergoing continuous cropping, and the proportion of bacteria capable of converting external nitrogen sources is increased. In addition, the relative abundance of aromatic compound degradation is significantly upgraded, indicating that continuous cropping increases the organic contaminants in the soils.
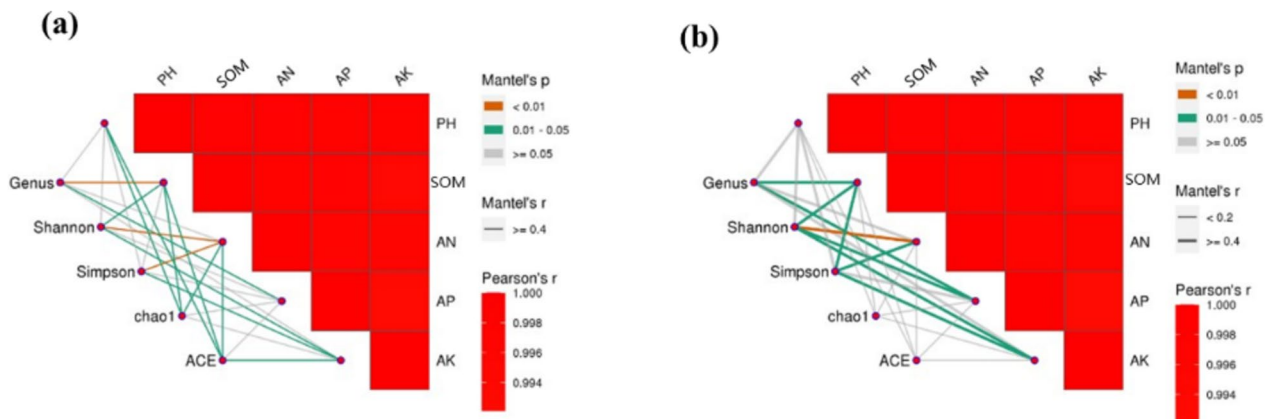
Fig. 4 A correlation heatmap and network map of environmental factors with alpha diversity index and bacterial and fungal communities at the genus level. **a** Bacteria. **b** Fungi. Mantel's *p* is the *p*-value of correlation of soil physicochemical properties with genus and Alpha index. Mantel's *r* is the *r* value of Mantel analysis of soil physicochemical properties with genus and Alpha index. Pearson's *r* is the *p*-value for correlation of soil physicochemical properties with genus and Alpha index
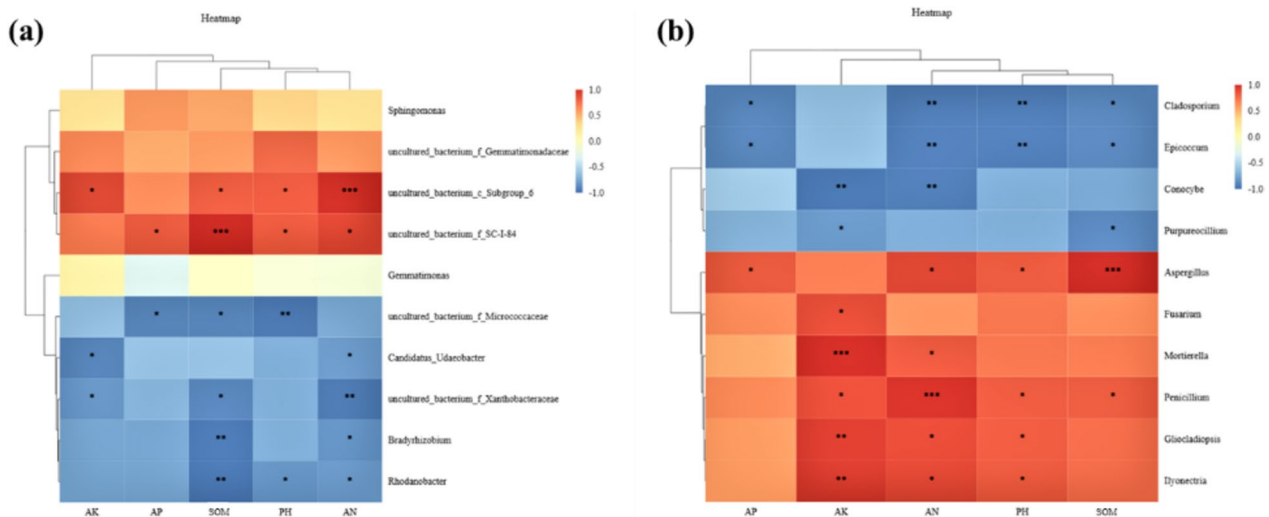

