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Research Article

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Mechanism by which cinnamic acid regulates the fungal community structure in the rhizosphere soil and exacerbates soil-borne diseases in Lanzhou lily under consecutive replant conditions: a case study of Lanzhou lily wilt disease

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Abstract

Aims Consecutive replant problems (CRPs) in Lanzhou lily are linked to soil degradation, autotoxin accumulation, and pathogen proliferation. This study investigated how cinnamic acid, a model autotoxin, interacts with soilborne pathogens to influence plant health, soil properties, and rhizosphere fungal communities under simulated CRP conditions.

Methods An artificial simulation was conducted by adding cinnamic acid to soil to induce autotoxic stress and concurrently inoculating lily plants with pathogens (*Fusarium oxysporum* and *Ilyonectria sp.*) to induce wilt. Growth parameters, disease incidence, root activity, leaf defense enzymes, proline, soil properties, and fungal community composition were analyzed.

Results Cinnamic acid at 0.24–2.40 mg/L promoted pathogen growth in vitro and soil colonization. In planta, it induced autotoxic stress, inhibiting growth and root activity while elevating leaf defense responses. Co-application with pathogens significantly increased disease index and incidence compared to pathogen-only treatments. Rhizosphere soil properties and fungal community structure were markedly altered, with enrichment of pathogenic genera (*Fusarium*, *Ilyonectria*, *Gibberella*) and species (*Fusarium oxysporum*, *Ilyonectria liriodendri*). RDA showed soil properties (OM, pH, AK) explained 63.92–66.97% of community variation, with stronger effects than cinnamic acid.

Conclusion Cinnamic acid exacerbates lily wilt by promoting pathogen proliferation and modifying soil properties, which collectively reshape the fungal community. Its indirect regulation of the microbial community via alterations in soil properties outweighs its direct effects. The synergy between autotoxin accumulation and pathogen enrichment drives CRP severity in lily.

Key words Lanzhou lily · Cinnamic acid · *F. oxysporum* · *Ilyonectria sp.* · Allelopathic effects · Fungal community · Consecutive replant problems

Introduction

Consecutive replant problems (CRPs) pose a serious threat to sustainable agricultural production. The degradation of soil physicochemical properties, the accumulation of pathogens, phytotoxic stress on plant roots, and imbalances in the soil microbial community are major causes of crop rotation problems [1]. Phenolic compounds are important secondary metabolites in plant root exudates and serve as major allelopathic substances. They are primarily synthesized via the shikimic acid pathway [2], and are therefore commonly present in higher plant tissues. In agricultural ecosystems, phenolic compounds can disrupt the defense mechanisms and structure of crops, reduce their inherent resistance and facilitate pathogen invasion, ultimately leading to the occurrence of soil-borne diseases [3]. With consecutive replant years increase, phenolic autotoxic substances accumulate in the soil, intensifying plant autotoxicity and soil-borne diseases. Therefore, phenolic autotoxic substances serve as key soil chemical factor contributing to the occurrence of pathogenic consecutive replant problems in crops.

Lanzhou lily (*Lilium davidii* var. *unicolor*) is the only sweet lily in China. It is suitable for cultivation only in the Diyi Mountain region south of Lanzhou, Gansu. It is a renowned specialty vegetable of Gansu province [4]. Due to its narrow distribution and perennial cultivation, the

production areas face severe consecutive replant problems and frequent soil-borne diseases, among which lily wilt is the most serious. The research team's previous studies indicate that in the main lily-producing areas, this disease begins to occur around late May, becomes severe when rainfall is heavy in June, and causes root rot in plants, along with above-ground yellowing, wilting, and death, leading to significant yield reductions [5]. Studies by our research group indicates that the accumulation of *Fusarium* and *Ilyonectria* is one of the important causes of consecutive replant problems in Lanzhou lily [6], among which *F. oxysporum* is the most important pathogen causing wilt disease in the main production areas of Lanzhou lily [7]. In summary, using wilt as a representative soil-borne disease to investigate the pathogenesis of plant pathogenic consecutive replant stress provides insights into the mechanisms revealing phenolic-mediated plant pathogenic consecutive replant problems.

Cinnamic acid is a model autotoxic phenolic acid widely found in crop rhizosphere soil. It possesses the dual characteristics of serving as a carbon source and exerting allelopathic regulation, making it a key soil chemical factor mediating crop consecutive replant problems and the occurrence of soil-borne diseases [8]. On the one hand, cinnamic acid can serve as a high-quality carbon source for saprophytic fungi, promoting their proliferation within a certain concentration range and significantly increasing their population, thereby aggravating disease incidence. This “low-concentration promotion, high-concentration inhibition” concentration allelopathic effect has been confirmed in studies on *Vicia faba*, peanut, and *Panax notoginseng* [9-11]. On the other hand, the accumulation of cinnamic acid is an important trigger for inducing autotoxic stress and exacerbating consecutive replant problems in plants. Previous studies on consecutive replant of medicinal plants such as ginseng and American ginseng [12], as well as peanut [13], have clearly demonstrated that the accumulation of phenolic acids such as cinnamic acid is a significant cause of consecutive replant problems. Consecutive replant leads to the accumulation of phenolic acids (e.g., cinnamic acid) in the rhizosphere secretions of Lanzhou lily. However, reducing chemical fertilizer application combined with microbial agent supplementation can effectively alleviate consecutive replant problems by significantly decreasing cinnamic acid content in the rhizosphere soil [14]. Among the various phenolic acid components, some degrade rapidly while others degrade slowly. Only those with slow degradation rates are likely to accumulate in the rhizosphere soil, where they serve as carbon sources for microorganisms, thereby persistently promoting pathogen proliferation and infection. Therefore, it becomes a key chemical factor driving the occurrence of pathogenic consecutive replant problems. In the previous studies, our research group detected various phenolic acid components such as cinnamic acid, aconitic acid, and phthalic acid in the consecutive replant soil of Lanzhou lily over 0 to 9 years. Among these, the content of cinnamic acid increased significantly with the extension of consecutive replant years (in the 0, 3, 6, and 9-year consecutive replant fields of Lanzhou lily, its contents were 0.27 mg/L, 0.36 mg/L, 0.405 mg/L, and 0.42 mg/L, respectively.) [15]. Although the association of cinnamic acid with consecutive replant problems and pathogen proliferation has been revealed in various crops, but the mechanism by cinnamic acid contributes to the occurrence of pathogenic consecutive replant problems in lily wilt disease has not been reported, which provides an entry point for this study.

The impact of phenolic acids on the occurrence of pathogenic consecutive replant problems depends, on the one hand, on their effect on the population size of pathogens, and is also closely related to the soil environment of the root. Soil fungi are widely distributed in terrestrial ecosystems and make significant contributions to ecosystem processes, including litter decomposition, nutrient

cycling, and soil development [16,17]. Under consecutive replant conditions, crop root exudates provide abundant nutrients for pathogens, leading to a continuous increase in pathogen populations and an imbalance in the microbial community structure in the crop rhizosphere [18]. Our research group investigated the characteristics of fungal community changes in the soil of the Lanzhou lily consecutive replant system. We found that with the increase in consecutive replant years, fungal diversity decreased, while the abundance of pathogenic fungal genera (such as *Fusarium*, *Ilyonectria* and *Gibberella*) increased, and the abundance of potentially beneficial fungal genera decreased, thereby exacerbating the consecutive replant problems of lily [6]. As mentioned above, cinnamic acid is an autotoxic substance, and its content increases with the duration of consecutive replant in lily soil. Therefore, is this substance the allelopathic regulatory factors underlying the pathogenic consecutive replant problems in Lanzhou lily? If so, what are the regulatory effects and underlying mechanisms of this substance on pathogen proliferation and soil fungal community structure?

Based on the above research background, this study first established different concentration gradients of cinnamic acid through in vitro culture experiments to investigate the effects of different concentrations of cinnamic acid on the colony proliferation of *F. oxysporum* and *Ilyonectria sp.*. On this basis, we established an artificially simulated pathogenic consecutive replant problems soil system: cinnamic acid was added to the soil to exert autotoxic stress on the plants, while simultaneously inoculating the lily plants with pathogens to induce wilt development. we evaluated the effects on lily plant growth, disease incidence, soil physicochemical properties, the population dynamics of soil-borne pathogens in the lily rhizosphere, and the fungal microflora. This study aims to elucidate the soil biochemical mechanisms underlying phenolic acid-mediated pathogenic consecutive replant problems associated with wilt in Lanzhou lily. The study aims to clarify: (1) Does the interaction between pathogens and cinnamic acid accelerate the degradation of the rhizosphere fungal community while simultaneously increasing the abundance of soil-borne pathogens? (2) If so, what are the soil biogeochemical factors associated with this regulatory effect? The research findings will provide a theoretical basis for a deeper understanding of the mechanisms underlying pathogenic consecutive replant problems in lily induced by *F. oxysporum* and *Ilyonectria sp.* mediated by cinnamic acid.

Materials and methods

Analysis of the effects of cinnamic acid on the chemotactic proliferation of two pathogens

In vitro test of the allelopathic effects of cinnamic acid on *F. oxysporum* and *Ilyonectria sp.*

The test fungal strains selected were *F. oxysporum* and *Ilyonectria sp.* of lily wilt disease. They were isolated, purified and preserved in the 4°C refrigerator of the laboratory of Horticulture College, Gansu Agricultural University. Cinnamic acid was purchased from Shanghai McLean Biochemical Technology Co., Ltd., with a purity of 99.50%.

