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Changes in Soil Nematode and Microbial Community in Cucumber Root-Zone Soil Shaped by Intercropping with Amaranth

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Abstract: Intercropping systems often contribute to soil health management including inhibiting root-knot nematode disease. The main purpose of this study was to investigate the potential effect of the cucumber-amaranth intercropping system on soil biota, specifically the nematode and microbial communities. Furthermore, the cucumber root-nematode disease was also evaluated. The study found significant effects of cultivation systems (cucumber-amaranth intercropping and cucumber monocropping) and growing seasons (winter-spring (WS) and autumn-winter (AW)) on both soil nematode and microbial community structures in cucumber root-zone soil. Intercropping resulted in a decrease in the relative abundance of *Meloidogyne* spp., which was consistent with the observed alleviation of root-knot nematode disease. Bacterivorous nematodes were dominant in the intercropping system. The microbial biomass and community-level physiological profiles (CLPP) were generally higher in the intercropping system. Beta diversity analysis showed that the composition of microbial communities varied widely among the treatments and growth seasons. These findings suggest that intercropping with amaranth can regulate soil biota, leading to decreased incidence of root-knot nematodes (RKNs) diseases.

Keywords: intercropping; cucumber; amaranth; soil nematode community; soil microbial community

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1. Introduction

The intercropping system, which is based on different crop species combinations, is a widely used agronomic practice in promoting crop productivity and suppressing soil-borne diseases [1–3]. It has been demonstrated that a cultivation system with high crop diversity showed effective and continuous control of crop diseases [4–6]. Different agricultural practices including intercropping have often been considered to apply ecological principles related to biodiversity, plant interactions, and other natural regulatory mechanisms [7–9]. Compared with monocropping, plant diversity-driven changes in the abundance and composition of soil biota cause feedback on plant performance [10]. Intercropping is a critical practice to increase biodiversity which can increase ecosystem function and stabilize food production [11]. Plant community variations greatly influence the community composition of root-associated organisms [12,13] which can improve plant health and control the populations of plant pests [14,15].

Soil biota play an important role in a variety of ecological processes associated with plant health and soil productivity. Each plant species harbors a specific rhizosphere community [16]. Intercropping can modify the composition of bacterial and fungal communities in desert ecosystems [17] and improve the soil environment [18]. Soil nematodes are involved in numerous soil processes and also enhance plant health [19,20]. The root-zone soil environment changes can affect the nematode community structure [21]. In an intercropping system, the inputs of main crops and intercrop roots shape the microbial community and

provide the resources for maintaining the soil nematode community [22]. Increasing aboveground plant diversity could also change the root-derived metabolites diversity which may affect soil biota [23,24].

However, the consequences of the biodiversity of these soil organisms have been rarely studied in intercropping systems, especially those suffering from RKNs disease. Root-knot nematodes especially *Meloidogyne incognita* is a devastating soil borne disease [25] and caused extensive damage to cucumber (*Cucumis sativus* L.) in China [17]. As sedentary endoparasites, Juveniles of *Meloidogyne incognita* locate host roots and penetrate them to establish permanent feeding sites in the vascular cylinder. The formation of giant cells for nutrient sources results in a deformed host root system and affects the root function which diminishes plant performance [26]. It was reported that the production of cucumbers in China accounted for about 59.5% of the worldwide production, which reached nearly 1.3 million hectares in 2021 (FAOSTAT). Although chemical nematicides have shown effective control they caused significant environmental risks. Conventional hybridization breeding method cannot confer *M. incognita* resistance to cucumber due to a lack of resistant cucumber cultivars germplasm resources [27] and candidate resistance genes [28]. According to the above, crop species-based intercropping systems may offer an alternative practice to contribute to the inhibition of RKNs disease through mobilizing soil biota shifts. The leafy vegetable amaranth (*Amaranthus tricolor* L.) could intercrop with a variety of crops including cowpea [29,30], maize [31] and cucumber [32]. In previous research, amaranth has shown the potential to increase cucumber production and decrease the population of plant-parasitic nematodes [18]. Therefore, amaranth may be used as a potential intercrop to control cucumber RKN disease.

The study aims to evaluate the potential influences of the cucumber-amaranth intercropping system on the cucumber root-zone soil nematode and microbial communities. The cucumber root-knot nematode disease was also evaluated. In the current study, a greenhouse experiment was conducted. We hypothesized that the soil nematode abundance and community would respond to the introduction of amaranth. The intercropping system would decrease the abundance of plant parasitic nematodes and suppress cucumber RKN disease. This study will focus on the shifting of soil biota mediated by the intercropping system.

2. Materials and Methods

2.1. Experimental Condition and Plant Materials

The experiment was carried out in the experimental station located in China Agricultural University, Beijing, from February 2020 to February 2021. The greenhouse had been planted with cucumbers for over a decade and had caused root-knot nematode disease. The chemical properties of the soil before the experiments have been described in Supplementary Materials and Methods. Cucumber (*Cucumis sativus* L. cv. 'Jinyou No. 35′) and Amaranth (*Amaranthus tricolor* L. cv. 'Jingxian No.1′) were used in the experiment. All seeds were sterilized using 70% ethanol and 3% sodium hypochlorite solution and rinsed with sterile water [33].

2.2. Cucumber-Amaranth Intercropping Experiment

Cucumber seedlings with two true leaves were transplanted into the greenhouse, with double rows of 40-cm row spacing and 20-cm plant spacing on the seedbed. Two treatments were set up including (i) Cucumber monocropping (C): Cucumber density was 40 plants per plot; (ii) Cucumber intercropping with amaranth (CA). Cucumber density was 40 plants per plot and about 20 amaranth seedlings were kept in the space of two rows between two cucumber plants. The distance between cucumber and amaranth is about 5 cm with no separation between the two crops' roots. Each plot (0.8 m × 5 m) was performed for a biological replicate and three biological replicates were set up for the treatments. After transplanting, the water and fertilizer management were consistent among different treatments.

