

Changes in Soil Bacterial and Nematode Communities during Long-term Continuous Cotton Cropping in arid Region

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Clarifying the effects of continuous cotton cropping (CC) on soil biological communities is essential for maintaining agricultural productivity. In this study, high-throughput sequencing was used to study the effects of different CC durations (0-yr, 5-yr, 10-yr, 15-yr, 20-yr, and 25-yr CC treatments) on soil microbial and nematode communities. The results showed that the dominant bacterial phyla were Actinobacteria and Proteobacteria, and the dominant nematode genus was *Helicotylenchus* in all CC treatments. The richness indexes (ACE and Chao1 index) and diversity index (Shannon index) of bacterial and nematode communities were the highest in the 15-yr and 10-yr CC treatment, respectively. Bacterial community was significantly correlated with soil pH and available potassium (AK), and nematode abundance was significantly correlated with microbial biomass carbon (MBC). Soil bacterial PICRUST analysis results showed that carbon metabolism and amino acid metabolism were the main metabolic functions of bacteria in the CC treatments. The composition and diversity of soil nematode communities were significantly related to the structure of soil bacterial communities, and the niche breadth of soil bacteria was negatively correlated with that of nematodes. *Panagrolaimus* and *Acrobeles* were the main genera of bacterial-feeding nematodes affecting bacterial communities, and their relative abundances were significantly positively correlated with the relative abundance of bacterial communities. Overall, long-term (10-

15 years) continuous cotton cropping negatively impacts soil biota and microecological environment in cotton fields in arid regions.

Key words: Cotton, Continuous cropping, Soil bacteria, Soil nematode, High-throughput sequencing

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BACKGROUND

Xinjiang Uygur Autonomous Region is the largest cotton-growing area in China, and its cotton acreage and output account for 82.8% and 89.5% of the country's total, respectively (National Bureau of Statistics 2021). Continuous cotton cropping (CC) is very common in Xinjiang. However, long-term continuous cropping brings a series of negative effects, causing crop yield and quality decline, soil nutrient imbalance, soil salinization, autotoxicity of secondary metabolites, and increases in soil pathogenic bacteria (Tan et al. 2010; Tan et al. 2011; Tan et al. 2013; Fu et al. 2017; Qin et al. 2017). This greatly restricts the sustainable agricultural development in Xinjiang (Qin et al. 2017).

Microbes and nematodes are the most active components of soil ecosystem and indicators of soil environmental changes (Bongers 1998; Pascual 2000; Romaniuk et al. 2011). The abundances of microbes and nematodes are greatly impacted by soil management (Coleman 2008). Some researchers found that CC could greatly affect soil microbial diversity through impacting the quality and quantity of soil resources (Li et al. 2010; Yang et al. 2018; Tian et al. 2020). Microbes and nematodes occupy key positions in soil food web (Bardgett and Van der Putten 2014; Manuel et al. 2016; Yurkevich et al. 2020), and play important roles in soil biogeochemical cycling and ecosystem services (Bardgett and Van der Putten 2014). Therefore, microbes and nematodes have great influences on crop growth and development (Ingham et al. 1985). Interestingly, selective predation by nematodes could stimulate microbial activity (Hu et al. 2019; Liu et al. 2019; Poromarto et al. 2019; Pawlowski et al. 2020), change the abundance and structure of microbial

community (Djigal et al. 2004), and inhibit diseases caused by bacteria, fungi, and pathogenic nematodes (Yeates et al. 1993a). In recent years, niche breadth has been widely concerned and applied in farmland ecosystem researches (Zhang et al. 2018a; Jiao et al. 2020). Some studies have found that microbiota with broad niche breadth are metabolically flexible at the community level (Pandit et al. 2009; Wu et al. 2018). However, it is still unclear that how soil bacterial and nematode communities respond to long-term continuous cropping.

In recent years, the application of high-throughput sequencing (HTS) technology provides valuable semiquantitative information for the study on nematode community ecology (Porazinska et al. 2012; Griffiths et al. 2018; Gao et al. 2021). On the one hand, the physiological structure of most soil nematodes meets the DNA extraction requirement for PCR reaction (Powers. 2004; Li et al. 2017). On the other hand, the identification of soil nematodes by morphological characteristics requires special taxonomic techniques and a significant amount of time (Mao et al. 2004), which limits the large-scale ecological study on soil nematodes (Lawton et al. 1998; Geisen et al. 2018). Treonis et al. (2018) showed that HTS annotated more nematode taxa than morphological identification, and HTS provided higher taxonomic resolution than morphological identification. Geisen et al. (2018) further confirmed this view. Therefore, high-throughput sequencing has great potential in nematode research. Kerfahi et al. (2016) and Moroenyany et al. (2016) studied the diversity of nematodes in tropical rain forests, Arctic regions, and mediterranean region using HTS. Du et al. (2020) also used HTS technology to quantitatively investigate the nematode community composition in the soil of a steppe-forest ecotone. However, HTS technology also has some problems to be solved. For example, different primers and databases may produce different results.

To reveal how soil bacterial and nematode communities respond to long-term CC in arid areas in Xinjiang, China. In this study, Illumina Miseq sequencing was used to study soil nematode and bacterial communities. The objectives were to clarify: (1) the responses of bacterial and nematode communities to long-term CC, (2) the changes in soil properties leading to the changes in soil nematode and bacterial communities, and (3) the relationship between soil nematode and bacterial community composition during long-term CC. This study will provide reference for soil biological regulation of agro-ecosystems, and is of great significance to maintain farmland ecosystem health.

MATERIALS AND METHODS

Study site

This experiment was conducted at Shihutan Town, Xinjiang, China (44°37'N, 86°8'E). This area has a temperate continental climate, with an average annual temperature of 6.6°C, an average annual precipitation of 110–200 mm, and an average annual evaporation of 1500–2000 mm. Continuous cotton cropping is a common practice in this area. Cotton yield was about 5250 kg ha⁻¹.

Experimental design

This experiment adopted randomized complete block design. Due to soil salinization, the experimental site was abandoned for 29 years. From 1996 to 2016, the abandoned farmlands were separately reclaimed to plant cotton, which were marked as 25-year CC treatment, 20-year CC treatment, 15-year CC treatment, 10-year CC treatment, and 5-year CC treatment, respectively. Besides, an area without cotton planting since 1996 was selected as the control (0-yr), which was also not affected by anthropogenic activities. Each treatment had three replicates/plots. The area of each plot was 5.2 × 7 m, and 2 m buffer rows were set around each treatment.

