Effects of Diseased Soil from Continuously Cropped Tomato Rhizosphere on Tomato Growth, Soil Nematodes, and Microorganisms (Postprint)

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Abstract

Investigating the induction effect of rhizosphere diseased soil from continuously cropped tomato plants infected with root-knot nematode disease on tomato root-knot nematode disease and the underlying microecological mechanisms causing continuous cropping obstacles can provide a scientific basis for elucidating the occurrence mechanisms of tomato continuous cropping obstacles and developing effective control strategies. In this study, pot experiments were conducted to determine changes in biological characteristics and root-knot nematode infection status of tomato plants grown in healthy soil and inoculated diseased soil, and to analyze and identify the species and quantities of microorganisms and nematodes in the soil. The results showed that inoculation with rhizosphere diseased soil from continuously cropped tomato plants infected with root-knot nematode disease significantly affected tomato growth and root-knot nematode infection: 1) The number of root galls on tomato roots at the seedling stage reached 9 per plant, while no galls were observed in healthy soil; the soil nematode population increased by 390.4% compared to healthy soil; at the harvest stage, the root-knot nematode infection rate of tomato reached 62.7%, with a disease index of 80.0%. 2) Tomato growth was inhibited, and leaf defense enzyme activities were suppressed. At the harvest stage, the fresh weight of shoots and roots decreased by 50.2% and 33.1%, respectively, compared to healthy soil. At the seedling stage, tomato leaf PPO activity decreased by 15.8% compared to healthy soil, while POD activity increased by 24.0% compared to healthy soil, with all differences reaching significant levels ($P<0.05$). 3) Tomato roots became more susceptible to harmful bacteria. The population of the pathogenic bacterium Pseudomonas brassicacearum in the roots increased by 463-fold compared to healthy soil, while the total populations of bacteria, fungi, and actinomycetes

in the rhizosphere soil increased by 46.3%, 94.5%, and 134.0%, respectively. 4) The populations of bacterivorous nematodes, fungivorous nematodes, and plant-parasitic nematodes were 3.3 times, 1.6 times, and 7.3 times those in healthy rhizosphere soil, respectively, with 95.6% of the plant-parasitic nematodes being root-knot nematodes. In summary, inoculation with rhizosphere diseased soil from continuously cropped tomato plants infected with root-knot nematode disease not only caused tomato plants to suffer from root-knot nematode infection, but also led to a substantial increase in total soil nematode populations and the proportion of plant-parasitic nematodes, and caused a significant increase in harmful bacterial populations within tomato roots, resulting in significant inhibition of tomato growth. Simultaneously, it affected the physiological and biochemical characteristics of tomato, with nematode-infected tomato showing decreased defense enzyme activities, making them more susceptible to infection by root-knot nematodes and pathogenic bacteria. The species and quantities of nematodes, microorganisms in tomato rhizosphere soil, and dominant bacteria within roots, as well as their interactions, were altered.

Full Text

Effect of Sick Rhizosphere Soil Under Tomato Continuous Cropping on Soil Nematodes, Microbes and Tomato Growth

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Abstract: Continuous cropping of tomato is a widespread practice that severely restricts sustainable tomato production. The interaction between soil nematodes, soil microbes and plants may be related to root-knot nematode disease due to continuous cropping. However, little attention has been paid to the interrelatedness of these factors or the effect of continuous cropping on the relationship among soil and other three factors. Thus, this study explored the effects of sick soil (root-knot nematode infecting soil) on tomato root-knot disease, mechanism of micro-ecological obstacles and plant growth of continuously cropped tomato with a pot experiment. The abundances and communities of microbes and nematodes in root-zone soils and the activities of defensive enzymes in seedling leaves of tomato were analyzed to determine what pathogenic mechanism existed in such cropping systems. Compared with healthy soil, sick soil caused the following changes: (1) at seedling stage, root-knot nematodes began to infect roots and therefore root-knots appeared on tomato roots. Compared with the healthy soil, sick soil increased the abundance of soil nematode by 390.4%. At maturity, the infection rate of root-knot nematode was 62.7% and the related disease index was 80.0%. (2) The growth of tomato was restrained and the activities of defense enzymes reduced. The dates of blossoming and