Fig. 5 A correlation heatmap between environmental factors and bacterial and fungal communities at the genus level. **a** Bacterial genera. **b** Fungal genera. Correlations between environmental factors and genera were indicated by different colors and "*"

Notably, alterations in the function of fungi were apparent in continuous cropping soils (Fig. 7b). The relative abundance of fungal parasites significantly increases, and that of plant pathogens decreases noticeably, indicating that the parasitic pathogenic fungi gradually increase in successional cropping soils. In contrast, the relative abundance of wood, dung, and plant saprotrophs declines significantly, along with a reduction in the relative abundance of symbiotrophs. This decrease implies that the beneficial functional fungi gradually decrease in the continuous cropping soil.

Discussion

Soil microorganisms are crucial in agroecosystems since they determine soil fertility, crop productivity, and resistance reversal (Hartmann and Six 2023). This study compares the diversity, structural characteristics, physicochemical properties, and correlations between the fungal and bacterial communities in the rhizosphere soil without continuous cropping and that of a tobacco plant affected by disease after 7 years of continuous cropping. The results indicate that continuous cropping leads to soil acidification in roots, nutrient imbalances, and reduced diversity within the microbial community and
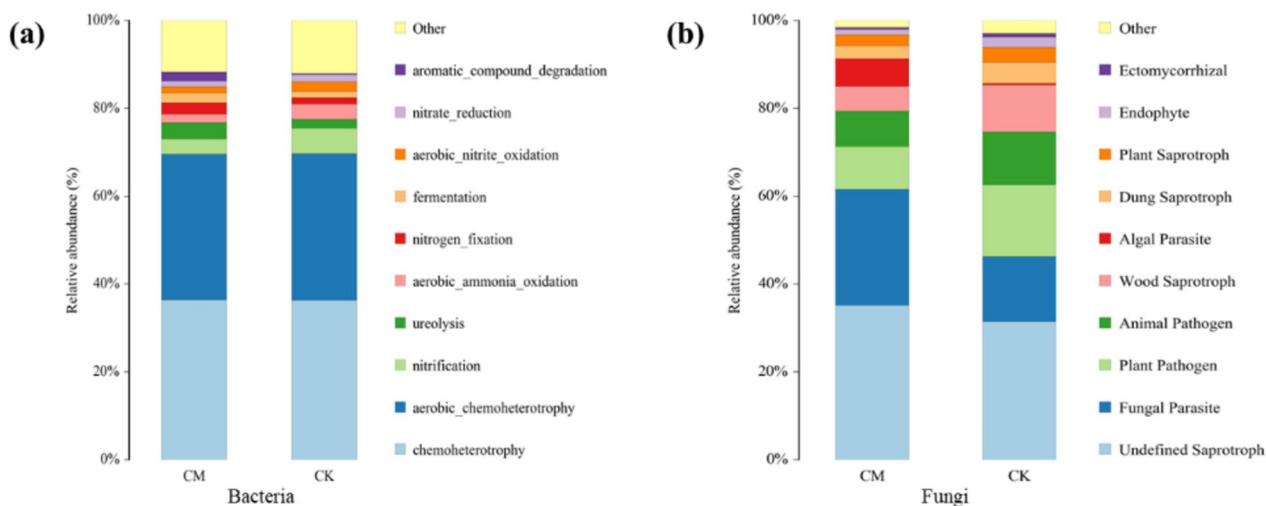
Fig. 6 Relative abundance of in functional groups on bacterial and fungal OUT data in soil samples. **a** Functional prediction of bacterial. **b** Functional prediction of fungi. CK: soil from non-continuous crop farmland. CM: soil from planting tobacco for 7 years

alters community structure. Alterations in soil nutrient levels in roots influence the rhizosphere microbial community composition. Such changes in microbial community characteristics cause tobacco morbidity.