The soil samples were randomly collected from the cucumber root-zone area at 0–20 cm depths using a soil core probe in the vigorous fruiting period of the winter-spring (WS) season (2020, 2–7) and autumn-winter (AW) seasons (2020, 9–2021, 1). Each replicate of two treatments contained six cores and was well mixed. After removing the plant residues and stones, the soil was passed through a 2 mm sieve and frozen at 4 °C until further analyses. Soil samples were separated into two parts: one part was kept moist to determine the soil mineral N and soil biota, and the other was air-dried for measurement of soil chemical properties.

2.3. Status of Cucumber Root-Knot Nematode Disease

For the identification of the *Meloidogyne* species that caused the cucumber RKN disease, the DNA of nematode egg masses on the surface of cucumber root galls was extracted for molecular identification using the marker Mi F/R [34]. The sedentary female nematodes were handpicked from root galls and the perineal patterns were examined and identified. Cucumber roots were sampled randomly from each experimental plot of two treatments. Five cucumber root systems were washed with deionized water and weighed to determine fresh weight. The status of root-knot nematode disease was evaluated by the galls per gram fresh root weight.

2.4. Soil Chemical Properties Analysis

Soil pH and EC were measured using a soil: water ratio at 1:2.5 (w/v) and a soil: water ratio at 1:5 (w/v) by a pH meter (FE28, METTLER TOLEDO) and an EC meter (FE30, METTLER TOLEDO), respectively. Soil water content was measured using the regular gravimetric technique. The soil organic C was analyzed by dichromate oxidation titration and soil total N was determined by Micro-Kjeldahl digestion procedure. Soil mineral N (nitrate N plus ammonium N) was measured using a continuous flowing analyzer [35], while available P was determined by the molybdenum-blue colorimetry [36]. Moreover, soil available K was measured using the flame photometry [36]. Soil ionome was detected by ICP-OES (ICP Optima 8000, PerkinElmer) and the extracted digested with a combined nitric acid (HNO_3), hydrofluoric acid (HF), and perchloric acid ($HClO_4$) method [37].

2.5. Soil Nematode Community Analysis

Soil nematodes were isolated from 100 g of fresh soil [38]. After counting the nematode abundance (expressed as the total number per 100 g dry soil), a total of 100 specimens were randomly selected and identified to the genus level under a stereomicroscope. Soil nematodes were further assigned to four trophic groups including bacterivores (Ba), fungivores (Fu), omnivore-predators (OP) and plant parasites (PP) as recommended by Yeates [39]. Moreover, diversity and maturity indices were also calculated based on the nematode communities which included the Shannon–Weiner diversity index (H') [40], Pielou evenness index (J') [41], Margalef richness index (SR) [42], Simpson dominance index (λ) [43], maturity index for c-p 1-5 members (MI) [44], maturity index for c-p 2-5 members (MI25) [33,44] and plant parasite index (PPI) [44].

2.6. Soil Microbes-Related Parameters Analysis

Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) were analyzed using the chloroform fumigation extraction method [45,46]. The community-level physiological profiling (CLPP) of the soil microbial community was evaluated using a Biolog EcoPlate method which contained 31 different carbon sources (Biolog Inc., Hayward, CA, USA) [47]. In detail, 5 g soil and 45 mL (1:9, w/v) sterilized water were placed in a 50 mL sterilized tube and vortexed for 1 h at 4 °C. Subsequently, the homogenized soil solution was diluted using sterilized water up to 10^{-3} fold. Finally, 150 µL soil suspension was added into the Biolog Ecoplate well and incubated at 25 °C in the dark. The optical density (OD) values of each well were measured at 590 nm every 24 h using a microplate reader. To eliminate the effects of inoculum density, the optical density value

from each well was normalized by the average well color development (AWCD). Plate readings at 96 h (the shortest incubation time that allowed the best resolution among treatments) of incubation were selected to evaluate the soil microbial community carbon source utilization. The normalized values were added up to get the proportion of the AWCD that is attributed to six different guilds (i.e., amides/amines, amino acids, carbohydrates, carboxylic acids, miscellaneous and polymers). In addition, to measure the diversity index of soil microbial community carbon source utilization, the Shannon–Weiner (SW) index and Simpson index (1/D) were calculated [18]. Further details of materials and methodology for the community-level physiological profiling (CLPP) are provided in Supplementary Materials and Methods.

2.7. Soil Microbial Community Analysis

Microbial community genomic DNA was extracted and purified from about 400 mg of soil using the PowerSoil DNA Isolation Kit (QIAGEN). To universally amplify the 292-bp fragment of V4 region in 16S rRNA gene, we used primers 515F (5′-GTGCCAGCMGCCGCG GTAA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) containing a variable 12 bp barcode sequence [48] and the primers ITS1F (5′-CTTGGTCATTTAGAGGAA GTAA-3′) and reverse primer ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) used for the amplification of 300-bp fragment of fungal ITS gene [49]. The PCR was performed under the following conditions: 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina) and subjected to a single sequencing run on the MiSeq platform (Illumina). For analyzing the soil microbial community, root-zone soil-based Illumina sequences of 16S and ITS were processed and sequentially quality-filtered using Fastp (version 0.19.6) [50]. Pair-end reads were merged with a minimum overlap using Flash (1.2.11) [51]. After removing chimeric sequences, the remaining sequences were binned into OTUs with 97% similarity and the representative sequence for each OTU was taxonomically classified via the Ribosomal Database Project's classifier [52] and the SILVA database (version 138) [53] for bacteria and UNITE (version 8.0) for fungi [54]. All OTUs identified as belonging to chloroplast and mitochondria were removed from the data set. Then, the representative sequences for each OTU were aligned using PyNAST [55] in QIIME [56] and carried out by Uparse software (version 11) [57]. Further details of materials and methodology for soil microbial community analysis are provided in Supplementary Materials and Methods.

