

Article

Continuous Cropping Alters Soil Microbial Community Assembly and Co-Occurrence Network Complexity in Arid Cotton Fields

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Abstract: This study examines the impact of continuous cropping (short-term: 1–8 years; medium-term: 9–15 years; long-term: 16–30 years) on soil microbial community diversity, co-occurrence networks, and assembly processes in Xinjiang’s cotton region, a globally recognized arid zone. The results are as follows. Soil physicochemical analyses showed that as continuous cropping duration increased, soil organic matter and total nitrogen significantly decreased, whereas available phosphorus and potassium increased, and the soil’s aggregate structure degraded. Microbial community analysis indicated that long-term continuous cropping notably increased the richness of bacterial species (Chao1 index) and altered fungal communities’ diversity and composition, especially increasing the relative abundance of *Cladosporium* and *Alternaria* in the long term (GY30). Co-occurrence network analysis revealed higher complexity in bacterial and fungal networks in the short term. As cropping duration increased, bacterial network complexity significantly decreased, while fungal networks partially recovered in the long term, indicating greater fungal adaptability to environmental changes. Assembly process analysis revealed that the assembly of bacterial and fungal communities was jointly regulated by stochastic and deterministic processes, but with increasing cropping duration, deterministic processes weakened while stochastic processes intensified. Soil available phosphorus, potassium, and pH were identified as key factors influencing microbial community succession and assembly. This study highlights the significance of co-occurrence networks and assembly processes for understanding the dynamics of continuous cropping’s impact on soil microbial communities, offering a theoretical foundation for improving agricultural management.

Keywords: continuous cropping; cotton; microbial community assembly; co-occurrence network; diversity



Academic Editor: Vito Armando Laudicina

Received: 12 May 2025

Revised: 7 June 2025

Accepted: 11 June 2025

Published: 12 June 2025

Citation: Chen, J.; Yang, X.; Zhong, D.; Huo, Z.; Sun, R.; Dong, H. Continuous Cropping Alters Soil Microbial Community Assembly and Co-Occurrence Network Complexity in Arid Cotton Fields. *Agriculture* **2025**, *15*, 1274. <https://doi.org/10.3390/agriculture15121274>

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1. Introduction

Continuous cropping refers to the agricultural practice of planting the same crop on the same piece of land for multiple consecutive years. In China, continuous cropping is widely adopted due to limited arable land and low agronomic costs, particularly in the Xinjiang region, where it is most prominent [1]. As the primary cotton-producing area in

China, Xinjiang accounts for 90% of the nation's total cotton output and represents 20% of worldwide cotton production [2]. Notably, Xinjiang, China, is the world's largest cotton production base in an arid region [3]. However, long-term monoculture has caused continuous cropping barriers, threatening the sustainable development of the cotton industry in this region [4]. Research indicates that continuous cropping alters abiotic soil properties, including soil moisture, pH, nutrient cycling, and organic matter, thereby impacting the composition and function of soil microbial communities [1,5,6], ultimately resulting in reduced crop yield and quality [7–9]. However, the interactions between abiotic and biotic (microbial) factors and their underlying mechanisms remain unclear in the context of long-term continuous cotton cropping in arid regions.

Soil microbes play an extremely vital role in element cycling and ecosystem functions, and their imbalance can serve as an indicator of anthropogenic activity, such as the application of chemical fertilizers and pesticides, changes in tillage practices, the promotion of crop rotation, and the use of organic fertilizers. [10]. Healthy soil is fundamental to crop growth and food security in agriculture [11], and soil microorganisms, as critical drivers of specific soil functions [12], must be included in studies on the response mechanisms of agricultural soil systems to continuous cropping. To date, the effects of continuous cropping on soil microbial communities have been assessed with regard to certain cash crops and ornamental plants [13]. However, studies on the response of soil microbial communities to continuous cropping in cotton cultivation are still limited. Research by Li et al. [14] demonstrated that continuous cropping significantly reduced the diversity of total bacteria and fungi in cotton soil. However, microbiome characteristics are not limited to the quantity and composition of microbial communities but also include the complex ecological interactions among their members [15]. Co-occurrence network analysis offers a novel approach to studying complex microbial communities [16]. For instance, microbial co-occurrence networks can predict potential interactions, such as competition, facilitation, and inhibition, among species [17]. Furthermore, network analysis can visualize the response patterns of different taxa to agronomic practices and identify key species assemblages associated with agricultural ecosystem functions [18]. Microbial network complexity serves as a key indicator of ecosystem stability and functionality [19]. However, it remains unclear how the network complexity of soil bacterial and fungal communities varies with the duration of cotton continuous cropping.

The assembly of microbial communities, influenced by both abiotic and biotic factors, plays a critical role in determining their functional outcomes [20]. Two primary theoretical frameworks, deterministic and stochastic processes, govern microbial community assembly [21,22]. According to niche theory, deterministic mechanisms, including environmental filters (e.g., salinity, pH, temperature) and biotic interactions (e.g., competition, predation, mutualism, and facilitation), shape microbial communities by leveraging variations in ecological traits among taxa [23–25]. In contrast, neutral theory posits that stochastic events, such as random births, deaths, and dispersal, drive community structure, assuming a dynamic balance between taxa loss and gain [26,27]. The neutral community model (NCM) proposed by Sloan [28] is particularly useful in quantifying the importance of neutral processes. The neutral community model (NCM) posits that abundant taxa are more likely to disperse randomly and become widely distributed across a metacommunity, whereas rare taxa are prone to local extinction due to ecological drift. The NCM is used to fit the relationship between the occurrence frequency of a taxon in a set of local communities and their abundance across the wider metacommunity by estimating the migration rate (m). Higher m values indicate that microbial communities are less dispersal-limited. R^2 indicates the fit of the parameter based on nonlinear least-squares fitting. Higher R^2 values indicate a higher contribution of stochastic processes to microbial community assembly [28,29].

Research has consistently demonstrated that both deterministic and stochastic mechanisms operate concurrently during community assembly [26,30,31]. Deterministic processes drive functional convergence among microbial taxa, whereas stochastic processes promote functional divergence [32,33]. Importantly, the relative roles of deterministic and stochastic processes in microbial community assembly can be evaluated using environmental variables [25,34,35]. While the influence of environmental factors on microbial assembly has been widely investigated, their effects differ based on the type of process [36]. Despite increasing interest in microbial community assembly mechanisms in recent years, knowledge of how these processes interact with ecological dynamics during cotton continuous cropping succession is still limited [37]. Identifying the factors that regulate the balance between stochastic and deterministic processes could offer key insights into microbial community assembly in cotton fields. Research indicates [38–40] that continuous cropping significantly affects soil nutrient availability and organic matter content. Thus, we hypothesize that continuous cotton cropping may shift the balance between stochastic and deterministic processes in microbial succession by modifying soil environmental conditions.