Continuous cropping leads to nutrient imbalances and soil acidification in the rhizosphere soil

Crops are characterized by selective uptake or enrichment of nutrients (Lu et al. 2020). Prolonged and continuous monoculture may disturb the nutrient equilibrium in the soil (Liu et al. 2002; Jin et al. 2002; Zhang et al. 2010). This study has shown that continuous cropping leads to a decline in soil nutrient status and an imbalance in the rhizosphere compared to the balanced and high-nutrient status found in non-continuous cropping soils. Effective nutrients in the continuous cropping soils exhibit significantly lower pH, SOM, AN, AP, and AK than those in non-continuous cropping soils. Prolonged continuous cultivation causes progressive soil acidification (Zhang et al. 2010; Tian et al. 2011). In this study, the pH of the soils between the roots of continuous cultivation decreased to 4.83, much lower than the pH range of high-quality tobacco (5.8–6.5) (Yang et al. 2022). The low pH could potentially affect the uptake and use of effective soil nutrients by the tobacco root system (Fig. 4). Soil acidification may be attributed to the prolonged continuous cultivation of tobacco, a result of the accumulation of phenolic acid-secreted substances in the root system (Zhang et al. 2018; Bai et al. 2019), and the imbalanced application of fertilizers (Yan et al. 2018, 2020). The SOM stability is critical

for maintaining soil fertility and crop yields (Githongo et al. 2023; Wood et al. 2018; Oldfield et al. 2019). The present study show that prolonged continuous cropping severely reduces SOM, consistent with the studies of Fujii et al. (2022) and Xie et al. (2023). Nitrogen is a crucial element for tobacco plant growth. Nitrogen deficiency can significantly affect physiological and biochemical processes essential for plant growth, such as plant cell synthesis, enzyme activity, and chlorophyll synthesis (Bai et al. 2020), ultimately influencing the intrinsic chemical qualities of tobacco (Lisuma et al. 2020). The AN content in soil subjected to continuous cropping is reduced below the threshold required for optimal growth of top-quality tobacco (Wu et al. 2014), aligning with the findings reported by Li et al. (2016). According to the above analysis, we speculate that soil alkaline nitrogen deficiency may be a triggering factor for tobacco plant susceptibility, consistent with the research results of Tian et al. (2011). Phosphorus is a limiting nutrient for plant growth in agricultural ecosystems (Hartmann and Six 2023). Our results indicate that continuous cropping can maintain the normal growth and development of tobacco despite the induced reduction in soil AP.

In summary, continuous cropping tends to acidify the soil and cause nutrient imbalances, depriving the roots of effective nutrients. Organic fertilizer and appropriate quicklime can improve the soil pH during tobacco cultivation. Growing application of organic fertilizers can also enhance the soil environment (Zhu et al. 2022; Zhang et al., 2022) and maintain fertility.
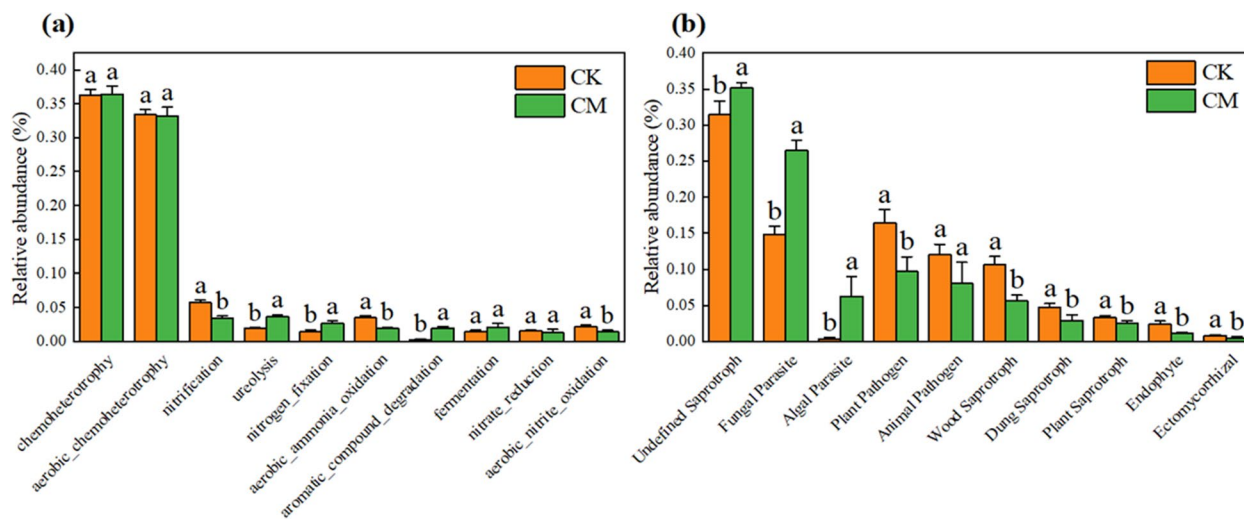
Fig. 7 An analysis of variance (ANOVA) of bacterial (**a**) and fungal (**b**) functional groups. **a** Bacteria. **b** Fungi. CK: soil from non-continuous crop farmland. CM: soil from planting tobacco for 7 years. Functional groups indicated by distinct letters exhibit notable differences at $P < 0.05$

