RESEARCH ARTICLE

The impact of pathogenic nematodes on the growth of medicinal plants and their symbiotic microbial communities

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Received: February 2, 2026; **accepted:** July 2, 2026.

The cultivation of medicinal plants is increasingly affected by pathogenic nematodes, but their multidimensional impact mechanisms on host growth, medicinal components, and rhizosphere microbial communities are still unclear. This study systematically investigated the multidimensional effects of *Meloidogyne incognita* infection on the growth, medicinal quality, and rhizosphere microbial community of *Salvia miltiorrhiza* and *Astragalus membranaceus* by using different inoculation gradients of *Meloidogyne incognita* and through biomass measurement, physiological and biochemical analysis, high-performance liquid chromatography, and high-throughput sequencing methods. The results showed that, under high inoculation treatment, the aboveground and root dry weights of *Salvia miltiorrhiza* decreased to 4.68 g/plant and 2.08 g/plant, while those of *Astragalus membranaceus* decreased to 7.48 g/plant and 4.18 g/plant, respectively. Meanwhile, malondialdehyde content in leaves increased to 59.36 nmol/g FW and 48.72 nmol/g FW, respectively. Key medicinal compounds, tanshinone IIA and salvianolic acid B, decreased to 0.45 mg/g and 2.41 mg/g in *Salvia miltiorrhiza*, while astragaloside and calycosin glucoside decreased to 0.06 mg/g and 0.04 mg/g in *Astragalus membranaceus*. Microbial community analysis indicated that nematode stress significantly increased the abundance of pathogenic bacteria in the *Salvia miltiorrhiza* rhizosphere, while beneficial bacteria declined sharply. In contrast, *Astragalus membranaceus* showed notable stability, and its beneficial microbial community maintained high abundance. This study revealed the mechanisms by which nematodes affected medicinal plant growth and microbial community structure and provided key theoretical support for green control strategies against nematode diseases in medicinal plants.

Keywords: medicinal plants; plant growth; symbiotic microbial community structure; pathogenic nematodes; medicinal compounds.

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Introduction

Medicinal plants are among the most economically significant crop categories, and their quality and yield are critical to the sustainable development of the traditional Chinese medicine (TCM) industry. [1, 2]. However, because of the current expansion of planting areas as well as the growing hindrances caused by continuous cropping, soilborne diseases can be stated to be a vital bottleneck that restricts the safe cultivation of medicinal plants [3, 4]. Among these soilborne diseases, pathogenic nematodes, particularly *Meloidogyne* and *Heterodera* species, exert severe impacts on high-value medicinal plants such as Panax

ginseng and *Astragalus membranaceus* due to their cryptic nature and widespread distribution. [5, 6].

In recent years, studies on plant parasitic nematodes have achieved significant accomplishments regarding the pathogenic mechanism and assessment of crop resistance [7, 8]. Zahoor *et al*. used molecular docking to discover that nematode pectin lyases formed two main evolutionary branches, revealing the core mechanism by which these enzymes degraded pectin and infect plant roots [9]. Mergaert *et al*. discovered that cyst nematodes secreted chitinase that specifically degraded key symbiotic signaling molecules released by rhizobia and arbuscular mycorrhizal fungi, thereby disrupting the mutualistic relationship between crops and beneficial microbes [10]. Pan *et al*. conducted host adaptation trials and showed that different medicinal plants exhibited significant variation in nematode sensitivity, providing important guidance for controlling nematode diseases in medicinal plants [11]. However, existing research appears to focus primarily on the effects of pathogenic nematodes on crop growth or interaction models. The mechanisms by which nematode infection affects the growth of medicinal plants and symbiotic microbial communities remain under-examined.

To fill these research gaps, this study selected two common medicinal plants, *Salvia miltiorrhiza* and *Astragalus membranaceus*, and conducted research under different inoculation gradients of *Meloidogyne incognita*. Combining physiological assays, high-performance liquid chromatography (HPLC) analysis, and high-throughput sequencing, the effects of nematode infection on plant growth, synthesis of key medicinal components, and the dynamic response of the rhizosphere microbial community were investigated. This study systematically elucidated the multidimensional effects of *Meloidogyne incognita* infection on the growth and rhizosphere microbial communities of *Salvia miltiorrhiza* and *Astragalus membranaceus*, integrated changes in medicinal compounds into the evaluation framework, establishing a new paradigm for understanding nematode-host interactions in medicinal plants. The results of this study provided certain scientific basis for the green control of nematode diseases in medicinal plants and the improvement of medicinal material quality, thereby promoting the sustainable development of the traditional Chinese medicine industry.

Materials and methods

Experimental materials

Salvia miltiorrhiza (Shandong Yellow River Delta Chinese Herbal Medicine Seedling Co., Ltd. (Dongying, Shandong, China) and *Astragalus membranaceus* (Ankou City Chinese Herbal Medicine Seedling Company, Ankou, Hebei, China) were employed as study materials of this research. Plant specimens were taxonomically identified by experts at Shandong Agricultural University (Taian, Shandong, China). *Meloidogyne incognita* was collected from the infected root zone soil of the medicinal plant cultivation base in Mudan District, Heze, Shandong, China and was purified into single oocysts, which was then propagated and preserved on susceptible tomato plants, “Zhongshu No. 4” variety (Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China).