2.8. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and the Kruskal–Wallis test with SPSS 26.0. Both soil properties and nematode trophic groups were analyzed by a two-way ANOVA with the factors growing season (GS), cultivation system (CS) and the interaction of GS × CS. PCA analysis was applied to the soil property and soil nematode community to see the separations between the groups which was performed in the OmicShare tools, a free online platform for data analysis (https://www.omicshare.com/tools, accessed on 5 May 2023). Co-occurrence networks were constructed by using the "picante" R package based on Spearman's correlation matrices of cucumber monocropping (C) and intercropping system (CA) (spearman's >0.8; after Benjamin and Hochberg FDR adjust, $p < 0.05$) [58]. Networks were visualized using the interactive platform Gephi (v.9.2) [59]. The false discovery rate (FDR)-adjusted *p*-values had a 0.05 cutoff and correlation coefficients were >0.8. The nodes and edges in the network represent nematodes genus and microbial OTUs and the correlations between pairs of nematodes genus and microbial OTUs, respectively.

3. Results

3.1. Soil Properties and Cucumber RKN Disease

Principal component analysis (PCA) revealed soil properties in cucumber-amaranth intercropping system were distinctly separated from the monocropping (Figure 1a) and

the distribution was largely separated between growing seasons along the x-axis (PCA1) (Figure 1a). A two-way analysis of variance showed that the growing seasons effect was greater than the cultivation system on soil chemical properties (Table S1). Furthermore, growing season and cultivation systems both alter soil chemical properties, especially for soil EC ($p < 0.001$) and soil available K ($p < 0.01$) (Table S1). That indicated soil nutrient accumulation and availability differed between monocropping and intercropping systems in two growing seasons. The specific amplification marker primers of *M. incognita* were used to characterize the root-knot nematode disease by sampling the egg masses on the surface of the cucumber root system. Combined with the perineal patterns of female nematodes, the results showed that the disease of the field is determined to be *M. incognita* (Figure S1). The RKN disease status was measured by recording root galls by fresh root weight. In this study, the number of cucumber root galls was declining in the intercropping system in both growing seasons (Figure 1b). Comparing the cucumber root system, the root system has only a few root knots in the intercropping system, and the lateral root development is not affected. In the monocropping system, a large number of root knots appeared (Figure 1b,c).

