Postprint: Effects of Continuous Cassava Cropping on the Succession of Rhizosphere and Non-rhizosphere Soil Fungal Community Structure

Authors: Huang Yanying, Peng Xiaohui, Ou Guining, Peng Xiaoxue, Gan Li, Huang Yuanhang, Yang Taiyi, Qin Fengyan, Shen Zhangyou, Wei Maogui

Date: 2024-05-15T00:00:00+00:00

Abstract

To elucidate the formation mechanism of cassava continuous cropping obstacles, this study conducted three-year fixed-point field continuous cropping of cassava and employed high-throughput sequencing technology and bioinformatics approaches to investigate the effects of continuous cropping duration on the succession of fungal community structure in cassava rhizosphere and bulk soils. The results showed that: (1) Continuous cropping significantly affected the alpha diversity and both taxonomic and phylogenetic beta diversity of soil fungal communities in rhizosphere and bulk soils. (2) The predominant fungal phyla in cassava were Ascomycota, SAR supergroup, Basidiomycota, Mucoromycota, and unclassified_{k__*}{Fungi}, with the main dominant classes being Sordariomycetes, Eurotiomycetes, Dothideomycetes, and Intramacronucleata. In bulk soil, the composition of Ascomycota changed substantially, evolving from Myrothecium, Sordariomycetes, and Dothideomycetes in the first year toward a single genus Knufia; in rhizosphere soil, fungi evolved from Hypocreales, Chaetothyriales, Myrothecium, Dothideomycetes, and Sordariomycetes of Ascomycota toward the genus Monosiga within the phylum norank*{d___}{Eukaryota}. (3) Soil pH, organic matter content, alkaline-hydrolyzable nitrogen content, available phosphorus content, readily available potassium content, and catalase activity significantly influenced soil fungal community changes, particularly affecting the distribution of Ascomycota, SAR supergroup, Basidiomycota, and Mucoromycota. In summary, cassava continuous cropping leads to accumulation of root exudates, alters soil physicochemical properties and the fungal living environment, thereby inducing changes in fungal community diversity and richness; specifically, in bulk soil, Ascomycota transitioned from Myrothecium, Sordariomycetes, and Dothideomycetes toward a single genus Knufia; in rhizosphere soil, the relative abundance of beneficial fungi such as Hypocreales, Myrothecium, and Sordariomycetes of Ascomycota decreased with increasing continuous cropping years, ultimately triggering cassava continuous cropping obstacles.

Full Text

Effects of Continuous Cropping on Fungal Community Structure Succession in Rhizosphere and Non-rhizosphere Soils of Cassava

HUANG Yanying[1], **PENG Xiaohui**[1], **OU Guining**[1], **PENG Xiaoxue**[1], **GAN Li**[1], **HUANG Yuanhang**[1], **YANG Taiyi**[1], **QIN Fengyan**[1], **SHEN Zhangyou**[2], ***WEI Maogui***[1]**,**[3]

[1]College of Agriculture, Guangxi University, Nanning 530004, China;
[2]Cash Crops Research Institute, Guangxi Academy of Agricultural Sciences, Nanning 530007, China;
[3]Guangxi Key Laboratory of Agro-environment and Agro-products Safety, Nanning 530004, China

Abstract

To reveal the formation mechanism of cassava continuous cropping obstacles, this study investigated the effects of continuous cropping duration on fungal community structure succession in cassava rhizosphere and non-rhizosphere soils using high-throughput sequencing technology and bioinformatics approaches in a fixed field experiment over three years. The results showed that: (1) Continuous cropping significantly affected the α-diversity and both taxonomic and phylogenetic β-diversity of fungal communities in cassava rhizosphere and non-rhizosphere soils. (2) The dominant fungal phyla were Ascomycota, SAR supergroup, Basidiomycota, Mucoromycota, and unclassified_{k__}*{Fungi}, while the dominant classes included Sordariomycetes, Eurotiomycetes, Dothideomycetes, and Intramacronucleata. In non-rhizosphere soils, Ascomycota composition changed substantially, evolving from Myrothecium, Sordariomycetes, and Dothideomycetes in the first year to a single genus* Knufia*. In rhizosphere soils, fungi evolved from Hypocreales, Capnodiales, Myrothecium, Dothideomycetes, and Sordariomycetes within Ascomycota toward* Monosiga *belonging to norank*{d___}{Eukaryota}. (3) Soil pH, organic matter content, available nitrogen content, available phosphorus content, available potassium content, and catalase activity significantly influenced soil fungal community changes, particularly affecting the distribution of Ascomycota, SAR supergroup, Basidiomycota, and Mucoromycota. In conclusion, continuous cassava cropping leads to accumulation of root exudates, altering soil physicochemical properties and the fungal living environment, thereby causing changes in fungal community diversity and abundance. Specifically, Ascomycota in non-rhizosphere soils evolved from Myrothecium, Sordariomycetes, and Dothideomycetes to a single genus *Knufia*, while the relative abundance of beneficial fungi such as Hypocreales, Myrothecium, and Sordariomycetes within

Ascomycota in rhizosphere soils decreased with increasing continuous cropping years, ultimately triggering cassava continuous cropping obstacles.