Experimental groups and treatments

A completely randomized design with ten treatments was employed in this study. *Salvia miltiorrhiza* treated with no inoculation was defined as a control group (CK), while treatments of 500, 1,000, 1,500, and 2,000 second-stage juveniles (J2s) of *Meloidogyne incognita* were designed as T1 - T4 groups, respectively. *Astragalus membranaceus* was also set with CK and T1 - T4 groups with the same inoculation amount as *Salvia miltiorrhiza*. The growth substrate consisted of loamy sands (Shandong Shouguang Woote Qiuzhi Co., Ltd., Shouguang, Shandong, China) with peat soil (Jilin Shennong Peat Technology Co., Ltd., Changchun, Jilin,

China) in a volume proportion of 2:1. After mixing, the substrate was sterilized at 121°C for 2 h using an autoclave to eliminate pathogens, nematode eggs, and weed seeds. Subsequently, the mixture was spread on a clean plastic mat to cool to room temperature. Healthy *Salvia miltiorrhiza* tissue-cultured seedlings of uniform height were selected. All the flowerpots were moved to the greenhouse in College of Food and Health Engineering, Zhengzhou University of Technology, Zhengzhou, Henan, China to adapt to the environment. After an acclimatization period of 1 week, when growth became normal, inoculation treatments were conducted. *Meloidogyne incognita* was maintained on susceptible tomato plants. J2s were isolated from infected roots using a Baermann funnel. To ensure accuracy and reproducibility, a stock calibration and dilution method was employed for inoculation. A high-concentration nematode suspension was first prepared, and its concentration was precisely measured with a counting chamber. The suspension was then diluted to fixed final volumes according to treatment levels to obtain 500 J2s/5 mL, 1,000 J2s/5 mL, 1,500 J2s/5 mL, and 2,000 J2s/5 mL treatments. The CK received an equal volume of sterilized distilled water. Following the acclimation period, each seedling was inoculated with the corresponding nematode suspension around the root zone, and CK plants received sterilized water to ensure thorough root infiltration. Leaf samples were collected before inoculation and during a series of post-inoculation sampling points spanning 30 to 150 days to evaluate dynamic changes in physiological indicators including malondialdehyde and proline under nematode stress. Upon completion of the growth cycle, *Salvia miltiorrhiza* and *Astragalus membranaceus* plants were harvested. Root and aboveground dry weights were measured to evaluate biomass accumulation. The key root medicinal compounds were quantified. For microbial community analysis, rhizosphere soil samples from each treatment were collected and analyzed by high-throughput sequencing and bioinformatics to systematically reveal microbial community structure and functional succession.

Measurement of growth and physiological indicators

The aboveground plant parts and whole roots were harvested. Biomass accumulation was determined by measuring dry weight. The physiological indices used to reveal plant stress responses were malondialdehyde and proline contents. Fresh leaf samples were mixed with a thiobarbituric acid-trichloroacetic acid solution, incubated in a boiling water bath for 30 min, and centrifuged at 10,000 × g for 10 min at 4°C. The absorbance of the supernatant was measured at wavelengths of 532 nm, 600 nm, and 450 nm using Evolution 201/220 ultraviolet-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Based on the measured absorbance values, the malondialdehyde content was calculated as $C = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. This content was then converted to the content per gram fresh weight (nmol/g FW) as $C \times \frac{V}{W}$, where C was the malondialdehyde content (μmol/L), V was the total volume of the extract (mL), and W was the fresh weight of the sample (g). A_{532}, A_{600}, and A_{450} were the absorbance values of the supernatant at 532 nm, 600 nm, and 450 nm wavelengths, respectively. The proline content was determined by the acidic ninhydrin method. Briefly, 0.5 g of fresh leaf tissue was homogenized and extracted in 5 mL of 3% p-dimethylaminobenzhydrazide solution. The mixture was then boiled in water bath for 10 minutes, cooled, and centrifuged to obtain the supernatant. Subsequently, 2 mL of the supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of acidic ninhydrin reagent prepared by dissolving 2.5 g of ninhydrin in 60 mL of glacial acetic acid and 40 mL of 6 mol/L phosphoric acid, and boiling for 30 min. After cooling, 4 mL of toluene was added to extract the red product following by measuring the absorbance at 520 nm using Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). The content was calculated based on the proline standard curve with the unit as μg/g FW [12].

Analysis of medicinal ingredients and microbial communities

To evaluate the effects of *Meloidogyne incognita* on medicinal quality of *Salvia miltiorrhiza*, high-performance liquid chromatography (HPLC) was employed to determine root tanshinone IIA and salvianolic acid B using a ZORBAX SB-C18 chromatographic column (4.6 mm × 250 mm, 5 μm) (Agilent Technologies, Santa Clara, California, USA). The tanshinone IIA was determined by gradient elution with methanol-water at the detection wavelength of 270 nm, while salvianolic acid B was measured using a C18 column with acetonitrile-methanol-formic acid solution gradient elution at 286 nm. For *Astragalus membranaceus*, key compounds astragaloside and calycosin glucoside were measured by using HPLC. Astragaloside was detected with an evaporative light scattering detector using a C18 column and acetonitrile-water isocratic elusion. Calycosin glucoside was detected with a C18 column using acetonitrile-0.2% formic acid water gradient elusion at 260 nm. To reveal the effects of *Meloidogyne incognita* on symbiotic microbial communities, the total DNA of the rhizosphere soil was extracted using the DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) following manufacturer's instructions. The polymerase chain reaction (PCR) amplification primers for the 16S rRNA gene V3-V4 region of bacteria were 341F (5'-CCT ACG GGN GGC WGC AG-3') and 805R (5'-GAC TAC HVG GGT ATC TAA TCC-3'). The amplification primers for the ITS region of fungi were ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS2R (5'-GCT GCG TTC TTC ATC GAT GC-3'). PCR amplification was carried out using the KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland) reagent kit following manufacturer's instructions. The amplified PCR products were purified and then sequenced in both ends on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Community α-diversity was evaluated by Chao1 richness index and Shannon diversity index with Chao1 index was shown below [13].

$$S_{chao} = S_{obs} + \frac{n_1(n_1-1)}{2(n_2+1)} \quad (1)$$

where S_{obs} was the number of observed species. n_1 was species occurring once. n_2 was species occurring twice. Shannon diversity index was shown below [14].

$$H = -\sum\left[(p_i)\times In(p_i)\right] \quad (2)$$

where p_i was the proportion of species i in the total sample. Community β-diversity was assessed by principal coordinate analysis (PCoA) based on Bray-Curtis distance to reveal differences and grouping patterns among samples and was calculated as follows.

$$Bc_{ij} = 1-\left(2*c_{ij}\right)/\left(S_i+S_j\right) \quad (3)$$

Where c_{ij} was the minimum sum of individuals of shared species. S_i was the total number of individuals. S_j was the total number of individuals.