Cotton seeds (cultivar *Zhuangjiahuan* 902) were sown (2.4×10^5 plants/hm²) in Mid-April. The row spacing was 66 cm + 10 cm, and the plant spacing was 9 cm. Plastic film mulch and drip irrigation were used. Irrigation were conducted 10–12 times during the whole growth period, with a total amount of 4500 m³/hm². Urea (150 kg/hm²), calcium superphosphate (450 kg/hm²), and K₂O (75 kg·hm⁻²) were basally applied, and topdressing was conducted six times by drip irrigation system from bud stage to boll opening stage (urea: 300 kg/hm²; potassium dihydrogen phosphate: 200 kg/hm²). Other field managements were the same as local practice. To reduce the adverse effects of pests and diseases on cotton growth and development and ensure the consistency of control variables in all treatments, each treatment adopted the same insecticide application time and dosage. That is, except for the duration of continuous cropping, all management measures were consistent for each treatment.

Soil sampling

On July 14, 2020 (full flowering stage of cotton), twenty sampling points were selected in each

plot along a "S"-shaped route. Before sampling, stones and fallen leaves on the soil surface were removed, and then soil samples (0–20 cm in depth) with a distance of 0–3 cm from cotton roots were collected. The twenty samples were mixed and used as the soil sample for the plot (500 g). A total of 18 soil samples were obtained. All soil samples were stored at 4°C, transported to the laboratory, passed through a 2-mm sieve, and divided into two portions. One portion was air-dried and stored at room temperature for soil property measurement, and the other was stored at -80°C for molecular analysis.

Determination of soil physicochemical properties

Soil pH was determined using a pH meter (PHS-3C, INESA Scientific Instrument CO., Ltd, Shanghai, China) (water: soil = 5:1). Soil electrical conductivity (EC) was determined using a conductivity meter (water: soil = 2.5:1) (Ciavatta et al. 1991). Soil organic matter (SOM) content was determined using the Walkley-Black dichromate oxidation method (Díaz-Zorita. 1999). Soil nitrogen content was determined using the Semimicro Kjeldahl method (Bremner and Mulvaney. 1982). Soil available phosphorus (AP) content was determined using the 0.5 mol L⁻¹ NaHCO₃ method (Page et al. 1982). Soil available potassium (AK) content was determined using the flame photometry (Shen et al. 2013). Soil nitrate nitrogen (NO₃⁻-N) and ammonium nitrogen (NH₄⁺-N) content were determined using a flow analyzer (Bran Luebbe, Germany) (McLeod, 1992). Microbial biomass carbon (MBC) was determined using the chloroform fumigation-extraction method (Vance et al. 1987). Table 1 shows soil properties under different CC durations.

Table 1. Effects of different continuous cropping spans on soil properties

Group	pH	EC ($\mu\text{S cm}^{-1}$)	SOC (g kg^{-1})	TN (g kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	MBC ($\text{mg}\cdot\text{kg}^{-1}$)
0-yr	9.15 ± 0.04^a	6855.56 ± 218.98^a	5.12 ± 0.31^e	0.30 ± 0.03^e	18.82 ± 0.48^f	509.81 ± 4.01^a	75.03 ± 1.94^b	51.67 ± 0.92^b	56.48 ± 3.29^e
5-yr	8.39 ± 0.09^f	534.00 ± 56.07^b	7.43 ± 0.61^{cd}	0.61 ± 0.00^c	25.41 ± 2.39^d	268.46 ± 2.03^c	73.06 ± 3.67^b	22.66 ± 1.75^d	143.24 ± 7.59^b
10-yr	8.92 ± 0.02^b	141.78 ± 6.53^d	11.11 ± 0.49^a	0.66 ± 0.02^b	33.10 ± 0.90^c	213.19 ± 0.45^d	97.19 ± 19.60^a	52.28 ± 1.78^b	222.82 ± 10.27^a
15-yr	8.55 ± 0.04^c	468.00 ± 10.16^b	9.44 ± 0.24^b	0.83 ± 0.01^a	83.69 ± 0.35^a	463.66 ± 2.74^b	85.13 ± 8.76^{ab}	93.94 ± 1.83^a	112.49 ± 14.35^c
20-yr	8.80 ± 0.02^d	256.11 ± 8.89^c	6.96 ± 0.09^d	0.57 ± 0.00^d	22.30 ± 1.23^e	207.19 ± 17.53^d	83.16 ± 10.10^{ab}	45.91 ± 2.21^c	84.22 ± 15.69^d
25-yr	8.85 ± 0.04^c	276.56 ± 11.33^c	8.02 ± 0.12^c	0.56 ± 0.00^d	38.68 ± 1.17^b	273.55 ± 1.36^c	81.76 ± 9.68^{ab}	8.78 ± 0.47^e	59.68 ± 6.24^e

Notes: Values are means \pm standard deviation ($n = 3$). Different lowercase letters in the same column indicate significant difference at $p < 0.05$. EC, Electrical conductivity; SOC, Soil organic carbon; TN, Total nitrogen; AP, Available phosphorus; AK, Available potassium; $\text{NH}_4^+\text{-N}$, Ammoniacal nitrogen; $\text{NO}_3^-\text{-N}$, Nitrate nitrogen; MBC, Microbial biomass carbo.

DNA extraction, PCR amplification, and Illumina MiSeq sequencing

The total DNA of nematodes and bacteria (from 0.5 g soil sample) were extracted using E.Z.N.A.[®] soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. Agarose gel (1%) was used to check the DNA extract, and the concentration and purity of DNA were measured using the UV-vis spectrophotometer (NanoDrop 2000, Thermo Scientific, Wilmington, USA).

The forward primer NF1-F (5'-GGTGTGCATGGCCGTTCTTAGTT-3') and the reverse primer 18Sr2b-R (5'-TACAAAGGGCAGGGACGTAAT-3') were used to amplify the variable region of soil nematode 18S rDNA gene V4 using PCR (Du et al. 2020). PCR amplification of the soil bacteria 16S rRNA gene was performed using the PCR primers specific for the 338-806 (V3-V4) regions, and the following primers were used to attach a barcode (an 8-base sequence unique to each sample): 338F (5'-barcod-e-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR mix (20 µL) contained 4 µL of 5x TransStart[™] FastPfu reaction buffer, 2 µL of 2.5 mM dNTP, 0.8 µL of forward primer (5 µM), 0.8 µL of reverse primer (5 µM), 0.4 µL of TransStart[™]FastPfu DNA polymerase, and 10 ng of ddH₂O template DNA. The visualization and purification of PCR products were conducted using 2% agarose gel electrophoresis and the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) following the manufacturer's protocol, followed by the quantification using the QuantiFluor[™] -ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio BioPharm Technology Co., Ltd. (Shanghai, China).