This study aims to systematically investigate the effects of different continuous cropping durations (short-term: 1–8 years; medium-term: 9–15 years; long-term: 16–30 years) on the structure, co-occurrence networks, and assembly processes of soil microbial communities in cotton fields. By analyzing soil samples from ten sites in the major cotton-growing regions of the arid area of Xinjiang, we aimed to reveal the significant impacts of continuous cropping duration on the diversity and composition of soil bacterial and fungal communities, explore its role in shaping the construction patterns of microbial co-occurrence networks, and analyze how continuous cropping alters the balance between deterministic and stochastic processes in microbial community assembly by regulating soil nutrient levels. In this context, we hypothesized the following: (1) different continuous cropping stages significantly alter the diversity and structural composition of soil bacterial and fungal communities; (2) the duration of continuous cropping influences the construction of bacterial and fungal co-occurrence networks in cotton field soil; (3) different continuous cropping durations regulate the relative contributions of deterministic and stochastic processes to bacterial and fungal community assembly by changing soil nutrient content.

2. Materials and Methods

2.1. Experimental Site

In the Xinjiang region of China (73°40′~96°18′ E, 34°25′~48°10′ N), cotton is primarily cultivated in the southern and northwestern areas. This study was conducted in the main cotton-growing regions of the arid zone in Xinjiang. All sampled cotton fields had been managed under a long-term irrigated oasis agricultural system. The climate in the arid zone of Xinjiang is characterized by temperate arid and alpine types. The soil types are mainly desert and mountain soils. Annual precipitation is low with significant variability, and evaporation rates are high. The region receives 2550–3500 h of sunshine annually, with long and intense sunlight exposure [41]. Vegetation is sparse, dominated by drought-tolerant herbaceous plants and shrubs. The soil is poor in quality, with low moisture content and saline–alkaline properties. The agricultural type is irrigated oasis farming. The Tianshan Mountains divide Xinjiang into southern and northern parts. Compared to the north, southern Xinjiang has less precipitation and higher annual average temperatures, while northern Xinjiang receives more precipitation and has lower annual average temperatures [42,43]. The sampling locations are shown in Figure 1, and Table S1 provides a comprehensive overview of the geographic and climatic data for each sampling site. Data on elevation, monthly average minimum and maximum temperatures, and monthly average precipitation during the experimental period were obtained from the

WorldClim global database (2020–2024; <https://www.worldclim.org/> (accessed on 29 May 2025)).

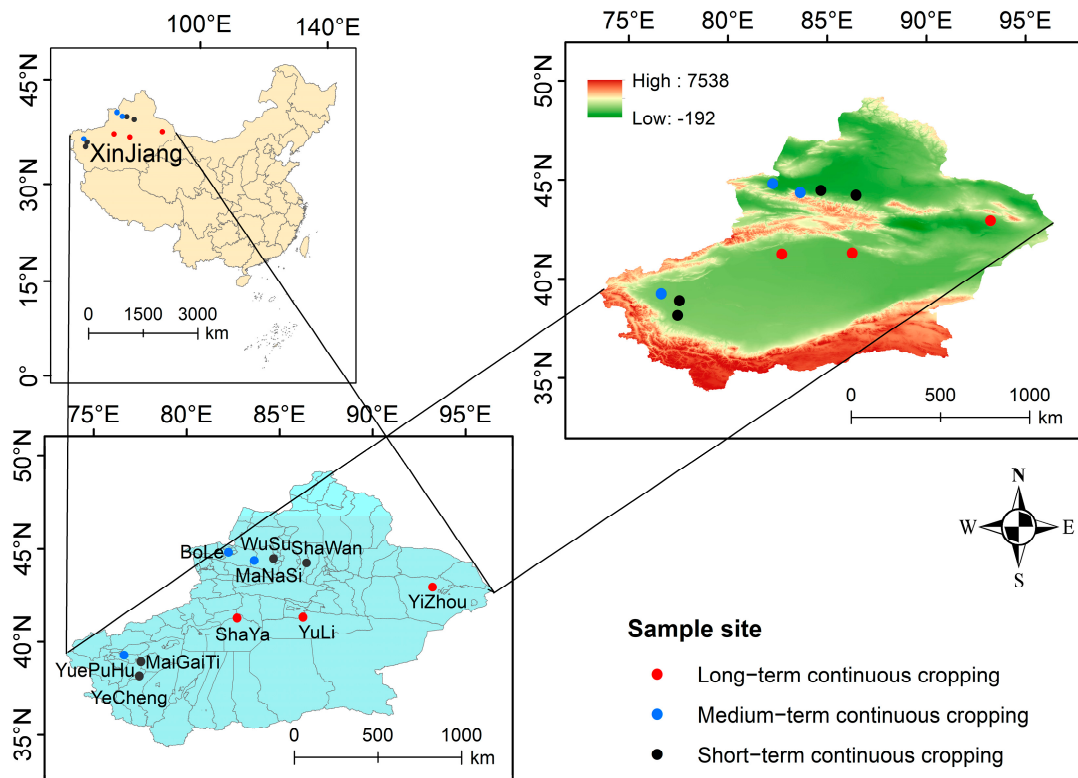


Figure 1. Study area and locations of sampling sites. The precise locations of cotton field sampling sites, classified by continuous cropping types, are indicated by dots in different colors.

2.2. Sample Collection

The soil samples analyzed in this study were sourced from the primary cotton cultivation areas in Xinjiang Uygur Autonomous Region, China (Figure 1). We collected cotton field soils with continuous cropping durations of 1, 4, 5, and 8 years from Manas County, Yecheng County, Maigaiti County, and Wusu City, respectively; soils with continuous cropping durations of 10, 13, and 15 years from Shawan City, Bole City, and Yuepuhu County, respectively; and soils with continuous cropping durations of 18, 20, and 30 years from Shaya County, Yizhou District of Hami City, and Yuli County, respectively. All sampling sites were from the same field experimental area, characterized by continuous cotton planting and straw return practices. The sites were classified into three gradients based on the duration of continuous cropping: short-term (GY8, 1–8 years), medium-term (GY15, 9–15 years), and long-term (GY30, 16–31 years).

This study was conducted in April 2024 in Xinjiang, China. Using an “S”-shaped five-point sampling method, soil samples from the 0–20 cm layer were collected at each sampling point, with each location sampled three times. The samples were sealed, transported to the laboratory, and divided into two portions; one was air-dried for physicochemical analysis, and the other was stored at -80°C for microbial community characterization. At 5–10 cm around the soil sample site, five 0–20 cm surface undisturbed soil cores were taken from each repeat point with a profile knife. Soil structure was not damaged as much as possible in sampling process. The samples were brought back to the laboratory for soil aggregate analysis. Meanwhile, five soil cutting ring samples were taken from each repeat point.

2.3. Soil Physicochemical Properties Analysis

Soil bulk density (BD, g/cm³) was determined using the ring knife method. Soil pH was measured with a glass electrode (PHS-3C, Shanghai Leici, Shanghai, China) at a soil-to-water ratio of 1:2.5. Water-stable aggregates were classified by particle size following the classical method outlined by Elliott and Cambardella [44]. Soil aggregates were initially screened using the dry sieving method, followed by wet sieving analysis on an aggregate analyzer (TTF-100, Shangyu Shunlong, Shaoxing, China) to determine the particle size distribution of water-stable aggregates. Soil electrical conductivity (EC, mS/cm) was assessed using a conductivity meter (DDS-307A, Shanghai Leici, Shanghai, China).