Statistical analysis

SPSS 26.0 (IBM, Armonk, NY, USA) was employed for statistical analysis of this research. Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc test. Permutation multivariate analysis of variance (PERMANOVA) for 999 permutations based on Bray-Curtis dissimilarity distance was used to evaluate the effects of plant species, nematode inoculation dose, and their interaction on the structure of the rhizosphere microbial community. *P* value less than 0.05 was defined as statistically significant difference.

Results

Effects of *Meloidogyne incognita* on growth indices of medicinal plants

(1) Biomass measurement results

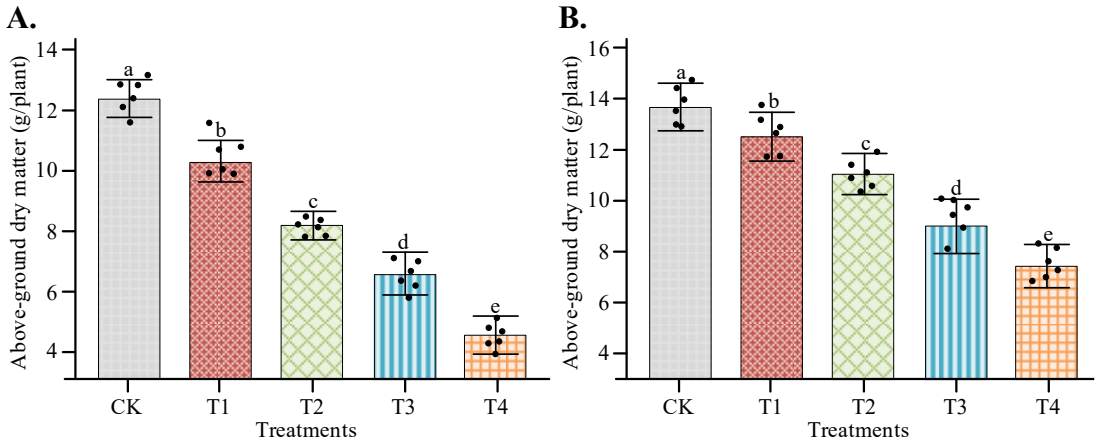


Figure 1. Aboveground dry weight of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) under different inoculation doses. Different lowercase letters (a - e) indicated significant differences ($P < 0.05$).

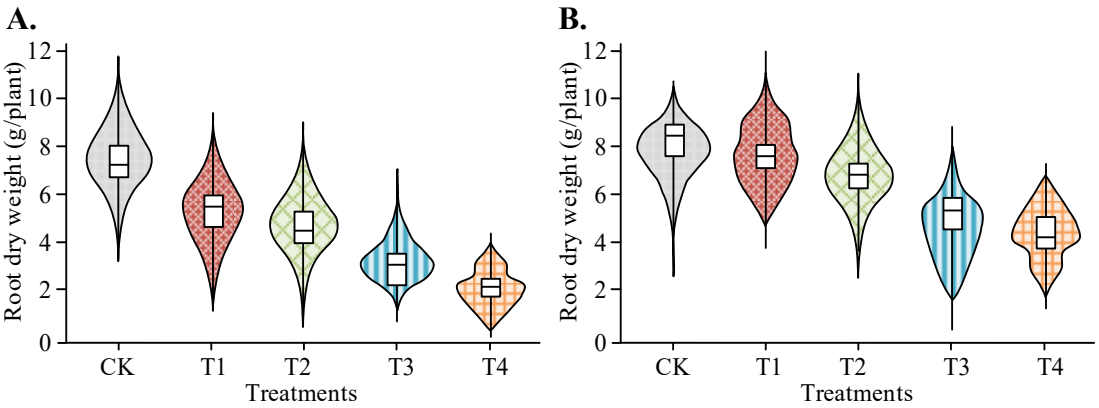
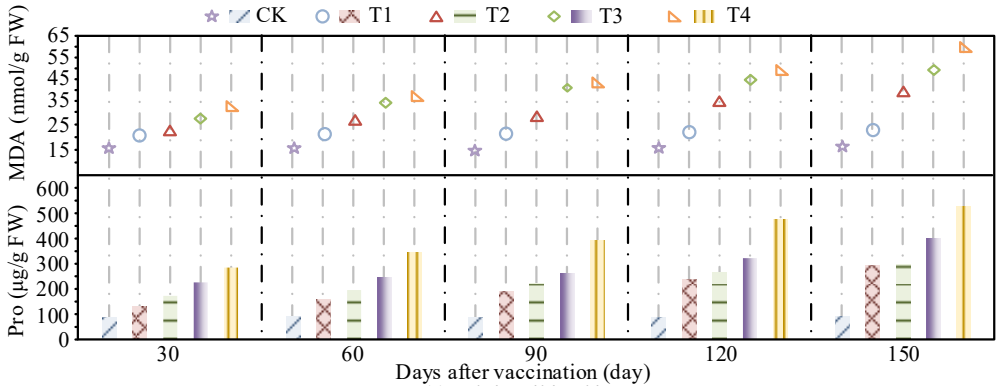


Figure 2. Root dry weight of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) under different inoculation doses.

To clarify the effects of *Meloidogyne incognita* infection on the growth of *Salvia miltiorrhiza* and *Astragalus membranaceus*, this study systematically analyzed the aboveground biomass accumulation of *Salvia miltiorrhiza* and *Astragalus membranaceus* under different stress intensities. The results demonstrated that the dry weight of the *Salvia miltiorrhiza* CK group was 12.36 g/plant. With the continuous increase of inoculation dose, the aboveground dry weight decreased successively with the dry weight under T4 treatment being only 4.68 g/plant (Figure 1A). The aboveground dry weight of *Astragalus membranaceus* showed that the dry weight of the CK group was 13.85 g/plant. The dry weights of each inoculation group (T1 - T4) were 12.56, 11.02, 9.25, and 7.48 g/plant, respectively (Figure 1B). These results demonstrated that *Meloidogyne incognita* infection exerted a significant inhibitory effect on the accumulation of aboveground dry weight in both species. The root dry weight of *Salvia miltiorrhiza* control group was 7.25 g/plant, while the dry weights of each *Meloidogyne incognita* inoculation treatment group (T1 - T4) were 5.82, 4.45, 3.18, and 2.08 g/plant, respectively, among which the root dry weight of the high dose (T4) was only 28.7% of the control group (Figure 2A). The root dry weight of *Astragalus membranaceus* also showed a significant decreasing trend with dose, but the root dry weight of each treatment group was significantly higher than that of *Salvia*

A.



