

Impacts of continuous melon cropping on soil properties and microbial network restructuring Postprint

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Abstract

Continuous cropping can lead to soil environment deterioration, cause plant health problems, and reduce crop productivity. However, the response mechanisms of soil microbial co-occurrence patterns to the duration of continuous melon cropping remain poorly understood. Here, we employed metagenomic techniques to comparatively investigate the bulk and rhizosphere soil microbial communities in major melon-producing regions (where the duration of continuous melon cropping ranges from 1 to 30 years) in the eastern and southern parts of Xinjiang Uygur Autonomous Region, China. The results showed that soil pH clearly decreased with increasing melon cropping duration, while soil electrical conductivity (EC) and other soil nutrient indices increased with increasing melon cropping duration (with the exception of AN and TK in the southern melon-producing region). The most dominant bacterial phyla were Proteobacteria and Actinobacteria, and the most abundant fungal phyla were Ascomycota and Mucoromycota. Redundancy analysis (RDA) indicated that soil pH and EC had no significant effects on bacterial communities. However, after many years of continuous melon cropping in the southern melon-producing region, fungal communities were significantly negatively correlated with soil pH and significantly positively correlated with soil EC ($P < 0.05$). Co-occurrence network analysis showed that continuous melon cropping increased the complexity but decreased the connectivity of cross-domain microbial networks. Moreover, the enrichment patterns of microorganisms in the main microbial network modules varied significantly with the duration of continuous melon cropping. Based on the analysis of keystone taxa, we found that continuous melon cropping increased some plant pathogens (e.g., *Fusarium* and *Stagonospora*) but decreased beneficial bacteria (e.g., *Mesorhizobium* and *Pseudoxanthomonas*). In conclusion, this study has greatly enhanced understanding of the effects of continuous

melon cropping on alterations in microbial community structure and ecological networks in Xinjiang.

Full Text

Preamble

Impacts of Continuous Melon Cropping on Soil Properties and Microbial Network Restructuring

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Ascomycota and Mucoromycota. Redundancy analysis (RDA) indicated that soil pH and EC had no significant effects on bacterial communities. However, after many years of continuous melon cropping in the southern melon-producing region, fungal communities were significantly negatively correlated with soil pH and significantly positively correlated with soil EC ($P < 0.05$). Co-occurrence network analysis showed that continuous melon cropping increased the complexity but decreased the connectivity of cross-domain microbial networks. Moreover, the enrichment patterns of microorganisms in the main microbial network modules varied significantly with the duration of continuous melon cropping. Based on the analysis of keystone taxa, we found that continuous melon cropping increased some plant pathogens (e.g., *Fusarium* and *Stagonospora*) but decreased beneficial bacteria (e.g., *Mesorhizobium* and *Pseudoxanthomonas*). In conclusion, this study has greatly enhanced understanding of the effects of continuous melon cropping on alterations in microbial community structure and ecological networks in Xinjiang.

Keywords: melon; continuous cropping; microbial community; ecological networks; metagenomic sequencing; redundancy analysis (RDA); keystone taxa

Introduction

Melon (*Cucumis melo* L.), an important horticultural crop cultivated worldwide including in China (Wang et al., 2023), is frequently grown in continuous cropping systems due to its significant nutritional and economic value. However, continuous cropping typically leads to severe soil degradation, accumulation of allelopathic substances, and ultimately inhibits plant growth (Hufnagel et al., 2020; Tan et al., 2021; Ku et al., 2022). Extensive research has demonstrated that continuous cropping substantially reduces both crop yield and quality while increasing the prevalence and severity of soil-borne diseases, primarily attributed to imbalances in the soil microecosystem (Pervaiz et al., 2020). For instance, Wang et al. (2024a) reported that continuous sesame cropping led to recurrent wilt disease outbreaks, which profoundly modified the structure and functional characteristics of rhizosphere soil microbial communities.

In relevant studies on other crops, soil bacterial diversity and richness clearly decline with increasing duration of continuous cropping (Guo et al., 2022; Chen et al., 2024), which contrasts with the fungal community (He et al., 2023). The main reason is the unidirectional evolution of the microbial community induced by prolonged continuous cropping (Chen et al., 2022). However, three years of continuous melon cropping in Jiashi County, Xinjiang Uygur Autonomous Region, China, has clearly reduced the diversity of both bacterial and fungal communities in the rhizosphere soil (Li et al., 2023b; Wang et al., 2023). Similar results were also reported in Turpan City, Xinjiang (Jia et al., 2024). These findings suggest that the long-term rhizosphere soil microbial effects of continuous melon cropping in Xinjiang are not clear.