Raw fastq files were demultiplexed and quality-filtered using QIIME (Quantitative Insights into Microbial Ecology, version 1.9.1). Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered using USEARCH (version 7.1), and chimeric sequences were identified and removed using UCHIME (Edgar et al. 2013). The bacterial taxonomy of each 16S rRNA gene sequence was analyzed using RDP classifier (version 2.2) based on the SILVA ribosomal RNA gene database (Release 123) (Wang et al. 2007). The representative sequences of nematode 18S rDNA were classified by RDP classifier (version 2.2) based on NCBI NT database. The confidence threshold was 70% (Du et al. 2020).

Statistical analysis

The data were statistically analyzed using SPSS software (version 20.0) (SPSS Inc., Chicago, IL, USA). The Chao1 richness index, ACE richness index, Shannon diversity index, and Simpson diversity index were determined using Mothur software (version 1.30.1) (Schloss et al. 2009). The similarities and differences among the communities were described using an Upset-plot diagram with common and unique OTUs. Principal component analysis (PCA) based on z-score normalization was performed using R software (version 3.6.1) (Mardia et al. 1979). Redundancy analysis (RDA) was performed using Vegan package in R software to determine relationship between soil bacterial and nematode communities, soil bacterial functional genes, and soil physicochemical properties (Dixon et al. 2003). Spearman correlation coefficients between the relative abundance of major genus and soil properties were also calculated to estimate the difference between CC treatments. Bacterial functional gene prediction was performed using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) (Langille et al. 2013). Functional genes were identified based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Ogata et al. 1999). Cluster heatmap was used to analyze the differences of pathway-3 functional genes among different treatments. To reveal habitat- and region-based species classification, dispersal limitations, and their impact on soil microbial communities, the “niche breadth” function in “spaa” package in the R software was used to estimate the niche breadth (B) of microbes (Levins 1968; Zhang et al. 2013). Community-level B-value (B_{com}) was calculated as the average of the B_{coms} for all taxa in a community (Wu et al. 2018). Mantel test was used to determine the correlation between nematode and bacterial communities (Sunagawa et al. 2015). SPSS 20.0 (SPSS Inc., Chicago, USA) was used for one-way ANOVA and Tukey 's HSD test for multiple comparisons.

RESULTS

Composition and diversity of soil bacterial and nematode community

A total of 867,354 and 384,208 optimized sequences were obtained from the 18 soil samples through HTS analysis of soil bacteria and nematodes, respectively, and 6290 and 51 OTUs were detected, respectively (Table 2).

Table 2. Effects of different continuous cropping spans on the richness and diversity of soil bacteria and nematode communities (similarity level: 97%)

Microbial and nematode community	Group	Sequencing results		Chao1 index	Shannon index (H)
		Total no. of sequences	Total no. of OTs		
Bacteria	0-yr	48696	3154	2928 ± 152 ^c	6.14 ± 0.08 ^c
	5-yr	41751	3606	3395 ± 148 ^{cd}	6.22 ± 0.05 ^{bc}
	10-yr	44746	3804	3533 ± 32 ^{bc}	6.37 ± 0.04 ^{ab}
	15-yr	40982	3872	3780 ± 83 ^a	6.42 ± 0.04 ^a
	20-yr	46884	3919	3662 ± 22 ^{ab}	6.38 ± 0.07 ^{ab}
	25-yr	40117	3531	3343 ± 33 ^d	6.12 ± 0.19 ^c
Nematode	0-yr	216	6	2.33 ± 1.53 ^b	0.35 ± 0.35 ^b
	5-yr	500	24	13.00 ± 6.08 ^a	1.49 ± 0.06 ^a
	10-yr	1952	28	14.00 ± 2.65 ^a	1.32 ± 0.33 ^a
	15-yr	224	17	8.83 ± 7.52 ^{ab}	1.25 ± 0.31 ^a
	20-yr	334	17	8.67 ± 3.51 ^{ab}	1.31 ± 0.56 ^a
	25-yr	797	14	7.33 ± 2.89 ^{ab}	1.16 ± 0.60 ^a

Notes: Values are means ± standard deviation ($n = 3$). Different lowercase letters in the same column indicate significant difference at $p < 0.05$. OTUs, operational taxonomic units.

Actinobacteriota and Proteobacteria were the dominant bacteria phyla, and the relative abundance were 21.32%–31.94% and 18.95%–27.16%, respectively (Fig. 1a, Table S1, $p < 0.05$). CC significantly decreased the relative abundance of Actinobacteria but increased the relative abundance of Proteobacteria, especially 25-yr CC treatment ($p < 0.05$). Other phyla with a higher relative abundance were Acidobacteria (2.61% - 24.07%), Chloroflexi (8.81%–14.05%), Firmicutes (2.93%–5.89%), and Bacteroidetes (1.98%–11.7%). The dominant genera were *Gp6*, *Arthrobacter*, *Gemmatimonas*, and *Gp4* (Fig. 1b, Table S2, $p < 0.05$). There were differences in the Chao1 and Shannon index among the six CC treatments, and the Chao1 and Shannon index began to decrease after 15-yr CC (Table 2, $P < 0.05$). The number of unique bacteria (genus level) in 0-yr, 5-yr, 10-yr, 15-yr, 20-yr, and 25-yr CC treatment were 57, 2, 1, 11, 4 and 1, respectively, and the total quantity of bacterial genera decreased after 15-yr CC (Fig. S1a).

