

Effects of allyl isothiocyanate fumigation on medicinal plant root knot disease control, plant survival, and the soil bacterial community

Yingbin Li

Yunnan Agricultural University

Daqing Lu

Yunnan Agricultural University

Yan Xia

Yunnan Agricultural University

Xinjing Xu

Yunnan Agricultural University

Huichuan Huang

Yunnan Agricultural University

Xinyue Mei

Yunnan Agricultural University

Min Yang

Yunnan Agricultural University

Jianqiang Li

China Agricultural University

Shusheng Zhu

Yunnan Agricultural University

Yixiang Liu

Yunnan Agricultural University

Zhiping Zhang (✉ m18788425134@163.com)

Yunnan Agricultural University

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Abstract

Background

Allyl isothiocyanate (AITC) is a natural product with high volatility that is used as a biofumigant to alleviate soil-borne plant diseases, and problems such as root knot nematodes (RKNs) that necessitate continuous cropping. However, little research has assessed the effects of AITC fumigation on medicinal plant.

Results

The current results indicated that AITC significantly reduced the population of RKNs in soil ($p < 0.0001$) and showed an excellent RKN disease control effect within 6 months after sowing *Panax notoginseng* ($p < 0.0001$). The seedling survival rate of 2-year-old *P. notoginseng* was approximately 1.7-fold higher after soil treatment with AITC ($p = 0.1008$). 16S rRNA sequencing indicated that the AITC treatment affected bacterial richness rather than diversity in the CC soil. Furthermore, biomarkers with statistical differences between AITC-treated and untreated CC soil showed that Pirellulales (o), Pirellulaceae (f), Pseudomonadaceae (f), and *Pseudomonas* (g) played important roles in the AITC-treated group. In addition, the microbiome functional phenotypes predicted using the BugBase tool suggested that AITC treatment is more conducive to improving CC soil through changes in the bacterial community structure. Crucially, our research confirmed that AITC soil treatment significantly increases soil organic matter ($p = 0.0055$), total nitrogen ($p = 0.0054$), and available potassium ($p = 0.0373$), which promotes the succeeding medicinal plant (*Polygonatum kingianum*) survival.

Conclusion

AITC as an ecologically friendly soil treatment method, which does not affect soil microbial diversity, but rather relative abundance. Also, it could be providing a basis for a useful agricultural soil management measure to alleviate soil sickness.

Background

Medicinal plants have important pharmacological activities and are valued as functional products that can be used as raw materials in the pharmaceutical and food industries. Because of increasing consumption and an associated shortage of raw materials, commercial cultivation of medicinal plants has been implemented in China since 2000 [1]. As an example, *Panax notoginseng*, for which > 20,000 ha has been dedicated for cultivation and the annual production amount in recent years has exceeded 20 million kg, provides ingredients for > 2,000 products [2, 3]. However, continuous cropping of medicinal plants in the field is limited by soil sickness, which is a complex phenomenon caused by a combination of biotic and abiotic factors that disturb the biological balance of the soil [4], including accumulation of

soil-borne pathogens, autotoxicity, soil microbial community imbalance, deterioration of soil physicochemical properties, unbalanced soil nutrients, and environment stresses [3, 5, 6]. Soil sickness is a form of negative plant–soil feedback that reduces crop yield and occurs when the same crop or a related species is cultivated successively in the same soil [5]. High seedling death rates due to root rot, as well as root-knot nematode disease, in many commercial medicinal plant production areas seriously affect plant growth and yield [3, 7]. These diseases also cause major changes in physicochemical and biological properties [8, 9].

Although soil sickness severely restricts sustainable development of intensive agriculture, it can be partially overcome by screening for low-autotoxic potential and disease-resistant genotypes, using resistant plant species as rootstocks, appropriate rotation, intercropping and soil and plant residue management, soil sterilization, and the introduction of beneficial microbes [5]. Soil sterilization using compounds such as dazomet, dimethyl disulfide, metam-sodium, methyl-bromide, and chloropicrin is an effective and common treatment for controlling soil-borne pests and diseases [10–12]. However, the use of most soil fumigants is forbidden or strictly restricted for resistance-related, environmental, and safety reasons [13, 14]. Pesticide residues have significantly reduced the quality of medicinal plants, thereby affecting human health [15, 16]. Therefore, there is an urgent need to identify a safe and effective soil fumigant to overcome soil sickness during medicinal plant cultivation.

Allyl isothiocyanate (AITC), which is derived from plant material in an ecologically friendly manner, has been used in agriculture [17] because of its soil-borne fungal or oomycetal, bacterial [18], nematocidal [19], and herbicidal [20] biological activities. The United States Environmental Protection Agency has licensed AITC for use as a biological pesticide [21].

Treatment of soil with chemical fumigants often has a significant impact on soil microbial communities. Chloropicrin greatly reduced soil biomass and bacterial species richness, influenced bacterial community structure, and affected non-target microorganisms [22, 23]. In addition, bacterial community diversity associated with biodegradation increased significantly, and denitrification was significantly promoted, suggesting negative effects on the environment [24]. Methyl bromide soil treatment resulted in a shift toward a community dominated by Gram-positive bacterial biomass [25]. However, some pesticides have suppressive or no effects on microorganisms. For example, diuron and chlorotoluron showed no differences between treated and nontreated soil, but linuron showed a strong difference [26]. Biofumigated mustard greens (*Brassica juncea*) caused much less damage to the soil bacterial community than chemical chloropicrin [22]. Biofumigation with rapeseed (*Brassica napus* 'Dwarf Essex') meal increased bacterial diversity but decreased fungal diversity [27].

Although AITC can control soil-borne diseases effectively, the effects on soil sickness in the context of medicinal plants require further evaluation. Thus, this study aimed to determinate the effects of AITC soil treatment on the survival of medicinal plants (*Panax notoginseng* and *Polygonatum kingianum*) in a consecutively cultivated (CC) soil system. In addition, the response of the soil bacterial community and changes of chemical properties associated with AITC fumigation were clarified.