A long duration of continuous monoculture cropping could cause the accumulation of plant pathogens, promote the spread of soil-borne diseases, decrease the proportion of beneficial microorganisms, and promote the enrichment of pathogenic fungi (Shi et al., 2021). For example, Huang et al. (2020) reported that pathogenic fungi, including *Alternaria* and *Fusarium*, significantly accumulated in the sugar beet rhizosphere soil environment while the abundances of beneficial bacteria such as *Actinomyces*, *Pseudomonas*, and *Bacillus* were markedly reduced. *Fusarium* is the primary pathogen that induces root rot diseases in melon (Ku et al., 2022). Three years of continuous melon cropping has been reported to increase the absolute abundance of *Fusarium* by ten times (Li et al., 2023b).

Autotoxicity is often referred to as the main reason for continuous cropping obstacles in melon (Liu et al., 2023). Owing to the long duration of continuous cropping of melon, autotoxicity has been reported to inhibit seed germination and affect seedling morphology (Zhang et al., 2022). Additionally, Zhang et al. (2020) highlighted the impact of autotoxicity on melon root growth and reactive oxygen species accumulation, which may lead to variations in soil microbial communities. Studies have shown that three years of continuous melon cropping reduces bacterial community diversity while increasing fungal abundance (Li et al., 2023b; Wang et al., 2023). The interactions among soil microbes (such as symbiosis, competition, or antagonism) constitute microbial networks, which can predict the keystone taxa that maintain community stability and reflect the structural stability of the microbial community. Research has demonstrated that long-term continuous cropping of cotton reduces the topological properties of soil bacterial co-occurrence networks and diminishes collaboration among species (Ma et al., 2023). Nonetheless, the long-term microbiological effects of continuous melon cropping remain unknown.

Xinjiang's arid and semi-arid areas are particularly notable for melon production because of their special climatic conditions (Zhu et al., 2021). Xinjiang has two major melon-producing regions: the eastern and southern Xinjiang. In the eastern region, the main melon-producing areas are Turpan City and Hami City, whereas Kashi Prefecture is the primary melon-producing area in the southern region. In 2023, the total planting area of melon in the two regions was approximately 382.13 km² (Yuan et al., 2024). However, such a large planting area still fails to meet the demand for melon cropping. As a result, continuous cropping is widely practiced. Continuous cropping of melon can lead to frequent soil-borne diseases, low yield, and poor melon quality due to soil nutrient imbalance and disruption of the rhizosphere environment, which is not conducive to the green and sustainable development of the melon industry in Xinjiang. To the best of our knowledge, few studies have examined the long-term effects of continuous melon cropping on soil microbial communities in Xinjiang's arid and semi-arid areas, especially at a spatial scale. Moreover, knowledge of the ecological networks in soil microbial communities in response to the duration of continuous melon cropping is still lacking. To address these knowledge gaps, we employed metagenomics technology to investigate both bulk and rhizosphere soil microbial

communities in the primary melon-producing regions of Xinjiang at a regional scale. We hypothesized that (1) the effects of continuous melon cropping differ between the two major melon-producing regions in Xinjiang and (2) continuous melon cropping alters soil ecological networks. The findings will provide foundational data for optimizing melon cultivation patterns and improving soil quality in Xinjiang.

2.1 Study Area and Soil Sampling

All soil samples (bulk and rhizosphere soils) were collected in June 2023 from two major melon-producing regions (eastern and southern Xinjiang) with different durations of continuous melon cropping. Automated water and fertilizer management systems were used at all sampling sites.

The eight sampling sites in the eastern region were located in Hami City and Turpan City, Xinjiang, which feature a temperate continental arid climate with an average annual precipitation of 33.4 mm and an average annual temperature of 9.8°C. In the eastern region, each sampling site was leased to large-scale growers by landowners through contracts, ensuring consistent melon varieties and management practices across sites. Typically, the total fertilizer application rate is 17,000 kg/hm² of organic manure, 300 kg/hm² of urea, 380 kg/hm² of potassium sulfate, and 380 kg/hm² of diammonium phosphate. All soil samples were divided into three distinct groups based on cultivation duration: short-term cropping (S) for fields with 4–9 years of continuous cropping, medium-term cropping (M) for fields with 10–19 years of continuous cropping, and long-term cropping (L) for fields with 20–30 years of continuous cropping.