fruiting delayed too. Fresh biomass of shoot and root at maturity significantly ($P < 0.05$) decreased by up to 50.2% and 33.1%, respectively. Also the quantity and fresh mass of fruit significantly ($P < 0.05$) decreased by up to 59.7% and 68.2%, respectively. While compared with healthy soil, PPO activity of seedling leaves significantly ($P < 0.05$) decreased (by 15.8%), and POD activity significantly ($P < 0.05$) increased (by 24.0%) for sick soil. (3) Tomato roots became easily infected by harmful bacteria (*Pseudomonas brassicacearum*). The number of pathogenic bacteria *P. brassicacearum* in tomato roots in sick soils was 463 times greater than that in healthy soils. The total number of bacteria, fungi and actinomycetes in rhizosphere soils increased by 46.3%, 94.5% and 134.0%, respectively. (4) The abundance of soil nematodes increased nearly 3 times, among which the abundance of fungi-feeding nematodes, bacteria-feeding nematodes and plant-parasitic nematodes increased by 1.6, 3.3 and 7.3 times, respectively. The abundance and diversity of plant-parasitic nematodes greatly increased, of which root-knot nematode accounted for 95.6%. In conclusion, the inoculation of sick soils from the root-zone under continuous tomato cropping had complex effects on root-zone soil ecology. It inhibited tomato growth and increased the incidence of root-knot nematode disease by influencing the abundance and diversity of microbes and nematodes in root-zone soils and also by influencing biochemical metabolism of tomato. Mass propagation of plant parasitic nematodes and plant pathogenic bacteria decreased defensive enzyme activity and stress resistant ability of tomato, which in turn led to more severe root-knot nematode infection with significant inhibitory effect on tomato production. The negative effects of continuous cropping on tomato growth were caused by the interactions among root-zone soil microbes, root endophytes and soil nematodes.

Keywords: Tomato; Continuous cropping; Root-knot nematode; Soil microbe; Defensive enzyme; Pathogenic bacteria

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely cultivated vegetables worldwide. In solar greenhouses in northern China, continuous tomato cropping is common, leading to increasingly prominent obstacles that cause soil quality degradation, imbalance in soil microbial population structure, and reduced tomato yield and quality [1-2], which severely restricts protected tomato production. Previous studies have shown that the main causes of continuous cropping obstacles include unbalanced soil nutrient consumption, deterioration of soil physical properties, accumulation of autotoxic substances secreted by plants, abnormal changes in soil chemical properties, and abnormal soil microbial flora and microecological imbalance [3-4]. The quantity, activity and community structure of soil microorganisms directly affect plant water and nutrient absorption and resistance to harsh environments [5]. Nayyar et al. [6] reported that continuous cucumber (*Cucumis sativus* L.) cropping significantly

altered rhizosphere soil microbial flora, with diversity indices and richness of soil microbial communities decreasing with increasing planting years. Sun et al. [7] and Xu et al. [8] demonstrated that continuous processing tomato cultivation significantly reduced the bacteria/fungi ratio, shifting the soil microbial flora from bacteria-dominated to fungi-dominated.

Changes in soil biological community structure can serve as early warning ecological indicators of soil changes [9]. Nematode communities, including plant-parasitic, bacteria-feeding, fungi-feeding, predatory and omnivorous nematodes, represent one of the most abundant and functionally diverse groups of soil fauna [10], influencing soil organic matter decomposition, nutrient cycling and crop growth, and serving as sensitive bioindicators of soil health [11]. Soil nematodes are primarily represented by microbivorous functional groups. Studies have shown that microbivorous nematodes affect soil ecological processes and plant growth by predating soil microorganisms and releasing nutrients immobilized in microbial biomass [12], and bacteria-feeding nematodes can promote plant growth [13].