Methods

Chemicals

Commercial 20% AITC EW and 0.5% avermectin GR were purchased from Jiangsu Teng-Long Biological Pharmaceutical, Ltd. and Guangdong Zhengze Biotechnology, Ltd., respectively.

Pot experiment

A pot experiment was conducted from December 2021 to July 2022 at the Agricultural Experimental Station of Yunnan Agricultural University, Xundian County, Kunming, China (25.521° N 103.286° E; altitude of 1,960 m). The test soil was collected from a *P. notoginseng* seedling base in Lancang County, where nematode disease was serious. The seeds were purchased from Wenshan Sanqi Trading Market. Healthy sanqi seeds were sown in bowls (0.05 m × 0.05 m spacing, 10 seeds per bowl) with a soil thickness of 0.15 m in December 2021. Each treatment had 10 replicates. Before sowing, 20% AITC EW was applied at doses of 0, 15, 45, or 75 L/ha for soil treatment; untreated soil (CK), soil treated by steaming at 80°C for 30 min, and soil treated with avermectin at a dose of 45 kg/ha were used as controls, respectively. The seedling survival rate (SSR) was recorded and the occurrence of root knot disease was investigated in July 2022 using previously described methods [28, 29].

AITC soil treatment

First, 30 L/m² of water was provided to ensure that the AITC could fully penetrate the soil fully. Second, black polyethylene film (thickness: 0.005–0.01 mm) was applied as a soil wrapping to create a tight seal. After 7 days of fumigation, the black polyethylene film was removed. Another 7 days of sun-curing was then necessary for the subsequent sowing or transplanting operations.

AITC soil treatment in the field

The effects of AITC treatment on the growth of 2-year-old sanqi were also assessed in the Agricultural Experimental Station of Yunnan Agricultural University, Xundian County, Kunming, China, where sanqi plants have been cultivated continuously from 2015 to 2017. The characteristics of the CC soil were as follows: pH, 7.49; electrical conductivity, 151.1 µs/cm; available phosphorus, 86.18 mg/kg; available potassium, 809.56 mg/kg; and organic matter (OM), 36.78 g/kg. Before seedling transplantation, 20% AITC EW was applied at a dose of 45 L/ha for CC soil treatment.

Healthy seedlings of *P. notoginseng* were transplanted into AITC-treated CC soil with a spacing of 0.15 m × 0.15 m. Each plot had an area of 5 m² and five replicates were used for each treatment. CC soil without AITC treatment was also transplanted as a control. To mimic the natural conditions for sanqi growth, the greenhouse was shaded with a polyethylene net that allowed 10% light transmission. The temperature was controlled at 18–30°C and strict moisture control was also implemented. The seedling emergence rate (SER) was recorded when the plant emergence rate in CC soil exceeded 50% (1 June). The SSR was recorded 2 months later (1 August). The “seedling survival fold value” was calculated as follows: fold

value = (number of seedlings in treatment group – number of seedlings in control group) / (number of seedlings in control group).

To evaluate the effect of AITC treatment on the growth of succeeding crops in CC soil, another traditional Chinese medicine *Polygonatum kingianum* was sown (spacing of 0.05 m × 0.05 m) in January 2022. Briefly, seeds were coated with fludioxonil, and a 2,000 m² area of soil was selected for AITC disinfectant treatment at a dose of 45 L/ha. AITC untreated CC soil was sown as a control. Three replicate plots (each with an area of 6 m²) were used. The greenhouse environmental conditions were consistent with those described above. The SSR per square meter was recorded and analyzed on June 17 and September 2, respectively.

Soil sampling

The CC soil samples treated with AITC at a dose of 45 L/ha were collected before seedling transplantation and untreated CC soil samples were collected as the control, according to a previously described method. Briefly, the soil samples were collected randomly from 20 pots and mixed into three biological replicates for each treatment. All samples were placed in 5 mL centrifuge tubes and stored at –80°C for DNA extraction and high-throughput sequencing.

DNA extraction and high-throughput sequencing

The total genomic DNA of each soil sample was extracted using the Fast DNA Spin Kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The V4–V5 regions of the bacterial 16S rRNA gene were amplified using the primer sets 27F (5'-AGRGTTCGATYNTGGCTCAG-3') and 1492R (5'-TASGGHTACCTTGTTASGACTT-3'), as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and 72°C for 10 min. The purified amplicons were sequenced using the single molecule real-time sequencing (SMRT) method and a PacBio Sequel sequencing platform; PacBio Menlo Park, CA, USA) according to the standard protocol. Data preprocessing was carried out using lima v 1.7.0, cutadapt 1.9.1, and UCHIME v 4.2. The aforementioned operations were completed by Biomarker Technologies Corporation (Beijing, China).

Bacterial community structure of rhizosphere soil

Raw circular consensus sequencing (CCS), clean CCS, and effective CCS data were obtained using lima v 1.7.0, cutadapt 1.9.1, and UCHIME v 4.2 software, respectively. The clean sequences were clustered into operational taxonomic units (OTUs) at the 97% identity threshold using Usearch software. Alpha diversity indices (Chao1, ACE, Shannon, and Simpson) and beta diversity were calculated using QIIME software (version 1.7.0). To compare samples, principal coordinates analysis (PCoA) was performed based on the Bray–Curtis distance at the OTU level. The samples were clustered hierarchically using the unweighted pair group method with arithmetic mean (UPGMA). The linear discriminant analysis (LDA) effect size (LEfSe) was used to identify biomarkers with statistical differences among different groups (the default

LDA score is 4.0). Microbiome functional phenotypes were predicted using the BugBase tool. All the data were obtained from BMK Cloud (www.biocloud.net).

