

RESEARCH

Open Access



Long-term garlic–maize rotation maintains the stable garlic rhizosphere microecology

Shidong He¹, Minghao Lv¹, Rongxin Wang¹, Ningyang Li², Taotao Wang³, Wenchong Shi¹, Zheng Gao¹ and Xiang Li^{1*}

Abstract

Background Crop rotation is a sophisticated agricultural practice that can modify the demographic structure and abundance of microorganisms in the soil, stimulate the growth and proliferation of beneficial microorganisms, and inhibit the development of harmful microorganisms. The stability of the rhizosphere microbiome is crucial for maintaining both soil ecosystem vitality and crop prosperity. However, the effects of extended garlic–maize rotation on the physicochemical characteristics of garlic rhizosphere soil and the stability of its microbiome remain unclear. To investigate this phenomenon, soil samples from the garlic rhizosphere were collected across four different lengths of rotation in a garlic–maize rotation.

Results There were notable positive associations between the total nitrogen and total phosphorus contents in the soil and the duration of rotation. Prolonged rotation could increase the maintenance of microbiome diversity. The number of years of rotation and the soil organic carbon (SOC) content emerged as principal determinants impacting the evolution of the bacterial community structure, with the SOC content playing a pivotal role in sculpting the species diversity within the garlic rhizosphere bacterial community. Additionally, SOC remains predominant in shaping the root-associated bacterial community's β -nearest taxon index. However, these factors do not have a notable effect on the fungal community inhabiting the garlic rhizosphere. In comparison with monoculture, rotation can amplify the interconnectivity and intricacy of microbial ecological networks. Long-term rotation can further maintain the stability of both microbial ecological networks and interactions between bacterial and fungal communities. It can enlist a plethora of beneficial *Bacillus* species microorganisms within the garlic rhizosphere to form a biological barricade that aids in safeguarding garlic against encroachment by the pathogenic fungus *Fusarium oxysporum*, consequently diminishing disease incidence. This study provides a theoretical foundation for the sustainable development of garlic through long-term crop rotation with maize.

Conclusions Our research results indicate that long-term garlic–maize rotation maintains stable garlic rhizosphere microecology. Our study provides compelling evidence for the role of long-term crop rotation in maintaining microbiota and community stability, emphasizing the importance of cultivating specific beneficial microorganisms to enhance rotation strategies for garlic farming, thereby promoting sustainability in agriculture.

Keywords Crop rotation, Rhizosphere microbiome, Rotation years, SOC, *Bacillus*, *Fusarium oxysporum*

*Correspondence:

Xiang Li

lixiang@sdau.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

Owing to the constraints of monoculture farming leading to continuous cropping obstacles, crop rotation has emerged as a focal point in agricultural cultivation practices [1]. As a sustainable agricultural system management approach, crop rotation promotes soil ecosystem balance, enhances soil quality, and reduces pest and disease pressures, thereby fostering agricultural sustainability. This method helps reduce the reliance on fertilizers and pesticides, enhances ecosystem services in farmlands, and aligns environmentally friendly and sustainable concepts in agricultural production. [2, 3]. For example, a meta-analysis suggested that, in contrast to continuous monoculture, crop rotation can increase crop productivity by an average of 20%, with the lasting advantages of crop rotation continuing to support future crop yields [4]. The crop rotation system modifies the soil physicochemical properties, altering the soil microbiome [5]. Among these organisms, the bacterial community is notably more delicate than the fungal community is, indicating a particularly heightened responsiveness to alterations in soil nutrient concentrations [6]. The rhizosphere delineates the boundary where plant roots interlace with the soil, establishing a sophisticated ecosystem teeming with diverse biotic and abiotic elements, including flora, microorganisms, and earth. Within the rhizosphere, a multitude of soil microorganisms engage in a spectrum of intricate biological and ecological activities, where the interplay among microorganisms, soil, and plants influences soil vitality, plant well-being, and productivity dynamics. The rhizosphere microbiome is frequently regarded as the second plant genetic blueprint of plants and the primary barrier against the incursion of plant pathogens [7]. For pathogens such as *Fusarium oxysporum* to invade plant vascular tissues, they must breach the plant rhizosphere microbiome barrier and compete for limited ecological niches [8]. Thus, manipulating the rhizosphere microbiome to achieve sustainable ecosystem functions is highly important [9, 10].

In China, garlic is one of the primary economic vegetables, with Shandong's Jinxiang County being renowned the "Garlic Capital of the World". The agricultural practices in this region revolve around a garlic-maize cropping system, emblematic of the local agricultural landscape. However, the production and quality of garlic are easily affected by soil-borne diseases, especially under monoculture practices. The pathogen *Fusarium* is a major constraint causing garlic root rot, with *Fusarium oxysporum* being one of the key pathogenic fungi [11]. Owing to the widespread use of chemical fungicides to mitigate soil-borne plant pathogens, concerns exist that their residues pose a risk to human and animal health, potentially generating fungal resistance and disturbing

the ecological balance [12, 13]. Crop rotation has demonstrated its ability to modulate the rhizosphere microbiome, erecting a barrier against pathogens and thereby furnishing significant antagonistic advantages [14, 15]. By recruiting beneficial microbes, crop rotation breaks the connection between plant hosts and pathogens, thus reducing the deleterious impact of pathogens on crop production. It is an economical disease management strategy [2, 14]. Nevertheless, there is limited research on how long-term garlic-maize rotation affects the garlic rhizosphere microbiome structure and soil physicochemical properties, and whether such rotation can recruit antagonistic bacteria in the garlic rhizosphere to regulate the abundance of garlic root rot pathogens remains unknown.

In this study, we procured samples of garlic rhizosphere soil from various durations of rotation to explore the impacts of extensive garlic-maize rotation on soil nutrient levels, the ecological composition of rhizosphere microbes, and the stability of rhizosphere microecology. Additionally, we sought to uncover whether prolonged crop rotations have the capacity to enlist beneficial bacteria to establish a barrier that suppresses pathogens within the garlic rhizosphere, thereby impeding the growth of *Fusarium oxysporum* and fostering the sustainable advancement of the garlic sector. The purpose of this study is to understand the impact of garlic-maize rotation on soil and plant ecosystems, providing scientific support for the sustainable development of garlic for agricultural production.

Materials and methods

Research location and sample collection

The study site lies within Jinxiang County, which is situated in Jining City, Shandong Province, China, and is positioned at 33° 02' N and 116° 23' E. This location has a warm temperate continental monsoon climate, characterized by an average annual temperature of 15.5 °C as of 2023, accompanied by an annual rainfall of 668.6 mm. The soil composition predominantly comprises natural brown soil. The agricultural practices in this region revolve around a garlic-maize cropping system, emblematic of the local agricultural landscape. Our survey of Jinxiang farmers revealed relative consistency in terms of fertilization and management practices across the entire area. On November 24, 2023, garlic at the seedling stage was randomly sampled at four locations in the region, with eight replicates per sampling site: (1) monoculture garlic as the control treatment (CK) in land that has never been planted with garlic or corn before; (2) 10-year crop rotation of garlic and maize (R10); (3) 20-year crop rotation of garlic and maize (R20); and (4) 30-year crop rotation