Current research on nematodes in continuous cropping soils has mainly focused on the effects of cropping years on soil nematode abundance [14] and community structure [15-16]. Studies have found that continuous cropping transforms soil nematode communities originally dominated by bacteria-feeding and fungi-feeding nematodes into communities dominated by plant-parasitic nematodes, severely damaging host plants [17-18]. Soil microbivorous nematodes primarily alter soil microbial quantity and activity through their feeding behavior [19], while microbial quantity also significantly regulates nematode community structure [20].

Soil nematodes, soil microorganisms and plants are all related to plant root-knot nematode diseases caused by continuous cropping, but few studies have examined the interrelationships among these three factors, and even fewer have investigated the effects of tomato continuous cropping sick soil on these relationships. The harmful phenomena of continuous cropping sick soil on tomatoes are widespread, but systematic studies on the degree of harm to continuous cropping tomatoes and the effects on soil microorganisms and nematodes are lacking. This study focused on investigating the effects of sick soil from the root zone of tomato plants infected with root-knot nematodes under continuous cropping on tomato root-knot nematode infection and tomato growth, and systematically studied nematodes and microorganisms in tomato root-zone soil and microorganisms within tomato roots after sick soil inoculation. The aim was to explore the effects of continuous cropping tomato root-zone sick soil on the micro-ecosystem of "tomato plant-tomato root-zone soil microorganisms-root endophytes-soil nematodes" and its induction effect on tomato root-knot nematode disease, providing a scientific basis for understanding the micro-ecological mechanisms of tomato continuous cropping obstacles and exploring remediation methods.

Materials and Methods

Experimental Materials

The tomato variety used was 'Baiguo Qiangfeng' , produced by Shenniu Seedling Business Department, Wuchang District, Wuhan City, suitable for protected and open-field cultivation. The sick soil was collected in September 2014 from the root zone of diseased tomatoes in a solar greenhouse that had been continuously cropped for more than 4 years in Mengjiazhai, Yangling District, Shaanxi Province. During the tomato fruiting peak, severely root-knot nematode-infected tomatoes were selected. After removing the diseased plants, surface soil (approximately 5 cm) was removed with a sampling shovel, and soil from the root distribution zone at 5-20 cm depth was collected in plastic self-sealing bags and thoroughly mixed as sick soil inoculation material for the greenhouse pot experiment. The nematode density in the sick soil was determined to be 800,000 individuals · kg ^{1} using the Baermann funnel method.

The potting soil was collected from 0-20 cm tillage layer of the same type of non-continuous cropping healthy soil, air-dried, crushed and passed through a 10 mm sieve before mixing.

Experimental Design

The experiment was conducted from October 2014 to May 2015 in the research greenhouse of the South Campus of Northwest A&F University. Two treatments were established: (1) Healthy soil (CK): each pot contained 6 kg of healthy tillage soil. (2) Healthy soil + sick soil: each pot contained 4 kg of healthy tillage soil inoculated with 2 kg of tomato root-zone sick soil containing nematodes, thoroughly mixed. Each treatment was replicated 3 times, with 30 tomato seeds sown per pot, and 10 tomato seedlings retained after emergence. The experiment was sown on October 10, 2014, and harvested on May 20, 2015.

During the experiment, compound fertilizer (N-P O -K O = 15-15-15) was applied 3 times at 1 g · pot ^{1} each time, dissolved in water and irrigated.

Tomato Root-Knot Investigation and Biological Characteristics Measurement

At the seedling stage (40 days after growth, February 1, 2015), after appropriate watering to moisten and loosen the soil, 6 tomato plants were completely excavated from each pot. Soil attached to roots was shaken off and roots were carefully rinsed with water until clean. Root surface water was absorbed with filter paper, and the number of fibrous roots and root-knots per plant were counted. Shoot and root fresh mass were measured using a 0.01 g precision balance.