The test design is as follows: the concentration of cinnamic acid was determined based on the results of our group's previous studies. Previous research found that in a 0-9 years consecutive replant system of Lanzhou lily, the cumulative concentration of cinnamic acid in the rhizosphere soil ranged from 0.27 to 0.42 mg/L [15]. To systematically investigate the dose-effect relationship of cinnamic acid on *F. oxysporum* and *Ilyonectria sp.*, and to simulate the rhizosphere accumulation effect of phenolic compounds that may result from long-term consecutive replant, this study established four concentration gradients: 0 mg/L, 0.24 mg/L, 2.40 mg/L, and 24.00 mg/L, representing the basal medium, the measured concentration in soil from a non-consecutive replant

field, ten times the measured concentration, and the stress warning concentration, respectively.

In this study, the addition of an equal volume of dd H₂O served as the control group, while the addition of different concentrations of cinnamic acid constituted the treatment groups. Cinnamic acid at concentrations of 0.24 mg/L, 2.40 mg/L, and 24.00 mg/L was added to the potato dextrose agar (PDA) medium during preparation. After the PDA medium solidified at room temperature, equal amounts of *F. oxysporum* and *Ilyonectria sp.* was inoculated.

Determination of colony diameter of *F. oxysporum* and *Ilyonectria sp.*. Fungal plugs ($\Phi=5$ mm) of two pathogens were inoculated onto PDA medium containing different concentrations of cinnamic acid and incubated at 28°C for 7 days. Colony diameters were measured starting from the third day after inoculation, with measurements taken every 24 hours. Colony diameter was determined using a vernier caliper (Ningbo Qichen Instrument Co., Ltd.). Because *F. oxysporum* colonies had grown to the edge of the Petri dish by day 7, the experimental period was set to 7 days.

Determination of spore counts for *F. oxysporum* and *Ilyonectria sp.*: on the 8th day of culture, a hemocytometer was used to measure the spore counts of *F. oxysporum* and *Ilyonectria sp.*.

Spore count (spores/mL) = (number of spores counted / 80) $\times$ 400 $\times$ 10000 $\times$ dilution factor
Effects of cinnamic acid on the colonization ability of *F. oxysporum* and *Ilyonectria sp.*

The treatment was the same as in 2.2.1.1, and the spore suspension was prepared according to the method of Mou et al. [7]. Colonization ability was determined following the method is as follows: Four portions of 150 g of substrate (vermiculite: perlite = 3:1) were placed in 500 mL beakers and sterilized at 121°C for 20 min. After irrigating with the spore suspension and mixing thoroughly, 10 g was taken and placed in a petri dish, sealed, and incubated at a constant temperature of 28°C. Each treatment consisted of 12 dishes and take samples for analysis on days 0, 2, 4, 6, 9, 12, 16, 20, 25, 32, 37, and 40. During sampling, 1 g was taken from the center of the substrate using a sterilized spatula under aseptic conditions, and diluted to 10⁷, 10⁶, and 10⁵ times. And 100 μ L of each dilution was spread onto Martin's medium, with three replicates for each concentration. After incubation at 28°C for 3 days, the colony count was recorded. The colony-forming units per gram of rhizosphere substrate (CFU/g) were calculated as the colonization density, which was used to represent colonization ability.

Effects of combined treatment with cinnamic acid and pathogens on lily plant growth and rhizosphere soil fungal microflora

Test materials

Lanzhou lily was used as the test material, with bulb weights ranging from 1.0 to 1.5 g. The test strains were the same as those mentioned above (2.2.1).

Test design

This study employed a pot-based experimental design with a total of 6 treatments: CK (no treatment), J (*F. oxysporum* inoculation group), T (*Ilyonectria sp.* inoculation group), R (2.40 mg/L cinnamic acid treatment group), JR (*F. oxysporum* inoculation+2.40 mg/L cinnamic acid treatment group), TR (*Ilyonectria sp.* inoculation+2.40 mg/L cinnamic acid treatment group), and set up three replicates of every treatment. Lily scales were sown in layers in vermiculite on July 26, 2024. On September 3, 2024, germinated seedlings were transplanted into nutrient pots (specifications: top diameter 6.8 cm, height 6.8 cm), with one plant per pot. Treatment began when plants reached 10 cm. For the R, J, and T treatment groups, 25 mL of the corresponding concentration of cinnamic acid solution or pathogen suspension was applied to each pot; For the combined treatment groups (JR and TR), 25 mL of the corresponding concentration of cinnamic acid solution and 25 mL of the

corresponding pathogen suspension were applied to each pot. Applications were made once every 5 days. Each treatment included 82 seedlings, and samples were collected on the 25th day after treatment for the determination of various indicators. Text management followed standard lily field management measures.

Determination of plant disease incidence, growth indices, and physiological and biochemical characteristics

Samples were collected 25 days after treatment. Ten lily plants were randomly selected from each treatment; Plant height was measured with a ruler and stem diameter with a vernier caliper, and calculate the seedling index. The classification of disease severity into orders is based on a previously published article by our team [7]. SOD activity was determined using the nitroblue tetrazolium method, POD activity was measured using the guaiacol method, CAT activity was measured using the ultraviolet absorption method, the proline content using the acid ninhydrin colorimetric method, and the root activity using the TTC method [19].

The formulas for calculating the healthy seedling index, disease incidence rate, and disease severity index are as follows:

Seedling index = stem diameter / plant height $\times$ whole-plant dry weight $\times$ 10;

Disease incidence (%) = (number of diseased plants / number of plants investigated) $\times$ 100%;

Disease severity index (DI) = [$\sum$ (disease grade value $\times$ number of plants with that grade) / (maximum disease grade $\times$ total number of plants investigated)] $\times$ 100

The CK control and R treatment were not inoculated with the wilt pathogen, and the plants did not show symptoms of wilt; therefore, disease incidence and disease index were not determined.

Soil physicochemical properties of Lanzhou lily under different treatments

Soil samples were collected at 25th days post-treatment. Ten plants were randomly selected for every treatment. And rhizosphere soil samples were obtained from the soil adhering to plants roots. Rhizosphere soil was obtained using the root-shaking method. The soils from the 10 plants were mixed to generate one soil sample; All 12 soil samples were collected in this same manner. Each soil sample was divided into two subsamples: one sample was placed in dry ice for 30 minutes, then stored at -80°C for downstream applications (DNA extraction). The remainder of the sample was air-dried for soil characteristic detecting (total phenols, cinnamic acid, pH, EC, organic matter, available nitrogen, available phosphorus, available potassium), and the data were described in our published work [20].

The cinnamic acid content was determined by high-performance liquid chromatography (HPLC), Beijing Omics Biotechnology Co., Ltd. Total phenols content was determined using the Folin-Ciocalteu colorimetric method [21]; The organic matter, conductivity, pH value, alkali-hydrolyzable nitrogen, available phosphorus and available potassium were examined with reference to our published study [6].

In addition, our previous study showed that the ratio of the number of cultivable bacteria/the number of cultivable fungi (B/F values) can be used as a reliable indicator for evaluating the soil quality [22], so we used the dilute plating method to determine the number of cultivable bacteria and fungi in the soil [23].

Data Analysis

The raw data was first screened and sequences were removed from the study if they were shorter than 130 bp, had a low-quality score (≤ 20), contained ambiguous bases, or did not exactly match the primer sequences and barcode tags. The software package Usearch (version 8.1) [search

and clustering orders of magnitude faster than BLAST] was then used to further filter out erroneous or chimeric sequences. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97 % similarity level using UPARSE [24] based on de novo genome assembly. Low-abundance OTUs (fewer than two reads, including singletons) that might influence the richness and diversity estimates were excluded from subsequent analyses. The alpha diversity index with Chao1 and the Shannon index [25] were calculated using the Mothur software [26] and plotted using Excel 2016 (Fig. 4A, 4B). The fungal feature sequences were annotated using the UNITE ([https://unite. ut. ee/](https://unite.ut.ee/)) and NT ([https://blast. ncbi. nlm. Nih. gov/Blast. cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi)) databases.

To examine the statistical significance of structural similarity among communities across sampling locations, both principal coordinates analysis (PCA) applying Euclidean distance metrics. To further assess the relationship between the composition of the microorganism community and the physicochemical properties under different soil treatments, a redundancy analysis (RDA) was performed, based on fungal and bacterial abundance at the genus level and physicochemical parameters, using the R package. To determine the significantly important microbial taxa, a linear discriminant analysis effect size (LEfSe) analysis was carried out. The LDA effect size analysis was carried out to detect species with significant differences in abundance at the genus level with groups with different treatments, with the threshold set at 3.0. To reveal significant differences among the treatments, single factor and two-factor analysis of variance (ANOVA) was performed using Duncan's multiple range test ($p < 0.05$) and independent samples t-tests were performed using IBM SPSS Statistics22.0.

Throughput sequencing by ILLUMINA Miseq

DNA extractions

DNA from the different samples was extracted using the E.Z.N.A. ®Soil DNA Kit (D4015, Omega, Inc., Norcross, GA, USA) according to manufacturer's instructions. The reagent, which was designed to uncover DNA from trace amounts of the sample, was shown to be effective for the preparation of DNA of most bacteria. Nuclear-free water was used for blank. The total DNA was eluted in 50 µL of elution buffer and stored at -80°C until measurement via PCR by LC-Bio Technology Co., Ltd., Hangzhou, China.