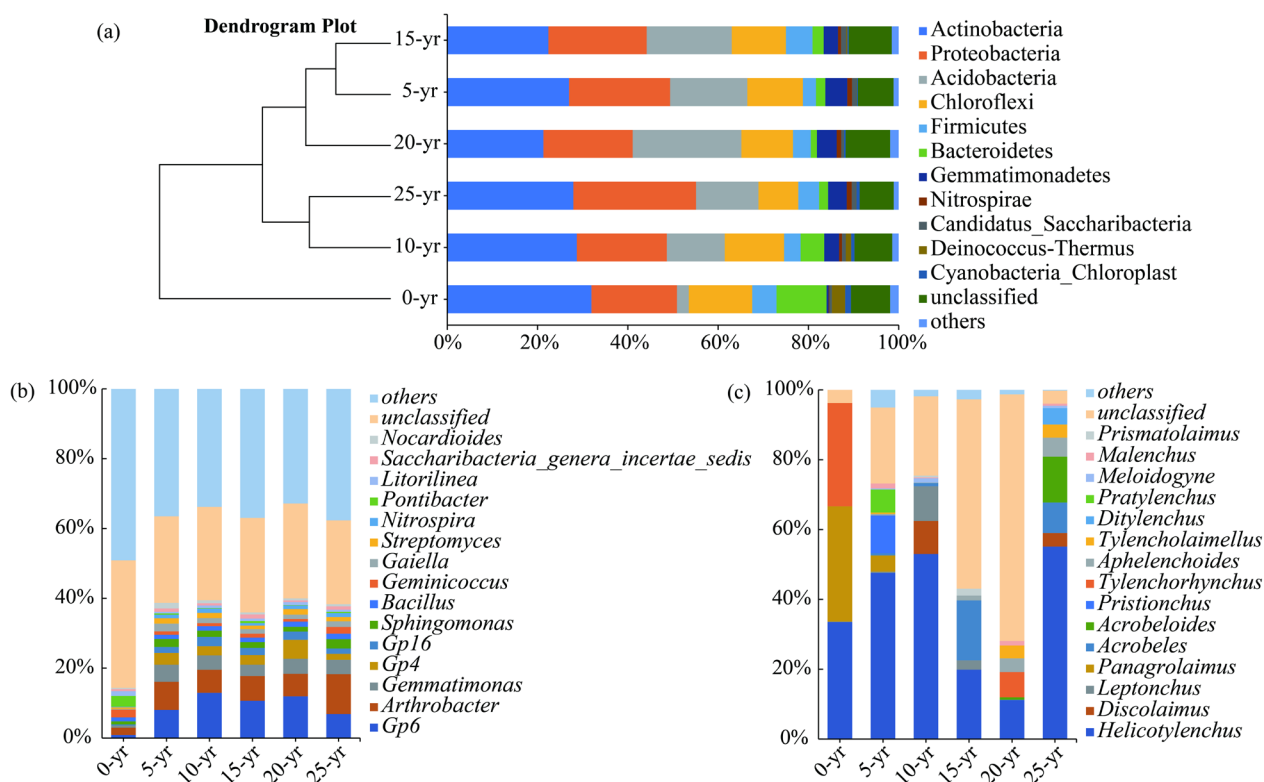


Fig. 1. Composition of soil bacterial community at the phylum (a) and genus (b) level and nematode community at the genus level (c) in different groups.

Helicotylenchus (relative abundance: 19.87%–55.13%) was the dominant nematode genus in all CC treatments except for the 20-yr CC treatment. The relative abundance of unclassified nematode genera in the 20-yr treatment was 70.59%; Especially, the relative abundance of OTU693 (28.1%), OTU282 (18.677%), and OTU281 (15.12%) were higher than that of other unclassified nematode genera (Table S4), and also higher than that of *Helicotylenchus* (11.18%) in the 20-yr treatment (Fig. 1c, Table S3 and S4, $p > 0.05$). *Leptonchus* was only detected in the 5-yr, 10-yr, and 15-yr CC treatments, *Panagrolaimus* was only detected in the 0-yr and 5-yr CC treatments, and *Acrobeles* was detected in all CC treatments except for the 0-yr CC treatment. The relative abundance of *Panagrolaimus* of the 5-yr CC treatment reduced by 85.81% compared with that of the control. Soil nematode Chao1 and Shannon index of the CC treatments increased compared with those of the control (Table 2, $p < 0.05$). The 10-yr CC treatment had the largest number of soil nematode genera, and the number of unique genera in the 0-yr, 5-yr, 10-yr, 15-yr, 20-yr, and 25-yr CC treatment were 0, 4, 3, 0, 0, and 1, respectively (Fig. S1b).

The analysis of similarities (ADONIS) of bacterial and nematode communities at the genus level showed that there were significant differences among the CC treatments (bacteria: $R^2 = 0.85$, p

= 0.001; nematode: $R^2 = 0.50$, $p = 0.002$), which was also confirmed by the PCA analysis results (Fig. 2). The PCA axis divided bacterial communities into three parts: (1) 0-yr CC treatment, (2) 15-yr CC treatment, and (3) other CC treatments (Fig. 2a). However, the nematode community in the 10-yr CC treatment was obviously separated from that in the other CC treatments (Fig. 2b). Redundancy analysis results showed that bacteria ($p = 0.001$, Fig. 3a) responded strongly to soil physicochemical properties. RDA1 and RDA2 explained 64.01% and 18.56% of the total variation, respectively. It was worth noting that both pH ($p = 0.007$) and AK ($p = 0.001$) had significant effects on bacterial communities, and soil MBC ($p = 0.004$) was the vital soil factor affecting nematode community composition (Table 3).

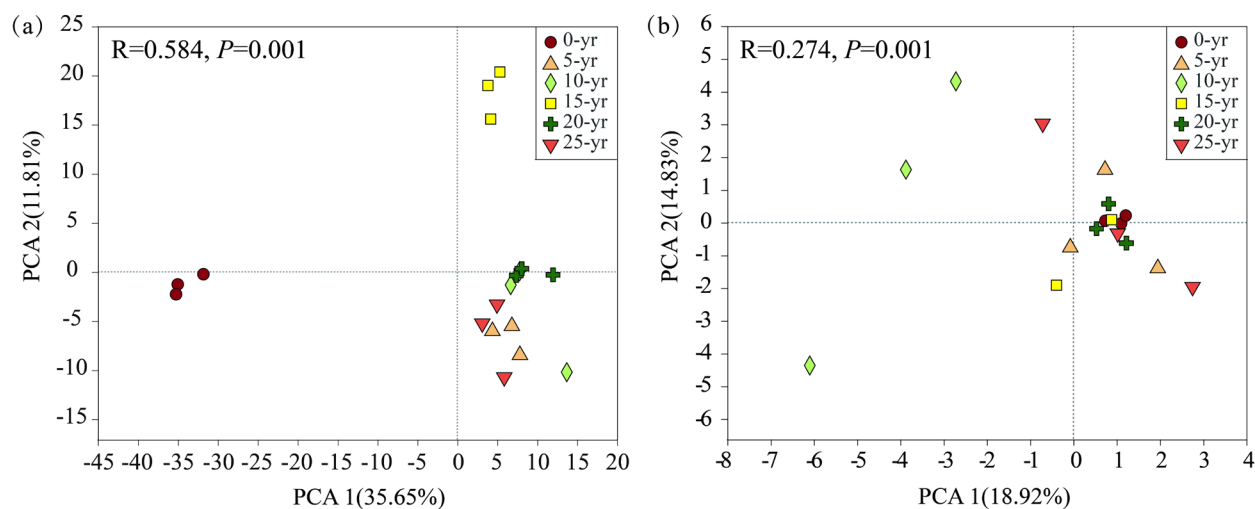


Fig. 2. PCA analysis of soil bacteria (a) and nematodes (b) at the genus level based on Bray-Curtis algorithm.