Soil chemical property analyses

The AITC-treated and untreated CC soils described above were sieved (2 mm diameter) and oven dried. The soil pH was determined using PHS-3E (Shanghai, China) in a 1:2.5 soil/water (w/v) suspension according to standard NY/T 1121.2–2006. Soil OM was assayed using dichromate wet combustion according to standard NY/T 1121.6–2006. Soil total nitrogen (TN) content was analyzed using an azotometer (SKD-1000, Kjeldahl, Shanghai, China) according to standard NY/T 53-1987. Soil available phosphorus (SAP) was analyzed using an ultraviolet–visible spectrophotometer (T6 series; PERSEE, Auburn, CA, USA) according to NY/T 1121.7–2014 standards, and soil available potassium was analyzed by flame atomic absorption spectrophotometry (Z-2310; Hitachi, Tokyo, Japan) according to standard NY/T 1121.7–2014. All soil samples from each treatment were tested with three replications.

Statistical analysis

Seedling survival, the alpha diversity index, the number of species types, and soil chemical properties were analyzed using GraphPad Prism 8.3 software (GraphPad Software Inc., La Jolla, CA, USA), which was also used for data visualization. The microbial taxa data were analyzed using R software (version 2.15.3; R Foundation for Statistical Computing, Vienna, Austria). Two-way analysis of variance (ANOVA) and the unpaired *t*-test were used for statistical analysis. All illustrations were created using Adobe Photoshop CS6 software (Adobe Inc., Mountain View, CA, USA).

Results

Effects of AITC soil treatment on seedling survival and the occurrence of root knot disease

As shown in **Fig. 1a**, the number of nematodes was significantly lower in the soil treated by the different methods ($p < 0.0001$) than in the untreated CK soil (23.00 ± 4.56). AITC treatment at doses of 15, 45, and 75 L/ha decreased the number of nematodes in the soil to 8.00 ± 1.79 , 3.33 ± 0.82 , and 2.50 ± 1.05 per 100 g soil, respectively. The 45 and 75 L/ha treatments were better than the 15 L/ha treatment. Avermectin and steam treatment also decreased the number of nematodes, to 3.33 ± 0.82 and 3.17 ± 0.98 , respectively. The SSR for CK was about 51%, whereas the AITC treatment groups achieved the highest SSR ($> 80\%$; $p < 0.0001$). Avermectin and steam treatment also maintained a high SSR of approximately 70% (**Fig. 1b and 1c**). Notably, AITC showed high ability to control root knot disease within 6 months after sowing ($p < 0.0001$) (**Fig. 1d**).

Effect of AITC soil treatment on sanqi seedling survival in the field

To evaluate the effect of AITC on the growth of 2-year-old *P. notoginseng* further, another field experiment was conducted. Compared to plants in untreated CC soil, AITC soil treatment promoted sanqi seedling survival (**Fig. 2a and 2b**). As shown in **Fig. 2c**, the SSR in June was approximately 1.7-fold higher in soil

treated with AITC ($p = 0.1008$) than in CC soil. Two months later, the seedlings of *P. notoginseng* in CC soil died rapidly due to root disease. However, the SSR was significantly higher when CC soil was treated with AITC at 45 L/ha. It was approximately 2.16-fold higher compared to CC soil in August ($p = 0.0061$).

Effects of AITC on the richness and diversity of soil bacteria

To clarify the disease-suppressing and growth-promoting effects of AITC soil fumigation, 16S rRNA amplicon sequencing was performed on the PacBio sequencing platform, and the number of circular consensus sequences was counted for each sample. The abundance heatmap showed that the soil bacteria in CC soil (CC1, CC2, and CC3) and CC soil treated with AITC (AITC1, AITC2, and AITC3) were divided into two clusters. In addition, most of the bacteria units showed lower richness ($p < 0.05$), with standardization Z values ranging from -2 to 0 , in the AITC group than in CC soil (Z value range of 0 to 2) (**Fig. 3a, Supplementary Tab. S1**); however, some bacterial genera showed higher richness (**Fig. 3a, Supplementary Tab. S2**) ($p < 0.05$). Furthermore, there were significantly fewer circular consensus sequences in both the raw CCS and clean CCS data ($p < 0.0001$) after AITC soil treatment at 45 L/ha than in CC soil, suggesting that AITC has strong killing activity against bacteria and causes DNA degradation in soil (**Fig. 3b**). Interestingly, AITC application did not affect soil bacterial richness at the phylum level ($p = 0.9932$) but did decrease richness at the order ($p = 0.0067$) and genus ($p < 0.0001$) levels (**Fig. 3c**). The alpha diversity indices, such as Chao1 and ACE indices, of bacteria were significantly lower after CC soil was treated with AITC (**Fig. 3d**), while there were no statistical differences in the Simpson ($p > 0.9999$) and Shannon ($p = 0.0904$) indices between the CC and AITC-treated soil (**Fig. 3e**). Taken together, these results indicate that AITC treatment affected bacterial richness rather than diversity in CC soil.

Biomarkers with significant differences between treated and untreated samples

Variations in the bacterial community of AITC-treated and untreated samples (CK) were evaluated using PCoA, in which the horizontal (PC1 axis) and vertical (PC2 axis) coordinates were the principal components contributing to the differences in soil bacterial community composition among all samples. The total variance explained in the bacterial community composition of the samples was 60.82% (PC1, 34.13%; PC2, 26.69%) (**Fig. 4a**), indicating that AITC treatment was the major factor contributing to the differences in bacterial community composition.

