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Effect of Aged Cherry Orchard Soil on the Potted Seedling Growth of *Malus hupehensis* (Pamp.) Rehd

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Abstract: Due to the aging of trees, aged apple and cherry orchards need to be rebuilt urgently. However, due to the limitation of land resources, it is inevitable to rebuild the apple orchard by taking the aged cherry orchard as a replacement, which will lead to replant disease and seriously affect the sustainable development of the horticulture industry. This study investigated the effect of aged cherry orchard soil on the growth of *M. hupehensis* seedlings grown in pots, and it was further verified that allelochemicals in soil were one of the reasons for this effect. Three treatments were implemented: aged apple orchard soil (ppl), aged cherry orchard soil (pyl), and aged cherry orchard soil after fumigation with methyl bromide (pyz). Compared with pyz, pyl treatment significantly decreased the biomass, root growth, and antioxidant enzyme activity of *M. hupehensis* seedlings, and increased the content of MDA. Compared with ppl, pyl contains a smaller number of fungi and bacteria, but the abundance of the four disease-causing *Fusarium* remained high. In addition, the levels of allelochemicals found in the soil of aged cherry orchards can inhibit the normal growth and development of *M. hupehensis* seedlings. Amygdalin most strongly inhibited these seedlings. In summary, directly planting *M. hupehensis* seedlings in the soil of the aged cherry orchards still inhibits their normal growth and development, although the seedlings grow better than in aged apple orchard soil. Therefore, it is not feasible to directly plant *M. hupehensis* seedlings in the soil of aged cherry orchards, and measures should be taken to eliminate allelochemicals such as amygdalin and harmful microorganisms.

Keywords: allelopathy; *Malus hupehensis* Rehd; replant disease; soil; allelopathic compounds; pathogens; *Rosaceae*; SARD

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1. Introduction

Apples and cherries, as the two main species of fruit trees in the world, are rich in nutrition and high in vitamin content. They can also be made into preserved fruits, canned products, and other processed products, which have extremely high economic and social benefits. However, due to the long history of cultivation, most of the old orchards have entered the aging stage, and the yield of fruit trees has become low and the benefit is poor. Therefore, a large number of old orchards need to be rebuilt every year [1,2]. However, due to the limitation of land resources, the new orchards can only be carried out on the original site of the old orchards, but both apple and cherry belong to the same *Rosaceae* family; this may be the cause of the problem of the "apple specific replant disease (SARD)", causing slow growth, disease susceptibility, root necrosis, and even death in severe cases [3,4]. This has become a worldwide problem.

Previous studies have shown that replant diseases are caused by a combination of biological and abiotic factors. The abiotic factors include soil physicochemical imbalances

and accumulation of allelochemicals [5–8]. The biotic factors include imbalance of soil microbial community structure caused by increasing harmful microbial such as fungi (*Fusarium* spp., *Cylindrocarpon* spp.) and nematodes (*Pratylenchus* spp.).

Allelopathic substances secreted by roots found in the aged orchard or after grubbing the trees are the cause of the inhibition of plant growth in replant soil [9]. Allelopathy refers to the phenomenon that allelopathic substances secreted by plants can produce harmful effects on the plant itself or other related plants, and then affect the growth and development. This effect participates in and mediates the replant disease, which is widespread in the ecosystem [10–12]. Previous studies have reported that some of the phenolic acids contained in root secretions may be allelopathic substances that negatively affect plant growth [13,14]. Chen et al. [15] found that amygdalin and benzoic acid in aged peach orchard soil were harmful substances that inhibited the growth of *M. hupehensis* Rehd seedlings. Zhu et al. [16] found that the growth and photosynthesis of peach seedlings were inhibited by phytotoxic extracts from peach root bark and benzoic acid. Many studies have proved that the occurrence of replanting diseases is closely related to the accumulation of allelochemicals in soil [17–19].

Biological factors such as the accumulation of pathogenic fungi are another important cause of replant disease. Xu et al. [20] found that with the extension of plant monoculture years, replant diseases became more and more serious, and the number of harmful bacteria in soil increased, while the number of beneficial bacteria decreased, and the soil microbial structure tended to develop negatively. Once pathogenic bacteria and other harmful microorganisms proliferate in the soil and increase in number, they will destroy the soil microecological environment and increase the incidence of diseases, thus affecting plants. Fusarium is considered to be the main pathogen causing "SARD" in China [21–24], and similar results were reported in orchards in South Africa [25].

In horticulture production, there is often a phenomenon of crop rotation among fruit trees, for example, planting apple seedlings in old peach orchards [26] and apple seedlings in old pear orchards [27]; however, the feasibility of planting apple seedlings directly after removing the cherry trees in the aged cherry orchard has not been reported. Therefore, it is of great significance for horticulture production to explore the influence of aged cherry orchard soil on apple seedlings and its mechanism.