For the S group, we collected a total of 9 rhizosphere soil samples and 6 bulk soil samples. For the M group, we collected 9 rhizosphere soil samples and 9 bulk soil samples. For the L group, we collected 5 rhizosphere soil samples and 6 bulk soil samples.

The six sampling sites in the southern region were located in Jiashi County and Shule County, Kashi Prefecture, Xinjiang, characterized by a temperate continental arid climate with an average annual precipitation of 80.9 mm and an average annual temperature of 12.3°C. In the southern region, melon varieties and management practices were also consistent across all sampling sites due to uniform cultivation. The total fertilization rate is 30,000 kg/hm² of organic fertilizer and 900 kg/hm² of compound fertilizer (percentage of N+P₂O₄+K₂O higher than 45%). During the sampling and investigation process, almost no melon fields were continuously cultivated for more than 10 years in the southern melon-producing region. Consequently, soil samples were divided into two groups: S for fields with 1–2 years of continuous cropping and M for fields with 3–5 years of continuous cropping. For the S group, we collected 9 rhizosphere soil samples and 7 bulk soil samples. For the M group, we collected 6 rhizosphere soil samples and 6 bulk soil samples.

After removing the surface litter and humus layers, bulk soil samples were collected from depths of 10–40 cm using a soil corer with an inner diameter of 5 cm. For rhizosphere soil, the shake-root method was employed. All rhizosphere soil samples were separated into two parts: one part was immediately cryopreserved in a 50 mL sterilized tube and stored at -80.0°C for deoxyribonucleic acid (DNA) extraction; the other part was placed in a self-sealing bag (approximately 500 g per sample) for soil chemical property analysis.

2.2 Determination of Soil Chemical Properties

All soil samples were naturally air-dried at room temperature and passed through a 2 mm sieve. Soil pH and electrical conductivity (EC) were measured in 1:2.5 and 1:5 soil/water mixtures, respectively. Total nitrogen (TN) and available nitrogen (AN) contents were determined using the semi-micro Kjeldahl method and the alkali-hydrolytic diffusion method, respectively. Total phosphorus (TP) was quantified using the $\text{H}_2\text{SO}_4\text{-HClO}_4$ -molybdenum-antimony resistance colorimetric method. Available phosphorus (AP) was measured through NaHCO_3 extraction followed by the molybdenum-antimony resistance colorimetric method. Total potassium (TK) and available potassium (AK) contents were determined using NaOH melt-flame spectrophotometry and ammonium acetate extraction with a flame spectrophotometer (YP-20, Shandong Youyunpu Optoelectronics Co., Ltd., Weifang, China), respectively. Soil organic carbon (SOC) content was quantified using the potassium dichromate external heating method.

2.3 Soil DNA Extraction and Metagenomic Sequencing

Frozen soil samples were sent to Biomarker Technologies Co., Ltd. in Beijing, China for DNA extraction and subsequent metagenomic sequencing. Briefly, we extracted soil DNA from 0.2–0.3 g of each sample using the TGuide S96 magnetic bead method genomic DNA extraction kit (DP812, Tiangen Biochemical Technology (Beijing) Co., Ltd., Beijing, China). After quality assessment and quantification, we fragmented the extracted DNA using the ultrasonic method and subsequently constructed a sequencing library. Metagenomic sequencing was performed on the Illumina NovaSeq 6000 platform.

2.4 Metagenome Assembly and Gene Annotation

First, we removed low-quality metagenomic reads (length <50 bp or mass value <20) (Mukherjee and Reddy, 2020). Using default parameters and a minimum overlap group size of 300 bp, overlap groups and scaffolds were assembled separately for each sample using Multiple_{Megahit}. MetaGeneMarker v3.26

was then employed to predict open reading frames (ORFs) for each sample and translate predicted ORFs 100 bp into amino acid sequences. We clustered all gene sequences with identity ≥ 0.9 and coverage ≥ 0.9 , constructing a non-redundant gene catalog using CD-HIT3. SOAPaligner4 was subsequently utilized to align high-quality reads with the non-redundant gene catalog to calculate gene abundance. Additionally, classification annotations for each sample were obtained by comparing the non-redundant gene catalog with the Nucleotide sequence database.