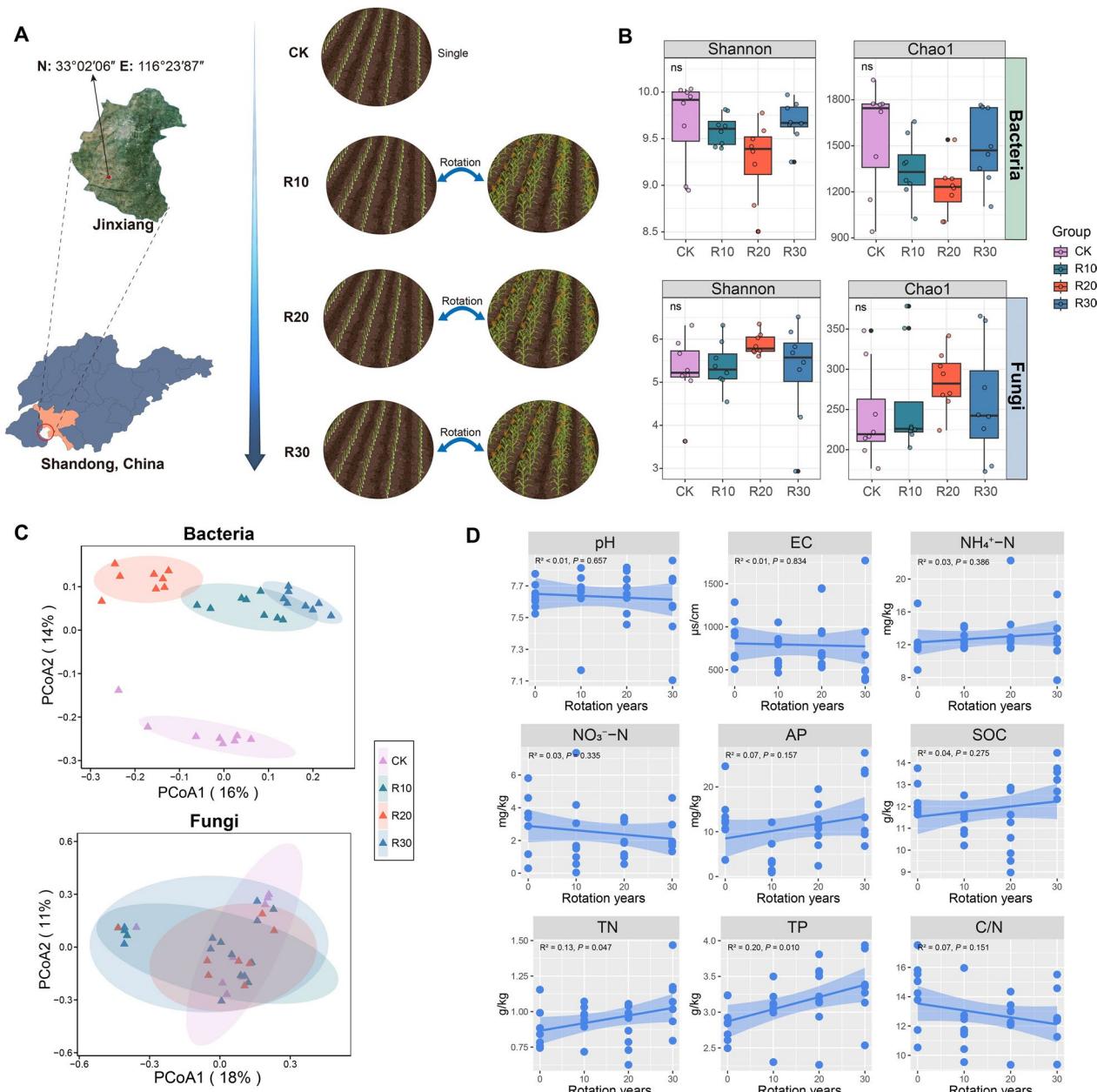


Fig. 1 **A** Schematic diagram of samples from different rotation years. **B** Shannon and Chao1 indices of bacteria and fungi from different rotation years. **C** PCoA of bacterial and fungal communities based on the Bray–Curtis distance. **D** Linear fitting of soil physicochemical properties and rotation years

of garlic and maize (R30) (Fig. 1A). During the growth of garlic, the roots were delicately unearthed using a shovel, with great care taken to preserve their integrity. The substantial clusters of soil enveloping the roots were subsequently gingerly dislodged. The stratum of soil approximately 1–2 mm in depth encasing the roots was identified as the rhizosphere soil [16]. Using soft-bristled brushes, we collected rhizosphere soil and

placed the samples into sealed bags, preserved them at a temperature of 4 °C, and then promptly transported them to the laboratory. A total of 32 rhizosphere soil samples were collected, which were divided into three portions: one for the determination of soil physicochemical properties, another for the isolation of cultivable bacteria, and the remaining portion stored at –80 °C for DNA extraction.

Determination of soil physicochemical properties

An autoAnalyser3 (Bran + Luebbe, Hamburg, Germany) was utilized for measuring total nitrogen (TN) and total phosphorus (TP) in the soil. The pH and electrical conductivity (EC) of the soil were determined using a pH meter (Leici, Shanghai). The nitrate nitrogen (NO_3^- -N), ammonium nitrogen (NH_4^+ -N), and soil organic carbon (SOC) contents in the soil were measured using the hydrazine sulfate reduction method, indophenol blue colorimetric method, and potassium dichromate volumetric method, respectively. The available phosphorus (AP) in the soil was determined using the molybdate/ascorbic acid method, with measurements conducted using a UV spectrophotometer (Eppendorf, Germany). The carbon/nitrogen ratio (C/N) is the ratio of SOC to TN [17].

DNA extraction and sequence analysis

The Fast DNA Spin Kit for Soil Assay (Omega, USA) was used for soil DNA extraction, followed by evaluation of DNA quality in a 1.2% agarose gel (Solarbio, China). The V5V7-1 region of 16S rRNA was amplified with specific primers (799F: 5'-AACMGGATTAGATAACCKG-3', 1193R: 5'-ACGTCACTCCCCACCTTCC-3') [18], and the ITS1–ITS2 region of the ITS region was also amplified using specific primers (ITS1F: 5'-CTTGGTCATTAGAGGAAGTAA-3', ITS2R: 5'-GCTGCGTTCTTCATCGATGC-3') [19]. Subsequently, paired-end sequencing was conducted at Guangdong Magigene Biotechnology Company, Limited, with the Illumina NovaSeq 6000 platform (located in Guangzhou, China).

Following the official instructions for QIIME2, the raw fastq files were subjected to sequence analysis [20, 21]. The DADA2 [22] plugin was utilized for sequence quality filtering, denoising, merging, chimera removal, and alignment, resulting in the generation of nonsingleton amplicon sequence variants (ASVs). The ASVs were subsequently taxonomically annotated using the SILVA(v138.1) and UNITE (v8.2) databases [23, 24], followed by the removal of contaminants such as mitochondria and chloroplasts. To address diversities arising from variations in sequencing depth, rarefaction was performed based on the minimum sequencing depth across all samples, resulting in rarefied counts of 31,740 for bacterial samples and 71,882 for fungal samples, providing an ASV table for downstream analyses. A total of 1,918,171 high-quality sequences were generated and assigned to 5866 ASVs in bacterial samples, whereas 4,154,583 high-quality sequences were generated and assigned to 1521 ASVs in fungal samples. In addition, rarefaction curves based on ASV numbers were close to the horizontal state (Fig. S1, Fig. S2), suggesting the sequencing data for each sample sufficiently reflected intact microbiological communities.

Isolation and identification of culturable bacteria

After gathering rhizosphere soil from garlic for the isolation of cultivable bacteria, the rhizosphere soil was immersed in water at a ratio of 1:10 and subsequently gradually diluted. The diluted suspensions with dilution factors of 10^{-5} , 10^{-6} , and 10^{-7} were plated onto solid culture media with different carbon–nitrogen ratios, and 100 μl was transferred using a pipettor. Individual colonies were then selected based on size, colour, and morphology for further purification and isolation, followed by sequencing of the 16S rRNA gene to identify beneficial bacterial strains. The bacterial strains were stored at -80°C . Species identification was conducted using the BLAST tool in the GenBank database (NCBI), and a phylogenetic evolutionary tree of all antagonistic bacteria and high-abundance ASVs (relative abundance $> 1\%$ and present in all samples) of bacterial data was constructed using MEGA (v6.0) [25].

Isolation and identification of potential fungal pathogens

In accordance with the methodology outlined in “[Isolation and identification of culturable bacteria](#)” section, pathogen screening was conducted. Suspensions diluted at 10^{-5} , 10^{-6} , and 10^{-7} were aliquoted by pipetting 100 μl onto Potato Dextrose Agar (PDA, Hopebio, China) medium for spread plating. Individual colonies were selected based on their size, colour, and morphology, followed by purification and ITS region sequencing for fungal identification [26]. Species identification was performed using NCBI, and the fungal strains were stored at -80°C .

Determination of the pathogenicity of potential fungal pathogens

The potential pathogen isolated from the rhizosphere soil of garlic was a strain of *Fusarium oxysporum*, which shares 100% sequence nucleotide identity with the fungus ASV30. Following the methodology of Zhou et al., *Fusarium oxysporum* fungal mycelia were inoculated into Potato Dextrose Broth (PDB, Hopebio, China) medium and cultured at 28°C for 7 days. The fermentation broth was filtered to remove mycelia, and the filtrate was centrifuged at 5000 rpm for 10 min to pellet the spores. The spores were subsequently resuspended in sterile water and adjusted to a concentration of 10^6 CFU/ml [2, 27]. To determine the pathogenic nature of *Fusarium oxysporum*, garlic was cultivated within a Plant Light Incubator (AS-R1060L2, Xunon, China) in a growth chamber set at 25°C , 40% relative humidity, and a light intensity of $500 \mu\text{M m}^{-2} \text{s}^{-1}$, following a 16/8 h day/night cycle. A volume of 10 mL of *Fusarium oxysporum* spore suspension was introduced into the rhizosphere of 10-day-old

garlic plants, and the rate of disease incidence was documented after a 30-day period postinoculation.

Biocontrol bacteria screening and inoculation validation

Seven-day-old *Fusarium oxysporum* PDA discs (6 mm) were placed in the center of a PDA plate. Potential antagonistic isolates were positioned 2.5 cm away from the center, and the assay was performed in triplicate. The mixture was incubated at 25 °C for 6 days in an inverted position for antifungal screening of the efficacy of each bacterial isolate against *Fusarium oxysporum* [27, 28]. In accordance with the aforementioned pathogenicity assay protocol, pathogenic fungal spores were collected. Subsequently, 8 strains of antagonistic bacteria were individually inoculated into Lysogeny Broth (LB, Hopebio, China) liquid medium for a 3-day fermentation period. The Strains were then centrifuged and resuspended in sterile water to an OD value of 0.8 for later use. The garlic was cultivated in a growth chamber set at 25 °C, with a relative humidity of 40%, a light intensity of 500 μM m⁻² s⁻¹, and a 16/8 h light/dark cycle. Around garlic seedlings grown in vermiculite for 10 days, 10 mL of *Fusarium oxysporum* spore suspension was inoculated, and those grown for 12 days received 20 ml of biocontrol agent mixture. Each treatment, including the control group without *Fusarium oxysporum* inoculation and the biocontrol agent, consisted of 20 plants, with disease incidence recorded after 30 days of inoculation.