PCR Amplification and ITS Sequencing

The ITS2 region of the small-subunit rRNA gene of the fungi was amplified with slightly modified versions of the primers ITS1-F (5'-GTGARTCATCGAATCTTTG-3') and ITS2-R (5'-TCCTCCGCTTATTGATATGC-3') [27]. The 5' ends of the primers were tagged with specific barcodes for each sample and universal sequencing primers. PCR amplification was performed in a reaction mixture with a total volume of 25 µL containing 25 ng of the template DNA, 12.5 µL of PCR Premix, 2.5 µL of each primer, and PCR-grade water to adjust the volume. The PCR conditions for amplification consisted of an initial denaturation at 98°C for 30 s; 32 cycles of denaturation at 98°C for 10 s, annealing at 54°C for 30 s, and extension at 72°C for 45 s; and then a final extension at 72°C for 10 min.

The PCR products were confirmed via 2% agarose gel electrophoresis. Throughout the DNA extraction process, ultrapure water, instead of a sample solution, was used as a negative control to exclude the possibility of false-positive PCR results. The PCR products were purified with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, Carlsbad, CA, USA). The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed on an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on a NovaSeq PE250 platform (Illumina, San Diego, CA, USA).

Results

Effects of cinnamic acid on the proliferation and colonization ability of two pathogens

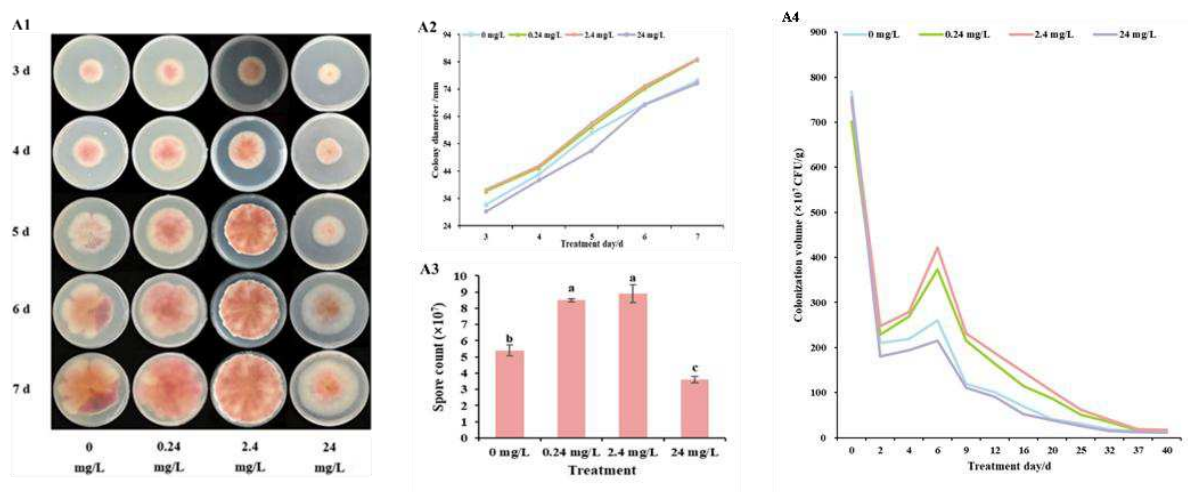
The colony sizes of *F. oxysporum* under different treatments from day 3 to day 7 are shown in

the Fig. (Fig. 1A1). With increasing cinnamic acid concentration, the colony diameter initially increased and then decreased. The colony diameter growth rate (Fig. 1A2) was higher at 0.24 mg/L and 2.4 mg/L cinnamic acid than at 0 mg/L, but lower at 24 mg/L cinnamic acid than at 0 mg/L. The spore counts of *F. oxysporum* under different treatments are presented (Fig. 1A3) showed that treatments with 0.24 mg/L and 2.4 mg/L cinnamic acid significantly increased the spore count, while the spore count at 24 mg/L was significantly lower than that of the control and other treatments.

The colony sizes of *Ilyonectria sp.* under different treatments from day 3 to day 7 are shown in the Fig. (Fig. 1B1). With increasing cinnamic acid concentration, the colony diameter initially increased and then decreased. The colony diameter growth rate (Fig. 1B2) was higher at 0.24 mg/L and 2.4 mg/L cinnamic acid than at 0 mg/L, but lower at 24 mg/L cinnamic acid than at 0 mg/L. The spore counts of *Ilyonectria sp.* under different treatments are shown in the Fig. (Fig. 1B3). The 2.4 mg/L cinnamic acid significantly increased the spore count, while the spore count at 24 mg/L was significantly lower than that of the control and other treatments.

To determine whether pathogens can colonize and proliferate in soil in the presence of cinnamic acid, the colonization ability of two pathogens in soil was assessed. Cinnamic acid at 0.24 mg/L and 2.4 mg/L promoted colonization of *F. oxysporum* (Fig. 1A4) and *Ilyonectria sp.* (Fig. 1B4), whereas 24 mg/L inhibited colonization, with a sustained effect. During the 0-40 day, the two pathogens number under different treatment showed a trend of first decreased, then increased, and then decreased again, with the overall order of 2.4 mg/L > 0.24 mg/L > 0 mg/L > 24 mg/L.

In summary, cinnamic acid at 0.24-2.4 mg/L promotes the growth of *F. oxysporum* and *Ilyonectria sp.*, but inhibits their growth at 24 mg/L.



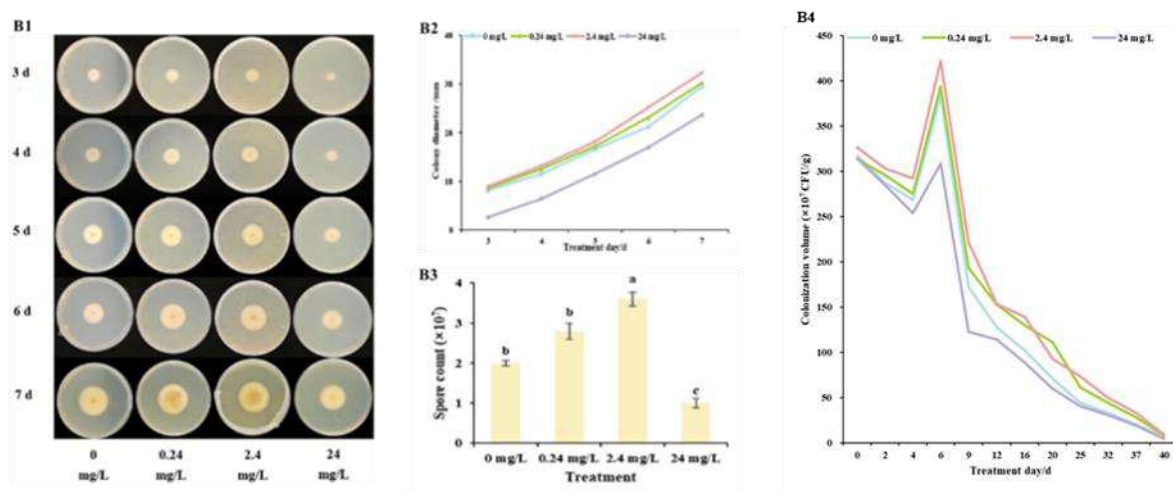


Fig. 1 Effects of cinnamic acid treatment on pathogen proliferation: Fig. A1, A2, A3 and A4 show the colony diagram, colony diameter, spore count and soli colonization ability of *F. oxysporum*, respectively; Fig. B1, B2, B3 and B4 show the colony morphology, colony diameter, spore count and soli colonization ability of *Ilyonectria sp.*, respectively. Spore counts were measured on day 8 of the treatment.

Effects of different treatments on lily growth and rhizosphere fungal microbiota

Assessment of plant disease, growth, and physiological indicators

Following inoculation with *F. oxysporum* and *Ilyonectria sp.*, the J、JR、T and TR treatments, all exhibited typical symptoms of wilt disease, with the lower leaves beginning to wilt and the wilting spreading upward; Following treatment with cinnamic acid (R treatment), the plants exhibited a significant decrease in leaf erectness, the leaves became soft and mostly drooped and spread out, resulting in a loose and disordered plant architecture (Fig. 2A0 and 2B0).

As shown in Fig. 3, the plant disease incidence in the J and JR treatments were 38.10% and 70.24%, respectively, with the disease incidence in the JR treatment significantly increased by 84.36% compared with J treatment (Fig. 2A1). The disease index in the J and JR treatments were 28.57% and 52.68%, respectively, with the disease index in the JR treatment significantly increased by 84.39% compared with J treatment (Fig. 2A2). Compared with the J treatment, the JR treatment resulted in significantly reduced seedling index (Fig. 2A3) and root activity (Fig. 2A4), significantly increased levels of SOD, POD, CAT, and proline contents (Fig. 2A5-2A8).

The plant disease incidence in the T and TR treatments were 59.52% and 63.10%, respectively, with the disease incidence in the TR treatment significantly increased by 6.01% compared with T treatment (Fig. 2B1). The disease index in the T and TR treatments were 44.64% and 47.32%, respectively (Fig. 2B2). Compared with the T treatment, the TR treatment resulted in significantly reduced seedling index (Fig. 2B3) and root activity (Fig. 2B4), and significantly increased levels of SOD, POD, CAT, and proline contents (Fig. 2B5-2B8).

The above results indicate that inoculation with *F. oxysporum* and *Ilyonectria sp.* significantly increased plant disease incidence and disease index, with more severe disease occurrence under the combined treatments (JR and TR). Inoculation with *F. oxysporum* and *Ilyonectria sp.*, as well as treatment with cinnamic acid, significantly inhibited lily plant growth, reduced root activity, and increased leaf defense enzyme activity; Moreover, the inhibitory effects of the combined treatments

(JR, TR) were even more pronounced (Fig. 3).