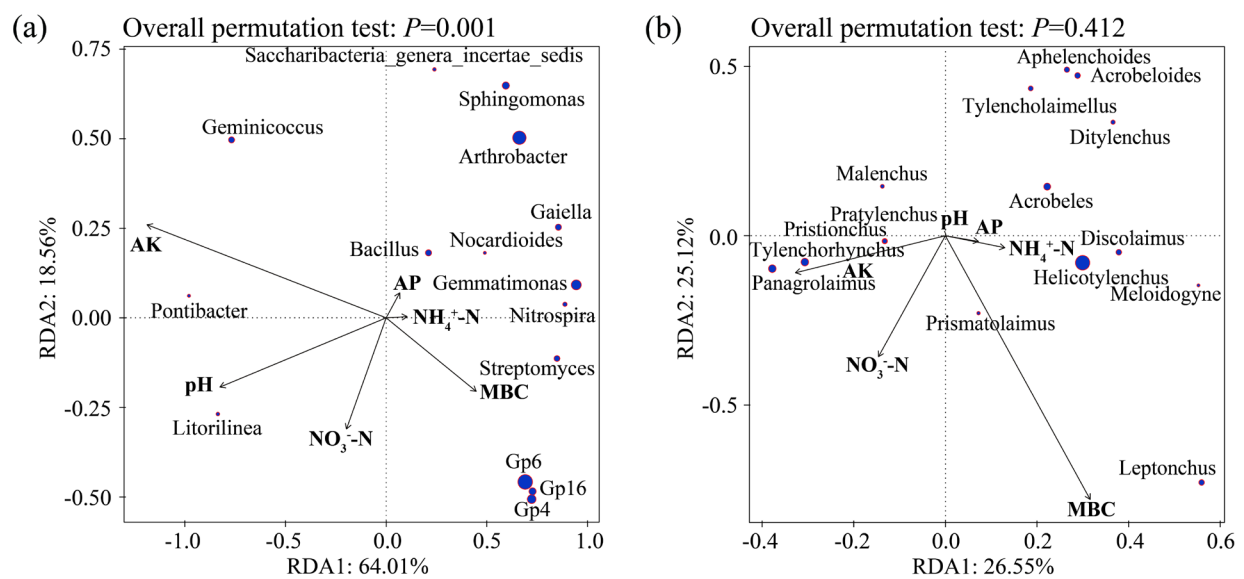


Fig. 3. Redundancy analysis of the top 15 bacteria (a) and nematodes (b) (at the genus level) with soil physicochemical properties. The colored dots represent relative abundance. The bars represent the total proportion of explained variations in the physicochemical properties by key bacteria and

nematodes. AP, Available phosphorus; AK, Available potassium; $\text{NH}_4^+\text{-N}$, Ammoniacal nitrogen; $\text{NO}_3^-\text{-N}$, Nitrate nitrogen; MBC, Microbial biomass carbon.

Table 3. Importance of soil physicochemical properties in explaining the bacterial and nematode community structure changes obtained by redundancy analysis

Soil properties	Bacteria				Nematode			
	RDA1	RDA2	R^2	Pr ($> r$)	RDA1	RDA2	R^2	Pr ($> r$)
pH	-0.9737	-0.2280	0.4786	0.007**	-0.6547	-0.7559	0.0027	0.982
AP	0.6945	0.7195	0.0548	0.687	0.9735	-0.2285	0.0494	0.685
AK	-0.9769	0.2138	0.6861	0.001**	-0.9484	-0.3169	0.2300	0.128
$\text{NH}_4^+\text{-N}$	0.9995	0.0315	0.0590	0.645	0.9647	-0.2632	0.0897	0.506
$\text{NO}_3^-\text{-N}$	-0.5363	-0.8440	0.2075	0.175	-0.3785	-0.9256	0.2572	0.108
MBC	0.9087	-0.4174	0.2777	0.093	0.3761	-0.9266	0.5598	0.004**

Notes: AP, Available phosphorus; AK, Available potassium; $\text{NH}_4^+\text{-N}$, Ammoniacal nitrogen; $\text{NO}_3^-\text{-N}$, Nitrate nitrogen; MBC, Microbial biomass carbon. *, $p \leq 0.05$, **, $p \leq 0.01$.

Potential functions of soil bacteria

The PICRUST analysis results of the metabolic functions of bacteria in CC soils showed that bacterial metabolism was the main pathway (level 1), and the relative abundance of genes in the bacterial metabolism pathway in each CC treatment was more than 79% (Fig. S2). The global and overview maps, carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, and energy metabolism were the major pathways enriched by functional genes (Level 2) (Fig. S2). Cluster analysis results showed that the metabolic functions of bacteria in the 0-yr and 25-yr CC treatments were similar (level 3) (Fig. 4a). There was no difference in the abundance of carbon metabolism genes among CC treatments ($p > 0.05$). The abundance of bacterial oxidative phosphorylation genes and carbon fixation genes in the 20-yr CC treatment was significantly higher than those in the 0-yr and 25-yr CC treatments. However, the relative abundances of methane metabolism, sulfur metabolism, and nitrogen metabolism genes showed an opposite trend, and those in the 20-yr CC treatment was the lowest (Fig. 4b). The two components in RDA analysis explained 94.05% of the total variation in the relationship between soil bacterial functional genes and soil physicochemical properties, and soil pH, AK, and $\text{NO}_3^-\text{-N}$ were the main influencing factors (Fig.

4C, Table 4).