At maturity (May 20, 2015), after appropriate watering, the aboveground parts were cut at the root-stem junction and numbered separately, with stems, leaves and fruits bagged. The entire pot soil was poured onto clean plastic film and

dispersed, and roots were carefully excavated to maintain integrity and keep attached soil from falling off. Roots with attached soil were placed in sterile self-sealing bags, and the soil shaken off and collected for analysis of root-zone soil microorganisms and nematodes. Roots were carefully washed and processed using the same method as at seedling stage to measure root fresh mass. For aboveground parts, shoot fresh mass, total fruit number and fruit fresh mass were measured separately.

Root-Knot Nematode Disease Index Determination

From the roots obtained in section 1.2.2, 5 representative root systems were selected per treatment. The total number of primary lateral roots and the number of primary lateral roots with root-knots were counted for each root system. The infection rate (K) was defined as the ratio of lateral roots with root-knots to total lateral roots, calculated using equation (1). The disease level for each root system was then determined based on K values: Level 0, K = 0%; Level 1, K 1%-25%; Level 2, K 26%-50%; Level 3, K 51%-75%; Level 4, K 76%-100%. The disease index for each treatment was calculated using equation (2).

Defensive Enzyme Activity Measurement in Seedling Leaves

From the seedling tomato plants obtained in section 1.2.2, the largest leaves from the 3rd to 5th lateral branches from top to bottom were collected, with 30 leaves each for CK and treatment. Polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) and malondialdehyde (MDA) were determined according to Gao [21]. POD activity was measured using the guaiacol colorimetric method [22].

Microbial Flora Analysis

Root-zone soil sample collection: During root collection at maturity in section 1.2.2, soil shaken off from roots was collected in self-sealing bags. This soil, from the dense root distribution zone, represented the root-zone soil where microorganisms and nematodes were closely related to root biological and physiological characteristics, accurately reflecting the microbial and nematode status in root-zone soil at tomato harvest.

Root and root-knot collection: Representative functional root systems were cut from the biomass-measured roots obtained in section 1.2.2. Root-knots and non-knot roots were separated, and root-knots were excised. Root-knots and roots were surface-sterilized with 1 $g \cdot L^{1}$ mercuric chloride for 30 s and rinsed 5 times with sterile water. Surface-sterilized root-knots and roots were ground separately in sterile mortars, appropriately diluted with sterile water, and analyzed for microorganisms in root-knots and roots [23].

Soil and root microbial isolation and counting: The dilution plate method was used [24]. Bacteria were cultured on beef extract peptone agar, fungi on

PDA, and actinomycetes on Gause' s No. 1 agar. Dominant bacteria, fungi and actinomycetes were purified and preserved on slants for further identification.

Dominant Microorganism Identification

Dominant microorganisms refer to those with relatively large quantities and high proportions in the total colony count on plates. Typical colonies of dominant strains were selected and purified. rDNA-ITS sequence analysis was used for dominant fungi identification according to Pryce et al. [25], and 16S rRNA sequence analysis was used for dominant bacteria and actinomycetes identification according to Xu et al. [26-27].

Soil Nematode Separation, Counting and Identification

Total nematode determination in sick soil: 100 g of root-zone sick soil from continuous tomato cropping with root-knot nematodes was weighed, and soil nematodes were separated and collected using the Baermann funnel method for counting with a nematode counting slide.

Root-zone soil nematode counting and identification: 100 g of tomato root-zone soil obtained in section 1.2.2 was weighed, and soil nematodes were separated using the Baermann funnel method. After 48 h, nematodes were collected and fixed in 4 mL of 4% formalin solution. Nematode species were identified and counted using the methods of Seinhorst [28] and Sohlenius et al. [29].