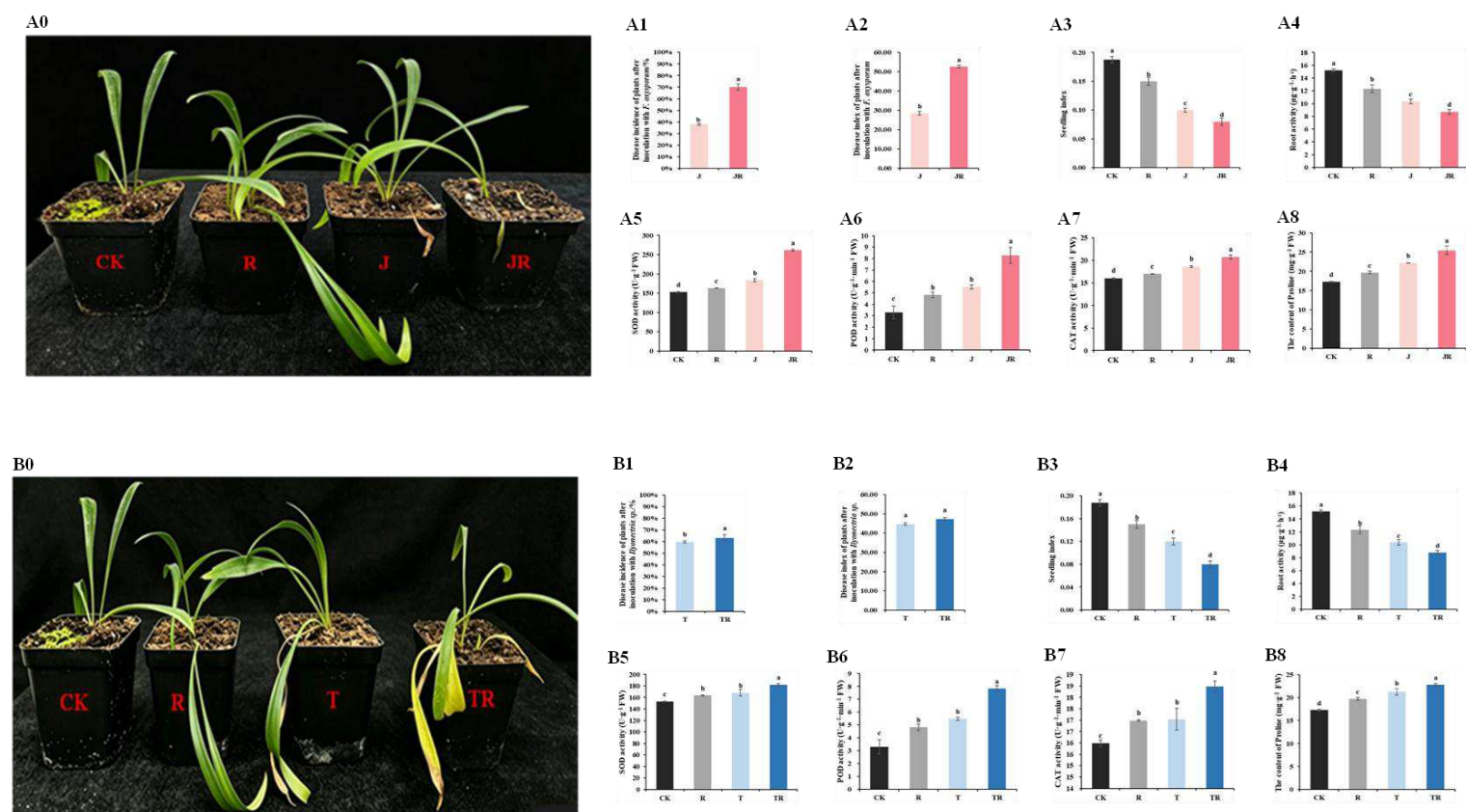


Fig. 2 Effects of pathogen inoculation, cinnamic acid addition, and combined treatments on disease incidence, growth, and physiology of Lanzhou lily plants: (A) inoculation with *F. oxysporum*, cinnamic acid addition, and combined treatments (CK, R, J, JR); (B) inoculation with *Ilyonectria sp.*, cinnamic acid addition, and combined treatments (CK, R, T, TR). A0-A4 and B0-B4 represent, in order: images of plant growth phenotypes, disease incidence, disease index, seedling index, and root activity; A5-A8 and B5-B8 represent leaf antioxidant parameters (SOD activity, POD activity, CAT activity, and proline content).

Diversity of soil fungi of Lanzhou lily under different treatments

Alpha diversity indices were assessed based on operational taxonomic units (OTUs). The Chao1 index and the Shannon index reflect species richness and diversity in individual samples, respectively. The results of α -diversity analysis indicate that, following inoculation with *F. oxysporum*, the Chao1 index of soil fungi decreased significantly (Fig. 3A1), while the Shannon index showed no significant change (Fig. 3A2); Following inoculation with *Ilyonectria sp.*, there was no significant change in the Chao1 diversity index of soil fungi (Fig. 3B1); the Shannon index increased significantly (Fig. 3B2); The Chao1 index of the fungi decreased significantly after treatment with cinnamic acid; the Shannon index showed no significant change (Fig. 3). Compared to the treatments involving inoculation alone (T treatment), the α -diversity indices of fungi in the treatments combining inoculation with exogenous cinnamic acid (TR treatment) significantly lowered. This indicates that both pathogen and cinnamic acid can reduce the biological traits of soil

fungi, but in the groups inoculated with *Ilyonectria sp.*, cinnamic acid addition and a combination of both treatments, cinnamic acid noticeably worsened the deterioration of these biological traits.

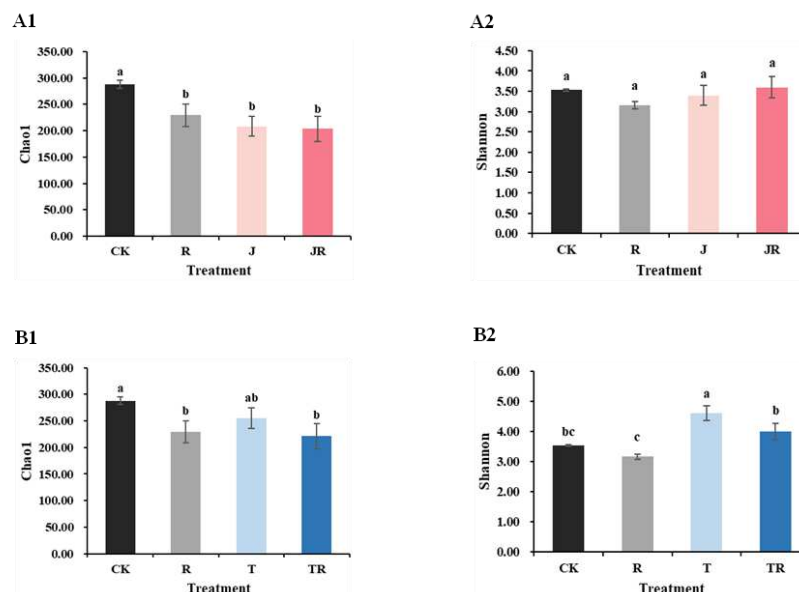


Fig. 3 Effects of pathogen inoculation, cinnamic acid addition, and a combination of both treatments on soil fungal diversity in Lanzhou lily: Fig. A1 and A2 show the changes in the soil Chao1 and Shannon indices following inoculation with *F. oxysporum*, cinnamic acid addition, and a combination of both treatments, respectively; Fig. B1 and B2 show the changes in soil Chao1 and Shannon indices following inoculation with *Ilyonectria sp.*, cinnamic acid addition, and a combination of both treatments, respectively.

The results of β -diversity analysis showed that, following inoculation with *F. oxysporum* and *Ilyonectria sp.*, the first and second principal component axes explained 84.82% and 87.50% of the total variation, respectively (Fig. 4).