The top 10 bacterial taxa in the community structure were analyzed further; the UPGMA was calculated through analysis of high-throughput sequencing results. As shown in **Fig. 4b**, the dominant bacterial phyla were uncultured_bacterium_c_Subgroup_6, uncultured_bacterium_f_Microscillaceae, Terrimonas, uncultured_bacterium_o_Saccharimonadales, uncultured_bacterium_f_Pedospaeraceae, uncultured_bacterium_f_TRA3-20, uncultured_bacterium_c_Alphaproteobacteria, Sphingomonas, uncultured_bacterium_f_Chitinophagaceae, and Methylobacter. Notably, although AITC-treated and untreated bacteria were obviously divided into two clusters, the bacterial community composition of each cluster was not significantly different. This result shows further that AITC treatment did not affect the bacterial diversity in CC soil.

Biomarkers with statistical differences between AITC-treated and untreated samples were subjected to LDA. As shown in **Fig. 4c**, the bacterial sequences were predominantly associated with Planctomycetacia (c), Planctomycetes (p), Pirellulales (o), Pirellulaceae (f), Proteobacteria (p), Pseudomonadaceae (f), and *Pseudomonas* (g). Among these bacterial taxa, Pirellulales (o), Pirellulaceae (f), Pseudomonadaceae (f), and *Pseudomonas* (g) played important roles in the AITC-treated group (**Fig. 4d**).

BugBase predictions of microbiome phenotypes in treated and untreated samples

The microbiome functional phenotypes in the treated and untreated samples, including aerobic, facultative anaerobic, anaerobic, biofilm-forming, oxidative stress tolerant, and mobile element-containing phenotypes, were predicted using the BugBase tool according to their relative abundance (**Supplementary Tab. S3**). Compared with CK, the abundances of aerobic (**Fig. 5a**), facultative anaerobic (**Fig. 5b**), biofilm-forming (**Fig. 5d**), oxidative stress tolerant (**Fig. 5e**), and mobile element-containing bacteria (**Fig. 5f**) increased, while that of anaerobic bacteria (**Fig. 5c**) decreased, after AITC treatment in CC soil. These results suggested that AITC treatment is able to improve CC soil by changing the bacterial community structure.

Effect of AITC treatment on the survival of succeeding crops in CC soil

To evaluate the effect of AITC on succeeding crop growth after the harvest of *P. notoginseng* in more detail, another traditional Chinese medicine, *P. kingianum*, was sown after AITC treatment in CC soil (**Fig. 6a**). As shown in **Fig. 6b**, seedling survival was significantly better in the AITC-treated than untreated CC soil. The SSR was approximately 1.3-fold higher after soil treatment with AITC ($p = 0.0665$) compared with CC soil (about 202 plants per square meter) in June. Five months later, the seedling survival rate of *P. kingianum* in CC soil had gradually diminished. However, the rate was significantly higher when CC soil was treated with AITC at a concentration of 45 L/ha. The SSR was about 2.0-fold higher compared with CC soil (about 337 plants per square meter) in August ($p = 0.0002$) (**Fig. 6c**).

Effect of AITC treatment on soil chemical properties

The soil chemical properties of AITC-treated and untreated CC soil were compared (**Fig. 6d**). The AITC soil treatments significantly increased the OM ($p = 0.0055$), TN ($p = 0.0054$), and SAP ($p = 0.0373$) values in comparison with the values in untreated CC soil. In addition, both soil pH and soil available potassium showed no significant difference between treated and untreated CC soil.

Discussion

The growth of medicinal plants is often restricted by soil diseases, and traditional prevention and control methods using chemical pesticides are not conducive to sustainable development [3]. However, soil fumigation is widely applied to increase crop yield and satisfy global food demand [30]. Biofumigation is an ecofriendly alternative to chemical fumigation that has been used to control soil-borne plant diseases [19, 27]. In this study, we first evaluated the ability of AITC fumigation to control medicinal root knot

disease and promote plant growth. Importantly, our research revealed, for the first time, the effects of AITC on the soil microbial diversity and community structure of medicinal plants. We found that both *P. notoginseng* seedlings and *P. kingianum* seeds showed higher SSR after CC soil was treated with AITC. Consistent with prior studies [22, 31], our research also revealed that AITC soil fumigation has less effect on soil bacteria and not have the ability of soil microorganisms destructive, it affects the richness rather than diversity of medicinal plant CC soil. Contrary to the negative effects (such as soil denitrification) of chloropicrin [24], AITC soil fumigation enhances beneficial functions such as aerobic, facultative anaerobic, biofilm-forming, oxidative stress tolerance, and mobile element functions. In addition, soil chemical properties including OM, TN, and soil available potassium were significantly improved. Thus, AITC soil treatment should have a positive effect on the environment.

AITC is the predominant isothiocyanate obtained from damaged *Brassica* tissues and has been used as a fumigant for controlling soil-borne diseases because of its low impact on the environment and low risk of persistence [32–34]. In our study, AITC was applied to control soil sickness of medicinal plants in a CC system. Previous studies reported mainly on soil diseases caused by pathogenic fungi, such as *Fusarium* spp. and *Ilyonectria* spp. [3]; we did not explore the impact of AITC fumigation on soil fungal communities, mainly because the composition and diversity of these communities are affected by various soil characteristics (pH, C/N) and vegetation types [35].

Previous studies have reported soil bacterial community dynamics in relation to the death rate of *P. notoginseng* in a continuous cropping system [36]. AITC decreased the relative abundances of dominant bacteria, *Planctomycetes*, *Acinetobacter*, *Pseudodeganella*, and RB41, but increased those of *Lysobacter*, *Sphingomonas*, *Pseudomonas*, *Luteimonas*, *Pseudoxanthomonas*, and *Bacillus*, at the genus level in pepper [37]. However, in tomato, there were significant increases in the relative abundances of probiotics, such as *Sphingomonas* and *Streptomyces*, following AITC fumigation [31]. On the contrary, *Planctomycetes*, *Acinetobacter*, *Pseudodeganella*, *Sphingomonas*, and *Streptomyces* were not detected by high-throughput sequencing in this study. Consistent with this, *Pseudomonas* and *Pseudoxanthomonas* were significantly enriched. One potential reason for this result is that the soil microbiota is shaped by native plants [38]; another possibility is that land-use history affects crop rhizosphere microbiomes, and the past planting record exerts a soil memory effect on microbial community diversity in the rhizosphere [39].