Data Processing

Root-knot density was defined as the number of root-knots per 100 fibrous roots, calculated using equation (3). The sick soil effect (ΔCK, %) was defined as the difference in nematode numbers between healthy soil + sick soil treatment and healthy soil, calculated using equation (4). The proportion of a certain dominant bacterium in the total bacteria of a sample (P, %) was calculated using equation (5). The proportion calculation for dominant fungi was the same as for bacteria. Duncan' s method was used for significance analysis.

Results

Tomato Root-Knot Disease

As shown in Table 1 , at the seedling stage, root-knots appeared on fibrous roots in the sick soil treatment, with a root-knot density of 21 knots $\cdot$ (100 roots) ^{1}. The number of fibrous roots increased by 44.4% compared with healthy soil, and soil nematode abundance increased by 390.4% compared with healthy soil, with significant differences ($P < 0.05$). At harvest, the root-knot infection rate reached 62.7% and the disease index was 80.0% in the sick soil treatment. Figure 1 [Figure 1: see original paper] shows that CK tomato roots had no root-knots at seedling stage, while the sick soil treatment showed numerous root-knots; at

harvest, the sick soil treatment exhibited poor root growth with many root-knots.

Effects on Tomato Leaf Defensive Enzyme Activities and MDA Content

As shown in Table 2 , sick soil inoculation had no significant effect on shoot and root fresh weight at seedling stage, but significantly inhibited shoot and root growth at harvest. At harvest, shoot fresh weight, root fresh weight, fruit fresh weight and fruit number per plant in the sick soil treatment decreased by 50.2%, 33.1%, 68.2% and 59.7% respectively compared with the control, with all differences reaching significant levels ($P < 0.05$).

As shown in Table 4 , PPO activity in seedling leaves of the sick soil treatment decreased by 15.8% compared with the control, while POD activity increased by 24.0% ($P < 0.05$). However, PAL and MDA showed no significant differences compared with the control ($P > 0.05$).

As shown in Table 3 , the numbers of flowers on March 20 and March 30, and fruits on March 30 in the healthy soil + sick soil treatment decreased by 80.7%, 23.9% and 62.3% respectively compared with the control, with all differences reaching significant levels ($P < 0.05$).

Nematode Species and Abundance in Continuous Tomato Cropping Root-Zone Soil

As shown in Table 5 , there were 3 species of fungi-feeding nematodes in tomato root-zone soil, mainly *Aphelenchoides dactylocercus*, followed by *Filenchus discrepans*. There were 11 species of bacteria-feeding nematodes, with 6 high-frequency species, among which *Acrobeloides maximus* had the highest abundance. There were 4 species of plant-parasitic nematodes and 1 species of omnivorous nematode. In the tomato root-zone soil inoculated with sick soil, the total nematode abundance was 29,680 individuals $\cdot$ (100 g) ^{1}, essentially consistent with the inoculation amount of 26,700 individuals $\cdot$ (100 g) ^{1}. Bacteria-feeding and fungi-feeding nematodes accounted for 49.3% and 23.2% of total nematodes respectively, while plant-parasitic and omnivorous nematodes accounted for 27.4% and 0.7% respectively. In tomato root-zone soil grown in healthy soil, bacteria-feeding and fungi-feeding nematodes accounted for 44.8% and 44.0% of total nematodes respectively, with plant-parasitic nematodes accounting for 11.2%. Sick soil inoculation increased the proportions of bacteria-feeding and plant-parasitic nematodes by 10.0% and 144.6% respectively compared with healthy soil, while decreasing the proportion of fungi-feeding nematodes by 48.6%. These results indicate that sick soil inoculation affected nematode composition in tomato root-zone soil, with plant-parasitic nematodes showing the largest increase.

Microorganisms in Tomato Root-Zone Soil

A total of 5 dominant strains were isolated from tomato roots and root-zone soils, including 2 dominant bacterial strains and 3 dominant fungal strains. The dominant bacteria and fungi were classified and identified using 16S rRNA and rDNA-ITS sequence analysis techniques, with results shown in Table 6 .