Soil chemical properties were analyzed according to the protocol by Bao [45]. Soil organic matter (SOM, g/kg) was determined using the K₂Cr₂O₇ external heating method. Total nitrogen (TN, g/kg) was measured via perchloric acid-sulfuric acid digestion, while total phosphorus (TP, g/kg) was assessed using the H₂SO₄-HClO₄ digestion-molybdenum antimony blue colorimetric method. Total potassium (TK, g/kg) was determined through HF-HClO₄ digestion and atomic absorption spectrometry. Soil available nitrogen (AHN, mg/Kg) content was measured using the alkaline hydrolysis diffusion method. Available phosphorus (AP, mg/Kg) was extracted using sodium bicarbonate and quantified via the molybdenum antimony anti-colorimetric method. Available potassium (AK, mg/Kg) was extracted using ammonium acetate and measured by atomic absorption spectrometry [46,47].

2.4. DNA Extraction

Total genomic DNA from the microbial community was extracted from 0.5 g of soil using the E.Z.N.A.TM MagBind Soil DNA Kit (Omega, M5635-02, Norwalk, CT, USA) as per the manufacturer's instructions. The concentration of the extracted DNA was determined using a Qubit 4.0 (Thermo, Waltham, MA, USA) to ensure the recovery of sufficient high-quality genomic DNA. The DNA was then stored at −80 °C for high-throughput sequencing analysis.

2.5. Illumina MiSeq High-Throughput Sequencing

High-throughput sequencing technology was employed to analyze the V3-V4 hyper-variable region of the 16S rRNA gene and the fungal ITS region, investigating the diversity and community composition of bacteria and fungi in the 0–20 cm soil layer under three different treatments. The bacterial 16S rRNA gene was amplified using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'), while the fungal ITS region was amplified with primers ITS3F (5'-GCATCGATGAAGAACGCAGC-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3'). Sequencing was conducted on the Illumina MiSeq platform by Sangon Biotech (Shanghai, China). Raw sequencing data were processed through assembly, filtering, and removal of chimeras, followed by OTU clustering at 97% sequence similarity. The resulting OTU data and taxonomic annotations were used for downstream analysis. The raw sequences were deposited in the NCBI database under accession numbers PRJNA1242761 and PRJNA1242994.

2.6. Statistical Analyses

To investigate the assembly mechanisms of soil microbial communities in cotton fields, two models were applied. The Neutral Community Model (NCM) was used to fit the relationship between the frequency of taxa occurrence in local communities and their abundance in a broader metacommunity by estimating the migration rate (*m*) [48,49]. Additionally, the normalized stochasticity ratio (NST) was used to quantify the relative contributions of deterministic and stochastic processes to bacterial and fungal community

assembly, with 50% as the threshold distinguishing more deterministic ($\text{NST} < 50\%$) from more stochastic ($\text{NST} > 50\%$) assembly [26,35,50].

To elucidate the impact of the duration of continuous cropping on the co-occurrence patterns of soil microbial communities, we constructed co-occurrence networks based on OTU abundance data. High-throughput sequencing detected a total of 37,335 bacterial operational taxonomic units (OTUs) and 2360 fungal OTUs. To reduce the complexity of the dataset, OTU abundance data were normalized, and low-abundance OTUs (relative abundance $< 0.005\%$) were filtered out to minimize noise. Subsequently, the SparCC algorithm was employed to calculate correlation coefficients between OTUs, and significant correlations were screened using a correlation threshold ($|r| > 0.6$, $p < 0.05$). An undirected network was constructed using the “ggClusterNet” package. Network topological parameters (such as the number of nodes, edges, average path length, clustering coefficient, etc.) were calculated using the igraph package in R. The complexity of soil microbial networks was evaluated using network topological features, such as nodes, edges, edge density, complexity, modularity, and betweenness centralization. Network visualization was performed using the Gephi0.10.1 platform (<https://gephi.github.io/> (accessed on 30 June 2024)) [17,51,52].

Soil physicochemical variables were analyzed in SPSS 27.0 using one-way ANOVA with Duncan’s post hoc test in SPSS. In R, the vegan package was employed to compute microbial diversity indices (Shannon, Chao1) and determine the relative abundance of microbial species. Heatmaps were generated using ComplexHeatmap and ggplot2. Redundancy analysis (RDA) was conducted to evaluate the impact of soil properties on bacterial and fungal communities. The R package “randomForest” was employed for random forest (RF) classification analysis to assess the relative importance of environmental variables in shaping bacterial and fungal community assembly [53]. Variable importance was assessed based on the percentage increase in mean squared error (MSE). Variables with higher MSE% values were considered more significant [54]. The “A3” package was applied to assess the significance of the models and the cross-validated R^2 values, with 999 permutations of the response variable performed for the analysis [55]. The “rfPermute” package (<https://cran.r-project.org/web/packages/rfPermute> (accessed on 18 January 2025)) was also employed to estimate the contribution of each predictor variable to the response variable.

3. Results

3.1. Soil Physicochemical Characteristics

Continuous cropping treatments notably altered soil physicochemical properties (Table 1). With long-term continuous cropping, there was a steady decrease in soil SOM, TN, EC, and BD, but AP and AK levels increased. Furthermore, significant changes were observed in soil aggregate structure. Cotton fields under mid-term planting management displayed improved soil structure characterized by higher macro-aggregate and fine macro-aggregate content, moderate micro-aggregate levels, and silt–clay fractions maintained at an optimal range.

3.2. Bacterial and Fungal Biomass and Diversity

Following sequencing, a total of 37,335 bacterial OTUs were identified, classified into 49 phyla, 158 classes, 432 orders, 754 families, and 1571 genera. Meanwhile, 2360 fungal OTUs were identified, classified into 17 phyla, 53 classes, 124 orders, 291 families, and 588 genera. Analysis of bacterial and fungal diversity (Figure 2) revealed no significant differences in the bacterial Shannon index across different planting durations ($p > 0.05$). However, significant differences were detected in the bacterial Chao1 index and the fun-

gal Shannon and Chao1 indices ($p < 0.05$). These results suggest that bacterial species richness in the soil significantly increased with prolonged continuous cropping, whereas fungal diversity and species richness exhibited changes associated with the duration of continuous cropping.

Table 1. Soil Physicochemical Indicators in Cotton Fields Under Different Continuous Cropping Stages.

	GY8	GY15	GY30
pH	7.49 ± 0.04 a	7.60 ± 0.04 a	7.66 ± 0.11 a
EC (mS cm ⁻¹)	0.79 ± 0.14 a	0.82 ± 0.29 a	0.40 ± 0.05 a
BD (g cm ⁻³)	1.34 ± 0.04 a	1.37 ± 0.06 a	1.27 ± 0.09 a
SOM (g kg ⁻¹)	15.89 ± 1.81 a	14.86 ± 1.07 a	12.15 ± 0.79 b
Total N (g kg ⁻¹)	1.03 ± 0.11 a	0.97 ± 0.03 a	0.84 ± 0.07 a
Total P (g kg ⁻¹)	0.78 ± 0.06 a	0.77 ± 0.04 a	0.79 ± 0.03 a
Total K (g kg ⁻¹)	21.83 ± 0.49 b	24.56 ± 1.15 a	22.78 ± 0.77 b
AHN (mg kg ⁻¹)	51.15 ± 4.12 a	41.45 ± 1.58 b	40.87 ± 2.35 b
AP (mg kg ⁻¹)	13.13 ± 1.55 b	23.53 ± 3.31 b	42.04 ± 8.30 a
AK (mg kg ⁻¹)	287.45 ± 36.81 a	158.73 ± 9.96 b	292.16 ± 22.48 a
LM (%)	0.04 ± 0.00 b	0.07 ± 0.01 a	0.03 ± 0.00 c
SM (%)	0.25 ± 0.03 a	0.22 ± 0.02 a	0.14 ± 0.02 b
MA (%)	0.30 ± 0.03 a	0.35 ± 0.01 a	0.28 ± 0.06 a
SC (%)	0.41 ± 0.05 ab	0.36 ± 0.03 b	0.56 ± 0.08 a

Note: Data are presented as mean ± standard error. Different letters within the same column denote significant differences at the 0.05 level. As defined in the materials and methods, the abbreviations for sampling points and physicochemical indicators are LM (large macroaggregates), SM (small macroaggregates), MA (microaggregates), and SC (silt and clay).