Soil microbes play a key role in nutrient cycling and carbon sequestration [40]. *Brassica* plants are the source of an enormous number of plant growth-promoting rhizobacteria (PGPR) that directly and indirectly promote plant growth. Some of the PGPR frequently isolated from *Brassica* species include *Agrobacterium*, *Pseudomonas*, and *Rhizobium* [30, 41]. Interestingly, many reported beneficial taxa in PGPR, such as *Pseudomonas* and *Pseudoxanthomonas* [42–46], were found to be more abundant after AITC fumigation.

In a prior study, the soil OM of mustard-eggplant treatment was 2.65 times greater than that of continually planted eggplant treatment [47]. OM was also increased after the AITC treatment in our study;

TN and soil available potassium were also significantly improved, possibly because AITC fumigation treatment promotes population and soil-aggregate restoration [30].

Conclusions

Soil sickness results in plant–soil feedbacks that reduce crop yield. The ecologically friendly method introduced herein provides new insight into soil-borne disease suppression and promotes medicinal plant growth for the current and subsequent crop. In addition, for the first time our study shows that AITC does not affect soil microbial diversity, but rather relative abundance. Importantly, soil bacteria were significantly enriched in soil after AITC fumigation, which could be provide a basis for a useful agricultural soil management measure to alleviate negative plant–soil feedbacks.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

YXL, ZPZ conceived and designed the research. YBL and DQL conducted the experiments. YBL, DQL, YX, and XJX analyzed the data. YBL wrote the manuscript. Manuscript preparation under the advisory of HCH, XYM, MY, JQL, and SSZ. All authors read and approved the manuscript.

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Availability of data and materials

The original contributions presented in the study are publicly available. These data can be found in the Short Read Archive (SRA) at NCBI database under accession number PRJNA917239 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA917239>). The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

Plant guideline statement

The plant materials (*Panax notoginseng* and *Polygonatum kingianum*) involved in this research belong to the category of traditional Chinese medicine, which have been widely cultivated in China. The plant collected and used in this study are permitted. All authors comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