2. Materials and Methods

2.1. Study Sites and Soil Sampling

The study was conducted at the National Apple Engineering Center Test Base (36°9′29″ N, 117°9′4″ E) on the Panhe Campus of Shandong Agricultural University (Tai'an, China) in 2022. The aged apple orchard soil was taken from a 32-year-aged apple orchard (the root stock was *Malus micromalus* Makino) in Manzhuang Town, Tai'an City, Shandong Province, China, and the aged cherry soil was taken from a 20-year-aged cherry orchard (the root stock was *Colt*) in Tianbao Town, Xintai City, Shandong Province, China. *M. hupehensis* Rehd (apple rootstock commonly used in China) seedlings were selected as the experimental materials. The chemical properties of soil in the cherry orchard were as follows: available potassium (AK) 21 mg kg^{-1}, available phosphorus (AP) 23 mg kg^{-1}, ammonium nitrogen (NH_4^+) 2 mg kg^{-1}, nitrate nitrogen (NO_3^-) 3 mg kg^{-1}, and organic matter (OM) 7 g kg^{-1}. The chemical properties of the apple orchard soil were as follows: AK 15 mg kg^{-1}, AP 12 mg kg^{-1}, NH_4^+ 2 mg kg^{-1}, NO_3^- 1 mg kg^{-1}, and OM 6 g kg^{-1}.

M. hupehensis seeds were stratified at 4 °C for approximately 40 d. After germination, they were placed in a seedling tray for growth, and the *M. hupehensis* seedlings were left to grow to six true leaves. Finally, those that had basically grown the same amount were transplanted into pots with different treatments (upper inner diameter 25 cm, lower inner diameter 17 cm, and height 18 cm), and one seedling was planted in each pot.

2.2. Pot Experiment

The first part of this study consisted of three treatments: aged apple orchard soil (ppl), aged cherry orchard soil (pyl), and aged cherry orchard soil that had been fumigated with methyl bromide (pyz). Before transplantation, methyl bromide was mixed with the aged cherry orchard soil and placed into plastic film for sealing and fumigation on 11 April 2022 for 21 d. The aged cherry orchard soil and the aged apple orchard soil were not treated. The three soils were potted separately on 2 May 2022, and the seedlings were planted on May 4. Ten pots were established for each treatment, and normal fertilizer and water management were unified after planting. On 15 August 2022, the *M. hupehensis* seedlings and corresponding soil samples were collected to determine the relevant indicators. Three pots were randomly selected for each treatment for use as three replicates. During sampling, the topsoil was removed from the pot and the rhizosphere soil was collected at a depth of about 10 cm, sifted through a 2 mm sieve, and placed into three sealed bags. Two of the bags were stored in a refrigerator at 4 °C and −80 °C for the determination to measure the quantity of microbes and DNA extraction. The last bag was naturally air-dried for the measurement of phenolic acid content. Finally, three seedlings were collected from each treatment; after being washed, samples were subject to root scanning, and biomass, root vitality, antioxidant activity, and malondialdehyde content measurements were taken.

The second experiment was conducted as follows: Under sand cultivation conditions, *M. hupehensis* seedlings were treated with four allelochemicals (gallic acid, benzoic acid, ferulic acid, amygdalin) and mixed solutions, The application concentration is the measured concentration in the soil of aged cherry orchards. The experiment was divided into six treatments: no allelochemicals (CK2), gallic acid (MP), benzoic acid (BP), ferulic acid (AP), amygdalin (KP), and a mixture of the four allelochemicals (HP), with six replicates per treatment. The concentrations of gallic acid, benzoic acid, ferulic acid, and amygdalin were 3.95 mg kg^{-1}, 9.96 mg kg^{-1}, 7.11 mg kg^{-1}, and 2.56 mg kg^{-1}, respectively. Based on the content of four types of allelochemicals measured, solutions of the four types of compounds were prepared. The treatment group was treated with 20 mL of solutions every 5 days, and the same amount of water was used on the CK2 treatment. After 30 days, three seedlings were collected from each treatment. After being washed, samples were subjected to root scanning, and biomass, root vitality, antioxidant activity and malondialdehyde content measurements were taken.

2.3. Measurement Indicators and Methods

2.3.1. Determination of the Seedling Biomass of *M. hupehensis*

The seedlings of *M. hupehensis* were sampled on 15 August and 15 September 2022, and the plant height and ground diameter of the seedlings were determined using a straightedge and Vernier caliper. The ground parts and root systems of the plants were completely removed, washed, and dried in a cool place. The fresh quality was determined using a balance, and then the green was deoxidized by oven drying at 105 °C for 30 min. After that, the dry weight was determined by a constant reuse balance at 80 °C.

2.3.2. Determination of the Growth of *M. hupehensis* Seedling Roots

The roots were spread on hard plastic sheets with water. A professional version of the WinRHIZO (2016 version; Ruifeng, Guangzhou, China) root analysis system was used to record the total root length, root area, root volume, and root tips.

2.3.3. Determination of the Root Vitality, Antioxidant Activity, and Malondialdehyde Content of *M. hupehensis* Seedlings

Roots were washed in clean water, and 0.5 g of fresh white roots was taken from the seedling roots and divided evenly into 0.1 cm segments. An Oxytherm oxygen electrode automatic measurement system (Hansatech, King's Lynn, UK) was used to determine the vitality of the roots, as described by Mao et al. [28].