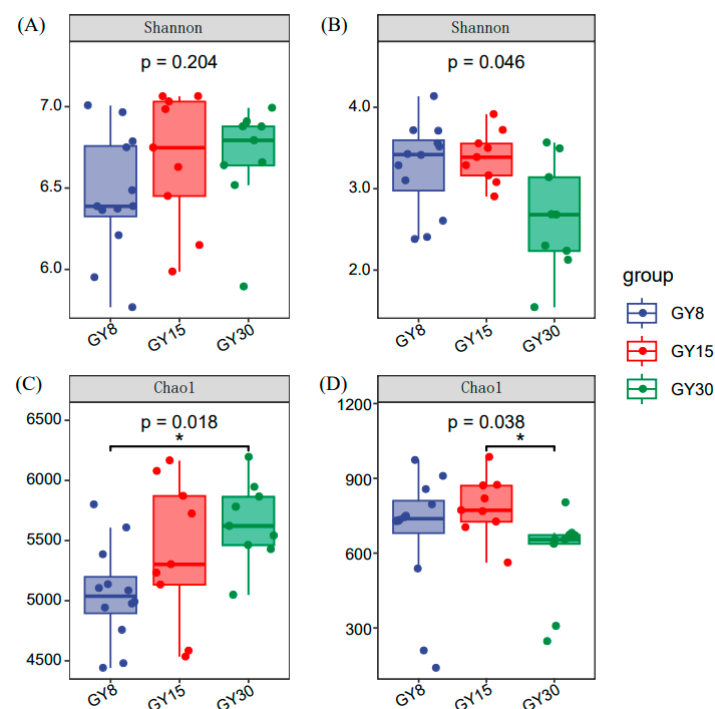


Figure 2. (A) Bacterial Shannon diversity index in soils under short-term (GY8), mid-term (GY15), and long-term (GY30) continuous cropping. (B) Fungal Shannon diversity index in soils under short-term (GY8), mid-term (GY15), and long-term (GY30) continuous cropping. (C) Bacterial Chao1 diversity index in soils under short-term (GY8), mid-term (GY15), and long-term (GY30) continuous cropping. (D) Fungal Chao1 diversity index in soils under short-term (GY8), mid-term (GY15), and long-term (GY30) continuous cropping. Significance between different continuous cropping stages is indicated by asterisks (*), * $p < 0.05$.

Specifically, soil under short-term continuous cropping (GY8) exhibited lower bacterial Chao1 and Shannon indices compared with soil under long-term continuous cropping (GY30). Conversely, soil under mid-term continuous cropping (GY15) displayed higher fungal Chao1 and Shannon indices compared with soils under the other two continuous cropping stages (Figure 2). These findings demonstrate that bacterial diversity and total species richness were lower in soils under short-term continuous cropping compared to long-term continuous cropping, whereas fungal diversity and total species richness were higher in soils under mid-term continuous cropping (GY15) than in short-term continuous cropping soils.

3.3. Compositional Differences in Soil Microbial Community Structure

At the bacterial phylum level, the top eight most abundant bacterial groups were identified as Proteobacteria, Actinobacteriota, Acidobacteriota, Bacteroidota, Planctomycetota, Chloroflexi, Gemmatimonadota, and Firmicutes. Among these, Proteobacteria, Actinobacteriota, Acidobacteriota, and Bacteroidota emerged as the dominant bacterial groups across the three soil groups (Figure 3A). In the GY8 group, the relative abundances of these dominant groups were 37.802%, 10.128%, 11.455%, and 10.013%, respectively. In the GY15 group, the values were 40.879%, 12.507%, 9.045%, and 10.584%, respectively. In the GY30 group, the relative abundances were 34.425%, 12.162%, 11.769%, and 7.805%, respectively. At the fungal genus level, the top 8 most abundant fungal genera were identified as *Lasiobolium*, *Cladosporium*, *Vishniacozyma*, *Botryotrichum*, *Cephalotrichum*, *Antarctomyces*, *Betamyces*, and *Alternaria*. As depicted in Figure 3B, significant differences were observed among the three groups. In the GY8 group, the dominant fungal genera were *Lasiobolium*, *Vishniacozyma*, *Botryotrichum*, *Cladosporium*, and *Cephalotrichum*, with relative abundances of 18.211%, 10.484%, 7.570%, 5.784%, and 3.024%, respectively. In the GY15 group, the dominant genera were *Cladosporium*, *Vishniacozyma*, *Betamyces*, *Cephalotrichum*, and *Lasiobolium*, with relative abundances of 10.454%, 9.599%, 9.034%, 8.778%, and 6.064%, respectively. In the GY30 group, the dominant species included *Lasiobolium*, *Cladosporium*, *Vishniacozyma*, and *Antarctomyces*, with relative abundances of 22.473%, 28.001%, 10.355%, and 9.889%, respectively. The color variations in the heatmap provide a clearer visualization of the differences in microbial community structure in cotton field soils across the three continuous cropping stages (Figure S1).

3.4. Associations Between Environmental Factors and Microbial Communities

Redundancy analysis (RDA) revealed that environmental factors accounted for 32.52% of the variation in fungal community structure. Specifically, AP and EC exhibited positive correlations with RDA1, whereas SOM, TN, TP, TK, AN, AK, pH, and BD showed negative correlations with RDA1. In contrast, SOM, TN, TK, AN, BD, and AK displayed positive correlations with RDA2 (Figure 4A). Correlation analysis between environmental factors and community structure (Figure 4B) demonstrated that soil organic matter (SOM, $r^2 = 0.732$) exerted the most significant influence on fungal community structure in cotton soils under different continuous cropping regimes ($p < 0.01$), followed by alkali-hydrolyzable nitrogen (AN, $r^2 = 0.534$) and available potassium (AK, $r^2 = 0.524$) ($p < 0.01$).

Redundancy analysis of bacterial communities (Figure S2) revealed that environmental factors accounted for 26.49% of the variation in fungal community structure. Specifically, SOM, TN, TP, AN, AK, and BD exhibited negative correlations with RDA1 but positive correlations with RDA2. AP and EC showed positive correlations with RDA1, while pH displayed negative correlations with both RDA1 and RDA2. Correlation analysis further indicated that total potassium (TK, $r^2 = 0.572$) exerted the most significant influence

on bacterial community structure in soils under different continuous cropping regimes ($p < 0.01$), followed by electrical conductivity (EC, $r^2 = 0.491$) and soil organic matter (SOM, $r^2 = 0.407$) ($p < 0.01$). The findings from these two redundancy analyses indicate that fungal communities may be more sensitive to the effects of soil physicochemical properties and continuous cropping duration.