Data analysis

All analyses and data presentation were conducted in R (v4.3.2; <http://www.r-project.org/>). The Picante package was used to compute the α diversity of the microbiome. The Vegan package was employed for principal coordinate analysis (PCoA) based on the Bray–Curtis distance to examine the differences among different rotation years by calculating P values. The correlations between soil physicochemical properties, years of rotation, microbial α diversity, and bacterial biomarkers were determined based on Spearman correlation coefficients. To identify a group of rotation year-sensitive microbes, a random forest regression model was established using the “randomForest” package. After 1000 iterations, the importance of classification units on model accuracy was reorganized, and the appropriate number of biomarkers was determined through 5 repeated tenfold cross-validations [29]. Linear regression analysis was conducted to analyze the linear relationships between SOC and microbial α diversity and β NTI. The influence and significance of soil physicochemical characteristics and years of rotation on microbial diversity and community composition were assessed using the rdacca.hp software package via redundancy analysis (RDA) and hierarchical partitioning

analysis, with a significance threshold of $P_{adj} \leq 0.05$. Mantel tests were performed using the “linkET” and “dplyr” software packages. The beta nearest taxon index (β NTI) was calculated based on zero models to infer community assembly processes, where β NTI < -2 indicated significant phylogenetic turnover that was lower than expected (i.e., homogeneous selection); conversely, β NTI > 2 indicated significant phylogenetic turnover that was greater than expected (i.e., variable selection). $|\beta$ NTI| < 2 indicated the dominance of random processes [30]. Microbial ecological network analysis was carried out using ASVs with an average relative abundance greater than 0.1%, and all possible pairwise Spearman rank correlations were calculated using the “ggClusterNet” package. To ensure comparability between different networks, a unified threshold ($|R| > 0.7, P < 0.05$) was used to filter significant interactions among microbes. The values of the edges, whether negative or positive, were determined based on the strength of the correlation, and the network layout was generated using the Fruchterman–Reingold algorithm in Gephi software (v0.9.2) [31]. Network robustness was defined as the proportion of nodes remaining in the network after 50% of the nodes were randomly removed [32]. The average variance index (AVD) was used to evaluate the stability of the soil bacterial and fungal communities [33]. MEGA (v6.0) was employed to construct phylogenetic trees, and the ChiPlot website (<https://www.chiplot.online/>) was used for visualization. The ggplot2 package was utilized for boxplots, heatmaps, bar graphs, ridgeline plots, and linear fit plots. SPSS Statistics software (IBM Corporation, New York, USA) was used for one-way analysis of variance (ANOVA) with Tukey’s HSD test ($P < 0.05$) to examine the experimental data, where letters above the bars indicate significant differences between groups, and “ns” denotes no statistical significance [34].

Results

The impact of rotation years on the physicochemical properties of soil and the diversity of microbial communities

To delve into the ramifications of varying durations of rotation on the physicochemical attributes and microbial ecosystems of garlic rhizosphere soil, we assessed the soil physicochemical properties under different rotation years and undertook an analysis of microbial community diversity. Discernible disparities were not detected within the bacterial and fungal realms across diverse crop rotation spans, as elucidated by the Chao1 and Shannon indices (Fig. 1B). Among the gamut of soil nutrients identified, rotation years had a negligible influence on the soil pH, EC, NH₄⁺-N, and NO₃⁻-N levels, whereas the levels of AP and SOC experienced sequential decreases followed

Table 1 Soil physicochemical determination of garlic soils with different rotation years

	CK	R10	R20	R30
pH	7.63±0.08 a	7.63±0.20 a	7.67±0.13 a	7.58±0.23 a
EC	831.50±261.30 a	716.38±199.18 a	887.63±380.36 a	726.88±459.25 a
NH ₄ ⁺ -N	12.08±2.25 a	12.59±1.11 a	13.92±3.46 a	12.69±2.97 a
NO ₃ ⁻ -N	3.09±1.76 a	2.42±2.40 a	2.04±1.12 a	2.45±1.09 a
AP	12.78±5.82 a	4.09±3.82 b	11.38±5.22 ab	15.38±8.08 a
SOC	12.31±0.72 a	11.14±0.78 b	10.79±1.30 b	13.25±0.68 a
TN	0.88±0.14 ab	0.94±0.11 ab	0.87±0.15 b	1.08±0.20 a
TP	2.86±0.27 a	3.04±0.34 a	3.20±0.47 a	3.37±0.45 a
C/N	14.28±2.26 a	11.99±1.91 a	12.55±1.48 a	12.55±1.88 a

The values are means±SDs (n=8). Significant differences ($P<0.05$) were calculated by one-way ANOVA with Tukey's HSD test and are shown with different lowercase letters

by increases in tandem with the lengthening of rotation years (Table 1). The use of a linear regression model to correlate crop rotation spans with soil physicochemical attributes yielded noteworthy findings, revealing a statistically significant positive association between TN and TP concentrations in the soil and the duration of crop rotation ($R^2=0.13$, $P=0.047$; $R^2=0.20$, $P=0.010$) (Fig. 1D). Through PCoA utilizing Bray–Curtis distance metrics, conspicuous dissimilarities in bacterial community configuration emerged across varying duration of rotation ($P<0.001$), whereas similar differences were not observed in fungal communities, with the bacterial community variability notably surpassing that of fungal communities (Fig. 1C, Table S1).

Soil physicochemical properties and rotation years affect microbial community composition

According to the findings derived from the ASV classification, Proteobacteria was the predominant phylum among the rhizosphere bacteria, constituting 50.96% of the total reads; meanwhile, Ascomycota was the dominant phylum among the rhizosphere fungi, representing 81.22% of the total reads. With increasing rotation years, there were discernible degrees of flux in both the composition and structure of the bacterial community. Among the top ten most prevalent taxonomic units, the relative abundances of *Chloroflexi* and *Nitrospirota* initially increased and subsequently decreased in tandem with increasing rotation years, whereas the relative abundances of the other phyla remained relatively constant throughout the rotation years (Fig. 2A, Fig. S3A). However, the configuration and constitution of the fungal community exhibited negligible alterations (Figs. 1B, 2B, Fig. S3B). Employing a random forest model, we endeavored to discern a cluster of rotation year-sensitive biomarkers at the bacterial genus level (Fig. 2C). The outcomes of tenfold cross-validation, which was repeated

five times, indicated that the error rate curve stabilized considerably upon the integration of the top 16 microbial attributes (Fig. S4, Table S3). Among these biomarkers, three features decreased with increasing rotation years, three features increased, five features remained consistent, and five features exhibited intricate fluctuations with increasing rotation years (Fig. 2D). Spearman correlation analysis between soil physicochemical properties, rotation years, bacterial and fungal α diversity, and bacterial biomarkers revealed significant associations between rotation years and SOC across most biomarkers, with the SOC content demonstrating a notable positive correlation with bacterial α diversity ($P<0.001$). The linear regression of the SOC content with the bacterial Shannon and Chao1 indices corroborated this finding, and hierarchical partitioning analysis underscored the predominant role of SOC in α diversity and biomarkers (Fig. 2E, Fig. S5).

Soil physicochemical properties and rotation years influence microbial community changes and assembly

We employed RDA to elucidate the influence of abiotic factors, such as soil physicochemical properties and rotation years, on the bacterial and fungal communities. The first two axes of the RDA explained 38.7% of the bacterial community variation, with rotation years and SOC exerting the most significant influence on the bacterial community structure ($R^2=0.8484$, $P=0.005$; $R^2=0.6271$, $P=0.005$), followed by TN ($R^2=0.2514$, $P=0.04$). The RDA results demonstrated that differences in rotation years, SOC, and TN drove the distribution of the bacterial communities, whereas the fungal community distribution remained unaffected by these abiotic factors (Fig. 3A, B; Table 2). Mantel tests were utilized to scrutinize the underlying determinants of microbial community composition over various rotation cycles, revealing notable impacts of SOC and