B.

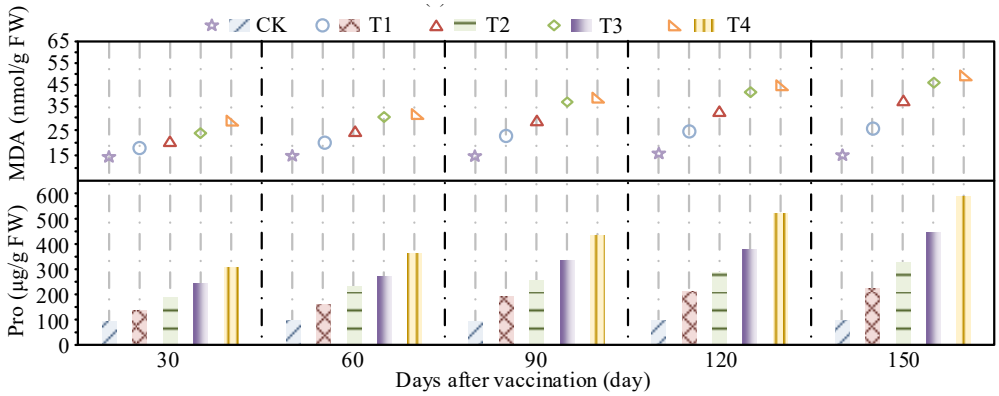


Figure 3. Changes in malondialdehyde and proline contents in leaves of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) over time.

miltiorrhiza group under the same treatment (Figure 2B). These results indicated that *Meloidogyne incognita* infection significantly inhibited the growth of the underground plant organs.

(2) Determination of malondialdehyde and proline content in leaves

As stress resistance indices, the malondialdehyde content in *Salvia miltiorrhiza* leaves showed a gradual increasing trend over time. After 150 days of inoculation, the malondialdehyde content in *Salvia miltiorrhiza* control group was 16.33 nmol/g FW, while the malondialdehyde contents in T1 group increased to 28.20 nmol/g FW and T4 group reached 59.36 nmol/g FW. The proline content in T4 group reached as high as 525.74 μg/g FW, significantly higher than other treatments after 150 days of inoculation (Figure 3A). Both malondialdehyde and proline contents in *Astragalus membranaceus* leaves showed a gradual increasing trend (Figure 3B). The results showed that infection by *Meloidogyne incognita* caused a significant rise in malondialdehyde and proline levels in the leaves of *Salvia miltiorrhiza* and *Astragalus membranaceus*, and the stress degree was positively correlated with inoculation dose.

(3) Determination of medicinal ingredient content

With the increase of *Meloidogyne incognita* dose, the content of tanshinone IIA, a medicinal component of *Salvia miltiorrhiza*, showed a gradual decreasing trend. Under high dose treatment (T4), the tanshinone IIA content decreased to 0.448 - 0.466 mg/g (Figure 4a). The salvianolic acid B content also decreased with the increase of *Meloidogyne incognita* inoculation dose. Under high dose treatment (T4), the

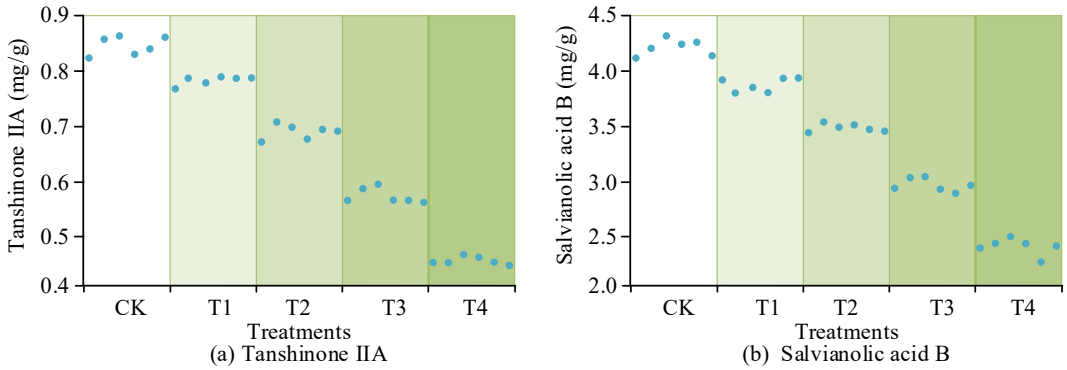


Figure 4. The contents of tanshinone IIA (**a**) and salvianolic acid B (**b**) in *Salvia miltiorrhiza*.

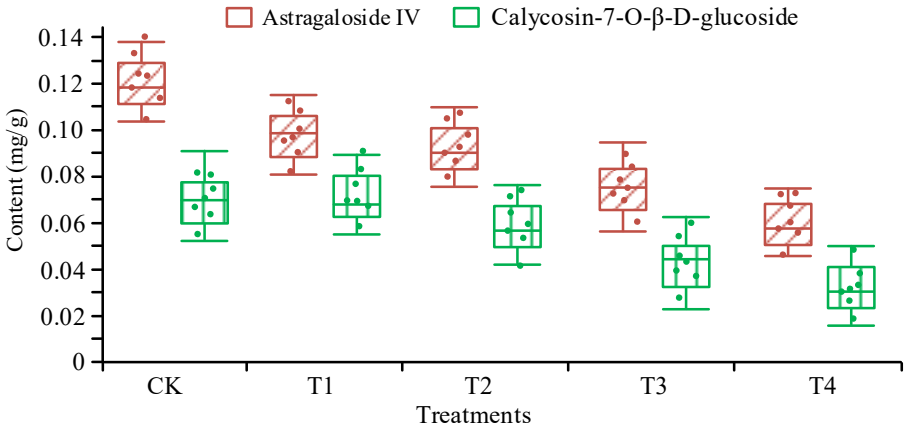


Figure 5. The contents of astragaloside IV and calycosin-7-glucoside in *Astragalus membranaceus*.