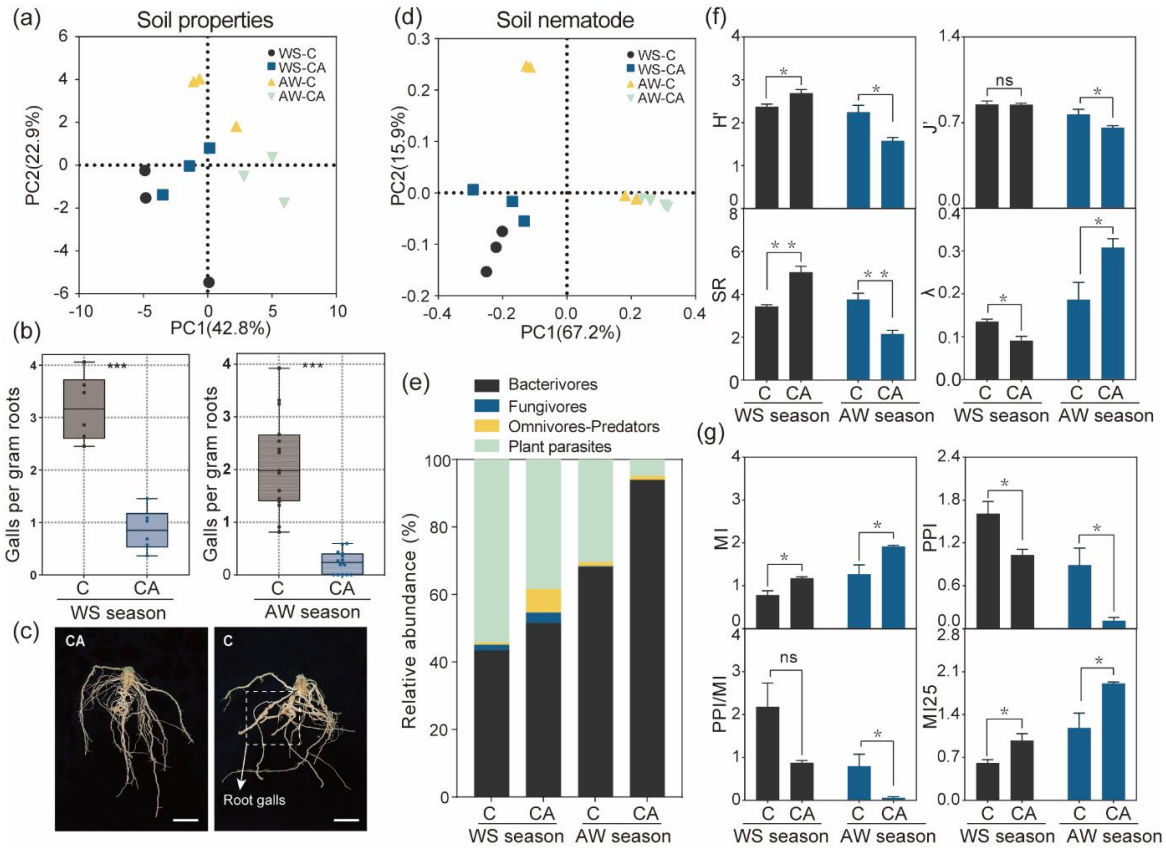

Figure 1. Intercropping changed the cucumber root-zone soil nematode community and decreased the RKNs disease. C: Cucumber monocropping; CA: Cucumber-amaranth intercropping; WS: Winter-spring season; AW: Autumn-winter season. (**a**) Soil chemical and ionome profiles analyzed by PCA. (**b**) Numbers of cucumber root galls in monocropping (C) and intercropping system (CA) in two growing seasons. *p*-values calculated using Student' *t*-test, *** $p < 0.001$, n = 5. (**c**) Representative pictures of cucumber root planted in monocropping (C) and intercropping (CA) system, bar: 2 cm. (**d**) Partial Canonical analysis of soil nematode community. (**e**) Distribution of soil nematodes by trophic groups. (**f**) The diversity indices of soil nematode community. *p*-values calculated using Student's *t*-test, * $p < 0.05$, ** $p < 0.01$, ns: not significant, n = 3. (**g**) The maturity indices of soil nematode community. *p*-values calculated using Student's *t*-test, * $p < 0.05$, n = 3.

3.2. The Soil Nematode Community Shaped by Intercropping System

The density of soil nematodes had the same trends in the two growing seasons (Figure S1). Especially in the AW season, the total numbers of nematodes per 100 g dry soil were significantly higher in intercropping plots (Figure S1) which reached 434. The nematode trophic groups fluctuated obviously in the two growing seasons. The percentage stack histogram showed the proportion of each trophic group (Figure 1e). In the WS season, the dominant trophic group was bacterivores which occupied 43.5% and 51.5% in the monocropping and intercropping systems, respectively. The percentage of plant parasites in monocropping was higher than in the intercropping system (CA) which reached 54.2% of the total population (Figure 1e). The dramatic changes in the nematode community appeared at AW season, bacterivore nematodes occupied 93.9% of the intercropping system and only 4.9% of plant parasites nematodes which is much less than nematode proportion in monocropping. The same trend appeared in fungivores and omnivores-predators which is the higher proportion in intercropping system except that Fungivore nematode was not detected in the CA treatment at AW season (Figure 1e). The reverse trend of nematode community diversity indices is shown in two growing seasons (Figure 1f). Significant higher H′, SR and λ were detected in intercropping in the WS season and these indices were lower in the AW season (Figure 1f). The same differences were shown in maturity indices in which the MI, PPI and PPI/MI were significantly lower in the intercropping system and had a significantly higher value in MI25 (Figure 1g) in both growing seasons.

3.3. Soil Microbial Biomass and Community-Level Physiological Profiling

The intercropping system (CA) enhanced soil microbial biomass carbon and nitrogen. The trend of soil microbial biomass in two growing seasons is consistent (Figure 2c,d). The results indicated that CA increased the microbial biomass carbon (MBC), especially in the AW season which showed a significant increase compared with cucumber monocropping ($p < 0.05$) (Figure 2a). Microbial biomass nitrogen (MBN) of the intercropping system was significantly increased in the WS season (Figure 2b). The results of community-level physiological profiling (CLPP) showed the Shannon–Weiner index (H) was significantly increased in CA soils at both WS and AW seasons (Figure 2c). The reciprocal of the Simpson's index (1/D) also showed an increase especially in the root-zone soils at AW season (Figure 2d). These results indicated that the diversity of soil microbial community in cucumber root-zone soils of the intercropping system was changed due to the amaranth root disturbance. The heatmap showed the metabolic diversity of the soil microbial community for the capacity to degrade 31 sources of carbon (Figure 2e). Based on the utilization of six kinds of carbon sources, the cucumber root-zone soil of CA made greater use of all kinds of carbon sources during both growing seasons (Figure 2f). Especially for the carbohydrates and miscellaneous at WS season and Amino acids and Polymers at AW season which were significantly higher in the cucumber intercropping system than in the monocropping system ($p < 0.05$) (Figure 2f). These results indicated that introducing amaranth in cucumber cultivation shaped the cucumber root-zone microbial community and increased the carbon utilization capacity.