2.5 Statistical Analyses

All statistical analyses were conducted using R v4.3.1, and plots were generated with the “ggplot2” package unless otherwise specified. Prior to significance testing, the Shapiro-Wilk test assessed normality, and the F test analyzed homogeneity of variance. Differences in soil properties between treatments were analyzed using one-way analysis of variance (ANOVA) and the minimum significant difference test ($P < 0.05$).

Microbial community composition and alpha diversity were assessed using the “microeco” package, while beta diversity (including principal coordinate analysis (PCoA), redundancy analysis (RDA), and permutational multivariate analysis of variance (PERMANOVA)) was performed using the “vegan” and “pairwiseAdonis” packages. Bacterial and fungal cross-domain networks were constructed to uncover complex microbiome interactions. This study selected soil microorganisms with relative abundance greater than 0.01% at each sampling site and calculated correlations among microorganisms (with Spearman’s $r > 0.7$ or $r < -0.7$ and $P < 0.01$) using the “ggClusterNet” software package (Wen et al., 2022). The network was evaluated by calculating its topological features and visualized using Gephi 0.10.1 (Bastian et al., 2009).

To further study the effects of continuous melon cropping on soil microbial co-occurrence patterns, we explored distribution patterns of enriched taxa in bulk and rhizosphere soil ecological networks. A likelihood ratio test conducted by the “edgeR” package analyzed bacterial and fungal species that clearly responded to different durations of continuous melon cropping (Robinson et al., 2010). To explore community structure within bulk and rhizosphere soil networks, we employed a greedy algorithm for modularity optimization to identify network modules. Network modules refer to substructures within the network that are more densely connected internally—groups of nodes (e.g., microbial species) with significantly higher connection density among themselves than with other nodes. Such structures may correspond to functionally related microbial groups in ecological networks. Co-occurrence networks were constructed using the Fruchterman-Reingold layout (Huang et al., 2020) with the “igraph” package.

To determine keystone species, we employed indicator species analysis (Hartman

et al., 2018) and specificity-occupancy (SPEC-OCCU) analysis (Gweon et al., 2021). Briefly, indicator species were calculated using the “indicspecies” package with 9999 permutations ($P < 0.01$) (de Cáceres et al., 2010; Dang et al., 2024). For SPEC-OCCU analysis, species with both specificity and occupancy ≥ 0.7 were identified as specialized species. Taxa identified by the intersection of both methods were regarded as keystone taxa.

3.1 Impacts of Continuous Melon Cropping on Soil Chemical Properties

Continuous melon cropping led to significant alterations in soil chemical properties (Table 1). As the duration of continuous melon cropping increased, soil pH in both major melon-producing regions decreased significantly ($P < 0.05$). Conversely, EC, SOC, AP, AK, TN, and TP all significantly increased ($P < 0.05$).

3.2 Soil Microbiome Under Different Continuous Melon Cropping Systems

In this study, almost 29,428 sequences were classified into bacterial phyla. A total of 154 bacterial phyla were identified across the eastern melon-producing region (Fig. 1 [Figure 1: see original paper]). The predominant phyla were Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes, accounting for 60.62%–81.15% of total bacteria. In bulk soil, the relative abundances of Proteobacteria and Actinobacteria in the S group were 2.25% and 4.13% greater than in the L group, respectively. Similarly, in rhizosphere soil, the relative abundances of Proteobacteria and Actinobacteria were 15.46% and 4.32% lower, respectively, in the L group than in the S group. Alpha diversity results indicated that bacteria were more abundant in rhizosphere soil than in bulk soil ($P < 0.05$). However, continuous melon cropping had no clear effect on bacterial alpha diversity in the eastern melon-producing region (Fig. S1). Similar predominant phyla were detected in the southern melon-producing region, with no clear difference in alpha diversity observed between bulk and rhizosphere soils in the S group.

In the study area, 10 fungal phyla were identified. Three phyla—Ascomycota, Mucoromycota, and Basidiomycota—were dominant, accounting for 75.32%–86.57% of total fungal sequences. In the eastern melon-producing region, Ascomycota abundance increased gradually, which was the inverse trend of that in the southern melon-producing region. Regarding alpha diversity, the Shannon index of fungal communities clearly increased with increasing duration of continuous melon cropping in the eastern melon-producing region, whereas no significant difference was observed in the southern melon-producing region.