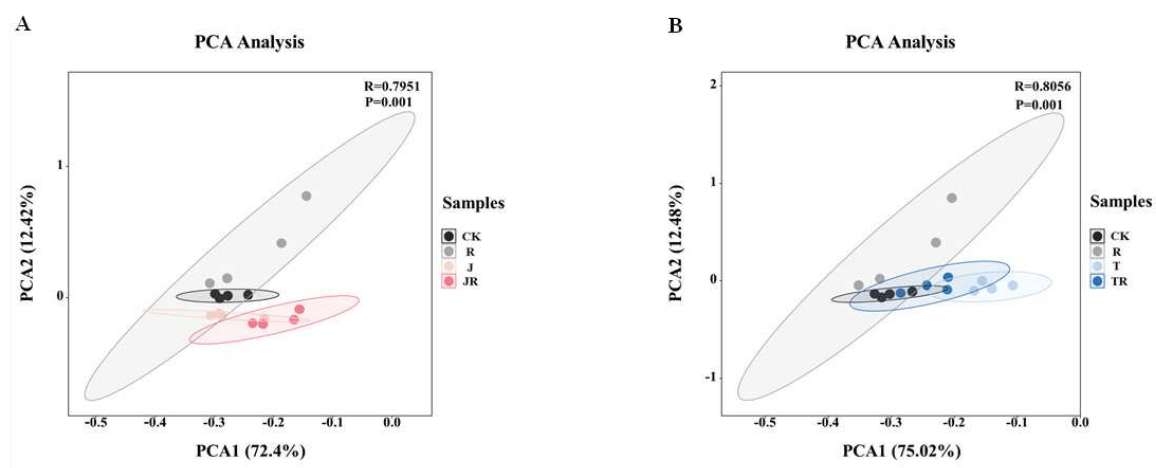


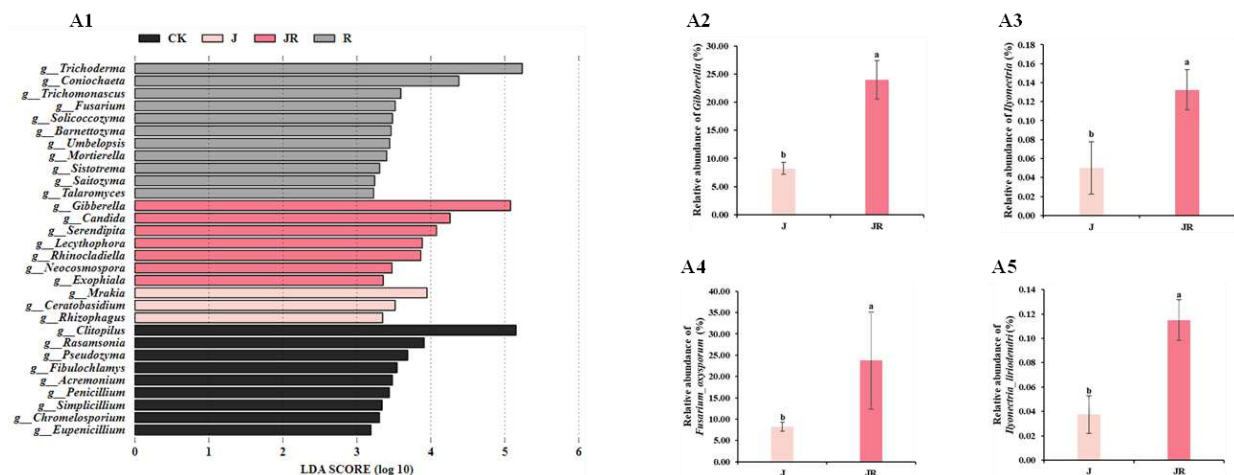
Fig. 4 Principal component analysis (PCA) based on Illumina MiSeq data: (A) Overall changes in the fungal community structure at the OTU level following inoculation with *F. oxysporum*, cinnamic acid addition, and a combination of both treatments and (B) overall changes in the fungal community structure at the OTU level following inoculation with *Ilyonectria sp.*, cinnamic

acid addition, and a combination of both treatments

Specific fungal microorganism groups under different treatments

For the groups treated with *F. oxysporum*, cinnamic acid, and a combination of both treatments, a total of 14 fungal phyla were identified, comprising 40 classes, 86 orders, 171 families, 283 genera, and 448 species. LDA analysis revealed taxonomic groups that were significantly enriched in each treatment, we focused on the genus and species levels: at the genus level, compared with CK, treatments J resulted in the enrichment of *Mrakia*, *Ceratobasidium*, *Rhizophagus*, and others, while treatment JR resulted in the enrichment of *Gibberella*, *Candida*, *Serendipita*, and others (Fig. 5A1). Since *F. oxysporum* was the inoculated pathogen, and *Ilyonectria* and *Gibberella* have been confirmed as major pathogens causing lily crop rotation problems [6], we further analyzed the abundance of these pathogens at the genus and species levels. The results showed that the abundances of *Gibberella*, *Ilyonectria*, *Fusarium_oxysporum*, and *Ilyonectria_liriodendri* in the JR treatment were significantly higher than those in the J treatment by 191.13%, 160.00%, 189.40%, and 200.00%, respectively (Fig. 5A2-5A5).

Similar to the analysis described above, a total of 14 fungal phyla, comprising 40 classes, 86 orders, 171 families, 283 genera, and 448 species, were identified in the groups treated with *Ilyonectria sp.*, cinnamic acid, and a combination of both treatments. LDA analysis revealed the taxonomic groups significantly enriched in each treatment. Compared with CK, *Trichomonascus*, *Penicillium*, and *Mrakia* were significantly enriched in the T treatment, *Candida*, *Ilyonectria*, *Serendipita*, and *Gibberella* were significantly enriched in the TR treatment (Fig. 5B1). The abundance of *Gibberella* in the TR treatment increased by 36.67% compared with T treatment (Fig. 5B2), the abundance of *Ilyonectria* in the TR treatment increased significantly by 59.05% compared with T treatment (Fig. 5B3), the abundance of *Fusarium_oxysporum* in the TR treatment increased by 7.69% compared with T treatment (Fig. 5B4), and the abundance of *Ilyonectria_liriodendri* in the TR treatment increased significantly by 60.28% compared with T treatment (Fig. 5B5).



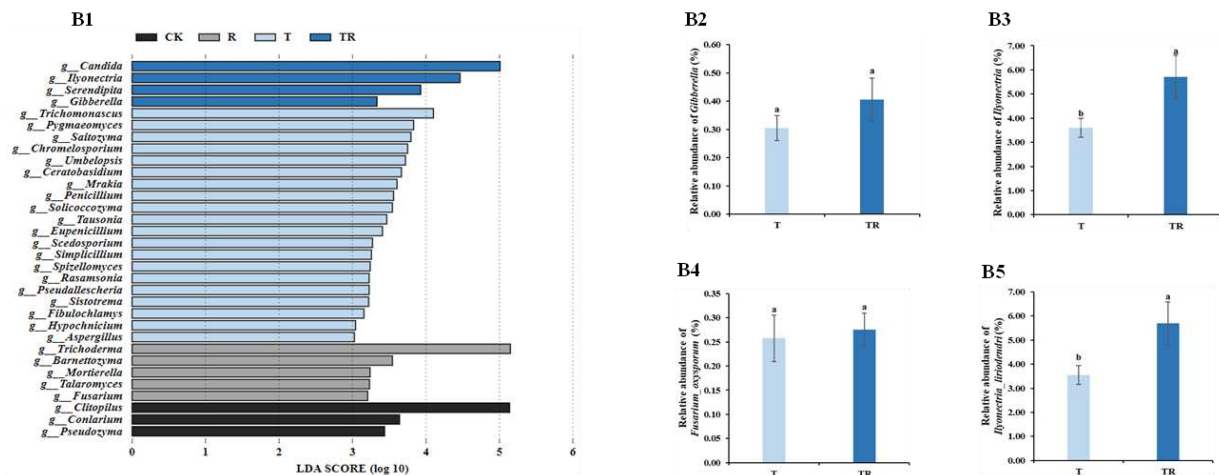


Fig. 5 LDA analysis revealed significantly enriched microbial groups across the various treatments: (A) Differential enrichment analysis at the fungal genus level under treatments involving inoculation with *F. oxysporum*, cinnamic acid addition, and a combination of both (A1), and comparisons of key pathogenic microorganism (genus/species) abundances between the J and JR treatments (A2-A5); (B) Analysis of differential enrichment at the fungal genus level (B1) and comparison of the abundance of key pathogenic microorganisms (genus/species) between the T and TR treatments (B2-B5) under conditions of inoculation with *Ilyonectria sp.*, cinnamic acid addition, and a combination of both.

Reshaping of fungal communities by cinnamic acid and other soil physicochemical properties

There are significant differences in the physicochemical properties of the soil around lily roots under different treatments (Table 1). The results of cinnamic acid and total phenolic content analyses showed that, in the groups inoculated with *F. oxysporum*, cinnamic acid addition, and a combination of both treatments, the cinnamic acid content in the JR treatment was 1.85% lower than that in the R treatment, while the R treatment showed a significant increase of 8.00% compared with CK; The total phenolic content in the JR treatment decreased by 0.07% compared with R treatment. In the groups treated with *Ilyonectria sp.*, cinnamic acid addition, and a combination of both treatments, the cinnamic acid content in the TR treatment was 5.56% lower than in the R treatment; the total phenolic content in the TR treatment was 0.29% lower than in the R treatment. The results of the organic matter analysis show that the organic matter content in the JR treatment decreased significantly compared with J and R treatments, and the organic matter content in the TR treatment decreased significantly compared with T and R treatments; The pH test results showed that the pH in the R, JR, and TR treatments was lower than that in the CK, with the JR and TR treatments showing a significantly lower pH than the R treatment; there were significant differences in indicators such as hydrolyzable nitrogen and available potassium between the JR and J treatments, and between the TR and T treatments.

Table 1 The soil's physicochemical properties of Lanzhou lily under different treatments

Treatment	Total phenolics ($\mu\text{g/g}$)	Cinnamic acid ($\mu\text{g/g}$)	Organic matter (g/kg)	Electrical conductivity ($\mu\text{S/cm}$)	pH	Alkali- hydrolyzable nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)
CK	1.0579 $\pm$ 0.0002b	0.50 $\pm$ 0.00d	44.09 $\pm$ 0.69a	237.56 $\pm$ 3.51b	6.36 $\pm$ 0.04a	266.49 $\pm$ 13.56d	11.37 $\pm$ 0.74c	65.56 $\pm$ 1.26e

R	1.0573±0.0001bc	0.54±0.01b	40.03±1.66a	137.29±1.82e	6.27±0.06ab	286.23±8.28d	7.40±0.21d	72.56±0.75d
J	1.0600±0.0001a	0.51±0.01d	27.14±0.91c	176.00±1.94d	6.04±0.02c	440.86±16.56b	10.98±0.39c	79.70±1.12b
JR	1.0566±0.0007c	0.53±0.01bc	18.88±1.81d	174.35±1.27d	5.98±0.02c	329.00±7.60c	12.42±1.08bc	83.94±0.59a
T	1.0601±0.0006a	0.58±0.01a	35.05±2.80b	204.22±2.05c	6.18±0.02b	658.00±10.74a	13.66±0.57b	77.09±0.54c
TR	1.0542±0.0002d	0.51±0.00cd	26.28±0.96c	272.91±1.80a	6.00±0.00c	407.96±7.60b	22.48±0.93a	81.22±0.38b

Note: Different lowercase letters indicate significant differences among different treatments ($P<0.05$) (the same below).