salvianolic acid B content decreased to 2.225 - 2.506 mg/g (Figure 4b). The results indicated that the infection pressure of *Meloidogyne incognita* not only inhibited the growth of *Salvia miltiorrhiza* plants but also interfered with the accumulation of its medicinal components. The astragaloside IV and calycosin-7-glucoside contents of *Astragalus membranaceus* in the healthy control group were 0.117 mg/g and 0.070 mg/g, respectively. Under the medium dose (T2) treatment, the contents of both components further decreased to 0.090 mg/g and 0.056 mg/g, respectively. Under high dose (T4) stress, the astragaloside IV content had decreased to 0.057 mg/g, while calycosin-7-glucoside decreased to 0.030 mg/g (Figure 5), which indicated that these two components had a certain degree of sensitivity to *Meloidogyne incognita* stress. The results indicated that *Meloidogyne incognita* infection not only inhibited the biomass formation of *Astragalus membranaceus* but also directly interfered with the generation of two key medicinal components of these traditional Chinese medicines, thereby affecting the quality and value of the medicinal materials.

Effects of *Meloidogyne incognita* on symbiotic microbial community structure

(1) Analysis of microbial community diversity

The Chao1 richness index of *Salvia miltiorrhiza* control group remained stable throughout the experimental period, indicating that its microbial community richness was not affected. However, with the increase of inoculation dose, the richness of each *Salvia miltiorrhiza* group showed a decreasing trend. After 120 days of inoculation, the Chao1 richness index of *Astragalus membranaceus* T4 group decreased to 310.08

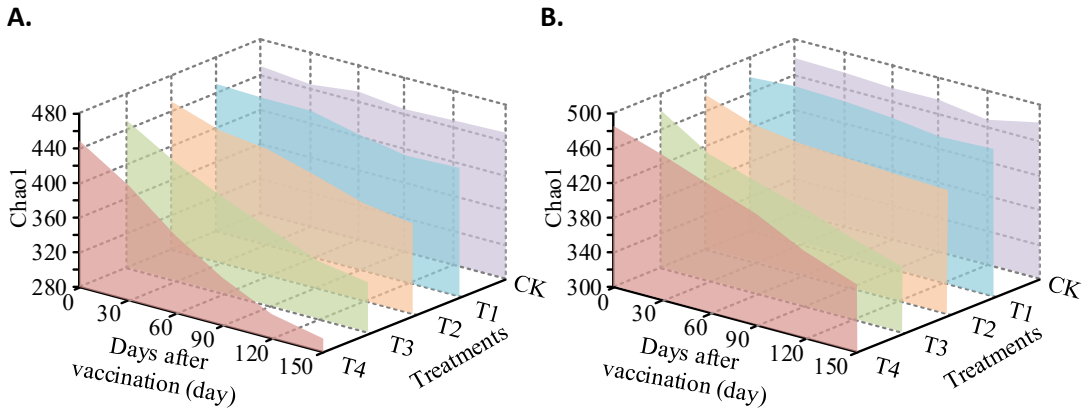


Figure 6. Comparison of Chao1 richness indices of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) under *Meloidogyne incognita* infection.

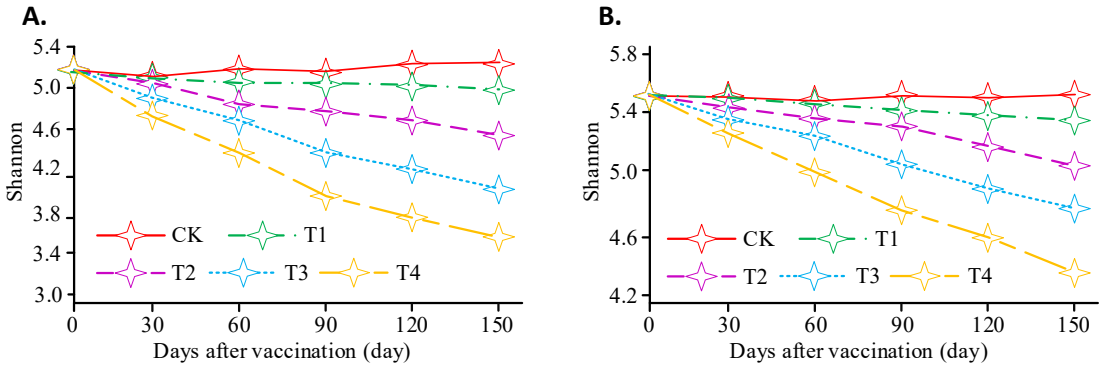


Figure 7. Comparison of Shannon diversity indices of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) under *Meloidogyne incognita* infection.

(Figure 6A). The decreasing trend of the Chao1 richness index in *Astragalus membranaceus* groups was relatively slow. On day 150, the Chao1 richness index value for the control group was 460.00. During the same period, the value for the group infected with a low dose of *Meloidogyne incognita* (T1) dropped to 450.52, whereas under high dose stress (T4), the index value was only 358.64 (Figure 6B). The results indicated that *Meloidogyne incognita* infection had a significant negative effect on the species richness of rhizosphere microorganisms in medicinal plants, which correlated closely with the deterioration of the medicinal plants' physiological status. The Shannon diversity index of *Salvia miltiorrhiza* control group remained stable between 5.20 - 5.25 throughout the experimental period. All inoculation treatment groups showed different degrees of decline starting from day 30 with the high-dose (T4) group showing the most significant decline after 150 days to 3.61 (Figure 7A). The Shannon diversity index of the *Astragalus membranaceus* control group remained between 5.49 - 5.51. Under the stress of *Meloidogyne incognita* infection, T1 group decreased to 5.35 on 150 day, while T4 group decreased to 4.28 (Figure 7B). The PCoA analysis results demonstrated that all *Salvia miltiorrhiza* samples were concentrated in the negative interval of PCo1, forming an independent left cluster. The PCo1 axis explained 38.5% of community variation, and the PCo2 axis explained 24.2% of variation. Among them, the control group was located at the positive high position of the PCo2 axis with values concentrated in the interval of 0.22 - 0.28. As