Key words: cassava (Manihot esculenta), continuous cropping, fungi, rhizosphere and non-rhizosphere soils, microbial diversity

Introduction

Cassava (Manihot esculenta), belonging to the family Euphorbiaceae, is one of the world's three major root crops. It exhibits tolerance to barren, drought, and acidic soil conditions. As a C3 plant with relatively high photosynthetic efficiency, cassava has a theoretical yield potential of 120 $t \cdot ha^{-1}$ and high fresh tuber yield potential (Zhang et al., 2012), serving as an important raw material for starch and alcohol processing in China. Currently, China's cassava industry faces challenges including large raw material deficits, high trade dependency, extensive cultivation management, and low economic benefits (Liang et al., 2016). Guangxi, as China's primary cassava cultivation and processing region, accounts for 60% of national planting area and processing output (Yang, 2020). However, continuous cropping of cassava is widespread in Guangxi. Research by Liang et al. (2017) demonstrated a negative correlation between cassava yield and continuous cropping duration: farmers in the lowest 25% yield bracket (average yield 27.00 $t \cdot ha^{-1}$) had an average continuous cropping history of 14.29 years, while those in the highest 25% yield bracket (average yield 55.38 $t \cdot ha^{-1}$) averaged only 2.54 years of continuous cropping. This indicates that continuous cropping obstacles represent a critical factor limiting yield improvement in China's major cassava production regions. Therefore, investigating the formation mechanisms of cassava continuous cropping obstacles and developing mitigation measures is essential for enhancing cassava yield in China.

Continuous cropping obstacle refers to the phenomenon where continuous cultivation of the same or related crops on the same land leads to weakened plant growth, increased pests and diseases, and reduced yield and quality (An et al., 2019). Previous research has attributed continuous cropping obstacles to deteriorating soil physicochemical properties, accumulation of autotoxic substances, and changes in soil microbial flora (Yang et al., 2011). These three factors are closely linked to soil, interact with each other, and form interactive relationships. Among them, microorganisms play a crucial role in continuous cropping obstacle formation. Allelochemicals accumulated in continuously cropped soils drive microbial community succession, ultimately leading to pathogen proliferation, beneficial bacteria reduction, and soil-borne diseases (Hou et al., 2016). Beyond inducing soil-borne diseases, soil microorganisms, as essential components of soil ecosystems, decompose organic matter, participate in nutrient cycling, and play key roles in ecosystem energy flow and material cycling (He et al., 2022). Their biodiversity is primarily influenced by vegetation, soil type, temperature, moisture, and management practices (Zhou and Lei, 2007). Numerous studies have shown that the quantity, species, metabolic activity of rhizosphere soil microorganisms, and their interactions can enhance plant resistance and promote growth, or parasitize plants and cause diseases, thereby affecting plant health (Berendsen et al., 2012; Sun et al., 2015; Terhonen et al., 2019; De et al., 2019).

Extensive research indicates that continuous cropping transforms soil microorganisms from bacterial-dominated to fungal-dominated communities, marking one indicator of soil fertility decline caused by continuous cropping. For example, continuous potato cropping leads to enrichment of specific root exudates, reducing rhizosphere soil microbial richness with increased fungal proportion and decreased bacterial proportion (Ma et al., 2015). In continuously cropped tobacco fields, bacterial populations decrease annually while fungal populations increase (Wang et al., 2008). In tomato soils, bacterial quantity negatively correlates with continuous cropping years, while fungal quantity shows the opposite trend (Sun et al., 2010). Long-term continuous soybean cropping significantly increases soil fungal abundance, whereas continuous maize cropping significantly decreases it, and continuous wheat cropping shows no significant change in fungal abundance. These varying trends suggest that different crops affect soil microbial communities differently, likely due to variations in root exudate composition and quantity, as well as residual root and litter components (Liu, 2019). However, the mechanisms underlying the effects of cassava continuous cropping on soil microbial community succession remain unclear.

Recent studies on cassava continuous cropping obstacles have focused primarily on impacts on soil physicochemical properties and soil microorganisms (Zhou, 2017; Liu, 2020). Cassava continuous cropping obstacles are closely related to deteriorating soil physicochemical properties, declining available nitrogen-phosphorus-potassium content, altered soil microbial composition, and accumulation of organic acids from cassava root exudates. Zhou (2017) reported that cassava continuous cropping deteriorates rhizosphere soil three-phase ratios, inhibits soil catalase activity, increases phenolic acid content, and reduces pH. Liu (2020) found that continuous cassava cropping increases soil fungal population quantity but decreases community abundance and diversity, with reduced soil available nitrogen and potassium content.

Since plant root exudates directly regulate rhizosphere microbial ecosystems, they inevitably cause differential changes between rhizosphere and non-rhizosphere soil microbial communities. Therefore, systematic investigation of the effects of cassava continuous cropping on fungal community succession in both rhizosphere and non-rhizosphere soils is necessary. Based on a fixed-site continuous cassava cropping experiment, this study employed bioinformatics and high-throughput sequencing technology to analyze the impacts of continuous cropping on fungal community succession in cassava rhizosphere and non-rhizosphere soils, providing a theoretical basis for systematically elucidating the formation mechanisms of cassava continuous cropping obstacles.