To identify differences among subgroups of soil microbial communities, we performed bacterial and fungal beta diversity analyses based on the Bray-Curtis index. PCoA showed that microbial communities were significantly different between bulk and rhizosphere soils, as well as among various durations of continuous melon cropping (Tables S1 and S2; Fig. S2). RDA subsequently identified potential factors influencing bacterial and fungal communities in the two distinct melon-producing regions (Table 2 ; Fig. S3). In the eastern melon-producing region, detected soil chemical parameters explained 56.33% and 50.29% of variances in bacterial and fungal communities, respectively. In the southern melon-producing region, soil chemical parameters explained 73.32% and 69.19% of variances in bacterial and fungal communities, respectively.

Strikingly, although soil pH and EC are widely recognized as primary factors shaping microbial communities (Fierer, 2017; Philippot et al., 2024), they had no clear effects on bacterial communities but dramatically affected fungal communities in the southern melon-producing region.

3.3.1 Bacterial and Fungal Cross-Domain Networks

Cross-domain co-occurrence patterns among taxa were analyzed to investigate dynamics of bacteria and fungi in response to varying durations of continuous melon cropping (Fig. 2 [Figure 2: see original paper]). Topological characteristics of co-occurrence networks indicated that as continuous cropping duration increased, networks became more complex. In the eastern melon-producing region, 6,309 and 9,506 edges were identified in bulk and rhizosphere soils of the L group, respectively, whereas 3,225 and 1,250 edges were identified in those of the S group, respectively. Similar results were found in the southern melon-producing region (Table S3).

Nonetheless, connectivity of soil microbial networks gradually declined in both bulk and rhizosphere soil environments. Notably, the proportion of fungi in rhizosphere co-occurrence networks exhibited opposite trends in the two regions. In the eastern melon-producing region, the ratio of bacterial to fungal nodes increased from 0.93 in the S group to 6.85 in the L group. Conversely, this ratio declined from 7.44 in the S group to 2.64 in the M group. Additionally, rhizosphere soil microbial networks exhibited longer path lengths (Table S1), suggesting these networks were more complex than those in bulk soil.

3.3.2 Distribution Patterns of Enriched Taxa in Different Cross-Domain Networks

We investigated different distribution patterns of enriched bacterial and fungal taxa in bulk and rhizosphere soil microbial associations. Three modules exhibited high cumulative relative abundances in each cross-domain network,

primarily consisting of bacterial taxa. Enriched taxa distribution in these modules clearly responded to continuous melon cropping duration. In bulk soil of the eastern melon-producing region, enriched taxa assigned to the S group were predominantly located in Module 1 and separated from Module 2 and Module 3 (Fig. 3a [Figure 3: see original paper]). However, in rhizosphere soil, Module 2 and Module 4 contained more enriched taxa in the S group, whereas Module 5 harbored more taxa enriched in the L group (Fig. 3b). In the southern melon-producing region, cumulative relative abundance of enriched taxa was not significantly different among groups in Module 1. Additionally, species abundance enriched in the M group was significantly greater than in the S group within Module 2 (Fig. 3c and d).

3.3.3 Correlation of Keystone Taxa and Soil Chemical Properties

Keystone taxa play crucial roles in stabilizing microbial communities by establishing symbiotic interaction systems between plants and microbial communities, as well as among host plants, through life processes including growth, reproduction, and death (Li et al., 2023c). Therefore, both indicator species analysis (Fig. S4) and SPEC-OCCU analysis (Figs. S5 and S6) were performed. Taxa identified by the intersection of both methods (Fig. 4 [Figure 4: see original paper]) were regarded as keystone taxa for further study. Ultimately, 7 bacterial genera and 4 fungal genera were identified as keystone taxa in bulk soil from the eastern melon-producing region (Figs. S7 and S8). For rhizosphere soil, 38 bacterial genera and 2 fungal genera were determined as keystone taxa (Figs. S9 and S10). In the southern melon-producing region, only 1 fungal genus was classified as a keystone taxon in bulk soil, whereas 2 fungal genera were classified as keystone taxa in rhizosphere soil (Figs. S11 and S12). Additionally, only 2 bacterial genera were identified as keystone taxa in bulk soil, and 30 bacterial genera were identified in rhizosphere soil (Figs. S13 and S14).