The content of MDA was determined using the thiobarbituric acid method [29]. A total of 0.5 g of frozen roots was homogenized with 5 mL 10% trichloroacetic acid in a mortar and pestle and then centrifuged for 10 min at 4000 rpm at 4 °C. Two-component spectrophotometry was then used to directly calculate the concentration of MDA in plant samples.

The activity of root superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were determined as previously described [30]. The activity of SOD was determined using nitro blue tetrazolium (NBT) photoreduction. The activity of POD was determined by measuring the oxidation of guaiacol. The activities of CAT were determined using ultraviolet spectrophotometry. The test was conducted in triplicate.

2.3.4. Determination of the Contents of Allelochemicals in the Soil

The soil allelochemicals content was measured using the method of Yin et al. [31]. A sample of dry soil (100 g) was passed through a 12-mesh-sized sieve, mixed with diatomaceous earth, and placed into a 100 mL extraction tank. The ASE 350 Fast Solvent Extractor (Sunnyvale, CA, USA) was used to perform the extraction. First, absolute ethanol was used as the extraction solvent, and static extraction was performed for 5 min at 120 °C and 10.3 MPa two times, followed by a purge volume of 60% and a purge time of 90 s. Next, the same sample was extracted again under the same conditions using methanol as the extraction solvent. After the extraction was completed, the two solvents were mixed and concentrated under reduced pressure at 34 °C to near dryness, and the sample was then reconstituted with 1 mL methanol and passed through a 0.22 μm organic phase filter membrane for HPLC analysis.

The HPLC procedure followed that described by Xiang et al. [32], with some modifications. An UltiMate 3000 HPLC system (Dionex) with an Acclaim 120 C18 column (3 μm, 150 mm × 3 mm) and a column temperature of 30 °C were used for quantification. The mobile phase A was acetonitrile, and the mobile phase B was water (adjusted to pH 2.6 with acetic acid). The flow rate was 0.5 mL min^{-1}, the automatic injection volume was 5 μL, and the detection wavelength was 280 nm. All reagents were chromatographic grade.

2.3.5. Determination of the Culturable Microorganisms in Soil

Soil microbial numbers were determined by a conventional plate count method. Briefly, 10 g of soil was added to 90 g of sterile distilled water and mixed at an appropriate shaker speed. Bacteria on Luria broth/AGAR plates and fungi were cultured on potato dextrose agar (PDA plates). The bacteria were cultured at 28 °C for 24 h and the fungi cultured at 28 °C for 48 h before observing and counting colonies.

2.3.6. Determination of the Gene Copy Number of the Soil Fungi

The gene copy numbers of four species of *Fusarium* species in the soil were determined from the different treatments, as described by Duan et al. [33] and Whelan et al. [34]. The CFX Connect system (Bio-Rad, Hercules, CA, USA) was used to determine the expression levels of four species of *Fusarium* species genes in the soil by real-time quantitative PCR. The primer pairs used in this experiment were as follows: JR (5′GGCCTGAGGGTTGTAATG-3′) × JF (5′CATACCACTTGTTGTCTCGGC-3′) for *F. oxysporum*; CHR (5′GACTCGCGAGTCAA ATCGCGT-3′) × CHF (5′GGGGTTTAACGGCGTGGCC-3′) for *F. moniliforme*; CR (5′GATCG GCGAGCCCTTGCGGCAAG-3′) × CF (5′CGCCGCGTACCAGTTGCGAGGGT-3′) for *F. proliferatum*; FR (5′CGAGTTATACAACTCATCAACC-3′) × FF (5′GGCCTGAGGGTTGTAA TG-3′) for *F. solani.* The reactions were performed according to the instructions of the SYBR Premix Ex Taq kit (TaKaRa Biotech Co., Ltd., Dalian, China). Each 25 μL reaction contained 1.5 μL of DNA, 12.5 μL of SYBR Premix Ex Taq II, 1 μL of each primer, and 9 μL of sterile double-distilled water. The thermal cycling parameters were as follows: predenaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s. A final extension at 72 °C for 10 min was also included.

2.3.7. DNA Extraction and High-Throughput Sequencing Analysis

DNA was extracted using the Fast DNA SPIN Soil kit (MP Biomedicals, Solon, OH, USA) and quantified using the NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The fungi ITS region was double-end sequenced using the Illumina MiSeq platform (www.i-sanger.com) URL (accessed on 5 October 2023). PCR amplification of the 16S rRNA gene was conducted using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGTCAATTCMTTTGAGTTT-3′). The sequences of the primers were ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′).

2.4. Statistical Analysis

All statistical analyses were performed with IBM SPSS 20.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA). Different lowercase letters represent significant differences between treatments (one-way ANOVA, $p < 0.05$) according to Duncan's multiple range test. The figures were plotted using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism 7.0 (GraphPad software, Inc., San Diego, CA, USA).

The R statistical platform (v.4.1.1) was used for principal component analysis (PCoA), Venn plots, and fungus community bar plot analysis to study differences in community composition among samples. Differences among samples were calculated using Bray–Curtis dissimilarity, and analysis of similarity (ANOSIM) was performed to identify significant differences among the fungal communities.