Following inoculation with *F. oxysporum*, cinnamic acid addition, and a combination of both treatments, the first and second principal component axes together explained 63.92% of the variance; following inoculation with *Ilyonectria* sp., cinnamic acid addition, and a combination of both treatments, the first and second principal component axes together explained 66.97% of the variance. Among all soil physicochemical parameters, cinnamic acid showed the weakest correlation with the microbial community, while TP, pH, OM, and others showed stronger correlations with the microbial community (Fig. 6A1 and 6B1); In addition, we examined the correlation between cinnamic acid and *Fusarium*, *Ilyonectria*, and *Gibberella* (since we had previously demonstrated that the abundance of these three genera is positively correlated with consecutive replant problems and that they are major pathogens responsible for consecutive replant problems in Lanzhou lily). The results showed that: in the groups treated with *F. oxysporum* inoculation, cinnamic acid addition, and a combination of both treatments, cinnamic acid showed a significant positive correlation with *Fusarium* (Fig. 6A2). In contrast, the other physicochemical parameters (TP, CA, OM, EC, AN, AP, and AK) all showed significant correlations with *Ilyonectria* or *Gibberella* (Fig. 6A2 and 6B2). This indicates that cinnamic acid plays a lesser role than other soil physicochemical properties in the establishment of the dominant fungal community in the rhizosphere of lily; its regulatory effect on the structure of the fungal microbial community is achieved indirectly through the regulation of physicochemical properties such as total phosphorus (TP), pH, and organic matter (OM).

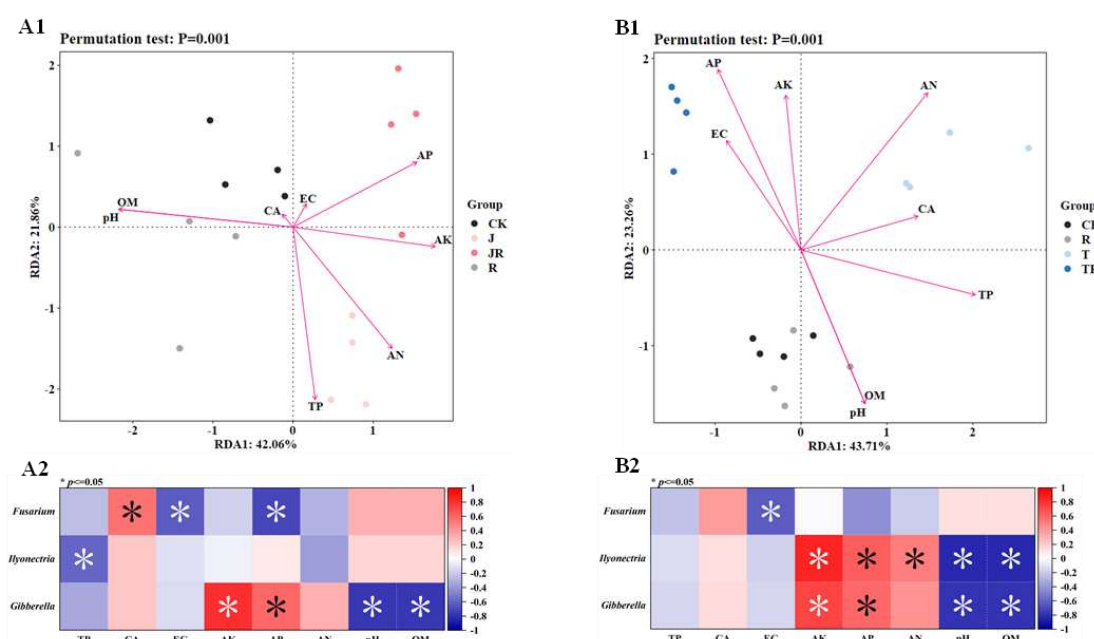


Fig. 6 (A) Groups inoculated with *F. oxysporum*, cinnamic acid addition, and a combination of both treatments (CK, R, J, JR): A1 and A2 show, respectively, the RDA plot and correlation plots

between *Fusarium*、*Ilyonectria*、*Gibberella* and soil physicochemical parameters; (B) Groups inoculated with *Ilyonectria sp.*, cinnamic acid addition, and a combination of both treatments (CK, R, T, TR): B1 and B2 are RDA plots showing the correlations *Fusarium*、*Ilyonectria*、*Gibberella* and soil physicochemical parameters, respectively. TP, CA, OM, EC, AN, AP, and AK represent total phenols, cinnamic acid, organic matter, electrical conductivity, alkali-hydrolyzable nitrogen, available phosphorus, and available potassium, respectively.

Number of cultivable microorganisms in the soil of Lanzhou lily under different treatments

The ratio of cultivable bacteria to cultivable fungi (B/F values) serves as an important microbiological indicator for assessing soil quality; an increase in this ratio typically indicates an improvement in soil quality [28-30]. Statistical results for cultivable microorganisms indicate that inoculation with *F. oxysporum* or *Ilyonectria sp.*, as well as cinnamic acid addition and a combination of both treatments, all significantly reduced the number of cultivable bacteria in the soil, and the reduction in the combined treatments (JR, TR) was significantly greater than that in the corresponding single treatments (Fig. 7A1 and 7B1); In contrast, all treatments significantly increased the number of cultivable fungi, with the increase being even more pronounced in the composite treatment (Fig. 7A2 and 7B2); the B/F value showed a significant decreasing trend across treatments, with the order CK > R > J > JR (Fig. 7A3) and CK > R > T > TR (Fig. 7B3). The above results indicate that inoculation with pathogens, cinnamic acid addition, and a combination of both treatments all led to a significant deterioration in soil quality, with the negative effects of the combined treatment being the most pronounced.

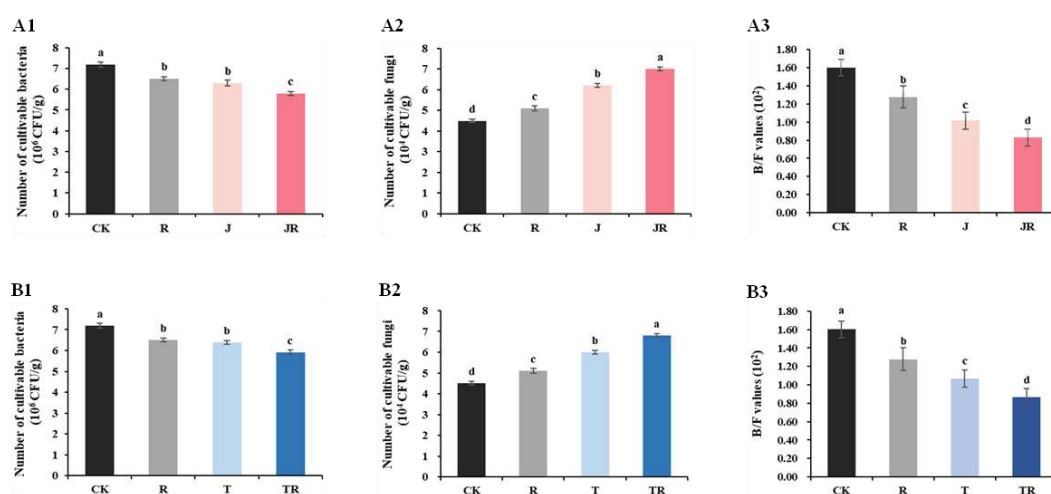


Fig. 7 Number of cultivable microorganisms in the rhizosphere soil of Lanzhou lily: (A) treatments inoculation of *F. oxysporum*, add cinnamic acid, and a combination of both (CK, R, J, JR); (B) treatments inoculation of *Ilyonectria sp.*, add cinnamic acid, and a combination of both (CK, R, T, TR). A1-A3 and B1-B3 represent, in order: number of cultivable bacteria, number of cultivable fungi, and B/F values.

Discussion

Analysis of the direct causes of cinnamic acid exacerbating Lanzhou lily wilt

Cinnamic acid, a typical phenolic phytohormone, has garnered considerable interest due to its involvement in lily continuous replant problems. In vitro and colonization assays in this study indicate that cinnamic acid exhibits a concentration-dependent “low-promoting, high-inhibiting” effect on the proliferation and colonization of *F. oxysporum* and *Ilyonectria sp.*. It promotes growth

and colonization within the range of 0.24–2.4 mg/L but significantly inhibits them at 24 mg/L (Fig. 1). The results of the pot experiments showed that inoculation with two pathogens and cinnamic acid addition significantly inhibited lily plant growth, reduced root vitality, and increased the activity of defensive enzymes in the leaves, with the inhibitory effects of the combined treatments (JR, TR) being even more pronounced (Fig. 2). At the soil microecological level, inoculation with two pathogens and cinnamic acid addition led to a decrease in soil fungal α -diversity (Fig. 3) and significant differentiation in community structure (Fig. 4). However, RDA analysis indicated that cinnamic acid exerts only a weak direct regulatory effect on fungal community structure, and primarily reshapes the community indirectly by influencing soil organic matter (OM), pH, and nutrient properties (Fig. 6). The number of cultivable fungi increased significantly, while that of cultivable bacteria decreased significantly; the B/F value showed a significant decreasing trend across treatments, with the order CK > R > J > JR and CK > R > T > TR (Fig. 7). Consistent with this, in the composite treatments (JR, TR), the abundance at both the genus and species levels of key pathogens—such as *Gibberella*, *Ilyonectria*, *Fusarium oxysporum*, and *Ilyonectria liriodendri*—which have been shown to be positively correlated with lily consecutive replant problems, was significantly higher than in the single treatments (Fig. 5).