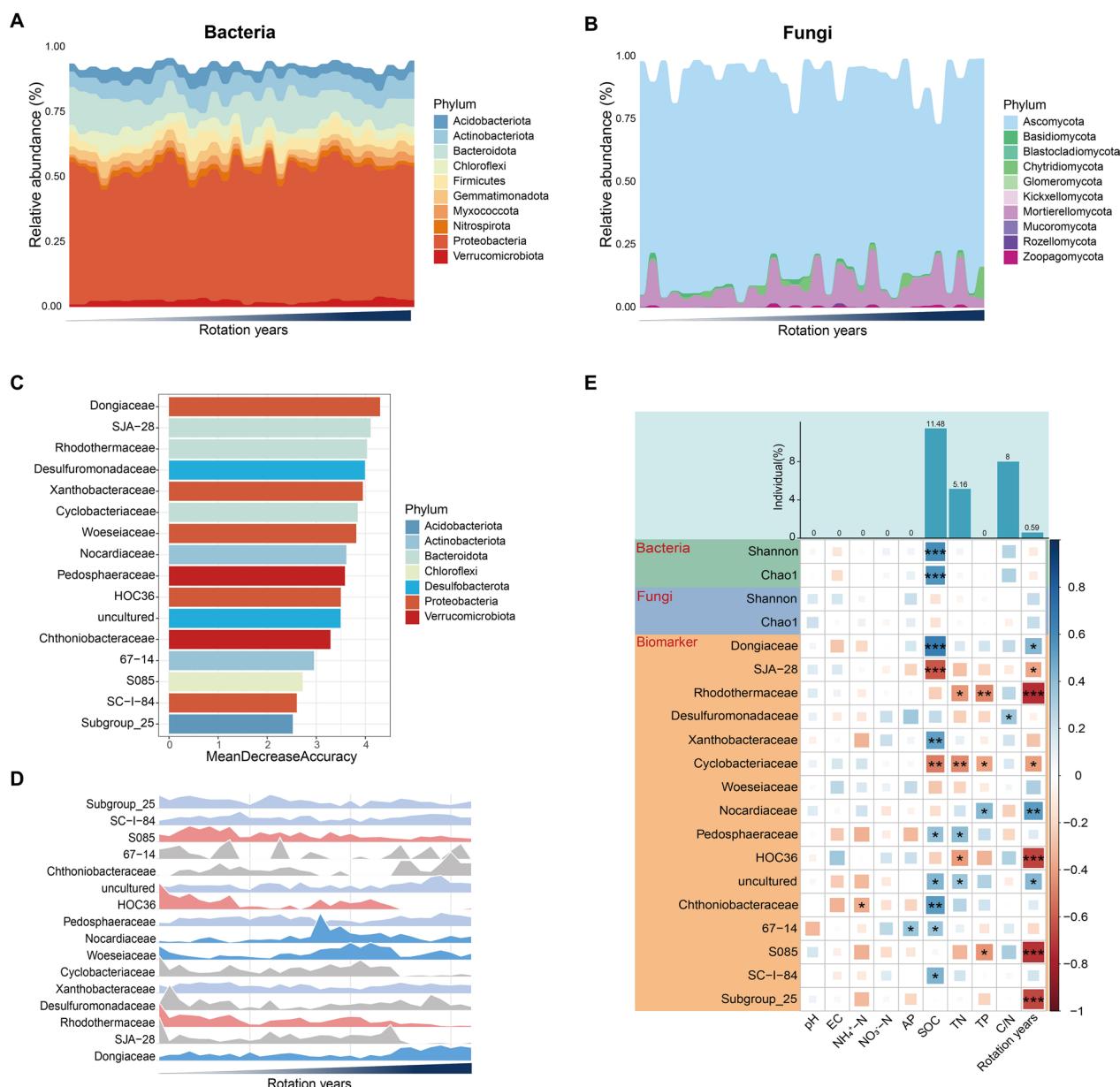


Fig. 2 **A** Turnover of the relative abundance of the top 10 bacterial phyla during the succession of rotation years. **B** Turnover of the relative abundance of the top 10 fungal phyla during the succession of rotation years. **C** Ranking of bacterial biomarkers in descending order of importance to the model. **D** Dynamic changes in the relative abundance of biomarkers with rotation years. **E** Spearman correlations between soil physicochemical properties, rotation years, bacterial and fungal α diversity, and bacterial biomarkers, as well as the contributions of soil physicochemical properties and rotation years to bacterial and fungal α diversity and bacterial biomarkers

rotation years on bacterial community composition, as well as C/N on fungal community composition, with rotation years emerging as the primary driver of shifts in bacterial community composition (Fig. S6, Table S2). The findings of the Mantel test revealed significant increases in microbial community heterogeneity with increasing crop rotation duration and SOC ($P < 0.05$).

Conversely, the rotation years and the SOC content had no substantial effect on fungal community heterogeneity (Fig. 3C, D). An evaluation of microbial community assembly through the β NTI index based on null models revealed that bacterial community assembly is mainly driven by variable selection, while fungal community assembly is governed mainly by stochastic processes (Fig. 3E). The results of the linear regression analysis

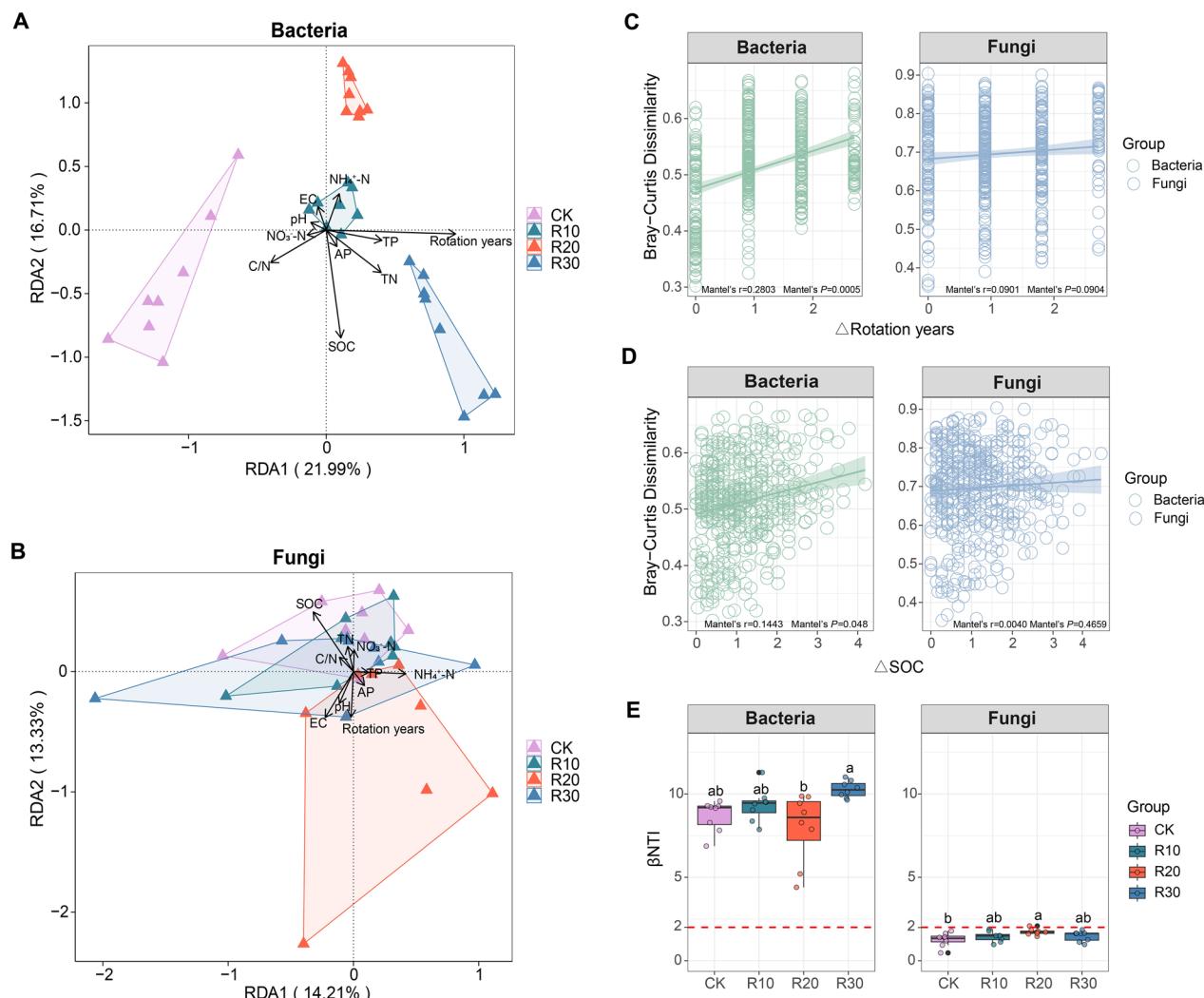


Fig. 3 **A** Relationships between all bacterial community ASVs and soil physicochemical factors and rotation years in the RDA. **B** Relationships between all fungal community ASVs and soil physicochemical factors and rotation years in the RDA. **C** Mantel tests of bacterial and fungal community diversity with rotation years. **D** Mantel tests of bacterial and fungal community diversity with SOC content. **E** Distribution of β NTI for bacteria and fungi in different rotation years

Table 2 RDA replacement test results

Name	Bacteria			Fungi		
	R ²	Padj	Individual (%)	R ²	Padj	Individual (%)
pH	0.015335	0.788	0	0.070558	0.678	0.23
EC	0.033022	0.788	0.04	0.165812	0.31	0
NH ₄ ⁺ -N	0.077324	0.487	0	0.141575	0.326667	0
NO ₃ ⁻ -N	0.019523	0.788	0	0.027936	0.841	0
AP	0.020523	0.788	1.5	0.018536	0.841	0
SOC	0.627094	0.005	2.88	0.301953	0.08	0
TN	0.251449	0.04	1.41	0.040192	0.841	0.23
TP	0.16324	0.162	0.94	0.011882	0.841	0
C/N	0.209172	0.1025	1.2	0.022882	0.841	0.42
Rotation years	0.848385	0.005	7.26	0.125334	0.36	0

revealed a notable correlation between the bacterial βNTI and the SOC content ($R^2=0.31$, $P<0.001$) (Fig. S7).