3. Results

3.1. Effects of Aged Cherry Orchard Soil on the Growth of M. hupehensis Seedlings

3.1.1. Effects of Aged Cherry Orchard Soil on the Biomass of *M. hupehensis* Seedlings

As shown in Table 1, the biomass of *M. hupehensis* seedlings planted in aged cherry orchard soil was significantly lower than that planted in aged cherry orchard soil fumigated with methyl bromide, but it was slightly higher than that planted in aged apple orchard soil. The plant height, ground diameter, fresh weight, and dry weight decreased by 20.06%, 26.09%, 42.65%, and 31.73%, respectively, in August compared with the methyl bromide treatment, and increased by 13.37%, 13.71%, 26.81%, and 33.81%, respectively, compared with the aged apple orchard soil treatment. In September, the plant height and ground diameter decreased by 12.75% and 12.66%, respectively, compared with the methyl bromide treatment and increased by 16.65% and 11.63%, respectively, compared with aged apple orchard soil treatment.

Table 1. Effects of different treatments on the parameters of *Malus hupehensis* Rehd. seedling growth.

Sampling Time Year-Month	Treatment	Plant Height (cm)	Ground Diameter (mm)	Fresh Mass (g)	Dry Mass (g)
2022-8	ppl	34.25 ± 1.96 c	4.45 ± 0.21 c	34.46 ± 0.96 c	13.99 ± 0.26 c
	pyl	38.83 ± 1.97 b	5.06 ± 0.24 b	43.70 ± 1.25 b	18.72 ± 0.65 b
	pyz	46.62 ± 2.23 a	6.38 ± 0.18 a	62.34 ± 1.13 a	24.66 ± 1.21 a
2022-9	ppl	58.61 ± 1.43 c	9.203 ± 0.43 c	——	——
	pyl	68.37 ± 1.36 b	10.27 ± 0.23 b	——	——
	pyz	77.09 ± 0.67 a	11.57 ± 0.42 a	——	——

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant soil from a 20-year-aged cherry orchard soil after fumigation with methyl bromide. SD, standard deviation.

3.1.2. Effects of Aged Cherry Orchard Soil on the Root System of *M. hupehensis* Seedlings

As shown in Table 2, although the root growth in aged cherry orchard soil (pyl) improved compared with the aged apple orchard soil (ppl), the growth of *M. hupehensis*

seedling roots was significantly inhibited compared with the aged cherry orchard soil that had been fumigated with methyl bromide (pyz). Compared with the aged apple orchard soil (ppl), the root length, root surface area, root volume, and root tip numbers of the aged cherry orchard seedlings increased by 16.32%, 35.17%, 18.46%, and 14.98%, respectively. However, the root length, root surface area, root volume, and root tip number decreased by 83.27%, 67.43%, 37.66%, and 44.84%, respectively, compared with methyl bromide fumigation and soil treatment (pyz).

Table 2. Effects of different treatments on the root architectural parameters of *Malus hupehensis* Rehd. seedlings.

Treatments	Length (cm)	Surface Area (cm^2)	Volume (cm^3)	Tips
ppl	467.58 ± 6.78 c	187.28 ± 3.27 c	7.80 ± 3.01 c	2083 ± 38.21 c
pyl	543.89 ± 3.48 b	235.15 ± 2.31 b	9.24 ± 2.86 b	2395 ± 16.54 b
pyz	996.77 ± 12.65 a	393.71 ± 7.46 a	12.72 ± 4.36 a	3469 ± 26.59 a

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant in a 20-year-aged cherry orchard soil after fumigation with methyl bromide. SD, standard deviation.

3.1.3. The Effect of Different Treatments on the Activities of Root Antioxidant Enzymes, Root Vitality and MDA Content

As shown in Table 3, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) from the *M. hupehensis* seedling roots increased by 17.62%, 15.84%, and 12.21%, respectively, in the soil of an aged cherry orchard compared with that in the soil of an aged apple orchard, and the content of MDA decreased by 26.92%. Compared with methyl bromide fumigation, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of *M. hupehensis* seedling in aged cherry orchard soil were reduced by 11.42%, 44.34%, and 48.7%, respectively. MDA content was increased by 50%. In terms of root vitality, compared with the aged apple orchard soil and the aged cherry orchard soil, the root vitality of *M. hupehensis* seedlings in the soil after fumigation with methyl bromide increased significantly, by 87.60% and 73.06%, respectively. The root vitality of the aged cherry orchard soil was slightly higher than that of the aged apple orchard soil.

Table 3. The effect of different treatments on the activities of root antioxidant enzymes, root vitality, and MDA content.

Treatments	SOD ($Umin^{-1}g^{-1}$, FW)	POD ($Umin^{-1}g^{-1}$, FW)	CAT ($Umin^{-1}g^{-1}$, FW)	MDA ($\mu molg^{-1}$, FW)	Root Vitality ($\mu molg^{-1}$, FW)
ppl	116.9 ± 0.93 c	18.3 ± 0.51 b	17.2 ± 1.04 c	1.32 ± 0.08 a	354.7 ± 11.64 b
pyl	137.5 ± 0.99 b	21.2 ± 0.42 c	19.3 ± 0.49 b	1.04 ± 0.13 b	384.5 ± 22.75 b
pyz	153.2 ± 0.58 a	30.6 ± 0.64 a	28.7 ± 1.60 a	0.52 ± 0.02 c	665.4 ± 16.41 a

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant in 20-year-aged cherry orchard soil after fumigation with methyl bromide. SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde; SD, standard deviation.