Keystone taxa distributions differed between the two major melon-producing regions. Most taxa were negatively correlated with soil pH and positively correlated with other soil nutrient indices ($P < 0.05$; Fig. 5 [Figure 5: see original paper]). In the eastern melon-producing region, abundance of certain salt-tolerant and salt-loving bacteria (such as *Gracilimonas* and *Salinimicrobium*) in bulk soil initially increased but then decreased with increasing continuous cropping duration (Fig. S7). Notably, both bacteria were not related to soil EC but were negatively related to soil nutrient indices. *Fusarium* was the most abundant fungus in the L group, whereas *Phialophora* was most abundant in the S group (Fig. S8). *Fusarium* was negatively related to soil pH but positively related to other nutrient indices.

Among key rhizosphere soil microbes, beneficial bacteria such as *Aequorivita*, *Dokdonella*, *Mesorhizobium*, *Methylobacillus*, *Pseudoxanthomonas*, *Rhodanobac-*

ter, *Streptomyces*, and *Thauera* were enriched in the S group. In contrast, plant pathogenic fungus (*Fusarium*) exhibited the highest abundance in the L group (Fig. S10). Similar results were found in the southern melon-producing region. The abundance of certain pathogenic fungi, such as *Stagonospora* and *Anncaliia*, increased in the M group (Fig. S12). Conversely, the abundance of beneficial bacteria, including *Daejeonella* and *Lysobacter*, decreased in the M group (Fig. S14).

4.1 Variations in Soil Chemical Properties in Response to Continuous Melon Cropping

Soil physical and chemical properties are essential indicators for assessing soil fertility, quality, and health status (Delgado and Gómez, 2024). Continuous cropping can lead to imbalances in soil nutrient contents (Li et al., 2022). In this study, as continuous cropping duration increased, all soil chemical properties significantly changed in both major melon-producing regions (Table 1), consistent with findings of Wang et al. (2023). Previous studies have reported dramatic decreases in soil pH under continuous cropping of peanuts (Wang et al., 2024b), cut chrysanthemums (Li et al., 2023a), and tobacco (Tan et al., 2021). The increase in soil pH might be attributed to organic acid accumulation (Pervaiz et al., 2020). Soil pH can influence nutrient availability, such as NO_3^- , NH_4^+ , H_2PO_4^- , and SO_4^{2-} , thereby affecting crop growth (Hartemink and Barrow, 2023). In Xinjiang's arid and semi-arid areas, although continuous melon cropping led to decreased soil pH, the overall soil remains alkaline, especially in southern Xinjiang. It has been reported that soil pH can shape bulk and rhizosphere bacterial communities (Lopes et al., 2021). However, in the present study, soil pH and EC had no clear influence on bacterial communities in the southern melon-producing region (Table 2). Additionally, except for AN and TK, other soil nutrient indices exhibited a gradually increasing trend in the southern melon-producing region ($P < 0.05$). The increase in soil nutrient indices may be attributed to overuse of inorganic fertilizers, reliance on traditional fertilization methods, and insufficient utilization of soil nutrients resulting from prolonged monoculture practices (Xiong et al., 2015; Arafat et al., 2019). Liu et al. (2020) reported that when application rates of nitrogen, P_2O_5 , and K_2O were 300, 150, and 180 kg/hm^2 , respectively, a balance that optimally promoted melon growth and yield was achieved. However, our investigation revealed that actual fertilization rates far exceeded these recommended levels, resulting in significant residual fertilizer in the soil.

4.2 Variation in Soil Microbial Communities in Response to Continuous Melon Cropping

Soil microorganisms are crucial components of the soil ecosystem and are closely linked to soil fertility and health (Su et al., 2015). In our study, the abundance of Proteobacteria and Actinobacteria gradually decreased with increasing continuous cropping duration in the eastern melon-producing region, consistent with Li et al. (2023b). Proteobacteria are among the most prevalent phyla in soil and play vital roles in global carbon, nitrogen, and sulfur cycles (Spain et al., 2009). Actinobacteria are very important for promoting plant growth, improving nutrient accessibility, and providing crop protection (Mittra et al., 2022). Similar results were observed in the southern melon-producing region. As the most abundant fungal phylum, Ascomycota exhibits high metabolic adaptability and can decompose organic residues and litter from various plant sources and hosts (Ekwomadu and Mwanza, 2023; Manici et al., 2024). In the present study, Ascomycota abundance increased with increasing continuous cropping duration in the eastern melon-producing region. However, in the southern melon-producing region, although Ascomycota was more abundant in the M group, the response pattern to long-term continuous cropping remains unclear.