Materials and Methods

1.1 Experimental Site Description

The experiment was conducted from 2019 to 2021 at the Wuming Lijian Experimental Base of Guangxi Academy of Agricultural Sciences in Wuming District, Nanning, Guangxi (107°49 26 E, 22°59 58 N). The experimental soil was latosol, previously abandoned farmland for many years with medium fertility. The pH value, organic matter content, available nitrogen content, available phosphorus, and available potassium content of the 0–20 cm soil layer before planting are detailed in Qin et al. (2022).

1.2 Experimental Materials

The test material was the industrial high-yield cassava cultivar ‘South China 205’, with planting stems provided by Guangxi Academy of Agricultural Sciences.

1.3 Experimental Design and Sampling

1.3.1 Experimental Design The field was prepared conventionally and divided into 12 plots of 70 m^2 each. Cassava was planted at 1 m × 1 m spacing, with 70 plants per plot. In 2019, three plots were randomly selected as experimental replicates, which were then fixed as annual replicates. Cassava was planted on April 16, 2019 (continuous cropping 0 season), April 10, 2020 (continuous cropping 1 season), and April 11, 2021 (continuous cropping 2 season). Consistent field management practices were applied throughout the experimental period. No artificial irrigation was applied; rainfall and monthly average temperatures are detailed in Peng et al. (2024).

1.3.2 Soil Sample Collection and Processing Soil samples were collected 250 days after cassava planting. In each plot, three uniformly growing cassava plants were randomly selected, and complete tuberous roots were excavated. After removing large soil clods, rhizosphere soil was collected using the “root-shaking method.” Non-rhizosphere soil was collected from five sampling points in each plot using an “S” pattern at 0–20 cm depth and mixed as the plot composite. After removing stones, plant litter, and roots, samples were mixed and divided into three portions: the first was snap-frozen in liquid nitrogen at -80°C for microbial community diversity analysis; the second was kept fresh in an icebox and stored at 4°C for soil enzyme activity determination; the third was air-dried, crushed, and sieved for soil physicochemical property analysis.

1.3.3 Soil Physicochemical Properties and Enzyme Activity Determination Methods for determining soil pH, available nitrogen content, organic matter content, available phosphorus content, available potassium content, and urease activity are detailed in Qin et al. (2022).

1.3.4 High-Throughput Sequencing and Bioinformatics Analysis of Soil Microorganisms Soil DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek). DNA concentration and purity were detected using NanoDrop2000, and quality was assessed using 1% agarose gel electrophoresis. The V4 region of the 18S rRNA sequence was amplified using specific primers SSU0817F (5 -TTAGCATGGAATAATRRAATAGGA-3) and 1196R (5 -TCTGGACCTGGTGAGTTTCC-3). PCR products were recovered using 2% agarose gel and purified according to the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) instructions, then detected using 2% agarose gel electrophoresis and quantified using Quantus™ Fluorometer (Promega, USA). Libraries were constructed using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA), and high-throughput sequencing was performed on the Illumina MiSeq PE300 platform by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. Raw data were uploaded to the NCBI SRA database (project accession PRJNA999067, sample accession SRR25439107-SRR25439124).

Raw sequences were quality-controlled and assembled using Trimmomatic (version 0.32) and FLASH (version 1.2.11). Operational taxonomic units (OTUs) were clustered at 97% similarity using UPARSE Version 7.1. Based on the Silva database, filtered non-chimeric sequences were taxonomically annotated on the RDP classifier (http://rdp.cme.msu.edu/) at a 70% alignment threshold. Fungal α-diversity and OTU richness were calculated using Mothur (version v.1.30.2) and Past 3.0 software (http://fold.uio.no/ohammer/past), respectively. Principal component analysis (PCA), permutational multivariate analysis of variance (PERMANOVA), correlation analysis, and redundancy analysis (RDA) were performed using the R (version 3.3.1) vegan package. The stats package was used for significance testing and plotting of inter-group differences in fungi at the genus level.

1.4 Statistical Analysis

Data were organized and analyzed using Microsoft Excel 2010 and IBM SPSS Statistics 25.0 software.

Results

2.1 Effects of Continuous Cropping on Fungal Community Diversity

2.1.1 Effects on α-Diversity High-throughput 18S sequencing analysis of rhizosphere and non-rhizosphere soil samples over three years yielded 713,640 effective sequences. Rhizosphere soils contained 395,429 high-quality sequences with an average length of 381.210 bp, while non-rhizosphere soils contained 402,223 high-quality sequences averaging 381.239 bp. Variance analysis of OTU richness across soil samples (Table 1) revealed significant interactive effects between continuous cropping years and soil type on fungal community Sobs index ($P < 0.05$). All treatments exhibited OTU library coverage exceeding 99.00% (Table 2). The Sobs index of rhizosphere soil in 2020 was significantly higher than that of non-rhizosphere soil. In non-rhizosphere soils, Shannon index was significantly greater in 2021 than in 2020, while Simpson index decreased annually. Conversely, rhizosphere soils showed a yearly decline in Shannon index and a yearly increase in Simpson index. These patterns indicate that fungal community diversity in cassava rhizosphere soils decreased progressively, whereas diversity in non-rhizosphere soils increased significantly after two continuous cropping seasons. Additionally, Ace and Chao1 indices of rhizosphere soils under 0 and 1 season of continuous cropping were greater than those of non-rhizosphere soils, though not significantly, suggesting that soil fungal communities began to be influenced by cassava root exudates after two years of continuous cropping.