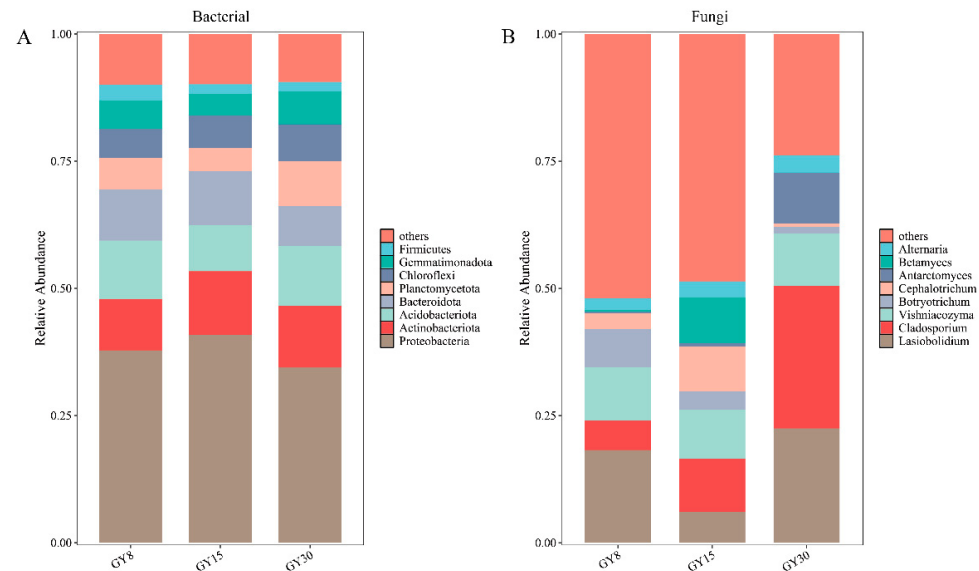


Figure 3. (A) Comparative analysis of bacterial relative abundances at the phylum level. (B) Comparative analysis of relative fungal abundance at the genus level.

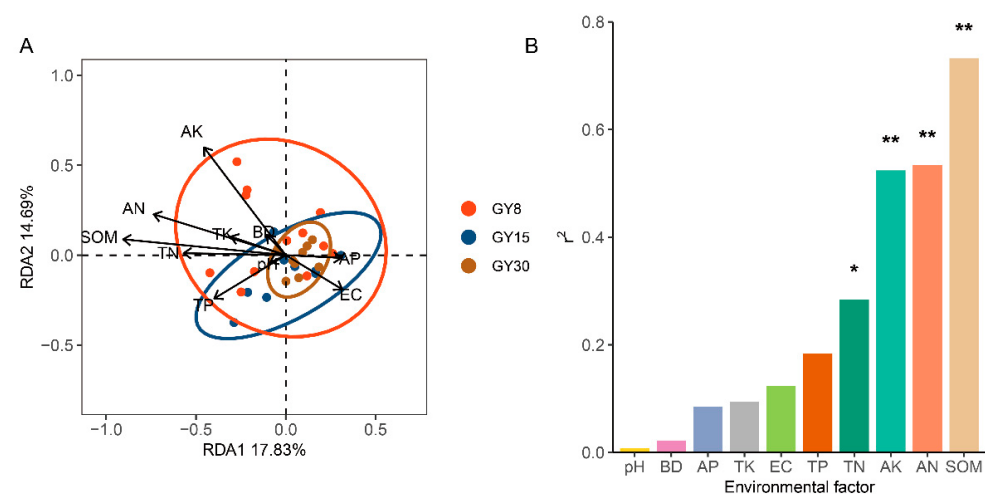


Figure 4. (A) Redundancy analysis (RDA) of fungi and soil factors across different continuous cropping stages. (B) Correlation analysis between environmental factors and fungal community structure. * $p < 0.05$, ** $p < 0.01$.

3.5. Bacterial and Fungal Co-Occurrence Networks

To investigate the co-occurrence patterns of microbial communities in the soils of GY8, GY15, and GY30 cotton fields, six network models were constructed based on the correlations between the screened bacterial and fungal OTUs (Figure 5). This study revealed that the co-occurrence network patterns of bacteria and fungi were significantly influenced by the duration of continuous cropping. This study employed key network topological properties (number of nodes, number of edges, average degree, and average clustering coefficient) to evaluate the complexity of soil microbial networks, while stability was assessed via connectivity. Higher values for the number of nodes, edges, average degree,

and average clustering coefficient reflect increased network complexity. In the GY8 group, bacterial and fungal networks exhibited higher numbers of nodes, edges, and average degrees compared with the other two groups. As the duration of continuous cropping increased, the number of nodes and edges in bacterial networks declined significantly, and the average degree also decreased markedly, particularly under long-term continuous cropping (GY30), leading to a further reduction in bacterial community complexity. The network diameter, defined as the longest path between any two nodes in the network, was smallest in GY8, further corroborating the idea that its network connections are more tightly knit (Table S2). In the fungal networks, the number of nodes, edges, and average degree exhibited an initial decrease followed by a slight rise, suggesting that fungal co-occurrence networks experienced reduced complexity under mid-term continuous cropping (GY15) but showed partial recovery after long-term continuous cropping (GY30). Connectivity exhibited a similar trend, demonstrating that the stability of fungal networks mirrored changes in their complexity.

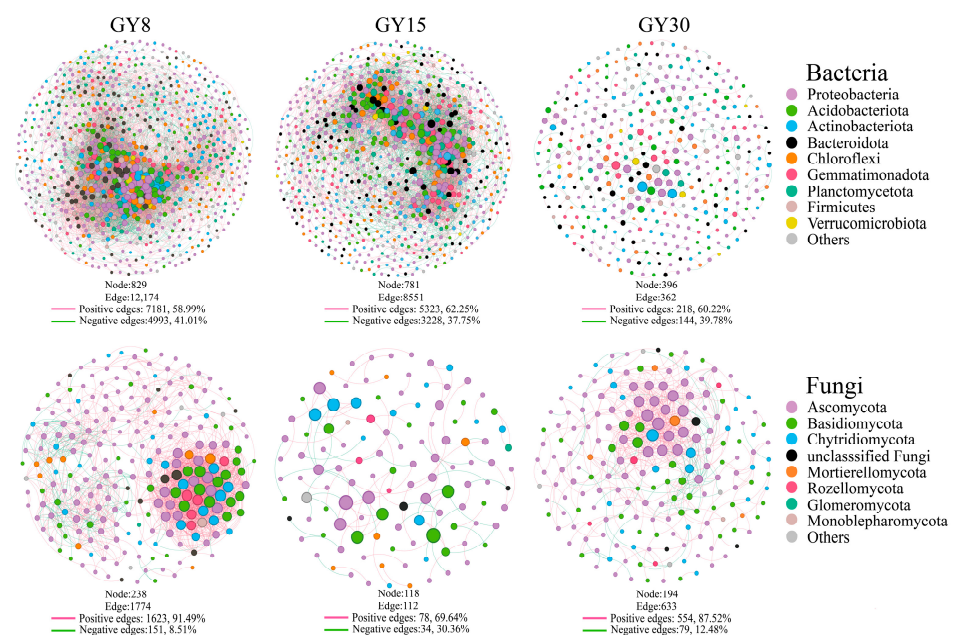


Figure 5. Bacterial and fungal networks in soils collected from short-term continuous cropping (GY8), medium-term continuous cropping (GY15), and long-term continuous cropping (GY30).