As shown in Table 7 , in healthy soil and healthy soil + sick soil treatments, total bacterial abundances in tomato root-zone soil were 60.06×10 CFU · g ^{1} and 106.4×10 CFU · g ^{1} respectively, with the dominant bacterium being *Stenotrophomonas maltophilia* in both treatments. Total fungal abundances were 13.07×10 CFU · g ^{1} and 25.42×10 CFU · g ^{1} respectively. There were 3 dominant fungal species in root-zone soil. The abundances of *Penicillium chrysogenum* and *Fusarium chlamydosporum* showed no significant differences between healthy soil and healthy soil + sick soil ($P > 0.05$), but the abundance of *Actinomucor elegans* in healthy soil was approximately 3.2 times that in sick soil, with significant difference ($P < 0.05$). Total actinomycete abundances were 9.77×10 CFU · g ^{1} and 22.86×10 CFU · g ^{1} respectively, with a 134.0% increase in healthy soil + sick soil compared with healthy soil.

Bacteria Within Root Systems

As shown in Table 7, total bacterial abundances within tomato roots in healthy soil and healthy soil + sick soil treatments were 2.73×10 CFU · g ^{1} and 1.44×10 CFU · g ^{1} respectively, with the dominant bacterium being *Pseudomonas brassicacearum* in both treatments. This pathogenic bacterium accounted for 100% and 87.7% of total bacteria within tomato roots in healthy soil and healthy soil + sick soil treatments respectively. However, the abundance of *P. brassicacearum* in tomato roots in the sick soil treatment was 463 times that in healthy soil.

Discussion

This study demonstrated that adding sick soil from the root zone of tomato plants infected with root-knot nematodes under continuous cropping at a 1:3 mass ratio to healthy soil produced complex effects on the tomato root-zone soil micro-ecosystem, thereby inhibiting tomato growth. The complex response of the "tomato root-zone soil microorganisms-root endophytes-soil nematodes-tomato plant" micro-ecosystem to sick soil addition manifested as: after sick soil addition, total bacterial abundance and plant pathogenic bacterial abundance within tomato roots increased to 527 and 463 times those in healthy soil tomatoes respectively; total bacteria, fungi and actinomycetes in sick soil tomato root-zone soil increased to 1.8, 2.3 and 2.6 times those in healthy tomato root-zone soil respectively, which in turn caused bacteria-feeding, fungi-feeding and plant-parasitic nematodes to increase to 3.3, 1.6 and 7.3 times those in healthy root-zone soil respectively, with root-knot nematodes accounting for 95.6% of plant-parasitic nematodes; root-knot nematode infection significantly increased root-knot numbers on tomato roots, with increased infection rate and disease

index at harvest. Additionally, tomato responses to sick soil addition included significant decreases in shoot and root fresh weight, flower number, and fruit number and weight, delayed flowering, and reduced defensive enzyme activity and disease resistance capacity (Figure 2 [Figure 2: see original paper]).

Based on the species and quantities of 3 fungi-feeding nematodes, 11 bacteria-feeding nematodes, 5 plant-parasitic nematodes and rare omnivorous nematodes in harvest-stage tomato root-zone soil, sick soil inoculation significantly increased total nematode abundance compared with healthy soil, similar to the effect of increased continuous cropping years [14]. Plant-parasitic nematode species increased and their abundance increased substantially, with root-knot nematodes accounting for 95.6% of total plant-parasitic nematodes. The addition of continuous cropping sick soil transformed the soil nematode community originally dominated by bacteria-feeding and fungi-feeding nematodes into one dominated by plant-parasitic nematodes such as root-knot nematodes, consistent with previous findings [17]. Dominance of plant-parasitic nematodes severely damages host plants [18], and root-knot nematode infection of tomato roots exacerbates root-knot disease.