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Figures

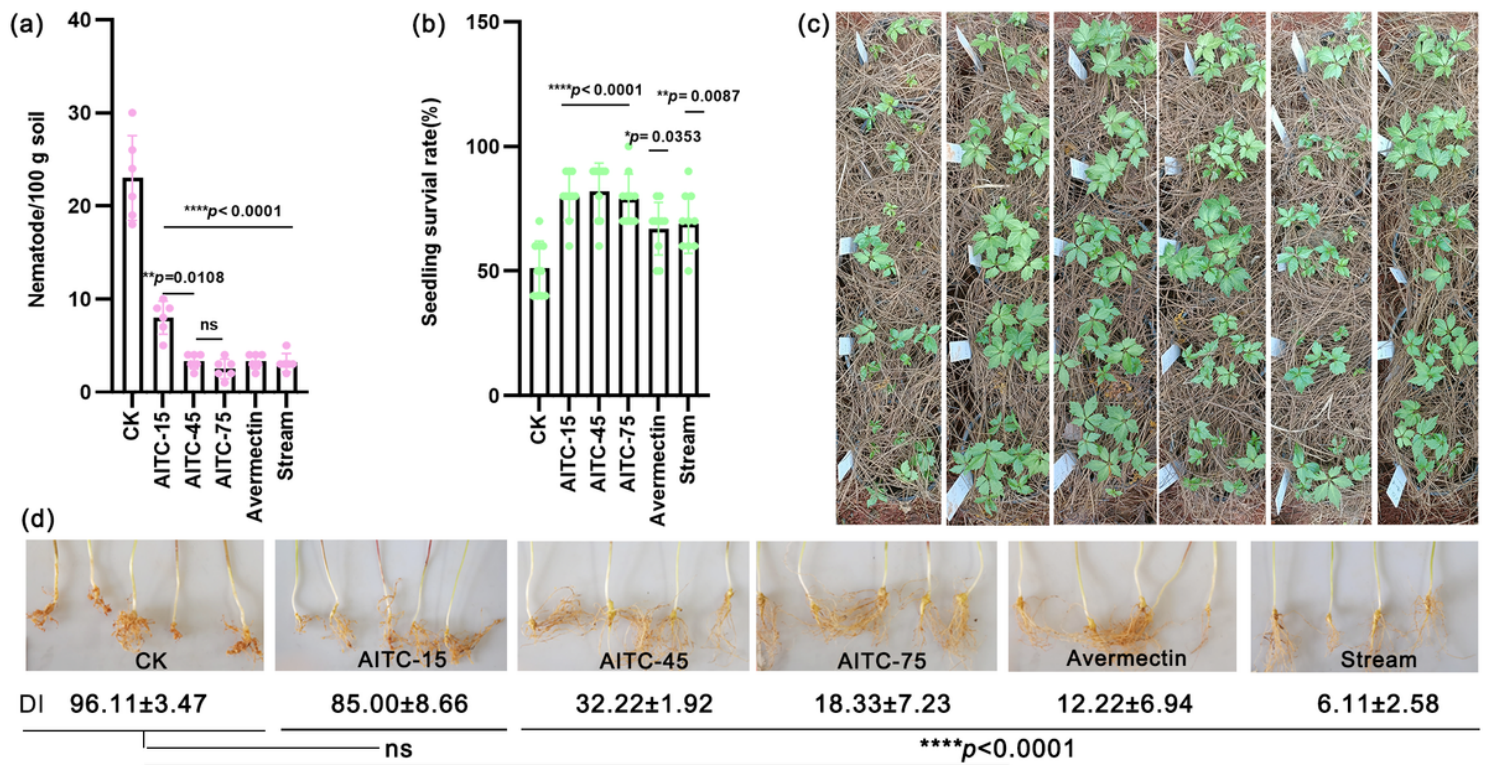


Figure 1

Seedling survival of 1-year-old *P. notoginseng*. **(a)** The number of nematodes ($n = 6$). **(b)** Seedling survival rate in July 2022 ($n = 10$). **(c)** Seedling growth in the study plot in July 2022. **(d)** Occurrence of root knot nematode disease ($n = 3$). Untreated soil (CK), avermectin treatment, and steam treatment were used as controls. AITC-“X” indicates soil treatment with AITC at a dose of 15, 45, or 75 L/ha. “DI”, means disease index; “ns”, no significant difference. All data are presented as mean $\pm$ standard deviation (SD) and were compared using two-way ANOVA.

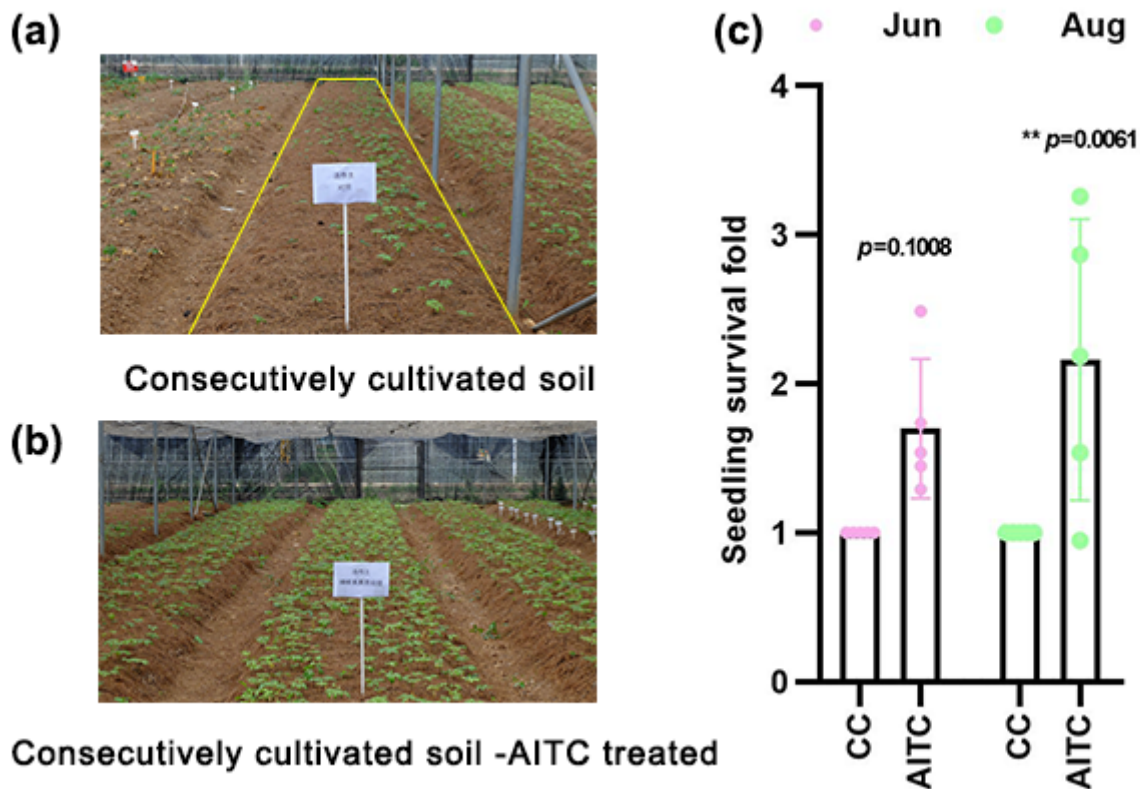


Figure 2

Survival of 2-year-old *P. notoginseng*. **(a)** Consecutively cultivated (CC) soil (the area marked with the yellow line). **(b)** AITC-treated CC soil. **(c)** Survival fold values in June and August. $n = 5$ for the CC/AITC treatment group. All data are presented as mean $\pm$ standard deviation (SD) and were compared using two-way ANOVA.