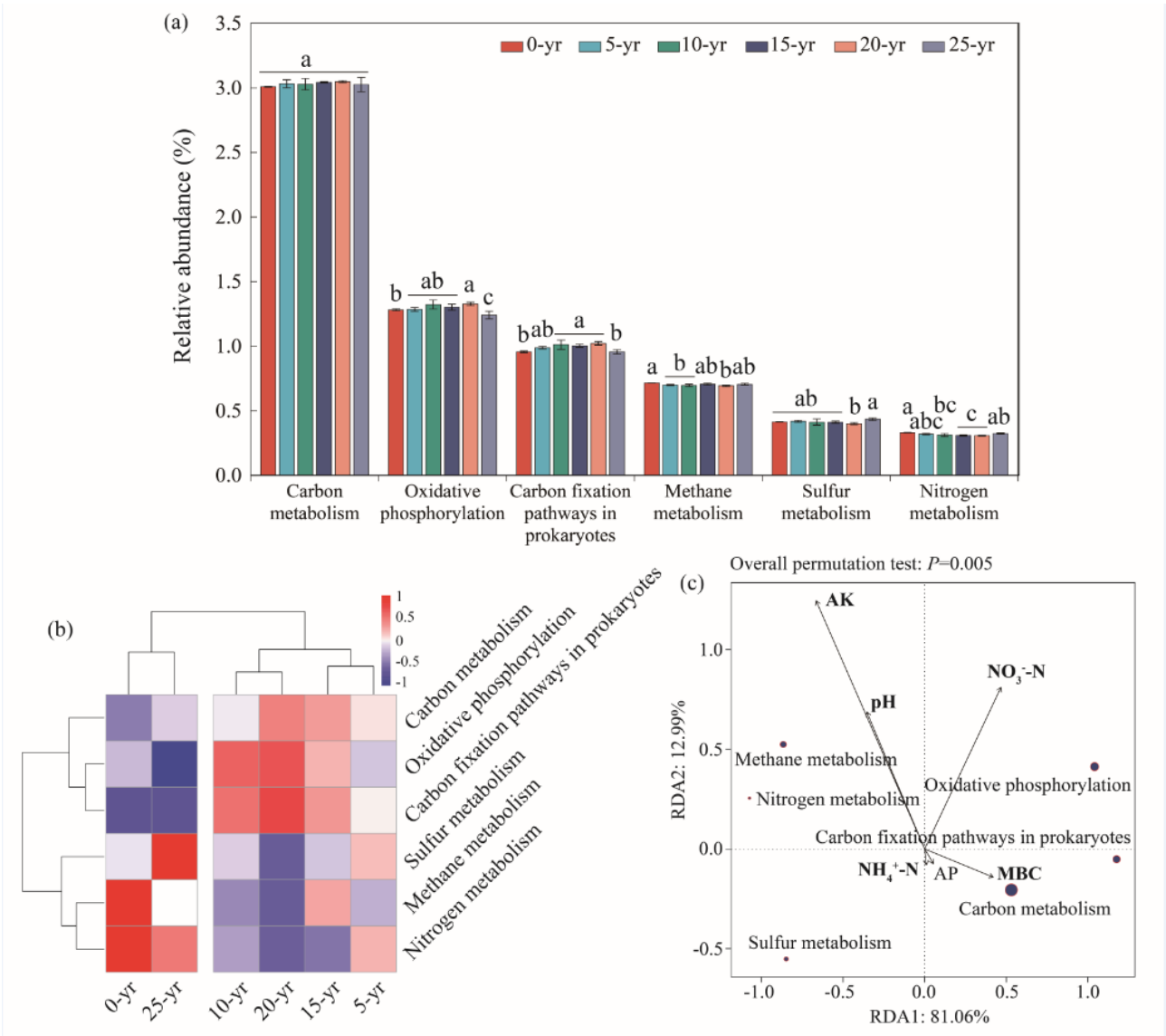


Fig. 4. Relative abundance (a) and cluster heatmap (b) of bacterial functional genes in different treatments based on pathway 3 of KEGG database, and RDA analysis of soil physicochemical properties and bacterial functional genes (c). Different lowercase letters indicate significant differences (Tukey's test, $p < 0.05$).

Table 4. Importance of soil physicochemical properties in explaining the relative abundance variances of bacterial KEGG functional genes obtained by redundancy analysis

Soil physicochemical properties	RDA1	RDA2	R^2	Pr (> r)
pH	-0.4590	0.8885	0.3329	0.049*
AP	0.5852	-0.8109	0.0396	0.770
AK	-0.4711	0.8821	0.6076	0.001**
NH ₄ ⁺ -N	0.1297	-0.9916	0.0345	0.793
NO ₃ ⁻ -N	0.4992	0.8665	0.4026	0.020**
MBC	0.9475	-0.3198	0.1908	0.203

Notes: *, $p \leq 0.05$, **, $p \leq 0.01$.

Relationship between bacterial and nematode communities

The niche breadths of soil bacteria and nematodes were different in different CC treatments ($p < 0.05$). The bacterial community in the 0-yr CC treatment had the highest *Bcom* value, while that in the 20-yr CC treatment had the lowest *Bcom* value (Fig. 5a). This was opposite to that of nematode community (Fig. 5b). Mantel test showed that nematode community diversity, composition, and niche breadth were the main factors affecting bacterial community composition. Besides, the diversity and niche breadth of nematode community also affected the niche breadth of bacterial community (Fig. 5c, Table S5, $p < 0.05$). It was also found that RDA1 and RDA2 explained 79.80% of the total variances in the relationship between bacterial-feeding nematodes and bacterial communities, and *Panagrolaimus* and *Acrobeles* were the two main bacterial-feeding nematode genera that affected soil bacterial communities (Fig. 5d, Table 5, $p < 0.05$). The correlation analysis results showed that the abundance of *Panagrolaimus* was significantly positively correlated with that of *Pontibacter*, *Euzebya*, *Nitriliruptor*, *Truepera*, *Blastococcus*, *Aliifodinibius*, *Rubellimicrobium*, *Sphaerobacter*, and *Gillisia*, and the abundance of *Acrobeles* was significantly positively correlated with that of *Saccharibacteria_genera_incertae_sedis* (Table S6, $p < 0.05$).