The study findings reveal that the combination treatments involving pathogen inoculation (*F. oxysporum* and *Ilyonectria* sp.) along with cinnamic acid addition (JR and TR treatments) exacerbated the occurrence of lily wilt (Fig. 2). This exacerbation can be attributed to two factors: firstly, cinnamic acid contributes to the proliferation of pathogens in the soil. Cinnamic acid, a phenolic autotoxin commonly found in soils undergoing consecutive replanting, has been extensively shown to significantly increase the number of pathogens in the rhizosphere soil of various crops: Dong Yan et al. [9, 31] reported that cinnamic acid, at concentrations of 100 and 200 mg/L, significantly increased the disease index of fava bean wilt and significantly increased the abundance of *Fusarium* in the rhizosphere soil. Similarly, Ye et al. [8] observed that pretreatment with cinnamic acid in the cucumber-*Fusarium oxysporum* system significantly increased the population of *Fusarium oxysporum* in the nutrient solution. On the other hand, cinnamic acid caused significant autotoxic stress in lily plants, reducing their resistance to wilt disease and thereby synergistically worsening the disease in conjunction with the pathogen. Cinnamic acid is one of the primary autotoxin phenolic acids in soil subjected to consecutive lily cultivation, and its concentration accumulates significantly as the number of consecutive replant years increases [32]. Previous studies have shown that cinnamic acid can significantly inhibit the growth and development of various crops—in watermelons, treatment with 1.0 mmol/L cinnamic acid reduced seedling root activity by 50%–90% and growth by 30%–50%, while the disease incidence and disease index of wilt increased by 30%–50% and 30%–40%, respectively [33]. In cucumbers, 0.05–0.25 mmol/L cinnamic acid significantly inhibited plant growth and resulted in a higher incidence of *Fusarium* wilt [34]. This is consistent with the findings in the present study, where plant height and stem diameter were significantly reduced under the composite treatments (JR, TR). In terms of antioxidant defense, cinnamic acid treatment significantly increased SOD and POD activities as well as proline content in both the aboveground and underground parts of peas [35]. Following treatment with 0.2 mmol/L cinnamic acid, SOD, POD, and ascorbate peroxidase (APX) activities in the roots of yardlong beans all increased significantly [36]. This is fully consistent with the results of this study, which showed significant increases in SOD, POD, and CAT activities as well as proline content (Fig. 2). More importantly, cinnamic acid disrupts the structure of root cell membranes and

reduces root activity [36]. It also significantly inhibits the activity of disease-resistance-related enzymes in cucumber roots, such as chitinase and β -1,3-glucanase [37]. Furthermore, cinnamic acid significantly inhibits the content of disease-resistant compounds in melons, disrupts the membrane permeability of melon roots, causes damage to root cells, reduces root activity, and increases the incidence of melon wilt [38]. This decline in defensive capabilities creates favorable conditions for pathogen invasion. In summary, on the one hand, cinnamic acid directly increases the infection pressure from pathogens by promoting their proliferation and increasing their population in the soil. On the other hand, it significantly weakens the plants' physiological and biochemical resistance to lily wilt by inhibiting lily plant growth, inducing oxidative stress, reducing root vitality, disrupting cell membrane structure, and suppressing the activity of disease-resistant enzymes. The combined and synergistic effects of these two mechanisms ultimately led to an increased incidence of lily wilt under the composite treatments (JR and TR).

The study findings reveal that cinnamic acid exerts an allelopathic effect on the proliferation of *F. oxysporum* and *Ilyonectria sp.*, promoting growth at low concentrations and inhibiting it at high concentrations. Within a certain concentration range of cinnamic acid (0.24–2.4 mg/L), it serves as a carbon source for the pathogens, thereby promoting their proliferation and colonization. As a typical phenolic allelocine in root exudates, cinnamic acid exhibits a significant concentration-dependent effect on soil microorganisms. The results of this study indicate that cinnamic acid exerts an allelic effect on the proliferation of *F. oxysporum* and *Ilyonectria sp.*, characterized by “low promotion and high inhibition”: within a concentration range of 0.24–2.4 mg/L, cinnamic acid significantly promoted colony growth, spore production, and soil colonization ability in both pathogens; however, when the concentration increased to 24.0 mg/L, it exhibited a marked inhibitory effect (Fig. 1 and Fig. 2). These results are consistent with the conclusions of previous studies on crops such as *Vicia faba*, peanut, and *Panax notoginseng* [9–11], further confirming the allelopathic mechanism by which phenolic acids mediate the occurrence of consecutive replant problems through direct regulation of pathogen populations. According to our group's previous field measurements of cinnamic acid concentrations in soils under consecutive Lanzhou lily cultivation (0.27–0.42 mg/L), the concentration of cinnamic acid accumulated through long-term consecutive replant in the field falls precisely within the range that promotes disease. It can serve as a sustained carbon source for *F. oxysporum* and *Ilyonectria sp.*, enhancing the pathogens' survival and colonization capabilities in the soil, increasing their population size, and creating favorable conditions for subsequent infection of lily root systems [15]. The results of soil organic matter content measurements also support the above conclusion: organic matter content in the JR treatment decreased significantly by 30.43% compared with J treatment, and organic matter content in the TR treatment decreased significantly by 25.02% compared with T treatment. These results provide evidence that the massive proliferation of pathogens depleted the carbon source (Table 1).

Effects of cinnamic acid on the structure of fungal microbial communities

Cinnamic acid affected fungal α -diversity (Fig. 3) and altered community structure (Fig. 6A and 6B). β -diversity results showed that, following inoculation with *F. oxysporum* and *Ilyonectria sp.*, the first and second principal component axes collectively explained 84.82% and 87.50% of the variation in fungal microbial community structure, respectively (Fig. 4). RDA analysis results indicated that the J and JR treatments, as well as the T and TR treatments, could be significantly clustered (Fig. 6A and 6B). This suggests that cinnamic acid treatment is the fundamental cause of changes in the fungal microbial community structure following pathogen inoculation.

The results of this study also indicate that cinnamic acid disrupts changes in soil physicochemical properties following pathogen inoculation, leading to a significant decrease in organic matter content, alkali-hydrolyzable nitrogen, and other parameters (Table 1). This suggests that the presence of cinnamic acid is also a key factor driving the deterioration of rhizosphere soil physicochemical properties. Previous studies have extensively investigated the role of cinnamic acids and phenolic compounds in reshaping the physicochemical properties of rhizosphere soil: Lv Weiguang et al. [39] found that the addition of exogenous phenolic acids (p-hydroxybenzoic acid and p-hydroxybenzoic acid) led to a decrease in soil organic matter content, alkali-hydrolyzable nitrogen, and other parameters; Zhuo Lifang [40] et al. found that under phenolic acid stress, the levels of soil organic matter, alkali-hydrolyzable nitrogen, and available potassium in the rhizosphere of tomato plants all decreased; Swain et al. [41] found that phenolic acids released by plant roots can activate insoluble nutrients in the rhizosphere soil, thereby promoting nutrient absorption and utilization. At the same time, research by Gauri et al. [42] indicates that phenolic acids can also inhibit plant uptake of mineral nutrients—as phenolic acids accumulate in the plant rhizosphere, the balance of available nutrients such as nitrogen, phosphorus, and potassium in the soil gradually becomes disrupted, leading to soil degradation. In addition, our research group's previous studies have found that reducing chemical fertilizer application by 30% and applying microbial inoculants in combination can effectively mitigate the deterioration of soil physicochemical and biological properties caused by consecutive cultivation of Lanzhou lily by significantly reducing the cinnamic acid content in the rhizosphere soil [14].

RDA analysis in this study also indicated that eight soil physicochemical factors, including cinnamic acid and total phenols, exert significant regulatory effects on the formation of fungal microbial community structure. Among these, OM (organic matter), pH (acidity/alkalinity), and TP (total phenols) were the most significant (Fig. 6). In contrast, the direct regulatory effect of cinnamic acid on fungal microbial community structure was significantly weaker than that of the aforementioned physicochemical factors. Other studies on how phenolic compounds indirectly regulate physicochemical properties to influence soil microbial community structure also support this view: for example, a study by Zhou et al. clearly demonstrated that phenolic acids (including cinnamic acid) do not exert direct toxic effects on microorganisms but rather indirectly influence microbial community composition and diversity through changes in physicochemical properties, specifically soil acidification [43].