These analyses demonstrate that the complexity and stability of bacterial and fungal co-occurrence networks in cotton soils are strongly associated with continuous cropping duration. In soils subjected to short-term continuous cropping, both bacterial and fungal networks demonstrated higher complexity and stability; in mid-term continuous cropping soils, network complexity and stability decreased significantly; in long-term continuous cropping soils, bacterial networks experienced further declines in complexity and stability, whereas fungal networks exhibited signs of recovery and reorganization (Figure 5; Table S2). These findings highlight the profound influence of continuous cropping on the co-occurrence networks of soil microorganisms in cotton fields and offer valuable insights into the functional and ecological adaptability of soil microbial communities across varying planting durations.

3.6. Microbial Community Assembly Processes

This study employed the neutral community model (NCM) to investigate the control mechanisms governing soil microbial community assembly. The results revealed that the R^2

values ranged from 0.368 to 0.778 (Figure S3), with taxa exceeding the external proportion dashed line (bacteria: 6.4~7.3%, fungi: 9.6~22.5%), suggesting that stochastic processes dominated community assembly. Notably, 77.5% to 93.6% of OTUs aligned well with the NCM (Figure S3). These results collectively highlight the minimal impact of deterministic processes on soil microbial community assembly in cotton fields, with stochastic forces emerging as the primary driver in this intricate ecological dynamic. Migration rate (m), a key measure of species dispersal ability, offers valuable insights into the assembly mechanisms of bacteria and fungi in cotton field soils. Significant differences were observed in migration rates between bacteria (0.41–0.69) and fungi (0.039–0.258). Notably, the lower migration rate of fungi indicates a more prominent role of stochastic processes in bacterial community assembly, as illustrated in Figure 6A. Furthermore, spatial analysis of migration rates across different durations was conducted. Migration rates for both bacteria and fungi reached their peaks in long-term continuous cropping (GY30) cotton soils (0.69/0.258), whereas bacterial migration rates exhibited a gradual increase from short-term to long-term planting (Figure 6A). These findings align with the network analysis, suggesting that long-term continuous cropping may reduce bacterial interactions, whereas changes in planting duration could modulate fungal connections, potentially enhancing biological interactions.

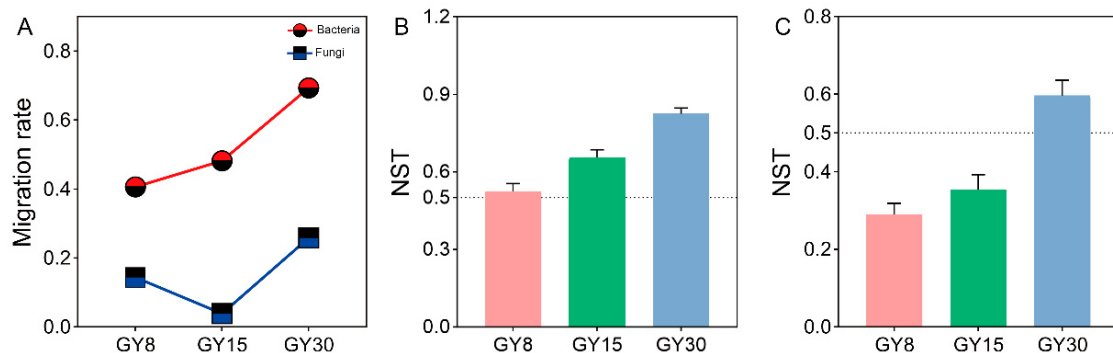


Figure 6. Mechanisms of microbial community assembly in cotton field soils across three continuous cropping periods. (A) Variations in the migration rate variations were estimated through the neutral community model (NCM). (B) The normalized stochasticity ratio (NST) for bacterial communities was calculated based on Bray–Curtis distance (NSTbray). (C) The normalized stochasticity ratio (NST) for fungal communities.

The normalized stochasticity ratio (NST) values revealed that the average bacterial community (NSTbray = 63.77%) across different treatments was primarily governed by stochastic processes, with a gradual increase observed during microbial assembly induced by long-term continuous cropping (Figure 6B). For fungi, deterministic processes were more influential than stochastic processes during short-term and mid-term planting stages, while stochastic processes became dominant in the long-term planting (GY30) stage (Figure 6C).

3.7. Predictors of Bacterial and Fungal Community Assembly Processes

Random forest modeling was employed to identify the key predictors of microbial assembly across three continuous cotton cropping systems (Figure 7A). The analysis revealed that AK content was the most significant variable for predicting fungal community assembly under the neutral community model (NCM), whereas AP content emerged as the primary predictor for NST-based bacterial and fungal community assembly as well as NCM-based bacterial community assembly, with pH and EC being secondary factors. Correlation analysis further demonstrated that bacterial community assembly was positively correlated with AP and negatively correlated with AHN. Similarly, fungal community assembly exhibited positive correlations with AP and AK (Figure 7B).

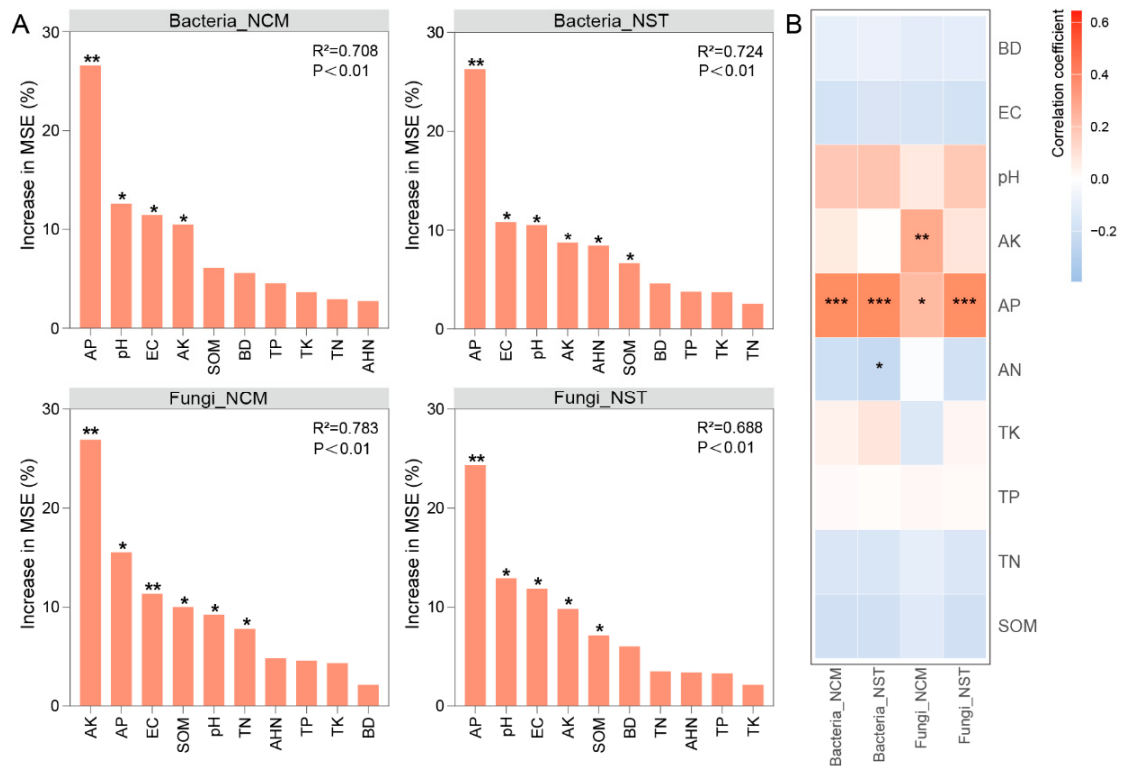


Figure 7. The Random Forest model illustrates the significance of predictors for bacterial and fungal community assembly (A); Regression analysis reveals the relationships between microbial distribution and soil physicochemical properties (B). Neutral community model (NCM); normalized stochasticity ratio (NST). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