3.2. Number of Culturable Microorganisms in the Different Soil Treatments

Table 4 shows that there were lower numbers of bacteria and fungi in the soil of an aged cherry orchard than in the soil of an aged apple orchard in August and September. However, looking at the two months of data separately, the ratio of bacteria to fungi did not differ significantly. There were fewer bacteria and fungi in the soil of an aged cherry orchard treated by methyl bromide. The ratio of bacteria to fungi was much higher than that in the previous two treatments.

Table 4. Effects of different treatments on the number of cultivatable microorganisms in the soil.

Sampling Time Year–Month	Treatments	Bacteria ($\times 10^5$ CFU g^{-1} Soil)	Fungi ($\times 10^3$ CFU g^{-1} Soil)	The Ratio of Bacteria and Fungi
2022–8	ppl	30.00 ± 2.00 a	24.00 ± 3.61 a	125
	pyl	17.67 ± 3.06 b	12.62 ± 0.58 b	140
	pyz	7.67 ± 1.53 c	1.00 ± 0.00 c	767
2022–9	ppl	44.66 ± 1.52 a	36.67 ± 2.08 a	161
	pyl	40.35 ± 1.92 b	25.67 ± 1.04 b	157
	pyz	30.33 ± 1.15 c	8.33 ± 2.31 c	364

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant in a 20-year-aged cherry orchard soil after fumigation with methyl bromide. SD, significant difference.

3.3. Effects of Different Treatments on the Soil Microbial Communities

3.3.1. Changes in the Gene Copy Number of Four Pathogens of Apple under Different Soil Treatments

The results of a real-time fluorescence quantitative analysis showed that after fumigation with methyl bromide, the growth of four pathogenic species of *Fusarium* could be effectively limited (Figure 1A). Among them, compared with aging apple orchard soil, the quantity of *F. oxysporum*, *F. solani*, *F. proliferatum*, and *F. moniliforme* in aging cherry orchard soil decreased by 158.89%, 17.09%, 807.92%, and 131.87%, respectively. Compared with bromo-fumigated aged cherry orchard soil, the quantity of *F. oxysporum*, *F. solani*, *F. proliferatum,* and *F. moniliforme* in aged cherry orchard soil increased by 3326.26%, 618.76%, 31.44%, and 99.17%, respectively.

3.3.2. Analysis of the Common and Endemic Communities of Soil Fungi after Different Treatments

Venn diagrams can clearly show the numerical composition, specificity, or similarity of the fungal community at the genus level in soil samples. As shown in Figure 1B, the soil samples of all treatments shared 197 OTUs, that is, 197 common species. In addition, the number of unique fungi in different soil samples showed a certain difference. A total of 323 genera were unique to the aged apple orchard soil treatment (ppl), and 335 genera were unique to the aged cherry orchard soil treatment (pyl). The aged cherry orchard soil treatment of fumigation with methyl bromide (pyz) had 312 genera; ppl and pyl had 227 genera; ppl and pyz had 223 genera; and pyl and pyz had 270 genera.

3.3.3. Effects of Different Treatments on the Soil Fungal Community Composition

At the genus level, the soil fungal community composition of the three soil samples was similar, but there were differences in the relative abundance of fungal genus. As shown in Figure 1C, the fungi genera with high relative abundance in the soil of aged apple orchard (ppl) included *Mortierella*, *Chaetomium*, *Solicoccozyma*, *Rozellomycota*, *Cryptococcus,* and *Fusarium*. Compared with the soil of aged apple orchard (ppl), the relative abundances of *Mortierella* and *Fusarium* in the aged cherry orchard soil (pyl) had decreased, while the relative abundances of *Chaetomium* and *Solicoccozyma* had increased significantly. Compared with aged cherry orchard soil (pyl), the relative abundance of *Mortierella* in aged cherry orchard soil (pyz) fumigated with methyl bromide increased significantly, while the relative abundance of *Chaetomium* and *Fusarium* decreased significantly. The results showed that the relative abundance of dominant fungi in soil vary under different treatments.