2.1.2 Effects on β-Diversity Principal component analysis based on OTU levels revealed that the first principal component (PC1) separated soil samples from 2021 from those of 2019 and 2020 in both non-rhizosphere and rhizosphere soils, explaining 22.32% and 21.50% of total variance, respectively (Figure 1 [Figure 1: see original paper]). This indicates substantial differences in fungal community structure and composition between soils under two seasons of continuous cropping versus those under 0 or 1 season. PERMANOVA analysis based on Bray-Curtis and unweighted Unifrac distances further demonstrated that continuous cropping years were the primary factor contributing to differences in taxonomic and phylogenetic β-diversity of fungal communities, while soil type had no significant effect (Table 3).

2.2 Effects of Continuous Cropping on Fungal Community Composition

2.2.1 Effects on Fungal Community Composition Fungal community composition at phylum and class levels was fundamentally similar between rhizosphere and non-rhizosphere soils across different continuous cropping years. Dominant phyla in all soil samples were Ascomycota (77.95%–89.43%), Basidiomycota (1.87%–8.37%), and Mucoromycota (1.55%–5.15%), collectively accounting for 89.95%–94.33% of total relative abundance. During the three-year experimental period, relative abundance of Ascomycota in rhizosphere soils decreased annually, while Basidiomycota and Mucoromycota increased (Figure 2 [Figure 2: see original paper]). At the class level, dominant fungal communities included Sordariomycetes, Eurotiomycetes, and Dothideomycetes (Figure 3 [Figure 3: see original paper]). Dominant phylum composition was similar between non-rhizosphere and rhizosphere soils under 1 and 2 seasons of continuous cropping, but differed interannually. Specifically, relative abundances of Sordariomycetes and Dothideomycetes in both soil types were lower under 2-season continuous cropping than under 1-season cropping, while Eurotiomycetes showed the opposite trend. Throughout the three years, relative abundance of Eurotiomycetes was consistently higher in non-rhizosphere than rhizosphere soils, while Dothideomycetes showed the reverse pattern. In summary, fungal community composition was fundamentally similar between non-rhizosphere and rhizosphere soils within the same year, but relative abundances differed. Fungal community composition under 2-season continuous cropping differed from that under the first two seasons in both soil types.

2.2.2 Analysis of Fungal Community Composition Differences In non-rhizosphere soils, the top 10 OTUs showing significant differences in fungal communities were classified as *Knufia* (OTU171), unclassified_{p_*}{Ascomycota} (OTU87), unclassified*{k_*}{Fungi} (OTU395), unclassified*{d_*}{Eukaryota} (OTU38), Colpodea family of Intramacronucleata subphylum (OTU14), Myrothecium (OTU319), Sordariomycetes (OTU47), Geosmithia (OTU284), Dothideomycetes (OTU166), and unclassified*{d_*}{Eukaryota} (OTU281) (Figure 4 [Figure 4: see original paper]). Relative abundances of* Knufia*, unclassified*{k_*}{Fungi}, and unclassified*{d_*}{Eukaryota} increased annually, while those of unclassified*{d___}{Eukaryota}, Colpodea, Myrothecium, Sordariomycetes, and Dothideomycetes decreased.

In rhizosphere soils, the top 10 significantly different OTUs were classified as Hypocreales (OTU304), Capnodiales (OTU11), *Monosiga* of norank_{d_*}{Eukaryota} (OTU91), Colpodea (OTU14), Myrothecium (OTU319), Dothideomycetes (OTU166), Salpingoeca (OTU122), unclassified*{k_*}{norank} (OTU183), Agaricales (OTU82), and Sordariomycetes (OTU47). Relative abundances of Colpodea, Hypocreales, Capnodiales, Myrothecium, and Sordariomycetes decreased annually, while* Monosiga *of norank*{d___}{Eukaryota} increased.

In summary, continuous cassava cropping caused annual reductions in soil fungal community composition. In non-rhizosphere soils, Ascomycota composition shifted substantially from Myrothecium, Sordariomycetes, and Dothideomycetes in the first year to a single genus *Knufia.* In rhizosphere soils, fungi evolved from Hypocreales, Capnodiales, Myrothecium, Dothideomycetes, and Sordariomycetes within Ascomycota toward *Monosiga* of norank_{d___}{Eukaryota}.