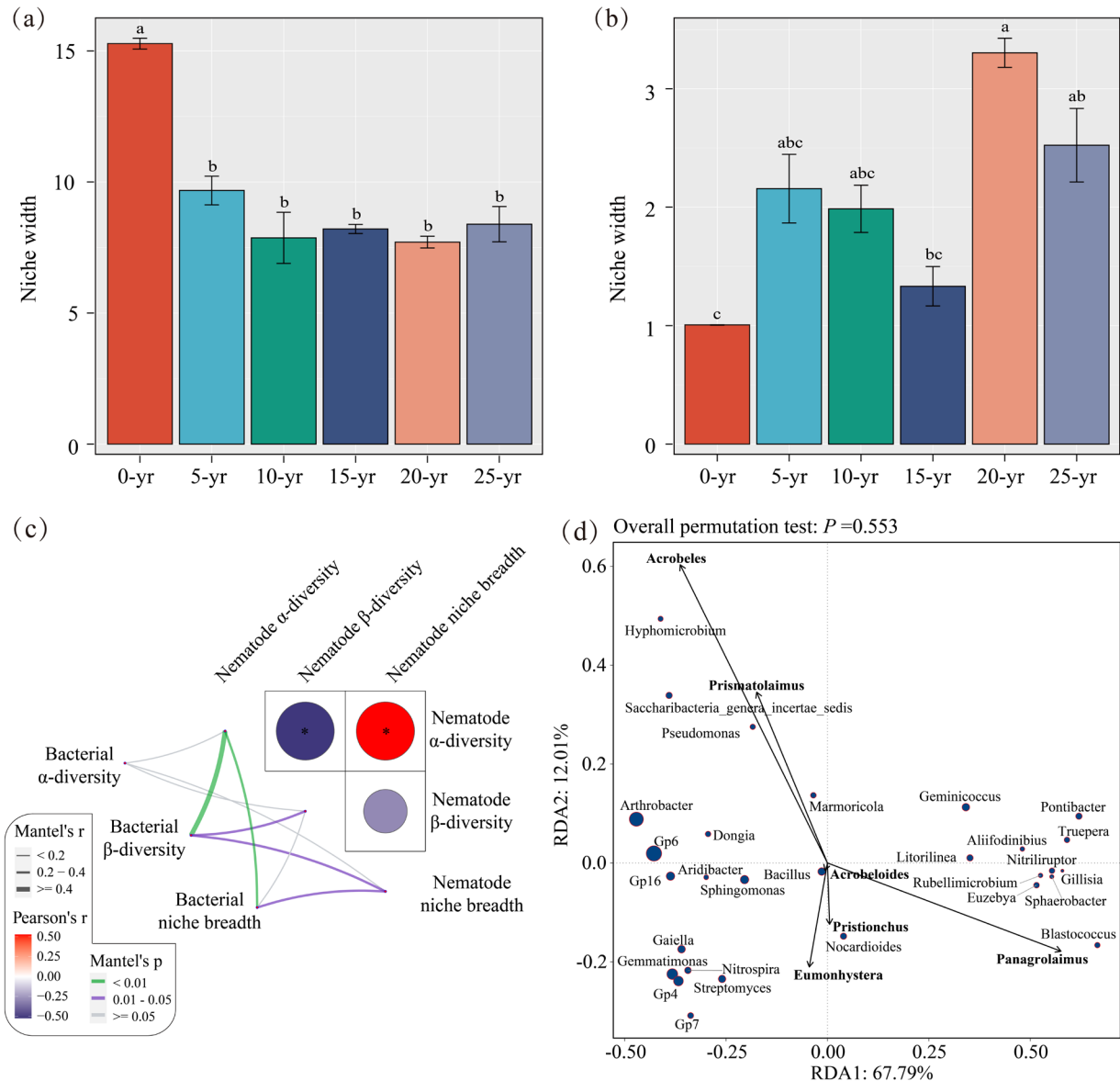


Fig. 5. Analysis of the niche width (*BCom*) of bacteria (a) and nematodes (b) (at the genus level) in each treatment based on correlation and optimal multiple regression model, Mantel test of community diversity and niche breadth of soil bacteria and nematodes (c), and RDA analysis of soil bacterial-feeding nematode and bacterial genera (d).

Table 5. Importance of soil bacterivores in explaining the bacterial genus variances obtained by redundancy analysis

Bacterivores	RDA1	RDA2	R^2	Pr (> r)
<i>Panagrolaimus</i>	0.9552	-0.2961	0.3596	0.018*
<i>Acrobeles</i>	-0.5157	0.8568	0.4198	0.025*
<i>Acrobeloides</i>	-0.3809	-0.9246	0.0095	0.954
<i>Pristionchus</i>	0.0432	-0.9991	0.0740	0.498
<i>Prismatolaimus</i>	-0.4506	0.8927	0.2308	0.161
<i>Eumonhystera</i>	-0.2083	-0.9781	0.1283	0.342

Notes: *, $p \leq 0.05$, **, $p \leq 0.01$.

DISCUSSION

Many factors affect the structure and diversity of soil bacterial community, such as climate, vegetation, and soil type (Xu et al. 2013; Gavrichkova et al. 2018; Sun et al. 2020). In this study, 25-yr continuous cropping significantly reduced the relative abundance of Actinobacteriota, but increased the relative abundance of Proteobacteria. Actinobacteriota and Proteobacteria are the dominant bacteria in most arid soils (Cheng et al. 2019). Doumbou et al. (2001) reported that Actinobacteria was an important microbe in rhizosphere soil, which could regulate plant pathogen resistance and soil microecological balance. The increase in the relative abundance of Proteobacteria may be due to that the increase in soil nitrogen content increases the uptake of other resources by soil bacteria (Fierer et al. 2012; Zhang et al. 2018b; Lin et al. 2019). Besides, continuous cropping significantly increased the diversity and richness of soil bacteria, especially 15-yr CC. This may be due to the changes in the soil environment, especially the changes in the nutrients required by soil bacteria (Cheng et al. 2018). Plant parasitic nematodes can induce inhibition to continuous cropping (Mweke et al. 2008). This study results showed that *Helicotylenchus* was the dominant nematode genus in all CC treatments except for the 20-yr CC treatment, and the abundance of *Helicotylenchus* increased in the 5-yr, 10-yr, and 25-yr treatments, compared with that in the 0-yr CC treatment. The relative abundances of unclassified OTUs were higher than that of *Helicotylenchus* in the 20-yr CC treatment. Therefore, the dominant nematode genus in the 20-yr CC treatment may be unclassified nematodes. This reflects the importance of the maturity of reference databases in the application of HTS technology to nematode community studies (Schenk et al. 2019; Sun et al. 2023). *Helicotylenchus* listed as one of the ten most important plant parasitic nematodes in the world (Sasser et al. 1989). Cotton is a good host for *Helicotylenchus* (Wrather et al. 2002; Schumacher et al. 2020). As early as 1994, Kinlock and Sprenkel (1994) reported that *Helicotylenchus* was detected in 76% of the cotton fields in each county of Florida. *Helicotylenchus* can invade the cortical tissue of crop roots, reduce the ability of the plant to absorb water and nutrients, and cause the development of plant shoots to be hindered (Yeates et al. 1993b; Riascos-Ortiz et al. 2020). Da Silva et al. (2023) also reported that the excessive density of *Helicotylenchus* put great pressure on the growth of cotton plants, resulting in yield loss. Moreover, continuous cropping significantly increased the richness and diversity of soil nematode communities compared with 0-yr CC treatment. This may be due to changes in nematode

hosts caused by cotton cropping (Nahar et al. 2006). It is worth noting that in our study, the richness and diversity of soil nematode community decreased after 10-yr CC. This may be due to that soil nematodes are sensitive to soil nutrients and environmental quality (Neher 2001). This indicates that the response of soil bacteria to the change of soil nutrients and environmental quality is hysteretic than that of soil nematodes. It must be mentioned that in agroecosystems, fertilization can affect soil nematode and bacterial communities by altering soil nutrient status (Liang et al. 2009; Zhou et al. 2015). However, a few studies have also shown no significant difference in soil bacterial and nematode abundance between fertilization treatment and non-fertilization control (Li, 2009; Hu, 2010; Hu et al. 2018). In this study, to reduce the impact of fertilization on soil nematode and bacterial communities and ensure consistency of control variables between treatments, consistent fertilization time, fertilization method, and fertilization amount were adopted across treatments. Besides, all field management measures were consistent except for the duration of continuous cropping.

Soil properties have great impact on soil microbial and nematode community structure (Buckley et al. 2006; Yang et al. 2021). Soil nematode community can reflect the degree of soil health and external interference (Wang et al. 2018). Our results showed that long-term CC led to significant changes in soil properties, impacting soil bacterial and nematode communities. The redundancy analysis results showed that soil pH was an important factor determining bacterial community structure. This is because soil pH affects the microbial community structure and diversity in surface soil by affecting the spatial distribution of microbial communities (Xu et al. 2020a; Kang et al. 2021). This study results also showed that soil AK was a factor greatly impacting soil bacterial community. This is consistent with the study results of Guo et al. (2019). Besides, MBC was a main environmental factor affecting soil nematode community structure. Nematode predation promotes the growth of microbial communities (Sun et al. 2017), while the excessive feeding of nematodes on microbes always leads to a decrease in soil microbes (Ingham et al. 1985; Jing et al. 2017), This is the reason for the decrease in MBC content in this study.