Long-term crop rotation maintains soil microbial ecological networks and community stability

In this study, a microbial ecological network of bacteria and fungi was constructed, and bacteria and fungi were combined according to different rotation years, to reveal

the changes in the coexistence patterns of microbial species under different rotation years. With increasing rotation years, the complexity of the network of bacteria and fungi first increased and then stabilized, reaching the highest complexity at R20: the nodes in the bacterial network numbered 248, with 3169 edges, and the nodes in the fungal network numbered 146, with 718 edges (Fig. 4A, B, D). Compared with those under monoculture, the connectivity and complexity of networks under

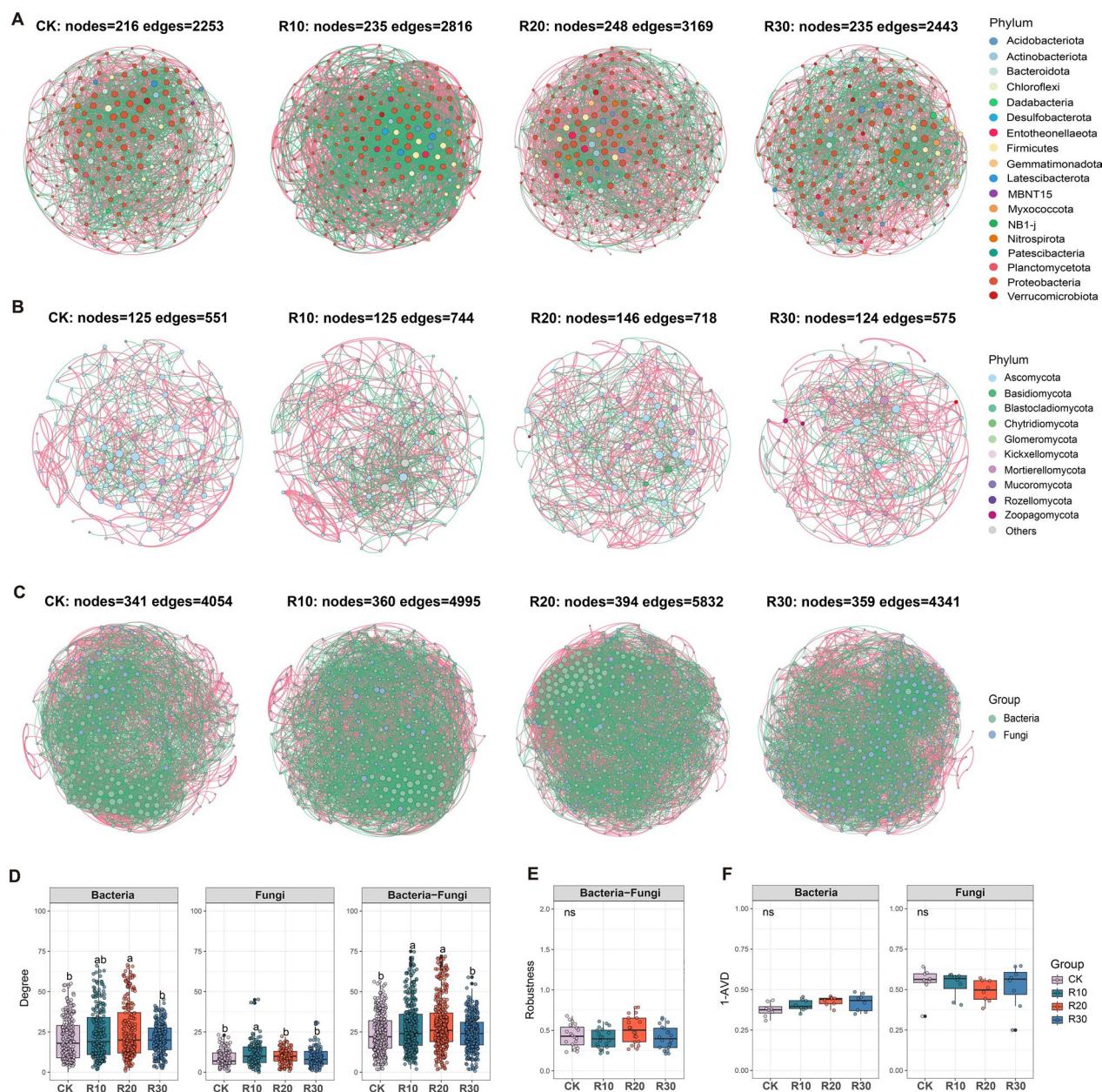


Fig. 4 **A** Bacterial ecological network with varying crop rotation intervals. **B** Fungal ecological network with varying crop rotation intervals. **C** Coexistence ecological network of bacteria and fungi with varying crop rotation intervals. **D** Degrees of freedom of network nodes. **E** Network stability of the bacteria-fungi coexistence network. **F** Stability of the bacterial and fungal communities

different numbers of rotation years were greater. The cross-domain network of bacteria and fungi still maintained a consistent trend, with the complexity peaking at R20, with 394 nodes and 5832 edges (Fig. 4C, D). The changes in nodes, edges, positive edges, and negative edges of the bacterial network, fungal network, and coexistence networks of bacteria and fungi first increased but then decreased. However, there was no clear pattern in the changes in the average degree, average path length, connectivity, centralization degree, centralization closeness, mean clustering coefficient, relative modularity, the number of keystone nodes among the three networks (Fig. S8). Additionally, it was found that the robustness of the bacterial–fungal coexistence network under different numbers of rotation years did not differ significantly (Fig. 4E). Long-term crop rotation between garlic and maize can sustain the stability of the garlic rhizosphere soil microbial ecology network. Furthermore, the community stability index (1-AVD) did not significantly differ between the bacterial and fungal communities across diverse rotation years (Fig. 4F), suggesting that continuous crop rotation of garlic and maize can maintain the stability of garlic rhizosphere bacterial and fungal communities.

Isolation and validation of a crop-enriched antagonist bacterium that inhibits *Fusarium oxysporum* in rotational crops

Through analysis, we found that 32 fungal ASVs were annotated to *Fusarium* (Fig. S9, Table S3). We isolated and identified *Fusarium* species pathogens in rhizosphere soil, and successfully identified a strain of *Fusarium oxysporum* (with a nucleotide identity of 100% to the fungal strain ASV30). *Fusarium oxysporum*, the primary pathogen responsible for garlic root rot, has been confirmed to be pathogenic (Fig. 5B). Our study revealed that there was no significant difference in the relative abundance of *Fusarium oxysporum* between monoculture and crop rotation ($P > 0.05$), and under crop rotation, its abundance remained relatively low (Fig. S9). Therefore, we infer that crop rotation can recruit and enrich specific core microorganisms in the garlic rhizosphere, serving as a functional barrier against *Fusarium oxysporum* invasion. We constructed a phylogenetic tree based on the top 1% relative abundance of ASVs present in all the soil samples, with the top 3 genera being *Sphingomonas* (3.53%), *Bacillus* (3.07%), and *Pseudomonas* (1.19%) (Fig. 5A, Fig. S10). To screen for antagonistic strains enriched by crop rotation, we isolated and purified 124 bacterial strains from garlic rhizosphere soil and conducted plate antagonism experiments with *Fusarium oxysporum*, 8 of which strongly inhibited pathogen proliferation (Fig. 5C). The 16S rRNA sequencing results,

revealed that these 8 antagonistic strains were mainly *Bacillus* species, including *Bacillus velezensis*, *Bacillus wiedmannii*, *Paenibacillus peoriae*, *Bacillus siamensis*, and *Bacillus tequilensis*. The results of the potted plants indicate that all eight strains of antagonistic bacteria had certain levels of biocontrol capability against garlic root rot, with strain R19 exhibiting the most effective biocontrol efficacy (Fig. 5D, E).

Discussion

In agricultural systems, long-term crop rotation can inspire variations in soil physicochemical properties and microbial diversity, leading to sustained impacts [35, 36]. Research has revealed that crop rotation markedly increases the levels of total nitrogen, total phosphorus, and total potassium in the soil [36–38]. In this study, long-term garlic–maize rotation can significantly increase the contents of TN and TP in the rhizosphere soil of garlic (Fig. 1D). A meta-analysis revealed that rotational practices increase the richness and diversity of microorganisms, with prolonged rotations exerting a more pronounced positive influence on microbial abundance [39]. Intensive rotation performs a crucial functions in the sequestration of soil carbon, enhancing the structure of soil microorganisms, and augmenting soil microbial diversity [40, 41]. In our investigation, it was observed that the α diversity of bacteria and fungi in the garlic rhizosphere remained relatively stable despite an extended duration of rotation. These studies prove that under the garlic–maize rotation model, with proper nutrient planning, agronomic management, and crop residue return, there are no obstacles to continuous planting [42]. In rotation systems, soil microorganisms establish distinctive community structures influenced by varying durations of rotation, resulting in notable variations in bacterial community composition across different rotation years, whereas fungi exhibit insignificantly different compositions (Fig. 1C), indicating that the variability of bacterial communities surpasses that of fungal communities as a result of variances in rotation periods within rotation systems. This may be because the rapid growth of bacteria differs mechanistically from the vigorous colonization of fungi. Bacteria are usually sensitive to changes in the soil environment and are able to respond and adjust their growth rapidly, which makes their populations and structures more susceptible to soil conditions. In contrast, fungi tend to form a more stable community structure through proliferation to maintain their growth and nutrient acquisition [43–45].