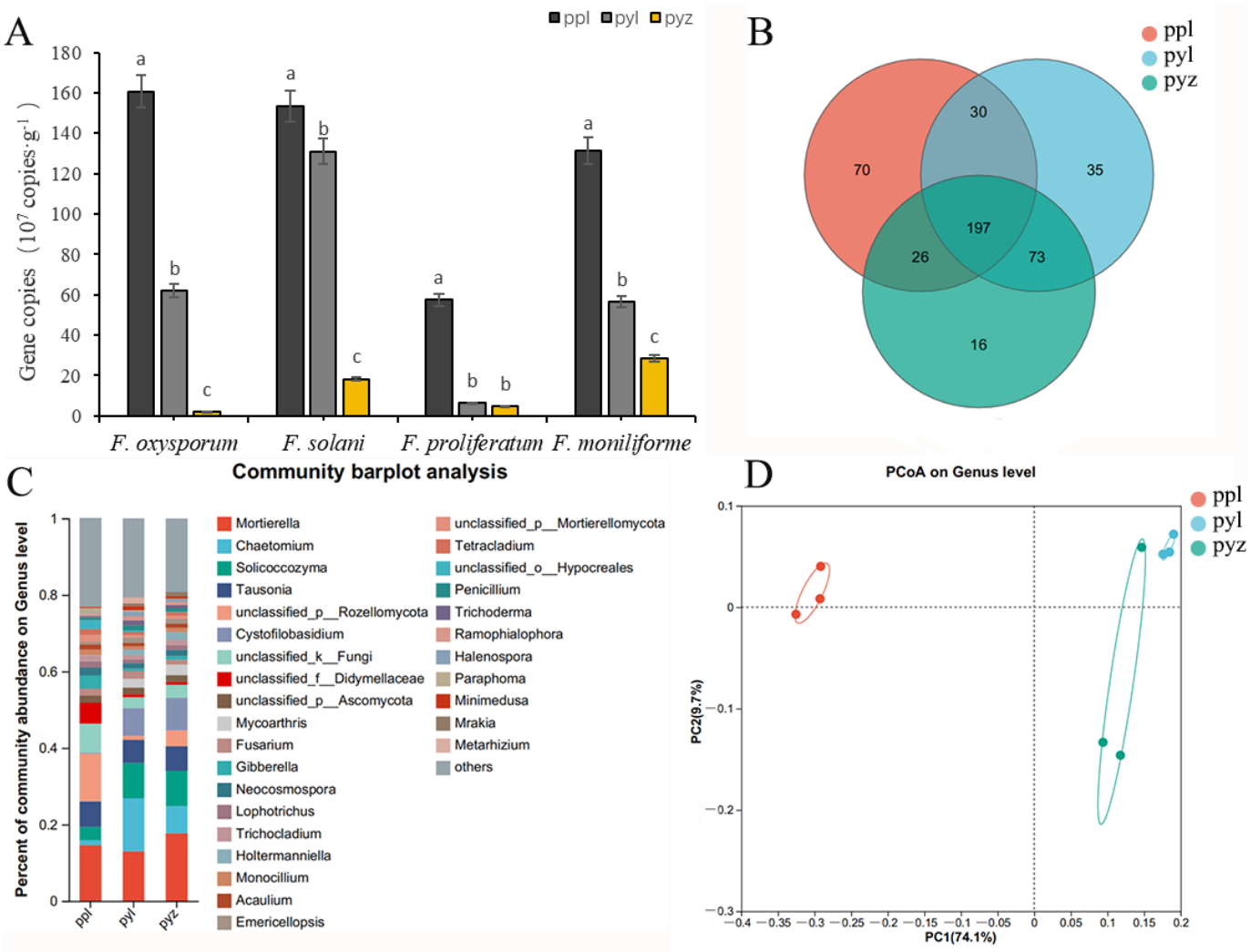

Figure 1. Effects of different treatments on the soil microbial communities. (**A**) Changes in the gene copy number of four apple pathogens under different soil treatments. (**B**) Analysis of the common and endemic communities of soil fungi after different treatments. (**C**) Effects of different treatments on the soil fungal community composition. (**D**) Principal coordinate analysis (PCoA) of the soil fungal community in different treatments. Lowercase letters indicate significant differences ($p < 0.05$). ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant soil from a 20-year-aged cherry orchard after methyl bromide fumigation.

3.3.4. Principal Coordinates Analysis (PCoA) of the Soil Fungal Community

A PcoA was used to analyze the differences in soil microbial diversity among the different treatments (Figure 1D). It showed that the treatment of aged apple orchard soil (ppl) was completely separated from and distant from that of the aged cherry orchard (pyl) and aged cherry orchard fumigated with methyl bromide (pyz). This indicates that there are significant differences between the aged apple orchard soil treatment (ppl) and the aged cherry orchard soil treatment (pyl) and the aged cherry orchard fumigated soil treatment (pyz), while the soil fungal community in the aged cherry orchard soil treatment (pyl) and the aged cherry orchard fumigated soil treatment (pyz) were closer. These results indicated that there was no significant difference in the diversity of fungal community between the aged cherry orchard soil treatment (pyl) and the treatment that had been fumigated (pyz). The PC1 values were 74.1%, and PC2 values were 9.7%, which accounted for 83.8% of the difference in diversity. These results indicate that different soil treatments may be one of the important factors related to the change in microbial community structure.