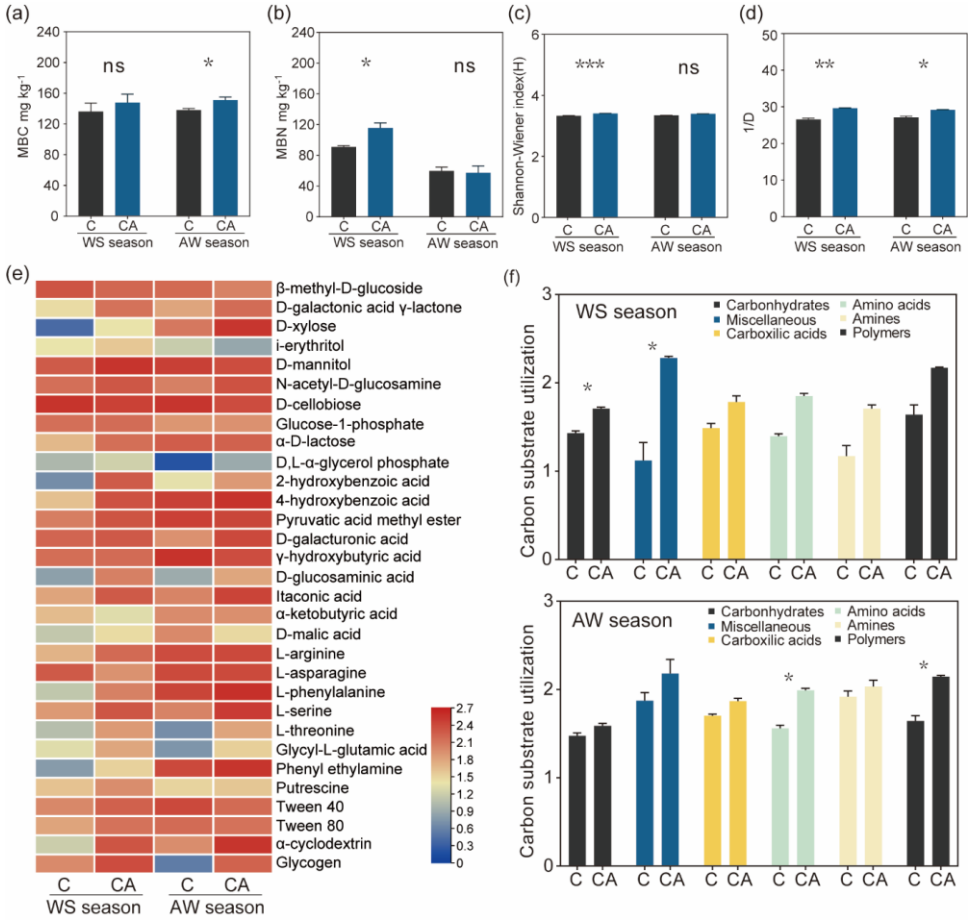

Figure 2. The community level physiological profiling (CLPP) of cucumber root-zone soil. C: Cucumber monocropping; CA: Cucumber-amaranth intercropping; WS: Winter-spring season; AW: Autumn-winter season. Soil microbial biomass carbon (**a**) and nitrogen (**b**) of cucumber root-zone soil in two growing seasons. MBC, microbial biomass carbon; MBN, microbial biomass nitrogen. (**c**) The Shannon-Weiner index and (**d**) the reciprocal of Simpson's index (1/D) of cucumber monocropping and intercropping system. (**e**) Heatmap showed the OD values of various 31 substrates. The observations of OD values at the 96 hr incubation points were used for drawing heatmaps. (**f**) The carbon substrates are grouped into six major carbon categories and we evaluated the carbon substrate utilization by OD values. *p*-values calculated using Student' *t*-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant, n = 3.

3.4. Intercropping System Altered Soil Microbial Community Composition

All rarefaction curves for bacteria and fungi community analysis tended to approach the saturation plateau which indicated the data volume of sequenced reads was sufficient for the species diversity (Figure S2). A total of 545,241 bacterial sequences and 850,926 bacterial numbers were detected. After double-end sequence data were spliced and filtered, 537,756 and 742,199 optimized sequences were generated for bacteria and fungi, respectively. The number of effective sequences of bacteria and fungi was 33,561–54,294 and 57,081–68,031, respectively, in different samples. And then, the number of sequences was normalized to 33,561 per sample for bacteria and 56,785 for fungi according to the minimum number of sequences. In total, 5571 OTUs of bacteria were detected in monocropping and intercropping cucumber root-zone soils, belonging to 49 phyla, 155 classes, 360 orders, 564 families, 978 genera and 1931 species (Table S10). A total of 1293 OTUs which belong to 13 phyla, 35 classes, 69 orders, 159 families, 297 genera and 483 species of fungi were obtained (Table S11). The intercropping system could not substantially alter the soil microbial α diversity (Figure 3); however, it strongly influenced the microbial community

structure. Nonmetric multidimensional scaling (NMDS) analysis based on Bray–Curtis distances were applied to determine the overall microbial community structure of root-zone soil and showed significant separation in both soil bacterial and fungal communities (Figure 4a,b). Furthermore, the growing seasons also displayed a substantial effect which varied by WS and AW growing seasons (Figure 4a,b). These results indicated that the cultivation system and seasonal changes both contributed to the shifts in the belowground microbial community. Venn diagram showing the overlapping microbes and the numbers of unique microbes (Figure 4e,f). Bacterial communities in soils at both growth seasons were primarily comprised of members of the phyla *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Planctomycetota* and *Crenarchaeota* accounting for about 80% of the relative abundance of all the identified phyla (Figure 4a). Fungal communities consisted of *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, *Rozellomycota* and *Chytridiomycota* (Figure 4a), and the *Ascomycota* were the main phylum accounting for more than 60% of all samples.