Continuous cropping clearly changes root exudates and might recruit specific microbes (Fu et al., 2023). The Shannon index of fungi in both major melon-producing regions increased with continuous cropping duration, consistent with previous findings (Dong et al., 2016). The increase in fungal alpha diversity may weaken community resistance to environmental disturbances (Wagg et al., 2019), indicating that soil microbial communities in the southern melon-producing region may be more vulnerable. PCoA and PERMANOVA results indicated that continuous cropping duration significantly drove separation of microbial community structure, consistent with PCoA results reported by Wang et al. (2023) on continuous cropping of Jiashi melons. Moreover, we found that soil chemical characteristics had significantly different effects on microbial communities. Correlation analysis between keystone taxa and environmental factors revealed that most microorganisms were negatively related to soil pH but positively associated with other soil nutrient indices (Fig. 5). Additionally, several beneficial keystone bacteria, such as *Arenimonas*, *Dokdonella*, *Psychrobacter*, and *Rhodanobacter*, were negatively correlated with soil EC. These bacteria are vital for wastewater treatment and bioreactor applications (Zhou et al., 2023). Our findings agree with results reported by Li et al. (2022).

A previous study revealed that soil EC was the most significant factor influencing fungal communities in saline-alkali soils (Ji et al., 2023). Soil pH has been reported to have a strong connection with bacterial community composition (Rousk et al., 2010). Numerous studies have confirmed that soil pH and EC are main drivers of microbial community composition and structure (e.g., Kim et al., 2016; Lund et al., 2020). In the eastern melon-producing region, soil nutrient indices are primary factors influencing rhizosphere bacterial and fungal communities. However, in the southern melon-producing region with medium-term

continuous cropping, soil acidification and increased EC drive formation of rhizosphere fungal communities; this might constitute a special adaptive strategy for crops in arid saline-alkali areas.

4.3 Impact of Continuous Melon Cropping on Microbial Network Topology and Keystone Taxa

In bacterial and fungal cross-domain networks, although the number of nodes and edges gradually increased with continuous cropping duration, microbial connectivity decreased (Fig. 2). Connectivity refers to the strength and density of connections among nodes within a network. Networks with high connectivity are typically considered more densely interconnected and exhibit greater stability. Research has shown that declining connectivity can lead to loss of microbial community diversity (Szabó et al., 2024). Our findings indicated that continuous cropping could reduce complexity of soil microbial communities. A high bacteria-to-fungi ratio in soil reflects plant ability to resist pathogens (Liu et al., 2015; Liu et al., 2021). With increasing melon cropping duration, the ratio of bacterial to fungal nodes in rhizosphere microbial networks increased in the eastern melon-producing region but decreased in the southern melon-producing region. This finding also partially indicated that continuous melon cropping in the southern melon-producing region might be more susceptible to pathogens. Additionally, we identified species significantly influenced by continuous cropping systems in both bulk and rhizosphere soils. These species were divided into different modules, each responding to different continuous cropping durations. These findings may be driving factors for community differences observed in PCoA ordination (Hartman et al., 2018).

In the study area, some salt-tolerant and halophilic bacteria, such as *Gracilimonas* and *Salinimicrobium*, were identified as key species that could play crucial roles in maintaining soil structure and fertility (Cho et al., 2013; Cao et al., 2020). The abundance of these bacteria initially increased, peaked at intermediate continuous cropping, and then gradually declined with prolonged continuous cropping. Additionally, several pathogenic microorganisms, including *Fusarium* (Ha et al., 2023), *Stagonospora* (Oliver et al., 2012), *Acidovorax* (Du et al., 2022), *Agrobacterium* (Hao et al., 2024), *Bdellovibrio* (Ezzedine et al., 2022), and *Phialophora* (Li et al., 2017), were identified as keystone taxa. These microorganisms have been reported as pathogenic agents responsible for various plant diseases, including root rot, leaf blight, and fruit spots. However, continuous melon cropping in Xinjiang's arid and semi-arid areas has led to reduced abundance of some beneficial bacteria, such as *Thauera* and *Arenimonas*, consistent with a previous study (Pang et al., 2021). These beneficial bacteria are pivotal in nitrogen cycling and degradation of organic pollutants (Lu et al., 2019), meaning continuous melon cropping could affect soil nutrient cycling. Collectively, our data concluded that continuous melon cropping could significantly alter structural and topological properties of soil microbial ecological

networks.