Effects of cinnamic acid treatment on the abundance of specific pathogens

The results of the LDA analysis indicate that cinnamic acid treatment induces the accumulation of various known pathogenic microorganisms in the soil around lily roots, which is another important factor contributing to the severe occurrence of pathogenic consecutive replant problems in lily. Our research group has demonstrated that, in addition to *F. oxysporum* and *Ilyonectria* sp., *Gibberella*, *Fusarium_oxysporum*, and *Ilyonectria_liriodendri* are also pathogens associated with lily consecutive replant problems [6]. In this study, compared with treatments involving pathogen inoculation alone (J and T treatments), the combined treatment of pathogen inoculation and cinnamic acid addition (JR and TR treatments) significantly increased the abundance of various known pathogenic microorganisms in the soil (For example, the abundances of *Gibberella*, *Ilyonectria*, *Fusarium_oxysporum*, and *Ilyonectria_liriodendri* in the JR treatment were significantly higher than those in the J treatment by 191.13%, 160.00%, 189.40%, and 200.00%, respectively (Fig. 5A2–5A5), while in the TR treatment, their abundances increased by 36.67%,

59.05%, 7.69%, and 60.28%, respectively, compared with T treatment (Fig. 5B2–5B5). This phenomenon may be related to the dual mechanism of action of cinnamic acid as an allelopathic substance: on the one hand, as mentioned earlier, low concentrations of cinnamic acid provide a carbon source for pathogens and stimulate their proliferation. This conclusion has been confirmed in numerous studies—Dong Yan et al. [31], in their study on cinnamic acid exacerbating faba bean wilt, showed that treatments with 50 and 100 mg/L cinnamic acid significantly increased the number of *Fusarium* in the rhizosphere by 40.35% and 108.07% compared to the control, respectively; Li Zibo [44] et al., after studying the allelopathic effects of phenolic acids in the rhizosphere soil of consecutive replant on the ginseng rust fungus, found that 0.05 mmol/L cinnamic acid significantly promoted the growth of ginseng rust fungal hyphae and spore germination, and significantly exacerbated the severity of ginseng rust disease. On the other hand, cinnamic acid may indirectly free up ecological niches for pathogens by inhibiting the activity of certain antagonistic or competitive microorganisms in the soil, thereby promoting their colonization and proliferation: Sun Xiaohan et al. [45] found that phthalic acid, p-hydroxybenzoic acid, ferulic acid, cinnamic acid, and mixed phenolic acids at concentrations of 10–50 mg/L all promoted the growth of *Fusarium oxysporum* watermelon-specialized strain and inhibited the biological activity of biocontrol bacteria; Tan Xiumei et al. [46] found that cinnamic acid accumulated in soils under consecutive replant inhibits the activity of beneficial microorganisms, such as cellulolytic bacteria.

Statistical results for cultivable microorganisms indicate that, compared with the J and T treatments, the JR and TR treatments showed a significant decrease in the number of cultivable bacteria (Fig. 7A1 and 7B1) and a significant increase in the number of cultivable fungi (Fig. 7A2 and 7B2). Numerous studies have shown that the ratio of cultivable bacteria to cultivable fungi (B/F value) can serve as a reliable indicator for assessing soil quality, a higher B/F value indicates better soil quality [28-30]: In this study, the B/F values exhibited a significant decreasing trend across treatments, with the order CK > R > J > JR and CK > R > T > TR (Fig. 7A3 and B3), indicating that the combined treatment of cinnamic acid and pathogenic fungi led to a significant deterioration in soil quality. This is consistent with the previously mentioned results showing inhibited plant growth and increased disease incidence (Fig. 2). In addition, the significant decrease in the number of cultivable bacteria and the significant reduction in the B/F value also indicate a shift in the soil microecosystem from a “bacterial-dominated” to a “fungal-dominated” state [47].

This study elucidated the soil biological mechanisms by which cinnamic acid exacerbates lily consecutive replant problems, however, certain limitations remain: (1) Under natural conditions, cinnamic acid in soil primarily originates from root exudates, and its secretion dynamics are jointly regulated by the growth stage, soil environment, and various external factors. However, this study employed an exogenous cinnamic acid treatment approach and was unable to continuously monitor the dynamic changes in root exudates, therefore, the analysis of cinnamic acid’s mechanism of action in natural soil over extended periods is subject to certain limitations. (2) There are numerous pathogens that cause lily wilt. Apart from *F. oxysporum* and *Ilyonectria* sp., other potential pathogens were not included in this study. Additionally, the response of some observed indicators to cinnamic acid treatment differed following inoculation with the two pathogens, which to some extent limits the generalizability of the study’s conclusions. In the future, long-term consecutive replant experiments (e.g., 0-9 years) could be conducted to further systematically evaluate the accumulation dynamics of cinnamic acid across a range of consecutive replant durations and its regulatory effects on lily wilt.

In summary, this study indicates that, on the one hand, low concentrations of cinnamic acid increase the number of pathogens by directly promoting the proliferation, spore formation, and colonization of *F. oxysporum* and *Ilyonectria sp.*. On the other hand, cinnamic acid causes significant autotoxic stress in lily plants, reducing their resistance to wilt disease, thereby synergistically exacerbating disease incidence in conjunction with the pathogens. Cinnamic acid treatment induces the accumulation of various known pathogenic microorganisms in the rhizosphere soil of lily, which is another major cause of severe pathogenic consecutive replant problems in lily. At the same time, cinnamic acid treatment generally has a negative effect on soil physicochemical properties, such as a decrease in soil organic matter content, by disrupting these properties, cinnamic acid indirectly reshapes the structure of the rhizosphere fungal microbial community. In summary, low concentrations of cinnamic acid directly promote the proliferation and colonization of pathogens, and act synergistically with them to exacerbate disease severity in lily plants and inhibit plant growth. At the same time, by driving the rhizosphere soil microecosystem toward a “fungal-dominated” state (as indicated by a decrease in the B/F value) and promoting the accumulation of key pathogens, it exacerbates the occurrence of consecutive replant problems. Based on this, we propose the following model to explain the mechanism by which cinnamic acid regulates the structure of the plant rhizosphere fungal microbial community under consecutive replant conditions, thereby exacerbating soil-borne diseases (Fig. 9).

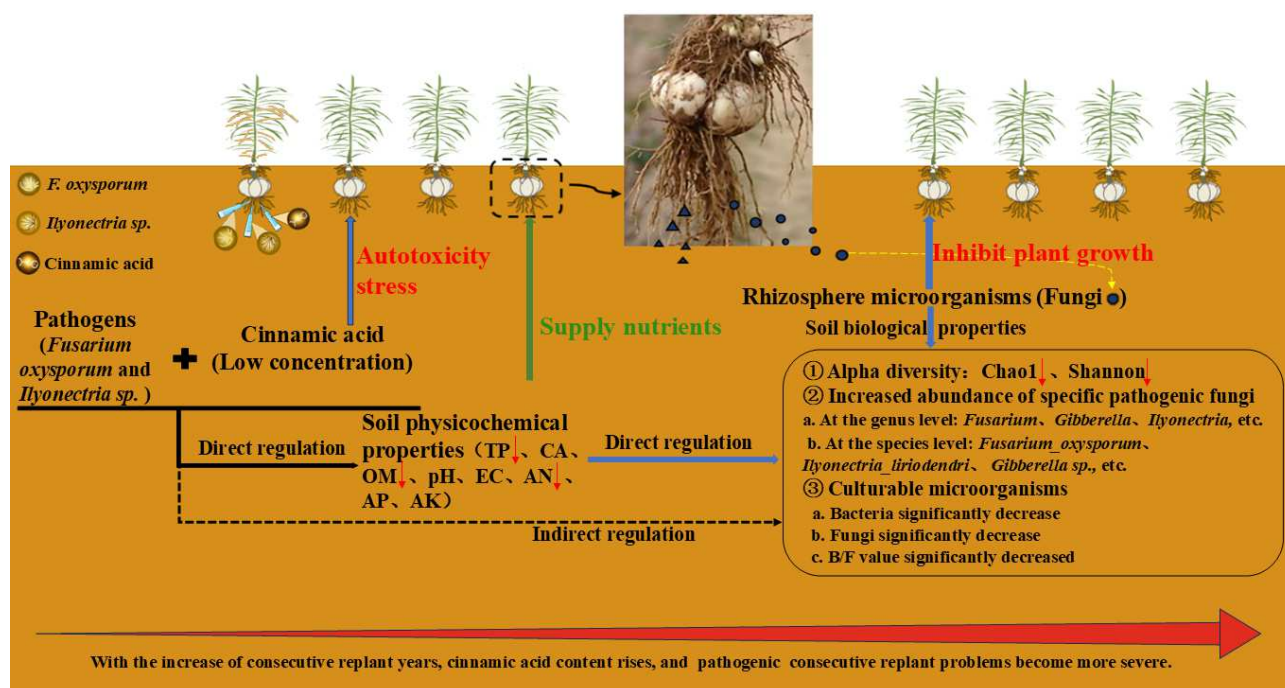


Fig. 9 Schematic mechanism diagram

Conclusions

Over a 0–9 year period of consecutive replant problems with Lanzhou lily, the cinnamic acid concentration in the rhizosphere soil showed a yearly increase, reaching cumulative concentrations between 0.27 and 0.42 mg/L [15]. This range is encompassed within the 2.4 mg/L concentration range. In summary, the following conclusions can be drawn: cinnamic acid increases the abundance of soil-borne pathogens associated with lily consecutive replant problems, and its interaction with

these pathogens accelerates the degradation of the rhizosphere fungal microbial community. Compared to the direct regulatory effects of cinnamic acid on fungal microbial community structure, its indirect regulatory effects—achieved by accumulating in the soil and altering its physicochemical properties—are more significant. Under the context of consecutive replant problems, phytotoxicity caused by the accumulation of cinnamic acid, the enrichment of pathogens such as *Fusarium*, *Ilyonectria*, and *Gibberella* and the interaction between these two factors are the soil biogeochemical factors that lead to the exacerbation of soil-borne crop diseases.

Author contributions All authors contributed to the study conception and design. Hui Li and Guiying Shi conceived the study, planned and designed the research. Li Wang, Jia Han, Xiangjun Fan, Fengfeng Guan, Chunxia Zhao, and Hongyu Yang participated in the revision of the manuscript. Hui Li analyzed the data, prepared the Fig., and wrote the article. Guiying Shi supervised the research. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations Not applicable.

Competing interests The authors have no competing interests to declare.

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