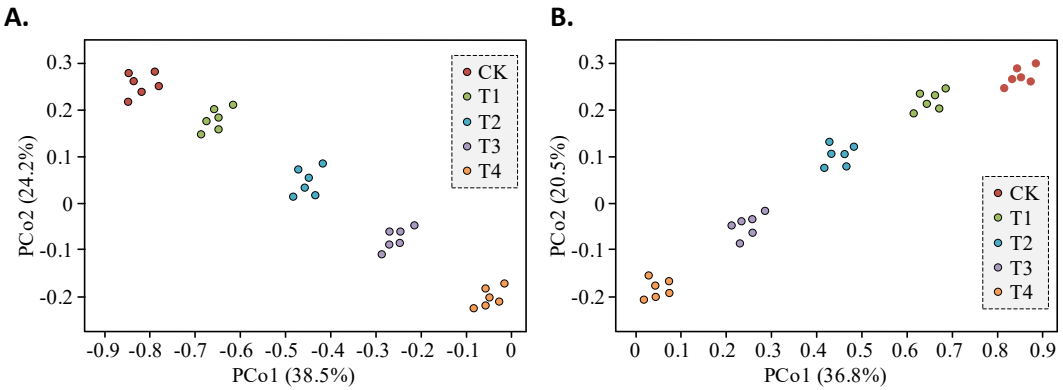


Figure 8. PCoA analysis results based on Bray-Curtis dissimilarity distance of *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**).

Table 1. Results of permutational multivariate analysis of variance.

Source of variation	Degrees of freedom (df)	Sum of squares (SS)	Mean squares (MS)	F value	R^2	*P* value
Plant species	1	5.82	5.82	85.36	0.452	0.001
Nematode inoculation dose	4	2.87	0.718	10.52	0.223	0.001
Plant species × dose	4	0.46	0.115	1.69	0.036	0.024
Residuals	40	2.73	0.068	-	0.289	-
Total	49	12.88	-	-	1.000	-

Meloidogyne incognita dose increased from T1 to T4 treatments, the sample points shifted successively along the negative direction of the PCo2 axis. The high-dose group was located at the most negative end of the axis with values in the interval of -0.17 - 0.23 (Figure 8A). All *Astragalus membranaceus* sample points were concentrated in the positive interval of 0.02 - 0.88 on the PCo1 axis. The PCo1 axis explained 36.8%, and the PCo2 axis explained 20.5% of variation. Compared with *Salvia miltiorrhiza*, the distribution of points of each dose group of *Astragalus membranaceus* on the PCo2 axis was more compact, and the coordinate values of the same treatment group were generally higher than those of the corresponding *Salvia miltiorrhiza* group, which suggested that the rhizosphere microbial community of *Astragalus membranaceus* possessed greater resilience when subjected to *Meloidogyne incognita* stress (Figure 8B). Based on PCoA ordination analysis, this study further applied permutational multivariate analysis of variance to reveal the relative contributions and interactive effects of *Meloidogyne incognita* infection and host plant species on their symbiotic microbial community structure. The results showed that the effects of different factors on rhizosphere microbial community structure demonstrated significant differences. Medicinal plant species was the most important factor explaining community variation, contributing about 45.2% of variation alone (F = 85.36, *P* = 0.001), indicating that *Salvia miltiorrhiza* and *Astragalus membranaceus* formed distinctly different rhizosphere microbial communities. *Meloidogyne incognita* infection dose also had an extremely significant effect, explaining about 22.3% of variation in community variation (F = 10.52, *P* = 0.001), confirming that *Meloidogyne incognita* infection was a key stress factor driving the changes of microbial community structure. Besides, there was a significant interaction between plant species and *Meloidogyne incognita* infection dose (F = 1.69, *P* = 0.024, R^2 = 3.6%), which suggested that the effect pattern of *Meloidogyne incognita* on microbial communities changed depending on host plants (Table 1).

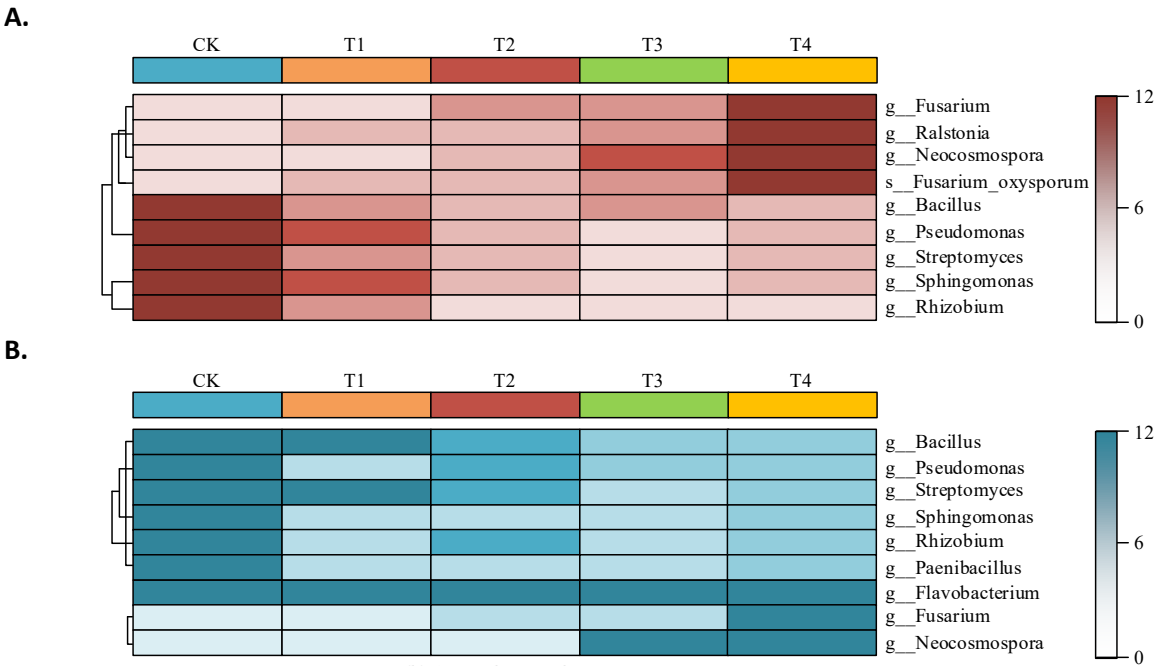


Figure 9. Differential abundance heatmap of marker species in *Salvia miltiorrhiza* (**A**) and *Astragalus membranaceus* (**B**) rhizosphere soils.