2.3 Effects of Continuous Cropping Years on Correlations Between Soil Nutrient Content and Fungal Community Composition

Soil fungal community composition was influenced by both soil physicochemical properties and enzyme activities, though the degree of influence varied. In non-rhizosphere soils (Figure 5 [Figure 5: see original paper]A), soil pH significantly affected relative abundances of Nucleariidae_{{{and}}*{{Fonticula}}}*{group}, norank_{d_*}{Eukaryota}, unclassified*{k_*}{norank}, unclassified*{k_*}{Fungi}, Chytridiomycota, and Aphelidea. Available nitrogen content significantly influenced relative abundances of SAR supergroup, Nucleariidae*{{{and}}*{{Fonticula}}}*{group}, norank_{d_*}{Eukaryota}, and unclassified*{k___}{norank}. Available phosphorus, available potassium, organic matter content, and urease activity also affected relative abundances of various fungal communities to different degrees. In rhizosphere soils (Figure 5 [Figure 5: see original paper]B), all soil physicochemical properties influenced fungal community richness to varying extents.

Redundancy analysis (Figure 6 [Figure 6: see original paper]) showed that RDA1 and RDA2 explained 36.44% and 9.80% of variation, respectively. The top five phyla by abundance were Ascomycota, Basidiomycota, Mucoromycota, SAR supergroup, and unclassified_{k_*}{Fungi}. Soil pH, organic matter content, available nitrogen content, available phosphorus content, available potassium content, and urease activity substantially influenced fungal community structure changes. Relative abundances of Basidiomycota, Mucoromycota, and unclassified*{k___}{Fungi} were negatively correlated with soil pH, organic matter content, available nitrogen content, and available phosphorus content, while SAR supergroup showed opposite correlations. Ascomycota relative abundance was positively correlated with soil pH, organic matter content, available nitrogen content, available phosphorus content, and available potassium content, but negatively correlated with urease activity.

Further correlation analysis between soil fungal communities (phylum level relative abundance $ $0.1%) and physicochemical properties across different continuous cropping years revealed that eight fungal phyla were significantly correlated with soil physicochemical properties (Table 4). Mucoromycota and norank_{k_*}{Fungi} were significantly negatively correlated with available nitrogen content. unclassified*{k_*}{Fungi} and Chytridiomycota were significantly negatively correlated with pH and available nitrogen content. Zoopagomycota was significantly negatively correlated with organic matter content. unclassified*{k_*}{Amoebozoa} and norank*{d___}{Eukaryota} were significantly negatively correlated with pH, organic matter content, available nitrogen content, and available phosphorus content, but significantly positively correlated with urease activity. These results indicate that pH, organic matter content, available nitrogen content, available phosphorus content, and urease activity are key physicochemical factors influencing fungal communities, while available potassium had relatively minor effects.

Discussion

3.1 Effects of Continuous Cropping on Fungal Community Diversity in Cassava Fields

Soil microorganisms play vital roles in soil nutrient availability, plant growth and development, and environmental quality (Liu et al., 2022). This study revealed significant differences in fungal community diversity between cassava rhizosphere and non-rhizosphere soils under continuous cropping, likely because cassava root exudates contain substantial amounts of sugars, amino acids, and allelochemicals (Han et al., 2023), creating differences in physicochemical properties and microbial communities between rhizosphere and non-rhizosphere soils. For instance, some microorganisms can produce oxidases that catalyze reactions using glycolaldehyde as substrate (Benjaphokee et al., 2012), and cassava continuous cropping leads to glycolaldehyde accumulation (Han et al., 2023), ultimately enriching microbial communities that use it as a catalytic substrate. Fungal community α-diversity in rhizosphere soils was higher than in non-rhizosphere soils under 0 and 1 season of continuous cropping, but the opposite pattern emerged under 2-season continuous cropping. This suggests that cassava continuous cropping began significantly affecting rhizosphere soil physicochemical properties after two seasons, thereby altering the fungal living environment, consistent with findings from potato studies (Tan et al., 2022). The yearly decline in rhizosphere soil fungal community α-diversity aligns with patterns observed in continuous cropping of *Pinellia ternata* (Liu et al., 2022) and *Gastrodia elata* (Cai et al., 2022), but contrasts with flue-cured tobacco (Rao et al., 2022) and tomato (Sun et al., 2017). This discrepancy may be attributed to differences in crop type (Solanaceae crops), soil type (yellow-cinnamon soil and gray desert soil in northern China), and climatic conditions. This study also found significant effects of continuous cropping years on soil fungal community β-diversity, consistent with research on *Pinellia ternata* (Liu et al., 2022) and cucumber (Li et al., 2020).

3.2 Effects of Continuous Cropping on Fungal Community Structure Succession in Cassava Fields

This study identified Ascomycota, SAR supergroup, Basidiomycota, Mucoromycota, and unclassified_{k___}{Fungi} as dominant phyla in both rhizosphere and non-rhizosphere soils under continuous cassava cropping, similar to findings in continuous cropping of ramie (Bai et al., 2021), potato (Ge and Sun, 2020), and *Lycium ruthenicum* (Li et al., 2018). As the largest fungal group, Ascomycota plays important roles in organic matter decomposition and beneficial metabolite production (Wang et al., 2020). Within Ascomycota, *Myrothecium* and *Hypocrea* can produce various decomposition enzymes and secrete antibiotics to inhibit pathogenic fungi (Zhu and Zhuang, 2014; Xu et al., 2019; Yang et al., 2023). Sordariomycetes are important secondary metabolite producers (Wang et al., 2021), and Hypocreales can reduce pest incidence (Li et al., 2022).