This study found that sick soil addition substantially increased microbial abundance in tomato root-zone soil and decreased the bacteria/fungi ratio, similar to results reported by Xu et al. [8] and Sun et al. [7]. However, Nayyar et al. [6] and Sun et al. [7] reported decreased total soil microbial abundance after continuous cropping. Previous studies have found that predation of bacteria by bacteria-feeding nematodes can increase bacterial biomass and activity [13]. In this study, both total bacteria-feeding nematodes and total bacteria increased significantly in the sick soil treatment, possibly due to adequate food resources for nematodes, though whether the increase in bacterial abundance represents positive feedback to nematode predation remains unclear. *Pseudomonas brassicacearum* is a plant pathogen [30] that can cause tomato bacterial pith necrosis [35]. Sick soil inoculation dramatically increased *P. brassicacearum* abundance within tomato roots, making it the dominant bacterium and rendering tomato roots more susceptible to harmful bacterial infection. *Stenotrophomonas maltophilia* can parasitize nematode surfaces, inhibit nematode growth, reduce nematode pathogenicity and control plant nematode diseases [31], while *Penicillium chrysogenum* helps tomato plants resist *Meloidogyne javanica* infection [32]. However, sick soil had no significant effect on the abundances of these two beneficial microorganisms and *Fusarium chlamydosporum*, but reduced the abundance of the dominant fungus *Actinomucor elegans* in tomato root-zone soil.

This study also found certain effects of sick soil inoculation on tomato biochemical characteristics: after sick soil addition, tomato leaf PPO activity decreased significantly, POD activity increased significantly, and MDA content increased, indicating that continuous cropping sick soil reduced tomato defensive enzyme activity and stress resistance capacity. Similar results were reported by Kang et al. [36]. PPO is widely present in plants and can promote oxidation of phenolic substances to form disease-resistant compounds such as caffeic acid and chloro-

genic acid, and can oxidize phloroglucinol to form more toxic compounds that kill pathogens [37]. Reduced PPO activity may render tomatoes more susceptible to root-knot nematode and pathogen infection. POD has dual protective and damaging effects: it can scavenge H O under stress or early senescence as a member of the reactive oxygen species protective enzyme system, but can also participate in reactive oxygen species production and chlorophyll degradation under stress or late senescence, inducing membrane lipid peroxidation and showing damaging effects, even serving as a senescence indicator [38]. MDA is produced from membrane lipid peroxidation in tissues or organs, and its content is closely related to plant senescence and stress damage [36]. The negative biochemical effects of sick soil on tomatoes led to significant growth inhibition, manifested as significantly reduced shoot and root fresh weight at harvest, delayed flowering with reduced flower number, and significantly decreased fruit number and weight.

Inoculating healthy soil with sick soil from the root zone of tomato plants infected with root-knot nematodes under continuous cropping produced complex effects on the tomato root-zone soil micro-ecosystem, inhibiting tomato growth and exacerbating root-knot nematode disease through effects on root-zone soil microorganisms, root endophyte species and abundances, soil nematode species and abundances, and tomato biochemical metabolism. The root-knot nematodes carried in sick soil completely altered the original nematode community structure in healthy soil, making plant-parasitic nematodes dominant. Sick soil inoculation made the plant pathogenic bacterium *Pseudomonas brassicacearum* the dominant bacterium within tomato roots. Mass propagation of harmful nematodes and microorganisms reduced tomato defensive enzyme activity and stress resistance, exacerbating root-knot nematode disease and severely inhibiting tomato growth and fruiting. The negative effects of continuous cropping sick soil on tomato growth were achieved through overall impacts on the "tomato root-zone soil microorganisms-root endophytes-soil nematodes-tomato plant" micro-ecosystem and interactions among various components within the system. Allelopathic inhibition caused by sick soil should also be considered in future research.

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Note: Figure translations are in progress. See original paper for figures.