PICRUSt has been proved to be an effective tool for the prediction of bacterial functions (Langille et al. 2013). In this study, carbohydrate metabolism, amino acid metabolism, and energy metabolism were the main metabolic functions of soil bacterial communities at Level 2. This indicates that soil bacterial communities are actively involved in fundamental metabolic processes. This is consistent with the study results of Ma et al. (2017) and Samaddar et al. (2019). Besides, Li

et al. (2021) showed that long-term CC reduced the relative abundance of bacterial functional metabolism in the cucumber rhizosphere soil, which was consistent with the change in bacterial community composition. In this study, cluster analysis results showed that there was difference in the abundance of carbon metabolism, oxidative phosphorylation, carbon fixation pathways in prokaryotes, methane metabolism, sulfur metabolism, and nitrogen metabolism genes between 0-yr/25-yr treatment and other treatments (Figure 4b). RDA analysis showed that the key factors causing this difference were soil pH, AK and NO_3^- -N contents. Soil pH impacts soil bacterial functions by affecting the structure of soil bacterial community (Xu et al. 2020b). This indicates that soil bacterial functions are closely related to the composition of soil microbial communities, and are regulated by a series of abiotic environmental factors (Bardgett et al. 2014). Due to the limitations of PICRUST, verification still need to be combined with metagenomic sequencing and other technologies in future research.

In our study, bacteria showed a broader niche breadth than nematodes, and the niche breadth of bacteria was significantly negatively correlated with that of nematodes. This indicates that compared with nematode communities, bacterial communities are less affected by environmental factors and have higher resource utilization ability, diversity, and competitiveness (Dolédéc et al. 2000; Pandit et al. 2009; Wu et al. 2018). This may be due to that the wide distribution and strong environmental adaptability bring a large niche breadth for key bacteria (Jiao et al. 2017), such as Actinobacteriota and Proteobacteria, the dominant bacteria phyla in all treatments in this study. Correlation analysis results showed that nematode community affected bacterial community mainly by affecting bacterial community structure. RDA analysis results also confirmed that the bacterial-feeding nematodes *Panagrolaimus* and *Acrobeles* were the main nematode genera that significantly affected soil bacterial communities in this study. On the one hand, selective predation by nematodes changes the size of different bacterial populations, altering biological interactions between bacteria, leading to changes in bacterial community composition (Xiao et al. 2014; Jiang et al. 2023). On the other hand, different predation intensities of soil nematodes have different effects on the structure of microbial communities, and moderate predation intensity of nematodes can maintain rapid growth of bacterial populations and increase bacterial metabolic activity (Ingham et al. 1985). To date, there has been much attention paid to the selective predation of bacterial-feeding nematodes (Zhang et al. 2005; Jousset et al. 2009). This study also found that the relative abundance of *Panagrolaimus* was positively correlated with that of *Pontibacter*, *Euzebya*, *Nitriliruptor*, *Truepera*, *Blastococcus*, *Aliifodinibius*, *Rubellimicrobium*, *Sphaerobacter*, and *Gillisi*, and the relative abundance of *Acrobeles* was positively correlated with that of *Saccharibacteria_genera_incertae_sedis*. The

decrease in the relative abundance of *Panagrolaimus* in the 5-yr CC treatment is mainly due to the significant reduction in the relative abundance of *Pontibacter*, *Euzebya*, *Nitriliruptor*, *Truepera*, *Blastococcus*, *Aliifodinibius*, *Rubellimicrobium*, *Sphaerobacter*, and *Gillisi*. As early as 1990, Sundin et al. reported that the feeding activity of nematodes promoted the proliferation of bacteria. Djigal et al. (2004) and Zubkov et al. (2000) also reported that selective predation by bacterial-feeding nematodes not only changed the soil microbial community structure, but also directly affected nematode growth and development. However, Wardle et al. (1993) found that the increase in the biomass of soil microorganisms did not increase the biomass of bacterial-feeding nematodes, possibly because bacterial-feeding nematodes were mainly controlled by the top-down regulation of nutritional levels in the food web. Mikola et al. (1998) and Groffmann et al. (1999) also found that the existence of bacterial-feeding nematodes reduced microbial biomass. This may be due to that the large over-feeding of nematodes masks the promotion effect on bacterial numbers (Ingham et al. 1985). Briar et al. (2007) pointed out that moderate nematode feeding intensity was most conducive to promoting microbial growth. It is worth noting that the interaction between bacterial-feeding nematodes and microorganisms is not generalized; The changes in microbial communities, especially key species, induced by nematode microbial communities have rarely been determined, and the results are not consistent. This study results further confirm that the selective predation by bacterial-feeding nematodes affects bacterial communities. This deepen our understanding of the relationship between the two and provide evidence on how nematodes play a role in altering soil biota.

CONCLUSIONS

In this study, high-throughput sequencing technology was used to determine the changes and interactions of microbial and nematode communities in the soils under different CC durations in Northwest China. Proteobacteria, Actinobacteria, and *Helicotylenchus* were the dominant bacteria and nematode in all CC soil samples. The main environmental factors affecting soil bacterial and nematode communities were different during CC, and nematode communities were more sensitive to environmental changes than bacterial communities during CC. Moreover, different CC durations not only had different effects on bacterial composition and diversity, but also affected the main metabolic functions of bacteria. The structure and diversity of soil nematode community, especially *Panagrolaimus* and *Acrobeles*, could significantly affect the structure of soil bacterial community.

The combination of high-throughput sequencing and function prediction provides new insights for understanding the relations of soil nematodes, bacteria, and their living environment.

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Supplementary materials

Fig. S1. Upset-plot analysis of the number of common and unique operational taxonomic unit (at the genus level) of bacteria (a) and nematodes (b). (download)

Fig. S2. Predicted functions of soil bacterial community using PICRUs at level 1 and level 2. *, $p \leq 0.05$; **, $p \leq 0.01$. (download)

Table S1. Effects of different continuous cropping durations on the relative abundance of bacteria at the phyla level. (download)

Table S2. Effects of different continuous cropping durations on the relative abundance of bacteria at the genus level. (download)

Table S3. Effects of different continuous cropping durations on the relative abundance of nematodes at the genus level. (download)

Table S4. Unclassified soil nematode OTUs under different continuous cropping durations. (download)

Table S5. Correlation analysis of soil bacterial and nematode communities. (download)

Table S6. Correlation analysis of soil bacterial-feeding nematode and bacterial communities. (download)