At the OTU level, this study revealed that under continuous cropping, Ascomycota in non-rhizosphere soils evolved from beneficial fungi including *Myrothecium* (OTU319), Sordariomycetes (OTU47), and Dothideomycetes (OTU166) toward a single genus *Knufia* (OTU171), possibly because *Knufia* adapts well to degraded soils with low organic matter and available nutrients following continuous cropping (Yang et al., 2022). In rhizosphere soils, relative abundances of beneficial fungi including Hypocreales (OTU304), *Myrothecium* (OTU319), and Sordariomycetes (OTU47) within Ascomycota decreased with increasing continuous cropping years, which may represent one of the inducing factors for cassava continuous cropping obstacles. Basidiomycota relative abundance increased in both soil types during the third experimental year, contrasting with strawberry research (Li et al., 2018). This difference may be because cassava is a small shrub leaving higher lignin residues in soil, and Basidiomycota possess lignin degradation capabilities, leading to their enrichment (Riley et al., 2014).

3.3 Correlations Between Physicochemical Properties and Fungal Communities in Cassava Fields

Soil chemical properties and enzyme activities are important indicators of soil function, while differences in fungal communities between rhizosphere and non-rhizosphere soils are often caused by pH and nutrient content variations. Rhizosphere microorganisms co-evolve with crops, as root exudates provide carbon and nitrogen sources for microbial physiological activities, while microorganisms decompose soil organic matter to release nutrients that promote crop growth (Gan et al., 2022). This study found that pH, available nitrogen content, and available phosphorus content were major factors influencing non-rhizosphere soil fungal community structure, while urease activity and organic matter content were primary factors affecting rhizosphere soil fungal communities. Different soil physicochemical properties had varying effects on fungal communities. Ascomycota relative abundance in rhizosphere soils was significantly positively correlated with organic matter content, consistent with research on *Codonopsis pilosula* (Yang et al., 2023) and mountainous apple (Ahmad et al., 2023). unclassified_{k___}{Fungi} relative abundance was significantly positively correlated with soil pH, organic matter, and available nitrogen content, similar to findings on *Atractylodes macrocephala* (Zhang, 2021). Mucoromycota relative abundance was significantly positively correlated with available nitrogen content, contrasting with studies on dark brown soil and chestnut soil (Chen et al., 2022; Zhang et al., 2023), possibly due to substantial differences in physicochemical characteristics between those soil types and the latosol in this study.

Preferential nutrient absorption by continuously cropped cassava leads to soil nutrient imbalance, with non-rhizosphere soil pH, organic matter, available nitrogen, and available phosphorus decreasing annually while urease activity increased. Rhizosphere soil organic matter, available nitrogen, available phosphorus, and available potassium decreased yearly, while pH and urease activity increased (Peng et al., 2024). These changes altered soil fungal community structure, reducing relative abundance of many beneficial fungi and inhibiting soil nutrient release, thereby affecting cassava growth.

Conclusion

Under continuous cropping conditions, accumulated cassava root exudates altered the soil ecological environment, changing rhizosphere soil pH, organic matter content, available nitrogen content, available phosphorus content, available potassium content, and urease activity, thereby modifying fungal community diversity in cassava fields. This resulted in greater α-diversity in non-rhizosphere than rhizosphere soils and significantly affected β-diversity. Fungal community structure succession in cassava fields under different continuous cropping years was primarily reflected in relative abundance changes. With increasing continuous cropping years, relative abundances of Ascomycota classes and genera changed significantly: *Myrothecium*, Sordariomycetes, and Dothideomycetes decreased substantially while *Knufia* increased dramatically in non-rhizosphere soils; Hypocreales, *Myrothecium*, and Sordariomycetes decreased in rhizosphere soils. Basidiomycota relative abundance increased in both soil types. As continuous cropping years increased, cassava rhizosphere soil fungal α-diversity gradually decreased and became lower than that of non-rhizosphere soils after two seasons, while relative abundance of beneficial fungi in rhizosphere soils gradually declined. Such fungal structural changes are unfavorable for cassava growth, development, and yield formation, ultimately contributing to cassava continuous cropping obstacles along with other factors. These changes are associated with alterations in soil physicochemical properties. Therefore, this study suggests that to maintain microecological stability in cassava fields and provide a suitable environment for cassava yield and quality formation, continuous cropping duration should not exceed two years. Cassava continuous cropping obstacles may be related to changes in soil physicochemical properties after two years of continuous cropping, leading to reduced rhizosphere soil fungal diversity and decreased relative abundance of beneficial fungi.

References

AHMAD R, GAO J, LI W, et al., 2023. Response of soil nutrients, enzyme activities, and fungal communities to biochar availability in the rhizosphere of mountainous apple trees[J]. Plant Soil, 487: 277-293.