(2) Species analysis of microbial community differences

The differential abundance heatmap of marker species in *Salvia miltiorrhiza* rhizosphere soil showed that beneficial functional groups such as *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Rhizobium* showed significantly high abundance in the control group. With the increase of *Meloidogyne incognita* infection doses, the relative abundance of these groups showed a systematically decreasing trend. The abundance of potential pathogenic groups such as *Fusarium*, *Ralstonia*, and *Fusarium oxysporum* significantly increased with the increase of stress intensity, and their relative abundance was significantly elevated in the high-dose treatment group (T4). The results indicated that *Meloidogyne incognita* infection caused the rhizosphere microbial community of *Salvia miltiorrhiza* to gradually shift from a healthy state dominated by beneficial bacteria to an imbalanced state dominated by pathogenic bacteria (Figure 9A). The abundance data of marker species in the rhizosphere microbial community of *Astragalus membranaceus* showed a distinctly different response pattern from *Salvia miltiorrhiza*. Beneficial functional bacterial groups that consisted of *Bacillus*, *Pseudomonas*, and *Streptomyces* showed a remarkably high abundance in the healthy *Astragalus membranaceus* root environment. Even in the high-dose T4 group, the abundance still sustained over half of that in the healthy control, which showed a much smaller reduction compared to that of *Salvia miltiorrhiza*. *Fusarium* and *Nectria* started to enrich at a relatively high level of *Meloidogyne incognita*, and their values of absolute abundance were much lower than that of *Salvia miltiorrhiza* group under the same treatment (Figure 9B). This analytical outcome suggested that the root microbial community of *Astragalus membranaceus* had higher stability and resilience than that of *Salvia miltiorrhiza*.

Discussion

This study used the medicinal plants *Salvia miltiorrhiza* and *Astragalus membranaceus* to establish different gradients of *Meloidogyne incognita* inoculation treatments and explored the comprehensive mechanisms of pathogenic

nematode effects on the growth of medicinal plant hosts and the structure of rhizosphere symbiotic microbial communities. The results showed that *Meloidogyne incognita* infection significantly inhibited the growth of both plants with *Salvia miltiorrhiza* suffering the most severe damage. The high dose *Meloidogyne incognita* treatment caused the aboveground and root dry weights of *Salvia miltiorrhiza* decreased, respectively, while *Astragalus membranaceus* showed relatively strong tolerance. This variation might be attributed to the distinct response patterns of the respective rhizosphere microbial communities. Nematode stress in *Salvia miltiorrhiza* rhizosphere led to the decline of beneficial bacterial groups, while the core beneficial bacterial groups in *Astragalus membranaceus* rhizosphere maintained relatively high abundance and stability. These findings were consistent with the results of Darling *et al*. who demonstrated that nematode infection led to a significant reduction in host biomass and systemic nutritional deficiencies [15]. At the physiological level, the malondialdehyde content in *Salvia miltiorrhiza* T4 group leaves increased after 150 days of high dose infection, which indicated that its leaf cell membrane system suffered severe oxidative damage. Meanwhile, its proline content reached high level, revealing a strong osmotic regulation response. This intense physiological stress response was closely related to nematode infection directly destroying root function, interfering with water and nutrient absorption, and subsequently triggering reactive oxygen species metabolism imbalance in leaves. Chihani-Hammas *et al*. reported that nematode infection led to increased protein and malondialdehyde contents in plant roots and caused nutritional imbalance in leaves [16]. Furthermore, the stress of *Meloidogyne incognita* greatly decreased the important medicinal compound content of *Salvia miltiorrhiza* and *Astragalus membranaceus*, which was primarily due to root system impairment caused by nematode infestation that interfered with secondary metabolite metabolism and photosynthetic product distribution. Wieczorek *et al*. indicated that the balance of resource exchange between plants and mycorrhizal fungi had been disrupted by the infestation of the pathogenic nematodes, which provided a solid theoretical basis for the core-activity of the pathogenic nematode impairment to plants [17]. Permutational multivariate analysis of variance further confirmed that, in disease systems, plant species were the key factor determining rhizosphere microbial communities, while nematode infection only exerted external pressure, thereby reshaping microbial communities. López *et al*. found that soil fungal community structure was simultaneously affected by both geographic region and nematode abundance and emphasized that geographic region and nematode abundance jointly shaped fungal communities [18]. However, the present study measured the main effects and interaction effects of host plants and nematode stress in a controlled setting, emphasizing the role of host in determining the rhizosphere microbial composition in disease model systems. The abundance of key functional species in the rhizosphere of *Salvia miltiorrhiza* in the presence of increased nematode doses indicated an acute rise in potential pathogenic bacteria and a systematic reduction in functional beneficial bacterial populations. The core functional beneficial bacteria populations in the rhizosphere of *Astragalus membranaceus* displayed strong tolerance, and the pathogenic bacteria enrichment level was significantly lower than that of *Salvia miltiorrhiza*, which was due to the differences in the inherent composition of their own root exudates and intensity of immune responses in the two plant species. The results of Gao *et al*. in yam were partly in agreement with the current findings. The positive effect of disease-induced rhizosphere microbial networks due to improved positive interactions and functional beneficial bacterial groups might contribute positively to the rearrangement of beneficial bacteria defense [19]. Zhao *et al*. explored the disease suppression mechanism of rhizosphere microbial communities against root-knot nematodes through metagenomic and metabolomic analysis and found that beneficial

bacteria such as *Bacillus amyloliquefaciens* had higher abundance in low infection soil with more complex microbial networks. They also found that acetophenone had strong repellent and nematicidal activity [20]. This research confirmed that the response strategies of different crop-microbe interaction systems to nematode stress at the functional level might differ. Although this study revealed the correlations among nematodes, microorganisms, and plants, it had not yet verified the direct role of specific microbial functions in disease resistance through causal experiments. Future research should employ multi-omics technologies to further elucidate the causal relationships between key microorganisms and host resistance, providing robust strategies for the sustainable control of nematode diseases.