3.4. Determination of the Contents of Allelochemicals in Different Soils

A number of allelochemicals were detected in the soil, including gallic acid, phloretin, phloroglucinol, ferulic acid, benzoic acid, phlorizin, amygdalin, and cinnamic acid among others (Table 5). The contents of gallic acid, ferulic acid and benzoic acid in the aged cherry orchard soil (pyl) were higher than those in the aged apple orchard soil (ppl) by 15.19-, 2.52- and 1.24-fold, respectively. In addition, there was a lower content of phloroglucinol, which was reduced by 87%. Amygdalin was not found in the soil of the aged apple orchard, while phloretin and phlorizin were not found in the soil of aged cherry orchard. The contents of gallic acid, phloroglucinol, ferulic acid, benzoic acid, amygdalin and cinnamic acid in the soil treated with methyl bromide fumigation (pyz) compared to unfumigated aged cherry orchard soil (pyl) decreased significantly by 0.22-, 0.65-, 0.72-, 0.54-, 0.57- and 0.94-fold, respectively.

Table 5. Determination of the contents of phenolic acids in different soils.

Types of Allelochemicals (mg kg^{-1})	ppl	pyl	pyz
Gallic acid	0.26 ± 0.05 c	3.95 ± 0.84 a	0.87 ± 0.02 b
Phloretin	3.23 ± 0.64	-	-
Phloroglucinol	7.25 ± 0.81 a	3.87 ± 0.74 b	2.51 ± 0.26 c
Ferulic acid	2.82 ± 0.58 c	7.11 ± 0.61 a	5.11 ± 0.61 b
Benzoic acid	8.02 ± 0.97 b	9.96 ± 0.43 a	5.41 ± 0.82 c
Phlorizin	4.56 ± 0.45	-	-
Amygdalin	-	2.56 ± 0.64	1.45 ± 0.09
Cinnamic acid	2.18 ± 0.02 a	0.54 ± 0.03 b	0.51 ± 0.05 b

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). Because amygdalin was not found in the soil of the aged apple orchard, while phloretin and phlorizin were not found in the soil of aged cherry orchard, these three substances were not analyzed for significance. ppl, replant soil from a 32-year-aged apple orchard; pyl, replant soil from a 20-year-aged cherry orchard; pyz, replant soil from a 20-year-aged cherry orchard soil after fumigation with methyl bromide; SD, standard deviation.

3.5. Effects of Allelochemicals in the Aged Cherry Orchard Soil on M. hupehensis Seedlings

3.5.1. Effects of the Primary Allelopathic Compounds in Aged Cherry Orchard Soil on the Biomass of *M. hupehensis* Seedlings

As shown in Table 6, compared with the control (CK2), the concentrations of allelochemicals in the soil of aged cherry orchards can inhibit the growth of *M. hupehensis* seedlings. The mixture of allelochemicals (HP) clearly had the strongest effects, and its plant height, fresh weight, and dry weight were 45.38%, 44.90%, and 23.52% of that of the control group (CK2), respectively. Compared with the single allelochemicals treatments (MP, BP, AP, and KP), amygdalin (KP) damaged the seedlings the most. The difference of other treatments was not significant, but compared with the control, they still had some inhibitory effects.

Table 6. Effects of the primary allelopathic compounds in aged cherry orchard soil on the biomass of *M. hupehensis* seedlings.

Treatment	Plant Height (cm)	Fresh Weight (g)	Dry Weight (g)
CK2	14.83 ± 1.79 a	3.93 ± 0.2 a	1.72 ± 0.08 a
MP	10.00 ± 0.82 b	3.14 ± 0.63 b	1.38 ± 0.05 b
BP	11.33 ± 0.47 b	3.16 ± 0.09 b	1.42 ± 0.04 b
AP	10.67 ± 0.28 b	3.23 ± 0.46 b	0.77 ± 0.08 c
KP	7.17 ± 0.24 c	1.99 ± 0.19 c	0.45 ± 0.07 d
HP	6.83 ± 0.25 c	1.41 ± 0.26 d	0.40 ± 0.01 d

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). CK2, no allelochemicals; MP, gallic acid; BP, benzoic acid; AP, ferulic acid; KP, amygdalin; HP, mixture of four phenolic acids; SD, standard deviation.

3.5.2. Effects of the Allelochemicals in Aged Cherry Orchard Soil on the Activities of SOD, POD, and CAT and the Vitality of *M. hupehensis* Seedling Roots

As shown in Table 7, the activity of root protective enzymes and vitality of the *M. hupehensis* seedling roots decreased significantly after treatment with allelochemicals. The mixed allelochemicals treatment (HP) inhibited the *M. hupehensis* seedlings the most, and of the single allelochemicals treatments, amygdalin (KP) was the strongest inhibitor. The activities of SOD, POD, and CAT and the root vitality were 44.45%, 43.38%, 70.34%, and 16.22% of that of the control (CK2), respectively. There was no significant difference in the degree of inhibition of the seedlings following treatment with the other allelochemicals.

Table 7. Effects of the primary allelopathic compounds in aged cherry orchard soil on the activities of SOD, POD, and CAT and the vitality of the *Malus hupehensis* seedling roots.