4.1. The Stage-Specific Impact of Continuous Cropping on Soil Physicochemical Properties and Microbial Diversity in Cotton Fields

The physicochemical properties of soil are essential for soil health, governing moisture availability, aeration, temperature, nutrient cycling, and root expansion. These elements directly affect agricultural productivity and environmental health [56–58]. Long-term continuous cropping has been demonstrated to disrupt soil nutrient balance and compromise soil structure [59–61], leading to persistent degradation of soil fertility [4,62]. Soil organic matter, aggregate structure, and nutrient content are essential metrics for assessing soil health, and alterations in these properties and structures in agricultural soils are significantly influenced by the duration of continuous cropping [63]. This study shows that different continuous cropping years have significant effects on the physicochemical properties and aggregate structure of cotton field soil. With increasing years of continuous cropping, soil organic matter, total nitrogen, and bulk density exhibited progressive declines, while available phosphorus and potassium contents showed an upward trend. These changes reflect the consumption of soil fertility and the alteration of soil structure by long-term continuous cropping (Table 1). Notably, during the long-term continuous cropping phase (GY30), the marked reduction in soil organic matter (SOM) and total nitrogen (TN) directly contributed to diminished soil fertility, subsequently affecting cotton growth and yield. The content of macroaggregates and microaggregates decreased again, and the content of silt-clay components increases significantly (Table 1), indicating that the soil structure tended to degrade. These alterations are likely to be closely associated with changes in soil organic matter and nutrient contents, further impacting soil physical structure and microbial habitats.

The soil microbiome is a complex mixture of microorganisms mainly composed of fungi and bacteria [64], and it plays a key role in regulating crop productivity and nutrient cycling through complex interactions with soil properties [65]. This study shows that the diversity and composition of bacterial and fungal communities exhibited different responses to different durations of continuous cropping (Figures 2, 3 and S1). Long-term continuous cropping significantly enhanced bacterial species richness (Chao1 index), whereas fungal diversity and species richness displayed more heterogeneous trends across different continuous cropping phases. Interestingly, the relative abundance of dominant bacterial taxa remained relatively stable across all continuous cropping stages, which may have been due to consistent agricultural management practices such as fertilization and irrigation, making these taxa core members of the soil microbial community. In contrast, fungal communities, which play a crucial role in organic matter decomposition and nutrient cycling [66], exhibited significant compositional shifts. For instance, *Lasiobolidium* and *Cephalotrichum* were prominent across all three continuous cropping phases, underscoring their critical roles in sustaining soil ecosystem functions. We hypothesize that the dominance of *Cephalotrichum* is associated with the annual return of crop residues to the farmland. It was also found that the relative abundance of *Cladosporium* and *Alternaria* was significantly higher in the long-term continuous cropping stage than in other stages, and they may act as secondary pathogens, increasing the incidence of cotton diseases and pests [67]. *Cladosporium* is a multifunctional fungal genus. It includes common endophytes, plant pathogens, and fungal hyperparasites [68]. The increase in its abundance may exacerbate the spread of plant diseases through its pathogenic characteristics [69]. At the same time, *Alternaria* is a typical plant pathogen. Its dominant growth directly promotes the occurrence and spread of diseases such as leaf spot. Studies have shown that the population dynamics of *Cladosporium* and *Alternaria* significantly influence the risk of diseases in various crops [70–72]. These are important factors in the mechanisms of disease epidemics, consistent with the observed increase in cotton diseases in long-term continuous cotton cropping systems in our study. This observation aligns with prior studies suggesting that long-term continuous cropping may promote the enrichment of pathogenic microorganisms and thereby intensify disease pressure within agricultural systems [4,6,73].

4.2. Continuous Cropping Decreased the Complexity of Bacterial Networks and Modified the Structure of Fungal Networks

Microbial co-occurrence network analysis (Figure 5) provided critical insights into the symbiotic patterns of microbial communities in cotton field systems across different continuous cropping stages [13]. Key network topological features, including nodes, edges, degree, clustering coefficient, and connectivity, serve as essential metrics for assessing interactions within microbial communities [74,75]. This study found that during the short-term continuous cropping stage, bacterial community networks exhibited higher complexity, with significantly greater numbers of nodes, edges, and average degrees compared to mid- and long-term continuous cropping treatments (Figure 5, Table S2). This indicates that in the early stages of continuous cropping, bacterial communities were characterized by intimate interactions, forming a highly connected co-occurrence network. However, as the duration of continuous cropping increased, network complexity significantly declined, particularly during the long-term (GY30) continuous cropping stage, where overall connectivity was markedly reduced. These changes may be closely linked to the deterioration of the soil environment induced by long-term continuous cropping, including reduced organic matter, structural degradation, and nutrient imbalances, which likely diminished niche differentiation among bacterial species and weakened competitive and cooperative relationships. Given that complex soil microbial communities are generally more resilient and adaptive to environmental disturbances compared with simpler communities [76], the

reduction in bacterial network complexity caused by long-term continuous cropping may increase the sensitivity of bacterial communities to environmental changes and reduce their recovery capacity. In contrast to bacterial co-occurrence networks, fungal co-occurrence networks also displayed higher complexity during the short-term continuous cropping (GY8) stage, but their complexity and stability decreased during the mid-term continuous cropping (GY15) stage, with reductions in node numbers, edge counts, and average degrees. However, during the long-term continuous cropping (GY30) stage, the complexity and stability of fungal networks partially recovered, indicating that fungal communities may possess certain adaptive and reorganization capabilities. This recovery may be attributed to the stronger tolerance of fungi to environmental changes, particularly under long-term continuous cropping conditions, where certain fungal taxa may sustain community stability by forming stable local subpopulations or enhancing interspecies interactions. Extensive research has demonstrated that microbial community interactions (i.e., cooperation and competition) weaken under favorable environmental conditions but may intensify in unfavorable soil environments [77,78]. We hypothesize that during long-term continuous cropping, agricultural management practices may provide transient favorable conditions for bacteria and fungi, resulting in reduced interspecies interactions and a tendency toward simplified co-occurrence networks. However, this hypothesis warrants further investigation.