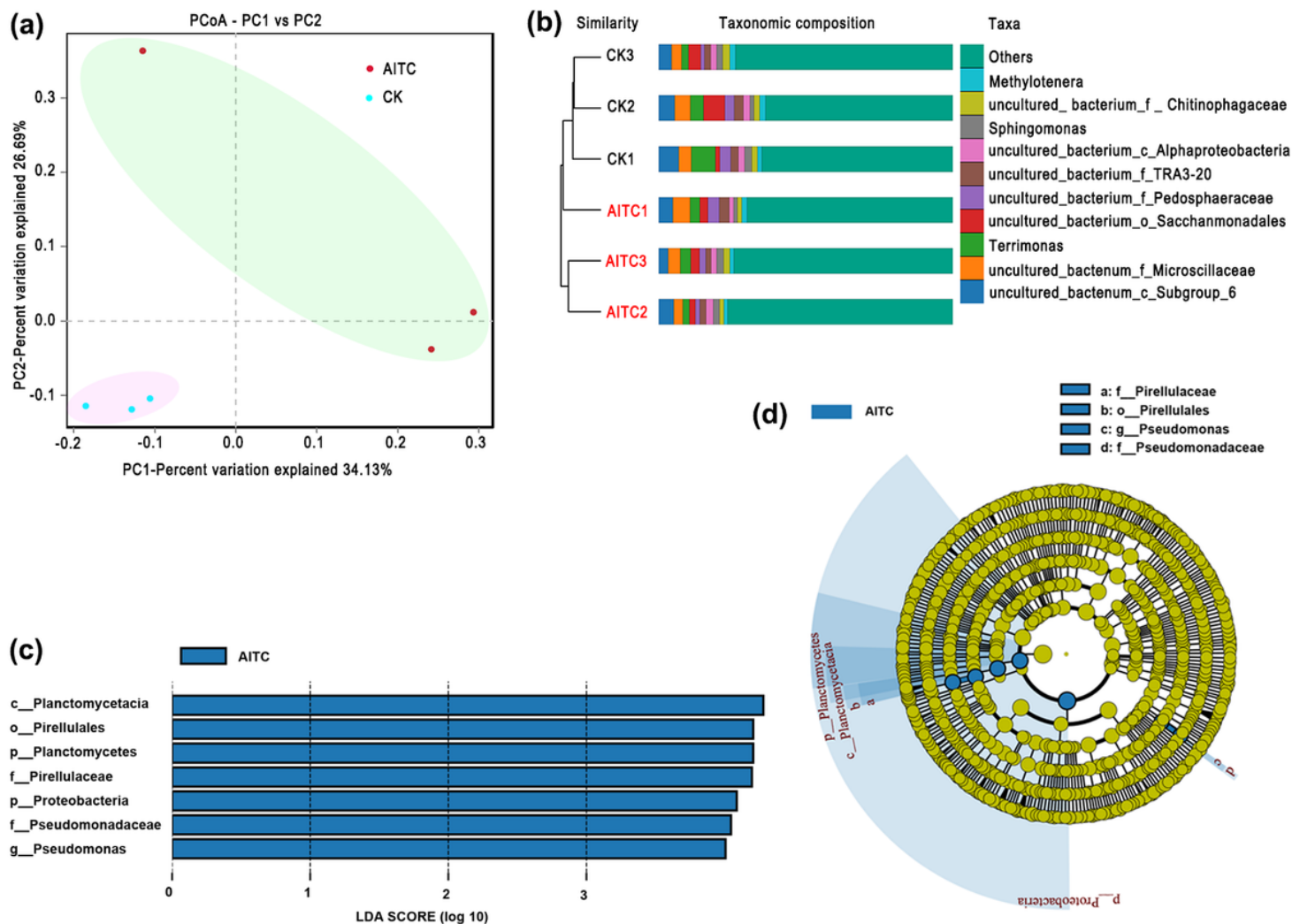


Figure 4

Results of beta diversity and LEfSe analyses. **(a)** Bray–Curtis principal coordinates analysis (PCoA) of bacterial communities with and without treatment with AITC. **(b)** Unweighted pair group method with arithmetic mean (UPGMA) clustering of the bacterial communities associated with all soil samples. **(c)** Species with a linear discriminant analysis (LDA) score greater than the set value (default setting is 4.0). The lengths of the histograms represent the impact of different species. **(d)** Phylogenetic dendrogram of bacterial biomarkers among all soil samples. $n=3$ for the CC/AITC treatment group.