Soil environmental factors (such as SOC, TN, and pH) play a crucial roles in shaping the composition of bacterial communities, resulting in significant impacts [46, 47]. Previous research has indicated that TN and SOC, as

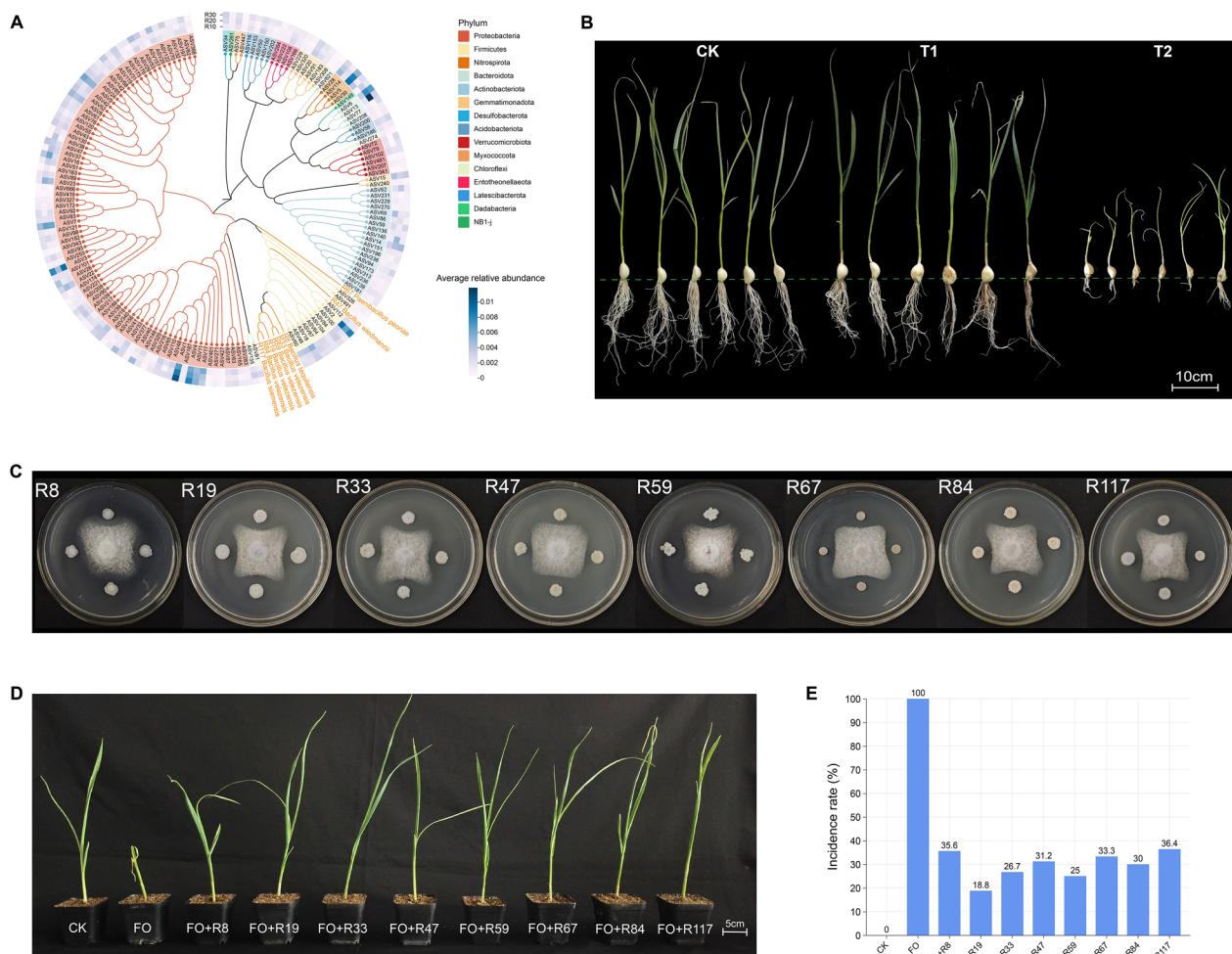


Fig. 5 **A** Systematic phylogenetic tree of ASVs present in the top 1% in terms of abundance across all soil samples, in relation to the 16S rRNA sequences of antagonistic bacteria on plates. **B** Pathogenicity experiment of *Fusarium oxysporum* on garlic, with CK as the control treatment, T1 representing the phenotype during the mild infection stage, and T2 representing the phenotype during the severe infection stage. **C** Eight strains of antagonistic bacteria inhibiting the growth of *Fusarium oxysporum*. **D** Eight strains of antagonistic bacteria aid garlic in resisting root rot in potted experiments. **E** Variation in the incidence of root rot with different treatments

nutrients for soil microbial metabolic activities, directly influence the diversity of microorganisms [48, 49]. Studies have indicated that fertilization can increase the richness and variety of bacteria within the soil by influencing the accumulation of SOC [50]. Our research revealed that SOC and rotation years had the greatest effects on the bacterial community structure, followed by TN (Fig. 3A). The SOC content was significantly positively correlated with the Shannon and Chao1 indices of the garlic bacterial community (Fig. 2E). Additionally, there was a linear positive correlation with the β NTI of the bacterial community (Fig. S3), indicating that SOC plays a dominant role in the assembly process of the garlic rhizosphere bacterial community. Furthermore, our research revealed that SOC content and rotation years are key factors

influencing the succession of bacterial communities in the garlic rhizosphere (Fig. 3C, D). These findings suggest that the SOC content and rotation years play significant roles in the species diversity of bacterial communities in the garlic rhizosphere [38, 47]. However, the rhizosphere is a specialized ecological region strongly influenced by plants, so our results may have several limitations.

The interplay of microorganisms plays a significant role in enriching the intricacy of community composition and maintaining the equilibrium of soil microbial populations, which is a pivotal factor in ensuring soil vitality [51]. Our investigation suggests that, in contrast to monoculture, crop rotation has the potential to augment the interconnectivity and intricacy of the microbial network. Rotations can increase the number of network

nodes and edges, with a 20-year rotation resulting in the highest network complexity (Fig. 4A, B, C), highlighting the importance of rotation in promoting strong interactions among garlic rhizosphere soil microbial communities. The highest network complexity and connectivity at the R20 time point may be because farmers have adopted significant changes in agrochemical inputs (herbicides, insecticides, fungicides) and mechanized use in the last decade with rapid economic development. These changes may be important reasons why network complexity and connectivity were not as high at the R30 time point as at the R20 time point. Consistent with previous studies, long-term diversified crop rotations increase the complexity of underground microbial networks [52]. In our study, the cause of this situation may be due to the legacy effects caused by the planting of maize, which alleviates the harm caused by continuous single garlic planting. It is well known that crop rotation can produce legacy effects, promoting niche sharing and interactions among microbial communities [53, 54]. Generally, the symbiotic ecological networks associated with crop rotation become more stable than continuous single planting [55, 56]. In our research, long-term garlic-maize rotations maintained the stability of the soil microbial ecological networks (Fig. 4E), as well as the stability of the bacterial community and fungal communities (Fig. 4F). Therefore, the long-term garlic-maize rotation pattern can effectively promote soil ecosystem health and sustainable agricultural development. When interpreting network analysis results, we must acknowledge that Spearman is not sensitive to the compositional nature of the data, and methods like SparCC can be used to address compositional nature concerns [57].

Continuous monocropping may exacerbate soilborne illnesses in agricultural land, culminating in diminished crop quality and yield, thereby severely impeding the productivity and sustainability of the agroecosystem [58, 59]. It is widely acknowledged that crop rotation may stimulate beneficial plant-associated microorganisms, which can persist and suppress pathogens in the following season [60, 61]. Under different rotation years, the microbial community clustering of rhizosphere soil bacteria became more similar, distinctly separating from that of single-crop, indicating community differentiation (Fig. 1C). In line with prior research, we have discovered that long-term rotation of garlic-maize can enrich *Sphingomonas*, *Bacillus*, and *Pseudomonas* in the garlic rhizosphere (Fig. 5A). Hong and colleagues research revealed that pepper-banana and eggplant-banana rotations enriched *Bacillus* and *Pseudomonas* core groups, aiding bananas in reducing *Fusarium oxysporum* invasion through stable residue effects [14]. Our study provides compelling evidence that long-term garlic-maize

rotation results in the recruitment of beneficial *Bacillus* in the garlic rhizosphere, forming a biological barrier to combat *Fusarium oxysporum* invasion (Fig. 5B, C), as demonstrated through plate antagonism and pot experiments. The underlying mechanism behind this phenomenon may involve the proliferation of beneficial microorganisms stimulated by specific plant chemicals in the root exudates of different plants [62], suggesting that crop rotation can recruit a plethora of beneficial indigenous species to counteract pathogen invasion [63, 64]. Further research is necessary to explore how rotation regulates exudates to recruit beneficial bacteria and support this hypothesis, and in future studies, the combination of antimicrobial substances with metabolomic analysis to elucidate the biocontrol mechanisms in rotation systems.