AN Y, YANG D, LI X, et al., 2019. Impacts of different cropping of *Pinellia ternata* on its yield, quality and enzyme activity in rhizosphere soil[J]. J Gansu Agric Univ, 54(2): 96-101.

BAI XH, CHEN XR, CHEN XF, et al., 2021. Diversity of microorganism in the rhizosphere of continuous cropping and non-continuous cropping on ramie[J]. Plant Fiber Sci China, 43(5): 222-230.

BENJAPHOKE S, KOEDRITH P, AUESUKAREE C, et al., 2012. CDC19 encoding pyruvate kinase is important for high-temperature tolerance in *Saccharomyces cerevisiae*[J]. New Biotechnol, 29(2): 166-176.

BERENDSEN RL, PIETERSE CMJ, BAKKER PAHM, 2012. The rhizosphere microbiome and plant health[J]. Trends Plant Sci, 17(8): 478-486.

CAI LQ, CHEN R, YANG DQ, et al., 2022. Analysis of fungal community diversity in rhizosphere soil of the continuous cropping *Gastrodia elata* based on the high-throughput sequencing[J]. Plant Fiber Sci Chi, 44(6): 321-330.

CHEN F, YU G, SUN YB, et al., 2022. Characteristics of microbial community structure in the surrounding farmlands of a mercury mining area and its environmental driving factors[J]. Environ Sci, 43(8): 4342-4352.

DE MEV, NOTARO KDA, DE BJA, et al., 2019. Soils from intercropped fields have a higher capacity to suppress black root rot in cassava, caused by

Scytalidium lignicola[J]. J Phytopathol, 167(4): 209-217.

GAN GY, CHEN FW, ZOU JL, et al., 2022. Effects of long-term different nutrient deficiency on the composition and diversity of soil microbial community in winter oil seed rape[J] Soil Fert Sci China, (4): 37-46.

GE YL, SUN T, 2020. Soil microbial community structure and diversity of potato in rhizosphere and non-rhizosphere soil[J]. Ecol Environ Sci, 29(1): 141-148.

HAN X, ZHANG JR, BEI LP, et al., 2023. Identification and allelopathic effect of root exudates of tissue-culture cassava under continuous cropping[J]. SW Chi J Agric Sci, 36(8): 1653-1661.

HE ZS, CHEN JJ, ZHU J, et al., 2022. Characteristics of microbial functional diversity and its influencing factors of forest soils at different elevations on the southern slope of Daiyun Mountain[J]. Acta Ecol Sin, 42(9): 3504-3515.

HOU H, DONG K, YANG ZX, et al., 2016. Advance in mechanism of continuous cropping obstacle[J]. Soils, 48(6): 1068-1076.

LI TT, DENG XH, LI RC, et al., 2022. Effects of *Ralstonia solanacearum* infection on soil fungal community diversity[J]. Biotechnol Bull, 38(10): 195-203.

LI XY, XIAO LK, LI QY, et al., 2018. Effect of continuous-cropping on rhizosphere soil fungal community structure of outdoor strawberry[J]. SW China J Agric Sci, 31(12): 2514-2519.

LI Y, HE, XM YANG XD, et al., 2018. The microbial community diversity of the rhizosphere and bulk soils of *Lycium ruthenicum* in different habitats[J]. Acta Ecol Sin, 38(17): 5983-5995.

LI YJ, LIU X, WU DF, et al., 2020. Effects of continuous cropping of greenhouse cucumber on soil fungal[J]. Acta Agric Boreal-Sin, 35(1): 194-204.

LIANG HB, HUANG J, AN FF, et al., 2016. Analysis of present situation of cassava industry in China[J]. Acta Agric Jiangxi, 28(6): 22-26.

LIANG HB, HUANG J, WEI YX, et al., 2017. Analysis of yield gap and limiting factors for cassava on the farmland[J]. J Yunnan Agric Univ, 32(6): 975-984.

LIU H, 2019. Impact of soil microbial community structure on rotation cropping and continuous cropping systems of typical crops in black soil[D]. Changchun: Northest Institute of Geography and Agroecology, Chinese Academy of Science.

LIU SR, WANG HL, SUN H, et al., 2022. Effects of continuous cropping of *Pinellia ternata* on rhizospheric microbial community[J]. Chin Tradit Herb Drugs, 53(4): 1148-1155.

LIU ST, 2020. Effects of cassava continuous cropping and rotation on soil physical and chemical properties, microbial community and yield[D]. Nanning: Guangxi University.

LIU ZG, LU HB, ZHAO HC, et al., 2022. Effects of methods for spring maize straw-returning to field on soil microbial biomass C, N, P and enzyme activities in dry farming area[J]. Acta Agric Boreal-Occident Sin, 31(2): 183-192.

MA L, MA K, YANG GL, et al., 2015. Effects of continuous potato cropping on the diversity of soil microorganisms[J]. Chin J Eco-Agric, 23(5): 589-596.