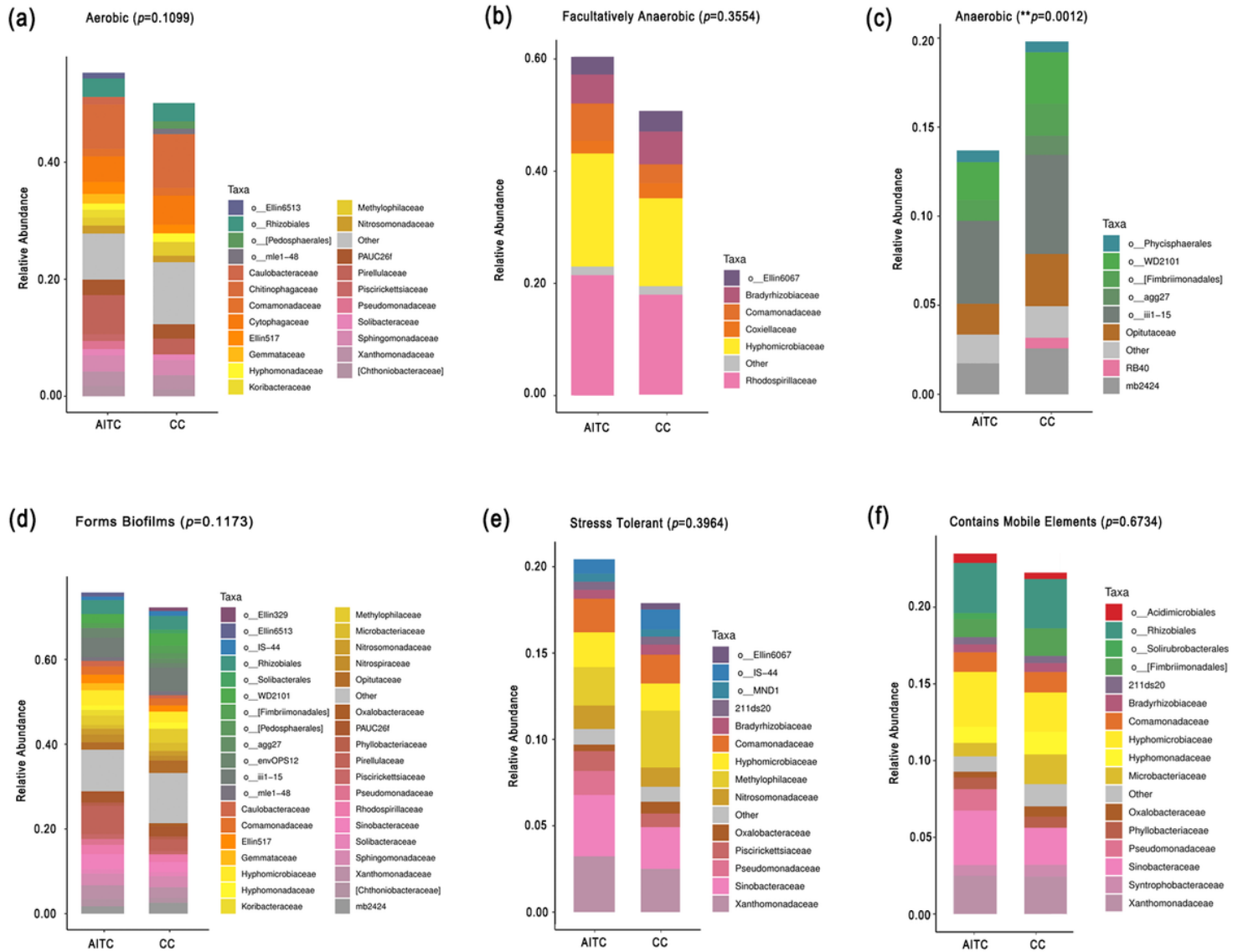


Figure 5

BugBase prediction of microbiome phenotypes in treated and untreated samples. **(a)** Aerobic. **(b)** Facultatively anaerobic. **(c)** Anaerobic. **(d)** Biofilm-forming. **(e)** Stress tolerant. **(f)** Mobile element-containing. $n = 3$ for the CC/AITC treatment group. All data are presented as means and were compared using the unpaired t test.

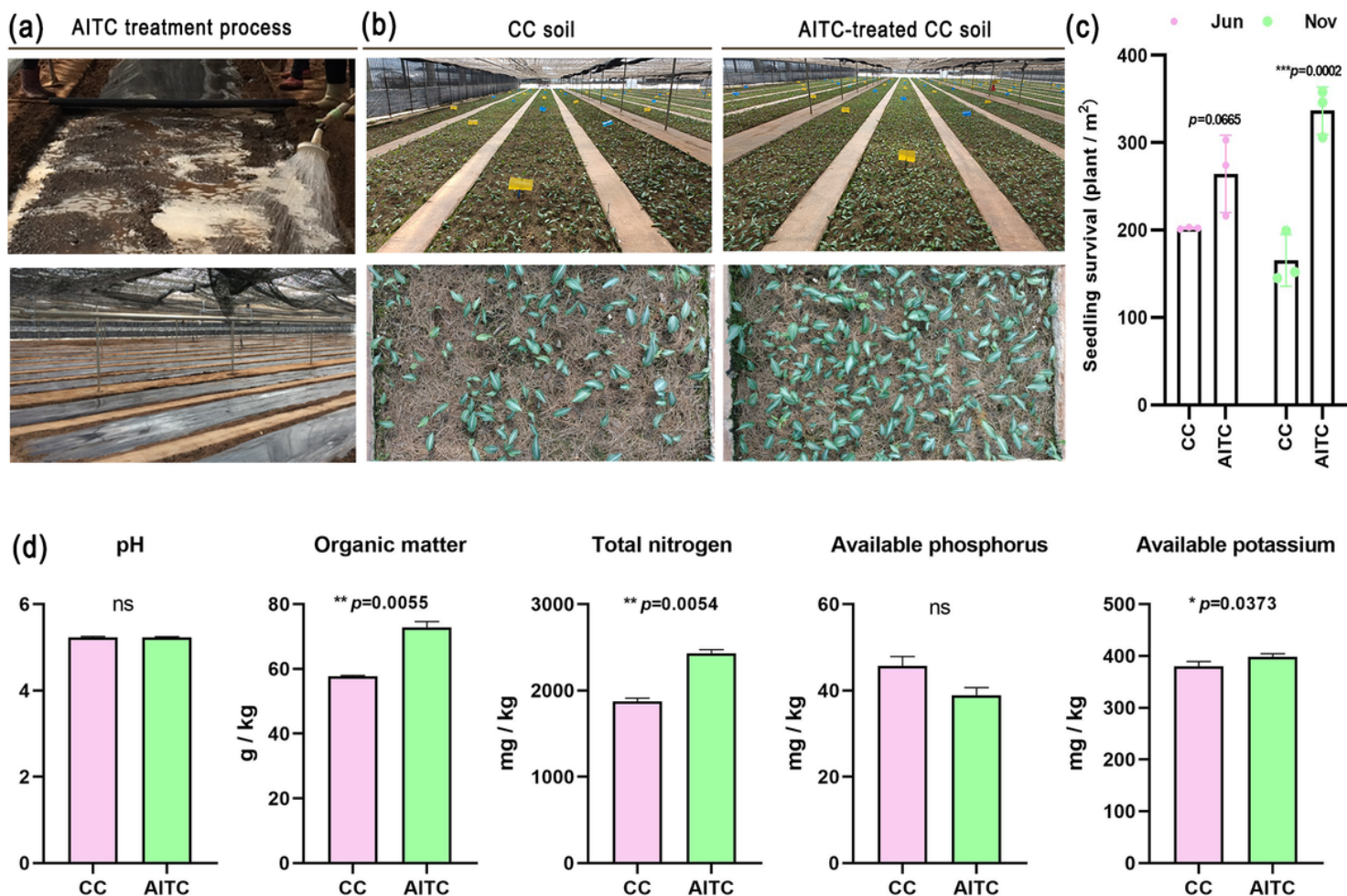


Figure 6

Seedling survival of *P. kingianum* and chemical changes in CC soil. **(a)** AITC treatment process. **(b)** Seedling growth in CC and AITC-treated CC soil. **(c)** Seedling survival in June and November, respectively. **(d)** Chemical changes of CC soil 7 days after fumigation with AITC. $n = 3$ for the CC/AITC treatment group. "ns", no significant difference. All data are presented as mean $\pm$ standard deviation (SD) and were compared using two-way ANOVA.

Supplementary Files

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