Conclusions

Garlic-maize long-term crop rotation maintains the stability of garlic rhizosphere bacterial and fungal community α diversity. Rotation years and SOC are crucial factors in shaping the succession of the garlic rhizosphere bacterial community structure, with the SOC content playing a critical role in shaping the species diversity of the garlic rhizosphere bacterial community, and the SOC also playing a leading role in the assembly process of the garlic rhizosphere bacterial community. However, these factors do not significantly impact the fungal community of the garlic rhizosphere. Network analysis of different garlic-maize rotation years revealed that, compared with monoculture, rotation can increase the connectivity and complexity of the microbial network, maintaining the stability of the microbial ecological network and community. Long-term crop rotation also helps recruit many beneficial *Bacillus* species to the garlic rhizosphere, forming a biological barrier to assist garlic in resisting infection by the root rot pathogen *Fusarium oxysporum*. Our study provides compelling evidence for the role of long-term crop rotation in maintaining microbiota and community stability, emphasizing the importance of cultivating specific beneficial microorganisms to enhance rotation strategies for garlic farming, thereby promoting sustainability in agriculture.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40793-024-00636-8>.

Additional file 1.

Additional file 2.

Acknowledgements

The authors extend profound appreciation to the two anonymous reviewers for their constructive critiques and perceptive suggestions, which have significantly enhanced the quality of this manuscript.

Author contributions

All authors were involved in the conception of this research. SD H, MH L and TT W assisted in the collection of microbial samples. SD H conducted most of the experiments. MH L and WC S assisted with metadata collation. SD H, RX W, MH L and NY L undertook data analysis. X L supervised the manuscript's drafting. All authors wrote and edited the manuscript.

Funding

This work was supported by the Key Research and Development Project in Shandong Province of China (2023CXPT045, 2023TZXD004, 2021TZXD001), the National Natural Science Foundation of China (42407427, 42377309, 42077027), the 'First Class Discipline' Construction Project of Shandong Agricultural University (SKL81103, SKL81110).

Availability of data and materials

The sequence data has been deposited in the NCBI SRA database under accession number PRJNA1085538.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors agree.

Competing interests

The authors declare no competing interests.

Author details

¹State Key Laboratory of Wheat Improvement, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China. ²College of Food Science and Engineering, Ocean University of China, Qingdao 266404, China. ³Shandong Engineering and Technology Research Center for Garlic, Jining 272200, China.

Received: 10 July 2024 Accepted: 7 November 2024

Published online: 13 November 2024

References

- Fang Y, Ren T, Zhang S, Liu Y, Liao S, Li X, et al. Rotation with oilseed rape as the winter crop enhances rice yield and improves soil indigenous nutrient supply. *Soil Tillage Res.* 2021. <https://doi.org/10.1016/j.still.2021.105065>.
- Zhou Y, Yang Z, Liu J, Li X, Wang X, Dai C, et al. Crop rotation and native microbiome inoculation restore soil capacity to suppress a root disease. *Nat Commun.* 2023. <https://doi.org/10.1038/s41467-023-43926-4>.
- Lv M-H, Shi W-C, Li M-C, Zhou B, Liu Y-X, Gao Z. Ms gene and Mr gene: Microbial-mediated spatiotemporal communication between plants. *Imeta.* 2024. <https://doi.org/10.1002/imt.2210>.
- Zhao J, Yang Y, Zhang K, Jeong J, Zeng Z, Zang H. Does crop rotation yield more in China? A meta-analysis. *Field Crops Res.* 2020. <https://doi.org/10.1016/j.fcr.2019.107659>.
- Soman C, Li D, Wander MM, Kent AD. Long-term fertilizer and crop-rotation treatments differentially affect soil bacterial community structure. *Plant Soil.* 2016. <https://doi.org/10.1007/s11104-016-3083-y>.
- Ai C, Zhang S, Zhang X, Guo D, Zhou W, Huang S. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma.* 2018. <https://doi.org/10.1016/j.geoderma.2018.01.010>.
- Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq BA. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules.* 2016. <https://doi.org/10.3390/molecules21050573>.
- Chapelle E, Mendes R, Bakker P, Raaijmakers J. Fungal invasion of the rhizosphere microbiome. *ISME J.* 2016;10:265–8.
- Ling N, Wang T, Kuzyakov Y. Rhizosphere bacteriome structure and functions. *Nat Commun.* 2022. <https://doi.org/10.1038/s41467-022-28448-9>.
- He S, Li L, Lv M, Wang R, Wang L, Shaowei Yu, et al. PGPR: key to enhancing crop productivity and achieving sustainable agriculture. *Curr Microbiol.* 2024. <https://doi.org/10.1007/s00284-024-03893-5>.
- Diaz R, Chavez EC, Delgado Ortiz JC, Velazquez JJ, Roque A, Ochoa YM. First report of clonostachys rosea causing root rot on garlic in Mexico. *Plant Dis.* 2022. <https://doi.org/10.1094/pdis-12-21-2658-pdn>.
- Bubici G, Kaushal M, Prigigallo MI, Gómez-Lama Cabanás C, Mercado-Blanco J. Biological control agents against fusarium wilt of banana. *Front Microbiol.* 2019. <https://doi.org/10.3389/fmicb.2019.00616>.
- Zhang N, Wang Z, Shao J, Xu Z, Liu Y, Xun W, et al. Biocontrol mechanisms of Bacillus: improving the efficiency of green agriculture. *Microb Biotechnol.* 2023. <https://doi.org/10.1111/1751-7915.14348>.
- Hong S, Yuan X, Yang J, Yang Y, Jv H, Li R, et al. Selection of rhizosphere communities of diverse rotation crops reveals unique core microbiome associated with reduced banana Fusarium wilt disease. *New Phytol.* 2023. <https://doi.org/10.1111/nph.18816>.
- Zhao J, Chen J, Beillouin D, Lambers H, Yang Y, Smith P, et al. Global systematic review with meta-analysis reveals yield advantage of legume-based rotations and its drivers. *Nat Commun.* 2022. <https://doi.org/10.1038/s41467-022-32464-0>.
- Li Z, Bai X, Jiao S, Li Y, Li P, Yang Y, et al. A simplified synthetic community rescues Astragalus mongolicus from root rot disease by activating plant-induced systemic resistance. *Microbiome.* 2021. <https://doi.org/10.1186/s40168-021-01169-9>.
- Yan K, Lu DS, Ding CJ, Wang Y, Tian YR, Su XH, et al. Rare and abundant bacterial communities in poplar rhizosphere soils respond differently to genetic effects. *Sci Total Environ.* 2023. <https://doi.org/10.1016/j.scitotenv.2023.168216>.
- Ritpitakphong U, Falquet L, Vimoltust A, Berger A, Métraux J, L'Haridon F. The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. *New Phytol.* 2016. <https://doi.org/10.1111/nph.13808>.
- Jiang TT, Shao T-Y, Ang WXG, Kinder JM, Turner LH, Pham G, et al. Commensal fungi recapitulate the protective benefits of intestinal bacteria. *Cell Host Microbe.* 2017. <https://doi.org/10.1016/j.chom.2017.10.013>.
- Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019. <https://doi.org/10.1038/s41587-019-0209-9>.
- Caporaso J, Kuczynski J, Stombaugh J, Bittinger K, Bushman F, Costello E, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods.* 2010;7:335–6.
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016. <https://doi.org/10.1038/nmeth.3869>.
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2013. <https://doi.org/10.1093/nar/gks1219>.
- Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 2019. <https://doi.org/10.1093/nar/gky1022>.
- Jiao S, Zhang B, Zhang G, Chen W, Wei G. Stochastic community assembly decreases soil fungal richness in arid ecosystems. *Mol Ecol.* 2021;30:4338–48.
- Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nat Rev Microbiol.* 2019. <https://doi.org/10.1038/s41579-018-0116-y>.
- Zhou X, Wang J, Liu F, Liang J, Zhao P, Tsui CKM, et al. Cross-kingdom synthetic microbiota supports tomato suppression of Fusarium wilt disease. *Nat Commun.* 2022. <https://doi.org/10.1038/s41467-022-35452-6>.
- Wen T, Yuan J, He X, Lin Y, Huang Q, Shen Q. Enrichment of beneficial cucumber rhizosphere microbes mediated by organic acid secretion. *Hortic Res.* 2020. <https://doi.org/10.1038/s41438-020-00380-3>.