The characteristics of microbial communities in rhizosphere soil were significantly altered due to continuous cropping

Rhizosphere soil microorganisms can reflect the arable ecosystem health and are considered a valuable indicator of soil quality (Yang et al. 2021). Rhizosphere microorganisms, known as the second genome of plants (Berendsen et al. 2012), contribute to the conversion of substances in the soil. This process is crucial for plant nutrient acquisition, development, and stress resistance (Zhang 2020; Raaijmakers and Mazzola 2016; Berendsen et al. 2012). With growing years of continuous cropping, soil microbial communities shift from "bacterial" to "fungal" soils, as observed in the works of Li et al. (2012) and Liu et al. (2016). This study found that the biodiversity of microorganisms (bacteria and fungi) in the rhizosphere with replant disease decreased, consistent with the findings reported by Liu et al. (2022) and Hou et al. (2016). When the abundance of fungi increases, the microbiota within the rhizosphere shifts towards a fungal-dominated soil.

In continuous cropping soils, the relative abundance of *Fusarium* and *Aspergillus* decreases significantly, while that of *Cladosporium* increases significantly. These substances are typically considered plant pathogens (Ge et al. 2022; Li et al. 2017; Wu et al. 2018; Rao et al., 2022; Heuchert et al., 2005; Prasannath et al., 2021). Furthermore, *Ilyonectria* and *Penicillium* are harmful fungi inducing plant diseases (Shen et al. 2018; Bhatta 2022; Xu et al. 2021; Stošić et al. 2021), and their relative abundance decreases slightly. The relative abundance of *Purpureocillium*, *Epicocum*, *Rhodanobacter*, and *Bradyrhizobium* elevates; by contrast, *Sphingomonas* decreases in relative abundance; these species are commonly considered beneficial colonies for plants (Zhang et al. 2021a, b; Taguiam et al. 2021; Huang et al. 2021b; Huo et al. 2018; Wu et al. 2022; Hartmann and Six 2023). In summary, long-term continuous cropping disrupted the balance of soil microbial communities, consistent with the study by Wu et al. (2018). The relative abundance of beneficial microbial communities increased and that of harmful pathogen communities decreased, contrary to the research results of Hou et al. (2016), Li (2022), and Wang et al. (2020). We proposed two possible explanations for this divergence: firstly, a reduction in effective soil nutrients results from prolonged continuous cropping (as shown in Table 1), leading to rapid proliferation of beneficial bacteria to transform the soil nutrients, meeting crop growth requirements. For example, *Rhodanobacter* can adapt to low-nutrient and acidic conditions and participate in the nitrogen nutrition cycle (Avijit Ghosh et al. 2022; Heuvel et al. 2010), *Bradyrhizobium*, nitrogen-fixing bacteria, participates in soil nitrogen transformation (Wu et al. 2022; Hartmann and Six 2023). It is hypothesized that the causal agent of root rot (*Fusarium*) prompts the rapid proliferation of its antagonistic bacteria and fungi to uphold the balance of soil microecology. Notably, *Purpureocillium* effectively suppresses *Fusarium*-induced root rot (Zhang et al. 2021), and *Rhodanobacter* reportedly delivers antagonistic effects against root rot pathogens (Huo et al. 2018), and both flora pronouncedly elevates in successive cropping soils, as illustrated in Fig. 1 (c and d). In conclusion, continuous cropping cultivation disturbs the equilibrium among microbial communities in rhizosphere soils and alters the microbiome configuration.

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The physicochemical properties impact the characteristics of rhizosphere soil microbial communities