PENG XH, LI LW, OU GN, et al., 2024. Effects of continuous cropping of cassava on soil physicochemical properties and bacterial community succession[J]. J Sorth Agric:1-17[2024-04-15]. http://kns.cnki.net/kcms/detail/45.1381.S.20240108.1807.002.html.

QIN FY, YANG WX, PENG XH, et al., 2022. Difference in the diversity of bacterial community structure in rhizosphere and non-rhizosphere soil of cassava in Fenlong tillage[J]. SW China J Agric Sci, 35(4): 729-739.

RAO DA, LIU PY, ZOU LY, et al., 2022. Effects of long-term continuous cropping and reductive soil disinfestation on fungal community in flue-cured tobacco rhizosphere[J]. Soil Fert Sci China, (4): 47-56.

RILEY R, SALAMOV AA, BROWN DW, et al., 2014. Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi[J]. Proc Natl Acad Sci, 111(27): 9923-9928.

SUN WQ, KANG YL, LIU JG, et al., 2017. Influence of continuous cropping of processing tomato on diversity of microflora in the rhizosphere soil[J]. Acta Agric Boreal-Occident Sin, 26(7): 1099-1110.

SUN XT, LI L, LONG GQ, et al., 2015. The progress and prospect on consecutive monoculture problems of *Panax notoginseng*[J]. Chin J Ecol, 34(3): 885-893.

SUN YY, JIANG GY, LI JG, et al., 2010. Effects of continuous cropping tomato for processing on soil enzyme activities and microbial flora[J]. Acta Ecol Sin, 30(13): 3599-3607.

TAN XL, GUO TW, HU XY, et al., 2022. Characteristics of microbial community in the rhizosphere soil of continuous potato cropping in arid regions of the Loess Plateau[J]. Acta Agron Sin, 48(3): 682-694.

TERHONEN E, BLUMENSTEIN K, KOVALCHUK A, et al., 2019. Forest tree microbiomes and associated fungal endophytes: functional roles and impact on forest health[J]. Forests, 10(1): 42.

WANG MS, JIANG CY, PAN WJ, et al., 2008, Study on physico-chemical properties and microbiological community in tobacco-growing soils under different continuous cropping years[J]. Anhui Agric Sci, 36(12): 5033-5034.

WANG R, ZHANG X, GAO Y, et al., 2020. An Investigation on Species Diversity of Macro-ascomycetes in Xingshan County, Hubei Province[J]. J Ecol Rural Environ, 36(3): 342-348.

WANG YY, LONG YX, LI Y, et al., 2021. Relationships of soil physicochemical properties to the distribution and the composition of microbial community under *Populus euphratica*'s crown[J]. Acta Ecol Sin, 41(14): 5669-5684.

XU ZC, WANG FZ, JIN YN, et al., Analysis of fungal communities in rhizosphere soil of flue-cured tobacco under different cropping years using 18S rDNA-PCR-DGGE[J]. Soil Fert Sci China, (4): 39-46.

YANG D, YANG Z, ZHU GX, et al., 2022, Characteristics of nutrient content and microorganism in different grades of rocky desertification soils in sourthweast Guizhou[J]. Jiangsu Agric Sci, 50(13): 256-264.

YANG HQ, 2020. Development situation of Guangxi's cassava industry and its countermeasures[J]. Agric Res Appl, 33(6): 74-77.

YANG XJ, ZHOU YY, PENG XY, et al., Comparative analysis of rhizosphere soil microbial community structure between root-knot nematode diseased and healthy cucumber[J]. J Agric Sci Technol, 25(1): 109-118.

YANG Y, LI HL, MA KL, et al., 2023. Effect of continuous cropping on the physicochemical properties, microbial activity, and community characteristics of the rhizosphere soil of *Codonopsis pilosula*[J]. Environ Sci, 44(11): 6387-6398.

YANG YH, CHEN DM, JIN Y, et al., 2011. Effects of different fertilizers on functional diversities of microbial flora in rhizospheric soil of monoculture tobacco[J]. Acta Agron Sin, 37(1): 105-111.

ZHANG HJ, LIU EP, LIU HQ, et al., 2012. Development status and countermeasures of cassava industry in Guangxi[J]. Guangdong Agric Sci, 39(5): 161-164.

ZHANG L, WANG Q, YANG XY, et al., 2023. Effect of the process of returning farmland to forest in the Loess hilly area on soil fungal communities[J]. Environ Sci, 44(3): 1758-1767.

ZHANG M, 2021. Effects of combined application of NPK fertilizer on soil microbial diversity and some physical and chemical indexes in the rhizosphere of *Atractylodes macrocephala*[D]. Guiyang: Guizhou University.

ZHOU GJ, 2017. The relationship between the continuous cropping obstacle and physical and chemical properties of cassava rhizosphere soil[D]. Nanning: Guangxi University.

ZHOU J, LEI T, 2007. Review and prospects on methodology and affecting factors of soil microbial diversity[J]. Biodivers Sci, 15(3): 306-311.

ZHU ZX, ZHUANG WY, 2014. Current understanding of the genus *Trichoderma*[J]. Mycosystema, 33(6): 1136-1153.

Note: Figure translations are in progress. See original paper for figures.