5 Conclusions

Continuous cropping of a single species type can exacerbate deterioration of the soil ecological environment, thereby affecting soil microbial communities and crop growth. Our study revealed that continuous melon cropping clearly changed soil chemical properties. In Xinjiang' s arid and semi-arid areas, continuous melon cropping led to significant decreases in soil pH and significant increases in soil EC and other soil nutrient indices (except AN and TK in the southern melon-producing region). Metagenomic sequencing analysis unequivocally confirmed that bulk and rhizosphere soil microbial communities were altered by continuous melon cropping. In the eastern melon-producing region, the Shannon index of fungal communities significantly increased with increasing continuous cropping duration. Moreover, microbial communities in both regions were distinctly different among various continuous melon cropping durations. Soil EC was the main factor shaping fungal communities in the southern melon-producing region ($R^2=0.88$, $P<0.001$). In particular, continuous melon cropping caused significant variations in microbial networks and keystone taxa. Some pathogens (such as *Fusarium*), identified as keystone taxa, clearly increased with continuous cropping duration. In summary, this study provides valuable insights into impacts of continuous melon cropping on soil health and offers a theoretical basis for future strategies to mitigate continuous cropping obstacles through microbial technology.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Supplementary Tables

Table S1. Pairwise Adonis test based on Bray-Curtis distance in bulk and rhizosphere soils of the eastern melon-producing region.

Table S2. Pairwise Adonis test based on Bray-Curtis distance in bulk and rhizosphere soils of the southern melon-producing region.

Table S3. Cross-domain network topological characteristics in bulk and rhizosphere soils of the two major melon-producing regions.

Supplementary Figures

Fig. S1. Microbial community alpha diversity in bulk and rhizosphere soils of the two major melon-producing regions with different durations of continuous cropping. (a) Shannon index of soil bacteria in the eastern region; (b) Shannon index of soil fungi in the eastern region; (c) Shannon index of soil bacteria in the southern region; (d) Shannon index of soil fungi in the southern region.

Fig. S2. Bray-Curtis principal coordinates analysis (PCoA) of soil bacterial and fungal communities in bulk and rhizosphere soils of the eastern (a and b) and southern (c and d) melon-producing regions with different durations of continuous melon cropping.

Fig. S3. Redundancy analysis (RDA) of soil bacterial and fungal communities in rhizosphere soil of the eastern (a and b) and southern (c and d) melon-producing regions with different durations of continuous melon cropping.

Fig. S4. Bipartite networks revealing specific species of bacterial and fungal communities in bulk and rhizosphere soils of the eastern (a and c) and southern (b and d) melon-producing regions with different durations of continuous cropping (based on indicator species analysis).

Fig. S5. Bacterial (a-f) and fungal (g-l) keystone taxa identified in bulk and rhizosphere soil microbial communities of the eastern melon-producing region with different durations of continuous cropping based on specificity-occupancy (SPEC-OCCU) analysis.

Fig. S6. Bacterial (a-d) and fungal (e-h) keystone taxa identified in bulk and rhizosphere soil microbial communities of the southern melon-producing region with different durations of continuous cropping based on SPEC-OCCU analysis.

Fig. S7. Abundance of keystone bacterial genera (a-g) in bulk soil of the eastern melon-producing region with different durations of continuous cropping.

Fig. S8. Abundance of keystone fungal genera (a-d) in bulk soil of the eastern melon-producing region with different durations of continuous cropping.

Fig. S9. Abundance of keystone bacterial genera (a-al) in rhizosphere soil of the eastern melon-producing region with different durations of continuous cropping.

Fig. S10. Abundance of keystone fungal genera (a and b) in rhizosphere soil of the eastern melon-producing region with different durations of continuous cropping.

Fig. S11. Abundance of the keystone fungal genus (*Penicillium*) in bulk soil of the southern melon-producing region with different durations of continuous cropping.

Fig. S12. Abundance of keystone fungal genera (a and b) in rhizosphere soil of the southern melon-producing region with different durations of continuous cropping.

Fig. S13. Abundance of keystone bacterial genera (a and b) in bulk soil of the southern melon-producing region with different durations of continuous cropping.

Fig. S14. Abundance of keystone bacterial genera (a-ad) in rhizosphere soil of the southern melon-producing region with different durations of continuous cropping.

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