The soil microbial community structure and plant growth are closely related to the physicochemical properties of soils (Liu et al. 2021a, b). Soil physicochemical properties can impact the structure and function of rhizosphere microorganisms (Song et al. 2019). The study reveals that soil acidification considerably decreases bacterial abundance and diversity, with minor changes observed in soil fungal communities. The changes reported here are consistent with the findings of the study conducted by Li et al. (2023). Organic matter and nitrogen are important components of microorganisms, providing nutrients for microorganisms (Obalum et al. 2017; Kuypers et al. 2018). In this study, it is found that SOM and AN significantly impact seven species (*Bradyrhizobium, Rhodanobacter, Purpureocillium, Aspergillus, Cladosporium, Epicoccum,* and *Conocybe*) between the two treatment groups, and *Fusarium* was only significantly positively correlated with AK. We hypothesize that decreased organic matter and nitrogen in continuous soils (refer to Table 1) enhances the dependence on parasitized tobacco for nutrients. Additionally, we propose that excessive *Fusarium* parasitism on tobacco can lead to diseases (i.e., root rot). It is demonstrated that *Sphingomonas* can degrade pesticide residues and organic contaminants in soils (Bhatia et al. 2022; Guo et al. 2010; Kant Bhatia et al., 2021; Zhang et al. 2021a), and soil physicochemical properties does not significantly impact the relative abundance of *Sphingobium*, which decreases in continuous cropping soils. Combining this observation with FAPROTAX analysis (Fig. 5), it is hypothesized that this reduction is due to the toxic effects of hazardous substance accumulation resulting from long-term continuous cropping. In addition, *Penicillium* and *Aspergillus* can exist in the soil as phosphate-solubilizing fungi (Li et al. 2023), requiring phosphorus as a nutrient. They decrease with the reduced AP, indicating a decrease in Phosphate-solubilizing content in the soil. In summary, the imbalance of soil physicochemical properties inhibits the proliferation of most rhizosphere soil microorganisms, as evidenced by the studies of Huang et al. (2021a), Yuan et al. (2019), and Zhong et al. (2020). Overall, changes in the physicochemical properties of the rhizosphere soils in continuous cropping are important factors contributing to rhizosphere microbial differences.

Significant changes were observed in the functional genes of rhizosphere soil bacteria and fungi

To elucidate the impact of continuous cropping on microbial activity, applying FAPROTAX functional prediction demonstrated a marked alteration in the relative abundance of genes implicated in the nitrogen cycle in the continuous cropping soils. The significant increase in the relative abundance of genes for nitrogen fixation and ureolysis suggests that bacteria actively contribute to nitrification. However, a pronounced reduction in the relative abundance of genes linked to nitrification is observed, specifically, aerobic ammonia, aerobic nitrite, and nitrite oxidation. This may be due to soil acidification arising from continuous cropping (Table 1) and the death of acid-intolerant bacteria. The notable rise in the proportion of aromatic compound degradation indicates a surge in soil organic pollutants, possibly attributed to pesticide residue and plastic remnants generated by the prolonged cultivation of tobacco crops for disease control.

Saprotrophic fungi represent the most significant fungal group within the fungal communities. They can degrade indissoluble residues of other organisms, including lignocellulose and chitin. This indicates that saprotrophic fungi are critical components of the carbon cycle's metabolic process (Várnai A et al. 2014). This study found a significant reduction in the relative abundance of saprophytic fungi in the continuous soil. Consequently, we hypothesized that the carbon cycle of the soil's metabolic processes had been negatively affected. Endophytic microorganisms are essential components of plants, residing within their tissues without inducing any disease (Porras-Alfaro et al., 2011). Importantly, fungal parasites are capable of transmitting viruses by contacting with the host plant through free spores and entering the plant by disrupting its cell walls (Gallet et al. 2018). Furthermore, they can attack all organisms utilizing a polysaccharide catabolic mechanism (Várnai A et al., 2014). The study found a significant increase in the relative abundance of fungal parasites in continuous cropping soils, which may be the cause of reduced levels of plant pathogens, animal pathogens and other pathogenic fungi, wood saprotrophs, dung saprotrophs, plant saprotrophs and other saprophytic fungi, and endophytes. Overall, continuous cropping has caused a shift in the rhizosphere soil fungi functions from saprotroph to pathotroph, resulting in a disturbance to the metabolic processes of the soil carbon cycle and an elevated risk of disease susceptibility in tobacco.

Conclusions

This study indicates that continuous cropping leads to rhizosphere soil acidification, nutrient imbalance, and carbon and nitrogen cycling involving microorganisms. It also reduces rhizosphere microbial diversity and increases pathogenic fungi abundance, major causes of continuous cropping disorder in tobacco. These findings complement the investigation of continuous cropping disorder in the Bijie city, Guizhou Province, providing an effective approach for managing continuous cropping in tobacco soils.

Availability of data and materials

All data generated and analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]College of Tobacco Science of Guizhou University, Guiyang 550025, China. [2]Guizhou Provincial Key Laboratory for Tobacco Quality, College of Tobacco Science, Guizhou University, Guiyang 550025, China. [3]Guizhou Tobacco Company Bijie Region Tobacco Company, Bijie 551700, China.

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