29. Zhang J, Liu Y-X, Zhang N, Hu B, Jin T, Xu H, et al. NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat Biotechnol.* 2019. <https://doi.org/10.1038/s41587-019-0104-4>.
30. Jiao S, Lu Y. Soil pH and temperature regulate assembly processes of abundant and rare bacterial communities in agricultural ecosystems. *Environ Microbiol.* 2019. <https://doi.org/10.1111/1462-2920.14815>.
31. Li C, Jin L, Zhang C, Li S, Zhou T, Hua Z, et al. Destabilized microbial networks with distinct performances of abundant and rare biospheres in maintaining networks under increasing salinity stress. *iMeta.* 2023;2:e79.
32. Yuan MM, Guo X, Wu L, Zhang Y, Xiao N, Ning D, et al. Climate warming enhances microbial network complexity and stability. *Nat Clim Change.* 2021. <https://doi.org/10.1038/s41558-021-00989-9>.
33. Xun W, Liu Y, Li W, Ren Y, Xiong W, Xu Z, et al. Specialized metabolic functions of keystone taxa sustain soil microbiome stability. *Microbiome.* 2021. <https://doi.org/10.1186/s40168-020-00985-9>.
34. Li J, He J-Z, Liu M, Yan Z-Q, Xing-Liang Xu, Kuzyakov Y. Invasive plant competitiveness is mediated by nitrogen use strategies and rhizosphere microbiome. *Soil Biol Biochem.* 2024. <https://doi.org/10.1016/j.soilbio.2024.109361>.
35. Bakker PAHM, Pieterse CMJ, de Jonge R, Berendsen RL. The soil-borne legacy. *Cell.* 2018. <https://doi.org/10.1016/j.cell.2018.02.024>.
36. Wang Y, Shi M, Zhang R, Zhang W, Liu Y, Sun D, et al. Legume-potato rotation affects soil physicochemical properties, enzyme activity, and rhizosphere metabolism in continuous potato cropping. *Chem Biol Technol Agric.* 2023. <https://doi.org/10.1186/s40538-023-00508-2>.
37. Zhang H, Luo G, Wang Y, Fei J, Xiangmin R, Peng J, et al. Crop rotation-driven change in physicochemical properties regulates microbial diversity, dominant components, and community complexity in paddy soils. *Agric Ecosyst Environ.* 2022. <https://doi.org/10.1016/j.agee.2022.108278>.
38. Ma T, Yang K, Yang L, Zhu Yi, Jiang B, Xiao Z, et al. Different rotation years change the structure and diversity of microorganisms in the nitrogen cycle, affecting crop yield. *Appl Soil Ecol.* 2023. <https://doi.org/10.1016/j.apsoil.2023.105123>.
39. Venter ZS, Jacobs K, Hawkins H-J. The impact of crop rotation on soil microbial diversity: a meta-analysis. *Pedobiologia.* 2016. <https://doi.org/10.1016/j.pedobi.2016.04.001>.
40. Liu Q, Zhao Y, Li T, Chen L, Chen Y, Sui P. Changes in soil microbial biomass, diversity, and activity with crop rotation in cropping systems: a global synthesis. *Appl Soil Ecol.* 2023. <https://doi.org/10.1016/j.apsoil.2023.104815>.
41. Zhou Q, Zhang P, Wang Z, Wang L, Wang S, Yang W, et al. Winter crop rotation intensification to increase rice yield, soil carbon, and microbial diversity. *Heliyon.* 2023. <https://doi.org/10.1016/j.heliyon.2023.e12903>.
42. Chen T, Hu R, Zheng Z, Yang J, Fan H, Deng X, et al. Soil bacterial community in the multiple cropping system increased grain yield within 40 cultivation years. *Front Plant Sci.* 2021. <https://doi.org/10.3389/fpls.2021.804527>.
43. Liu D, Liu Y, Li J, Mo Q, Tang J, Liu W, et al. Shaping the succession patterns of different soil nutrients, enzyme stoichiometry, and microbial communities through rotation systems. *CATENA.* 2023. <https://doi.org/10.1016/j.catena.2023.107740>.
44. Kandeler E, Gebala A, Boeddinghaus RS, Müller K, Rennert T, Soares M, et al. The mineralosphere: succession and physiology of bacteria and fungi colonising pristine minerals in grassland soils under different land-use intensities. *Soil Biol Biochem.* 2019. <https://doi.org/10.1016/j.soilbio.2019.107534>.
45. Yang X, Zhu K, Loik ME, Sun W. Differential responses of soil bacteria and fungi to altered precipitation in a meadow steppe. *Geoderma.* 2021. <https://doi.org/10.1016/j.geoderma.2020.114812>.
46. Gong X, Wang S, Wang Z, Jiang Y, Hu Z, Zheng Y, et al. Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. *Geoderma.* 2019. <https://doi.org/10.1016/j.geoderma.2019.03.043>.
47. Ji L, Wu Z, You Z, Yi X, Ni K, Guo S, et al. Effects of organic substitution for synthetic N fertilizer on soil bacterial diversity and community composition: a 10-year field trial in a tea plantation. *Agric Ecosyst Environ.* 2018. <https://doi.org/10.1016/j.agee.2018.09.008>.
48. Shen C, Ni Y, Liang W, Wang J, Chu H. Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. *Front Microbiol.* 2015. <https://doi.org/10.3389/fmicb.2015.00582>.
49. Singh D, Shi L, Adams JM. Bacterial diversity in the mountains of South-West China: climate dominates over soil parameters. *J Microbiol.* 2013. <https://doi.org/10.1007/s12275-013-2446-9>.
50. Kong C, Zhang S, Yuan S, Wang W, Song X, Guo D, et al. Soil bacterial community characteristics and its effect on organic carbon under different fertilization treatments. *Front Microbiol.* 2024. <https://doi.org/10.3389/fmicb.2024.1356171>.
51. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* 2012. <https://doi.org/10.1038/ismej.2011.119>.
52. Yang X, Hang-Wei Hu, Yang G-W, Cui Z-L, Chen Y-L. Crop rotational diversity enhances soil microbiome network complexity and multifunctionality. *Geoderma.* 2023. <https://doi.org/10.1016/j.geoderma.2023.116562>.
53. Jiang Y, Li S, Li R, Zhang J, Liu Y, Lv L, et al. Plant cultivars imprint the rhizosphere bacterial community composition and association networks. *Soil Biol Biochem.* 2017. <https://doi.org/10.1016/j.soilbio.2017.02.010>.
54. Zhang K, Maltais-Landry G, Liao H-L. How soil biota regulate C cycling and soil C pools in diversified crop rotations. *Soil Biol Biochem.* 2021. <https://doi.org/10.1016/j.soilbio.2021.108219>.
55. Wei S, Fang J, Zhang T, Wang J, Cheng Y, Ma J, et al. Dynamic changes of soil microorganisms in rotation farmland at the western foot of the Greater Khingan range. *Front Bioeng Biotech.* 2023. <https://doi.org/10.3389/fbioe.2023.1191240>.
56. Yang X, Cheng J, Franks AE, Huang X, Yang Qi, Cheng Z, et al. Loss of microbial diversity weakens specific soil functions, but increases soil ecosystem stability. *Soil Biol Biochem.* 2022. <https://doi.org/10.1016/j.soilbio.2022.108916>.
57. Friedman J, Alm EJ. Inferring correlation networks from genomic survey data. *PLoS Comput Biol.* 2012. <https://doi.org/10.1371/journal.pcbi.1002687>.
58. Ahsan T, Tian P-C, Gao J, Wang C, Liu C, Huang Y-Q. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping system. *J Adv Res.* 2023. <https://doi.org/10.1016/j.jare.2023.11.028>.
59. Cui R, Geng G, Wang G, Stevanato P, Dong Y, Li T, et al. The response of sugar beet rhizosphere micro-ecological environment to continuous cropping. *Front Microbiol.* 2022. <https://doi.org/10.3389/fmicb.2022.956785>.
60. Yuan X, Wang B, Hong S, et al. Promoting soil microbial-mediated suppressiveness against Fusarium wilt disease by the enrichment of specific fungal taxa via crop rotation. *Biol Fertil Soils.* 2021. <https://doi.org/10.1007/s00374-021-01594-w>.
61. Kabir AH, Baki MDZI, Ahmed B, Mostofa MG. Current, faltering, and future strategies for advancing microbiome-assisted sustainable agriculture and environmental resilience. *New Crops.* 2024;1:100013.
62. Thiergart T, Durán P, Ellis T, Vannier N, Garrido-Oter R, Kemen E, et al. Root microbiota assembly and adaptive differentiation among European *Arabidopsis* populations. *Nat Ecol Evol.* 2019. <https://doi.org/10.1038/s41559-019-1063-3>.
63. Kou C, Song F, Li D, Xu H, Zhang S, Yang W, et al. A necessary considering factor for crop resistance: precise regulation and effective utilization of beneficial microorganisms. *New Crops.* 2024;1:100023.
64. Tiemann LK, Grandy AS, Atkinson EE, Marin-Spiotta E, McDaniel MD. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol Lett.* 2015. <https://doi.org/10.1111/ele.12453>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.