Acknowledgements

This research was supported by the National First-class Undergraduate Curriculum Program (Document No. Jiaogao Han [2025] No.9) and the Construct Program of the Key Discipline in Zhengzhou University of Technology (0832).

References

1. Tiwari DC, Bahukhandi A, Dhyani V, Parihar N, Pandey V, Bhatt ID. 2025. Comparative assessment of morphological, physiological attributes of two high value medicinal herbs of Himalaya under different growth conditions. Vegetos. 38(4):1435-1444.
2. Chang KJ, Kim YB, Koo HJ, Baek HJ, Hong EG, Lee SB, *et al*. 2023. Effects on ginseng growth and ginsenoside content in ICT-based process cultivation and conventional cultivation. J Pract Agric Fish Res. 25(2):12-19.
3. Wang X, Xiang S, Wang J, Luo X, Du C, Sun X. 2024. Long-acting sustained-release hydrogel for soil-borne pathogen control in Chinese herbal medicine. J Polym Environ. 32(12):6311-6319.
4. Deng W, Gong J, Peng W, Luan W, Liu Y, Huang H, *et al*. 2024. Alleviating soil acidification to suppress Panax notoginseng soil-borne disease by modifying soil properties and the microbiome. Plant Soil. 502(1):653-669.
5. Zheng L, Ma H, Li Y, Ha B, Wu H, Long W, *et al*. 2025. Antagonistic effect of symbiotic bacteria from entomopathogenic nematodes and *Bacillus* spp. against Panax ginseng root rot pathogens. Can J Plant Pathol. 47(2):143-154.
6. Alkas FB, Kocadal K, Battal D, Çalış İ, Saygi S. 2023. Evaluation of the chemotactic and genotoxic effects of selected cycloartane-type glycosides on *Caenorhabditis elegans*. Rec Nat Prod. 17(4):648-663.
7. Patil M, Khan MR, Mondal S. 2023. Pathogenic variation among three major root-knot nematodes (*Meloidogyne* spp.) affecting guava (*Psidium guajava* L.) cv. Allahabad Safeda. Indian Phytopathol. 76(2):551-558.
8. Thiruchchelvan N, Kularathna M, Moukarzel R, Casonato S, Condron L. 2025. Population development, damage assessment, and susceptibility of maize hybrids to root-lesion nematodes (*Pratylenchus neglectus* and *P. crenatus*) under glasshouse conditions. Austral Plant Pathol. 54:333-342.
9. Zahoor A, Niaz Z, Jabran M, Azeem F, Joyia FA, Nawaz MA, *et al*. 2023. Computational insights into evolutionary and functional biology of plant cell wall degrading pectate lyases from plant parasitic nematodes of the order *Tylenchida*. Pak J Agr Sci. 60(1):95-106.
10. Mergaert P, Giraud E. 2024. Pathogenic nematodes exploit Achilles' heel of plant symbioses. Trends Parasitol. 40(10):873-875.
11. Pan S, Wei P, Li Y, Chen Z, Peng D, Wang L, *et al*. 2024. Identification of *Meloidogyne* species on traditional Chinese medicine plants in the Qinling Mountain area of China and their aggressiveness to different medicinal herbs. Plant Dis. 108(5):1202-1210.
12. Chen JN, Chen PL, Li Y, Xie MX, Li XB, Ding W. 2025. Mechanism of tobacco resistance to bacterial wilt induced by magnesium oxide nanoparticles. Scientia Agricultura Sinica. 58(16):3327-3344.
13. Li A, Lin P, Wang H, Chen J, He Y, Han S. 2024. Analysis of microbial community structure from rhizospheric soil of rapeseed ankang, Shaanxi, China. Bangladesh J Bot. 53(3):783-793.
14. Al-Kofahi SD, Al-Kafawin AM, Al-Gharaibeh MM. 2024. Investigating domestic gardens landscape plant diversity, implications for valuable plant species conservation. Environ Dev Sustain. 26(8):21259-21279.
15. Darling E, Núñez-Rodríguez L, Chung H, Zasada I, Quintanilla-Tornel M. 2023. The hop cyst nematode, *Heterodera humuli*: History, distribution, and impact on global hop production. Phytopathology. 113(2):142-149.
16. Chihani-Hammas N, Verdejo-Lucas S, Horrigue-Raouani N. 2023. *P ratylenchus* vulnus infecting apple rootstock MM106: Defense reaction, plant nutrient alterations and pathogenic potential. Eur J Plant Pathol. 167(2):169-181.
17. Wieczorek K, Bell CA. 2025. Exploited mutualism: The reciprocal effects of plant parasitic nematodes on the mechanisms underpinning plant–mutualist interactions. New Phytol. 246(6):2435-2439.
18. López MM, Lopez-Nicora HD, Ponce MSB. 2024. Fungal communities shift with soybean cyst nematode abundance in soils. Phytobiomes J. 8(4):568-577.
19. Gao J, Chen L, Wang J, Zhao W, Zhang J, Qin Z, *et al*. 2024. Response of the symbiotic microbial community of *Dioscorea opposita* cultivar tiegun to root-knot nematode infection. Plant Dis. 108(8):2472-2483.

20. Zhao X, Lin C, Tan J, Yang P, Wang R, Qi G. 2023. Changes of rhizosphere microbiome and metabolites in *Meloidogyne incognita* infested soil. Plant Soil. 483(1):331-353.