The co-occurrence patterns of soil bacterial and fungal taxa during continuous cropping succession demonstrate their adaptive responses and selection mechanisms to environmental changes [79,80]. Variations in the complexity and stability of bacterial and fungal co-occurrence networks highlight the divergent ecological strategies adopted by microbial communities across different continuous cropping durations. In the short-term continuous cropping stage, the soil environment is relatively favorable, with high species diversity in bacterial and fungal communities, frequent interspecies interactions, and high network complexity and stability. However, as the duration of continuous cropping increases, changes in the soil environment drive microbial communities to adopt different survival strategies to cope with environmental stress. Moreover, variations in network complexity may influence soil ecological functions. Higher network complexity typically signifies greater diversity and stability in the ecological functions of microbial communities, whereas reduced network complexity may result in functional simplification and heightened vulnerability. Thus, the decline in bacterial network complexity induced by long-term continuous cropping may adversely affect soil ecological functions, whereas the partial recovery of fungal networks offers potential for sustaining these functions. These findings provide critical insights into the effects of continuous cropping on soil microbial ecosystems and offer theoretical foundations for optimizing agricultural management practices to sustain soil health and sustainability.

4.3. Variations in the Assembly Mechanisms of Microbial Communities in Soils Subjected to Continuous Cropping

Elucidating the assembly mechanisms of soil microbial communities is essential for understanding their formation [81], particularly in cotton field ecosystems extensively cultivated in arid regions. The results of this study indicate that the assembly processes of bacterial and fungal communities are jointly regulated by stochastic and deterministic processes across different continuous cropping stages, but their relative contributions exhibit significant variations with continuous cropping duration (Figures 6 and S3). The divergent changes in the assembly processes of bacterial and fungal communities reflect their distinct adaptation strategies to continuous cropping environments and offer critical insights into the effects of continuous cropping on soil microbial community assembly mechanisms. The enhanced stochasticity in bacterial community assembly is likely to be closely linked to the deterioration of soil environments induced by long-term continuous cropping (e.g.,

nutrient imbalance, structural degradation), which diminishes niche differentiation and consequently amplifies the role of stochastic processes. Notably, many studies suggest that deterministic processes are the primary drivers of bacterial community assembly [82–84], but there are certain discrepancies between our findings and these observations. Additionally, some studies have shown that stochastic processes dominate bacterial community assembly in forest and urban ecosystems [35,85]. These discrepancies indicate that the relative significance of deterministic and stochastic processes in bacterial community assembly may differ among ecosystem types. In fungal community assembly, deterministic processes prevail during the initial and mid-term continuous cropping stages, whereas long-term continuous cropping transitions to stochastic processes. This shift may be attributed to the combined effects of environmental filtering and interspecies competition in relatively stable soil environments, which shape fungal community composition, allowing certain fungal taxa to dominate specific niches and establish stable interactions with other species. For instance, environmental factors such as available phosphorus (AP) and available potassium (AK) selectively filter and promote the proliferation of certain fungal taxa while suppressing others, thereby reconstructing complex co-occurrence networks. This transition from deterministic to stochastic processes underscores the dynamic adaptation mechanisms of fungal communities to continuous cropping environments.

Moreover, our results corroborate the third hypothesis that alterations in soil nutrients induced by continuous cotton cropping govern the succession of microbial community assembly processes. Available phosphorus (AP), as a direct nutrient source for cotton growth and soil microorganisms, is the most critical factor shaping bacterial and fungal community assembly processes (Figure 7). Earlier studies have demonstrated that soil nutrients, including soil organic matter (SOM) and dissolved organic carbon (DOC), play a pivotal role in shaping microbial community assembly processes [86–88]. Our results align partially with previous research by Li et al. [89], which revealed that extreme environmental conditions, including salinity stress, nutrient deficiency, and water scarcity, profoundly impact microbial community assembly processes. With increasing continuous cropping duration, the deterministic processes governing bacterial and fungal community assembly diminish, whereas stochastic processes become more pronounced. Studies have shown that increases in phosphorus and nitrogen (N) also enhance the stochasticity of microbial assembly processes [32]. This study also found significant differences in other nutrients such as available potassium (AK), soil organic matter (SOM), available hydrolyzable nitrogen (AHN), and pH in shaping bacterial and fungal community assembly (Figure 7). Similar patterns of microbial assembly processes have been observed in agricultural soils [25], grasslands [90], and forests [91], suggesting that the role of soil mineral nutrients in influencing microbial assembly may be universal across ecosystems [32]. These findings not only enhance our comprehension of the mechanisms underlying soil microbial community assembly under continuous cropping but also offer a theoretical foundation for refining agricultural management strategies to sustain soil health and ecosystem functionality.

5. Conclusions

This study reveals the significant impact of long-term continuous cropping on soil microbial communities in arid cotton fields, elucidating the dynamic evolution of diversity, co-occurrence network complexity, and assembly mechanisms of bacterial and fungal communities at different stages of continuous cropping. The study finds that during the initial stages of continuous cropping, both bacterial and fungal communities exhibit high diversity and complexity. Bacterial communities are mainly shaped by stochastic processes, while fungal communities are largely influenced by deterministic processes. With prolonged continuous cropping, bacterial communities experience a sustained reduction in diversity

and network complexity, accompanied by an increased contribution of stochastic processes. In contrast, fungal communities demonstrate partial adaptability during long-term continuous cropping, showing recovery of network complexity and a transition in assembly mechanisms from deterministic to stochastic dominance. Soil physicochemical factors, such as available phosphorus, available potassium, and pH, play a pivotal role in driving microbial community succession and assembly. The degradation of the soil environment due to long-term continuous cropping diminishes the influence of deterministic processes while amplifying the contribution of stochastic processes. This study highlights the critical roles of stochastic and deterministic processes in the assembly of bacterial and fungal communities, but further investigation is needed to uncover the underlying mechanisms.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture15121274/s1>, Figure S1: Heatmap illustrating the top 50 bacterial and fungal phyla or genera, highlighting intergroup differences; Figure S2: Redundancy analysis (RDA) of bacterial communities and soil factors across three continuous cropping periods, along with correlation analysis between environmental factors and community structure; Figure S3: Neutral community model of farmland soil microorganisms; Table S1: Summary of descriptions at sampling sites; Table S2: Topological properties of the co-occurrence networks of the bacterial community in soils of different groups.

Author Contributions: J.C.: Writing—original draft, visualization, software, methodology, investigation, formal analysis, data curation, conceptualization. X.Y.: Writing—original draft, visualization, software, methodology, investigation, formal analysis, data curation. D.Z.: Investigation, formal analysis. Z.H.: Investigation, formal analysis. R.S.: Investigation, resources, supervision, writing—review and editing. H.D.: Investigation, conceptualization, formal analysis, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (Grant No. 32460352), the Corps Guided Science and Technology Program Project (2023ZD051), the Tianchi Talent Introduction Program of Xinjiang Uygur Autonomous Region (CZ001613), and the High-level Talent Research Launch Project of Shihezi University (RCZK202365). We sincerely thank all authors for their significant contributions to this research and express our appreciation to the editor and anonymous reviewers for their valuable feedback, which greatly improved the quality of this manuscript.

Institutional Review Board Statement: Not applicable. This study primarily focused on the processes of farmland soil microbial communities (community assembly, Co-Occurrence networks) and soil property analysis, and did not involve human participants, animal experimentation, or endangered plant species.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the data are part of an ongoing study.

Conflicts 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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