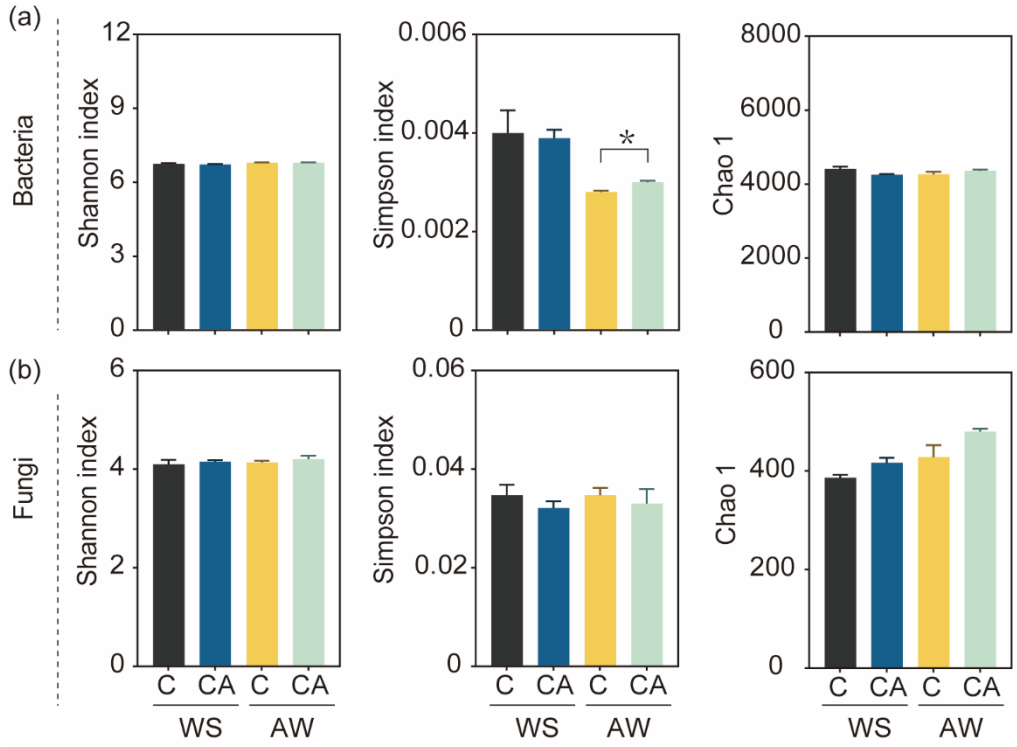

Figure 3. The α-diversity of cucumber root-zone soil community. C: Cucumber monocropping; CA: Cucumber-amaranth intercropping; WS: Winter-spring season; AW: Autumn-winter season. (**a**) Shannon, Simpson and Chao 1 index of bacterial community. (**b**) Shannon, Simpson and Chao 1 index of fungi community. *p*-values calculated using Student' *t*-test, * $p < 0.05$, n = 3.

Furthermore, the LefSe analysis showed that the number of genera of bacteria enriched in two cucumber cultivation systems was higher in monocropping than in intercropping (FDR-adjusted $p < 0.05$, Wilcoxon rank-sum test, the absolute LDA score > 2.5). Notably, both bacterial and fungal genera enriched by intercropping and monocropping are quite different. The class *Alphaproteobacteria*, the family *Micromonosporaceae* and the order *Rhodobacterales* were enriched in the intercropping system (Figure 4c). For the fungal community, the genera *Cladosporium*, *Fusicolla* and *Chordomyces* were enriched in the intercropping system (Figure 4c). Specifically, we conducted a non-parametric test (Kruskal–Wallis test) to determine the effect of the intercropping system on the abundance of bacteria and fungi at phylum and family levels. Based on phylum abundance analysis, the intercropping system increased the abundance of *Proteobacteria*, and *Acidobacteriota* in associated bacterial communities in the WS season (Figure 4a). The abundance of *Proteobacteria* in AW season had the same trend but the *Acidobacteriota* was decreased (Table S6). Associated with

fungal communities, *Ascomycota* as the main phylum did not change in the WS season; however, the abundance was significantly increased in the AW season ($p < 0.05$) (Figure 4a; Table S7). In both growing seasons, the abundance of *Basidiomycota* and *Mortierellomycota* was decreased in the intercropping system (Figure 4a; Table S7). At the family level, the bacterial abundance of *JG30KFCM45*, *Sphingomonadaceae* was significantly higher in the intercropping system and *Xanthomonadaceae* showed a significantly lower abundance in WS season (Table S8). The abundance of *Sphingomonadaceae* showed the same trend in the AW season which indicated that amaranth may recruit its community formation (Table S8). Introducing amaranth into the cucumber intercropping system had a significant effect on the abundance of fungi based on family level, especially in the AW season (Table S9).

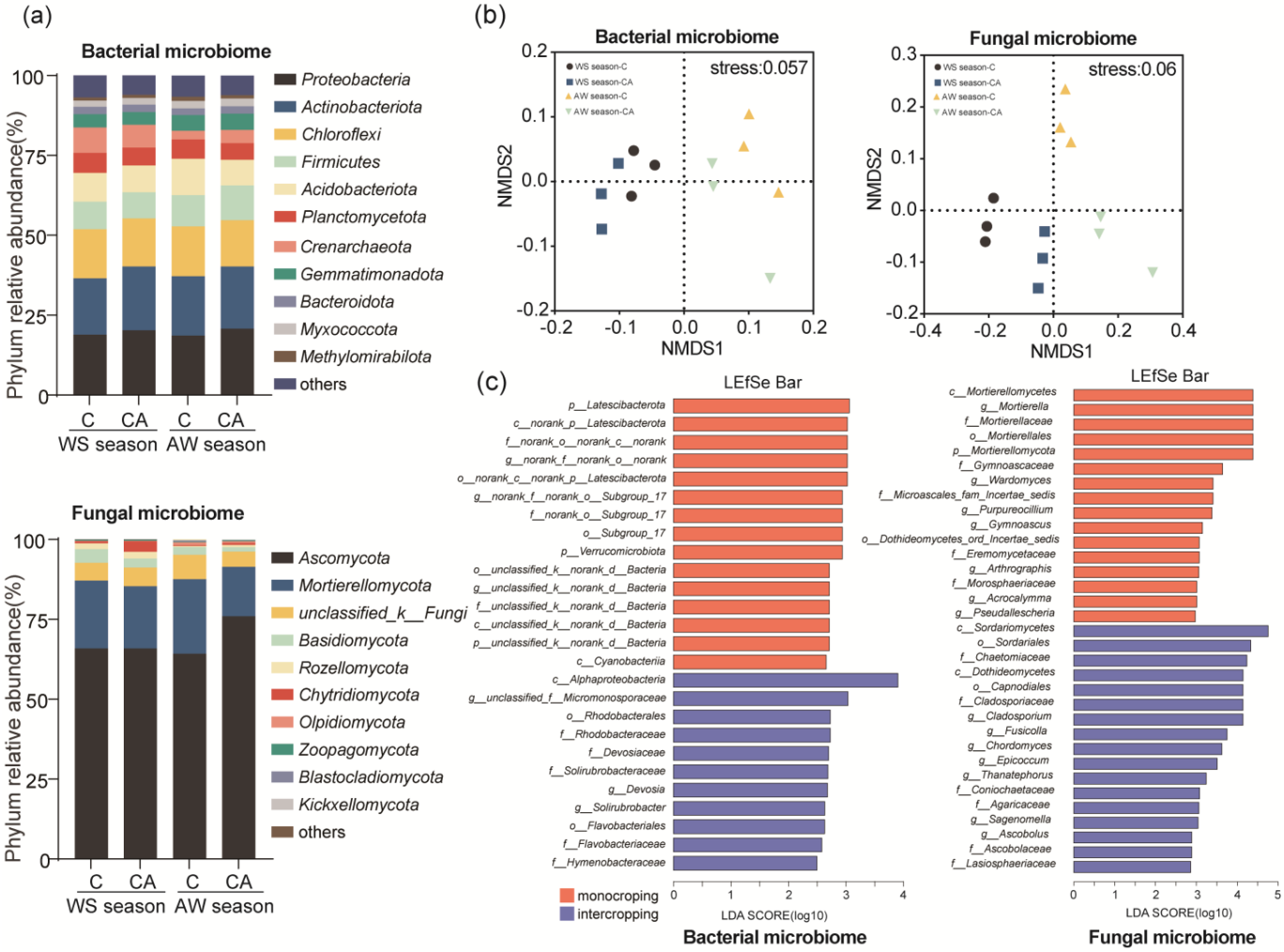

Figure 4. Intercropping system shaped cucumber root-zone soil microbial community structure. C: Cucumber monocropping; CA: Cucumber-amaranth intercropping; WS: Winter-spring season; AW: Autumn-winter season. (**a**) Microbial composition in root-zone soil displayed as stacked bar plot at phylum level of bacterial and fungal community. (**b**) Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity displaying bacterial and fungal community. (**c**) The linear discriminant analysis (LDA) scores to identify the intercropping system shaped taxa at the phylum (p) and genus (g) levels, detected by the LDA of effect size (LEfSe) analysis. Only taxa with the absolute LDA score > 2.5 are shown.

3.5. Co-Occurrence Network Soil Biota

We further explored co-occurrence patterns using network inference based on significant correlations (using non-parametric Spearman's correlations). The network of soil nematode and microbial communities at each site demonstrated distinct co-occurrence

patterns especially for nematode structure (Figure 5a–f). Here, we used the network topological parameters of node and edge numbers to assess soil microbial network complexity, with higher node and edge numbers representing greater network complexity.

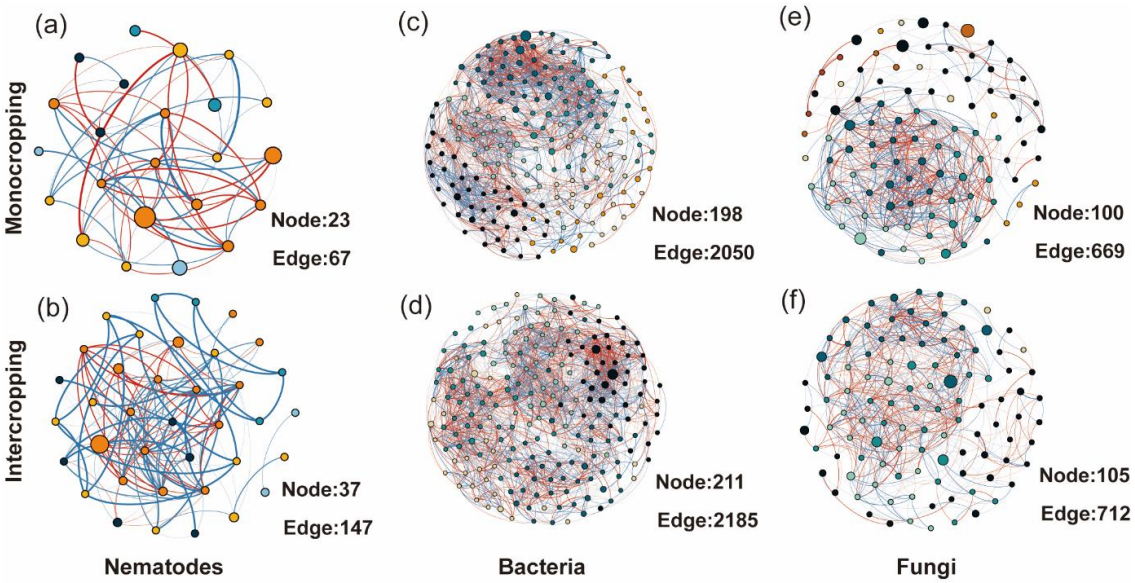

Figure 5. Co-occurrence patterns of soil nematode and microbial community in cucumber root-zone soil. Co-occurrence network of soil nematode community (**a**,**b**), soil bacterial (**c**,**d**) and fungal community (**e**,**f**) differed in two monocropping and intercropping systems. The sizes of the nodes (genus for soil nematode and OTUs for bacterial and fungal community) are proportional to the number of connections. Only nodes (OTUs) that were significantly correlated each other (spearman's > 0.8; after Benjamin and Hochberg FDR adjust, $p < 0.05$) were connected (edges). The numbers of nodes and edges showed in the network diagram.

The resulting soil nematode network consisted of 23 nodes and 67 edges (Figure 5a) in the cucumber monocropping system, but an increase appeared in intercropping soils which consisted of 37 nodes and 147 edges (Figure 5b). For the microbial network of cucumber root-zone soil, we generated four networks for bacteria and fungi under monocropping and intercropping systems. Collectedly, the introduction of amaranth in the cucumber intercropping system slightly improves the complexity of the soil microbial community (Figure 5c–f).

4. Discussion

Given that RKN disease is a global concern, it is crucial to investigate strategies that are both effective and environmentally safe for managing it. Intercropping systems have been shown to mitigate soil-borne diseases by influencing below-ground biodiversity. In the greenhouse experiment, the introduction of amaranth into the cucumber cultivation system resulted in distinct changes in the soil nematode and microbial communities. Our findings support the hypotheses of the intercropping system in suppressing RKN disease affected through affecting the soil biota.

Plants possess traits that regulate the abundance and composition of the soil organisms [60] including the soil nematode community [61]. Bacterivores were the major trophic group in this study which is in line with other agricultural systems [62–64]. Several studies demonstrated that root exudates stimulated microbial activities and improved the turnover of bacterivores and fungivores [65,66]. The significantly decreased indices include H′ and SR and the higher Simpson index (λ) indicated the lower diversity in soil nematode in the intercropping system (CA) at AW season (Figure 1f). This results from the decreased numbers of nematode taxa. However, the maturity indices of the soil nematode community

showed a consistent trend in the two cultivation seasons (Figure 1g). A lower index of MI and a higher index of PPI/MI indicated soil disturbance [67,68]. So, the significantly higher MI and lower PPI/MI index indicated the adaptability of the cucumber-amaranth intercropping system. Soil nematodes can serve as indicators due to their sensitivity to soil environmental variation [69]. Complex chemical signals in the root zone soil contribute to the formation of nematode community [70]. Moreover, non-host plants were used as intercrops to disrupt the chemical communication between RKN nematodes and host roots by the repellent metabolites released into the soil [71,72]. Therefore, the significant changes in bacterivorous and plant parasite nematode abundance (Figure 1e) implied the introduction of amaranth may change the belowground metabolic diversity in cucumber root-sone soil.

Plants host specific rhizosphere microbiomes that change with the neighborhood richness [73,74]. The intercropping system significantly affects the β-diversity soil microbial community in this study (Figure 4a,b). Studies have reported that the structure, composition and diversity of the soil microbial community could be affected by plant species [75–77]. Continuous cropping decreases the diversity and biomass of the soil microbiome which generally improves in intercropping and rotation systems [77–79]. The Biolog Ecoplate method was applied to evaluate the CLPP of the soil microbial community [80]. The significant difference in carbohydrates and miscellaneous utilization in the WS season and carboxylic acids and polymers in the AW season showed the improvement of the intercropping system (Figure 2f). Combined with the increasing microbial biomass N and C (Figure 2a,b), the intercropping system enhanced soil carbon sources metabolic utilization intensity and had positive effects on soil microbial biomass. The microbial community also showed separation following the cultivation system and growing season (Figure 4b), which implied the belowground effects of amaranth. Soil temperature is an important environmental factor affecting microbial and nematode communities [81–83]. That may also explain the variation in soil nematode communities due to the tight links with soil microbial communities. Generally, increasing soil microbial diversity is beneficial to soil function and health [84]. The number of nodes and edges (Figure 5c–f) indicated that the intercropping system could promote the connectivity and complexity of the soil microbial community and may cause positive interactions. Moreover, the soil nematode community responded more strongly to intercropping system than the microbial community which may lead to the inhibition of plant parasitic nematodes (Figure 5a,b). The specific members of the family *Sphingomonadaceae* and *Xanthomonadaceae* were significantly changed in the intercropping system (Table S8), which may be relative to the pathogen invasion due to its ability to produce secondary metabolites [85]. The two-way interactions between soil microbial communities and soil nematode communities were studied in different crop systems [86,87] which might decrease the abundance of plant-parasitic nematodes. We demonstrated that, similarly to the variations in the soil microbial community, the soil nematode community was also changed due to the intercropping system.

In this study, Plant parasites decreased in the intercropping system (Figure 1e; Table S4) and the decreasing populations of *Meloidogyne* sp. J2s were consistent with the alleviation of cucumber root-knot nematode disease in the intercropping system (Figure 1b,c; Table S4). The intercropping system was widely used to improve crop productivity and control root-knot nematode disease [3,15,88] which allows amaranth to serve as an intercrop to affect soil biota and promote soil health.

5. Conclusions

In this study, we have demonstrated the shifts in the soil microbial community and soil nematode community in the cucumber-amaranth intercropping system. Moreover, we indeed observed a significant reduction in cucumber RKN disease. The amaranth which served as an intercrop, enhanced the microbial biomass and soil microbial carbon source utilization capacity. The cucumber-amaranth intercropping system also increased the bacterivores and suppressed plant-parasitic nematodes in soils, thereby alleviating the

damage of RKNs to cucumber roots. This study demonstrated the potential of a cucumber-amaranth intercropping system for regulating soil biota and alleviating the cucumber RKN disease. Further research will focus on the direct mechanism of amaranth to suppress the RKN disease especially the metabolic profiling of root and rhizosphere in cucumber and amaranth and the relationship between specific compounds and RKN management in intercropping systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9080924/s1, Supplementary file [18,49,51–57]; Figure S1: Total numbers of soil nematode and the identification of *Meloidogyne incognita* in cucumber cultivation; Figure S2: Rarefaction curves of different soil microbial communities. Table S1: Effects of cultivation system and growing season on soil chemical property; Table S2: Effects of cultivation system and growing season on soil ionome; Table S3: Changes in the abundances (individuals per 100 g soil) of nematode functional guilds; Table S4: The mean proportion (%) of various nematodes on genus level in cucumber root-zone soil under monocropping and intercropping system; Table S5: Effects of cultivation system and growing season on soil nematode diversity and maturity indices; Table S6: Results of Kruskal–Wallis test the abundance of bacterial taxa at phylum level; Table S7: Results of Kruskal–Wallis test of the abundance of fungal taxa at phylum level; Table S8: Results of Kruskal–Wallis test of the abundance of bacterial taxa at family level; Table S9: Results of Kruskal–Wallis test of the abundance of fungal taxa at family level; Table S10: The OTU data of bacterial community; Table S11: The OTU data of fungal community.

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