Treatments	SOD ($Umin^{-1}g^{-1}$, FW)	POD ($Umin^{-1}g^{-1}$, FW)	CAT ($Umin^{-1}g^{-1}$, FW)	Root Vitality ($\mu molg^{-1}$, FW)
CK2	264.3 ± 17.40 a	58.50 ± 2.29 a	56.75 ± 0.22 a	571.33 ± 24.98 a
MP	158.4 ± 11.21 b	43.51 ± 1.54 b	46.40 ± 0.82 b	153.52 ± 12.16 c
BP	147.5 ± 12.05 b	31.54 ± 1.25 c	44.55 ± 0.27 b	352.14 ± 26.68 b
AP	163.2 ± 13.96 b	44.62 ± 1.03 b	46.55 ± 0.72 b	139.02 ± 8.10 c
KP	117.5 ± 12.37 c	25.38 ± 2.32 d	39.92 ± 0.47 c	92.67 ± 6.47 d
HP	104.8 ± 8.55 c	10.41 ± 1.75 e	34.825 ± 0.33 d	56.00 ± 4.12 e

Note: Data are presented as the mean ± SD, and different lowercase letters indicate significant differences ($p < 0.05$). CK2, control; MP, gallic acid; BP, benzoic acid; AP, ferulic acid; KP, amygdalin; HP, mixture of four phenolic acids; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; SD, standard deviation.

4. Discussion

Replant disease is a complex, multifaceted disease, which is common in the world and seriously restricts the development of horticulture production [35]. Others studies have shown that replantation disorders are caused by a combination of biological and abiotic factors, and chemical fumigation may be an effective way to solve this problem [36,37].

4.1. Effects of the Different Treatments on the Growth of M. hupehensis Seedlings

The size of biomass is a visual parameter that directly reflects the growth and development of the plant. Mazzola et al. [38] found that the distribution of microbial populations in the plant rhizosphere soil can affect the growth and development of other plants or their own plants. Simultaneously, many studies have also found that the accumulation of heterologous biomass in the soil owing to the previous crop can inhibit the accumulation of biomass in the next crop [39]. When plants are under stress, the generation and elimination of reactive oxygen species in the plant roots will become unregulated, which will result in a large accumulation of free radicals in the plants and a significant increase in the content of MDA [40]. This will accelerate aging in the plant roots. POD, SOD, and CAT in the plant can remove the free radicals in plant cells, reduce the damage to plant cell membranes, and improve the resistance of plants to stress [41]. In this study, the biomass and growth of *M. hupehensis* and the activities of its roots and their protective enzymes in seedlings that were directly planted in the soil of an aged cherry orchard were found to improve, compared with those planted in the soil of an aged apple orchard. However, these parameters were significantly lower than those from an aged cherry orchard soil that had been fumigated with methyl bromide or sterilized. The reason may be that the allelopathic compounds and pathogens in the soil of aged cherry orchard inactivated the SOD, POD, and CAT enzymes in the root cells, which resulted in the excessive accumulation of free radicals in the plants. This process caused stress on the roots. The root vitality and the activities of protective enzymes of the *M. hupehensis* seedlings decreased, and their growth and development were inhibited. In the aged cherry orchard soil fumigated with bromomethane, the strong oxidation of bromomethane will destroy the membrane structure of microorganisms and reduce the number of pathogens in the soil. Moreover, others studies have found that the phenolic acids secreted by plants in the soil can be oxidized and decomposed by oxidizing

chemicals to reduce toxicity [42,43]. These results were consistent with the findings of this study. Methyl bromide fumigation treatment significantly promoted the growth of *M. hupehensis* seedlings.

4.2. Effects of Different Treatments on the Soil Microbial Community Structure

Continuous cropping easily transforms the soil environment and provides a breeding site for pathogens to survive. The accumulation of harmful fungi and the increase in the number and types of autotoxic substances further aggravate the occurrence of soilborne diseases [44]. Others studies have shown that the accumulation of pathogenic fungi significantly reduces crop yields and increases the occurrence of diseases and pests [45]. Sun et al. [46] showed that a higher ratio of bacteria to fungi facilitated crop growth. In this study, it was found that although there were fewer fungi in the soil of aged cherry orchards than in that of aged apple orchards, the ratio of bacteria to fungi did not differ substantially. The numbers of bacteria and fungi in the soil of aged cherry orchard sterilized with methyl bromide were reduced, and the ratio of bacteria to fungi was significantly higher than that of the other two treatments. Thus, this indicated that more pathogenic fungi accumulated in the soil of aged cherry orchards. The soil had been transformed into "fungal-type" soil, which is no longer suitable for the growth of *M. hupehensis* seedlings.

Studies from China and other countries have shown that the primary pathogenic fungal genera of apple and continuous cropping disorder of apples include *Fusarium*, *Stylospora*, *Filamentus*, *Phytophthora,* and *Saprophyus*. The pathogenic fungi in the continuous cropping orchards of different countries and regions vary [47]. *Fusarium* is the primary pathogenic fungus in the continuous crop orchards in the Bohai Rim region of China, and pot experiments verified that four pathogens, namely, *F. oxysporum*, *F. monizonoides*, *F. proliferatum,* and *F. solani*, were highly pathogenic to *M. hupehensis* seedlings [48]. This study showed that, compared with the soil of an aged apple orchard, *F. proliferatum* was less abundant in the soil of aged cherry orchards. However, the other three pathogenic fungi were more abundant, which indicated that the soil of aged cherry orchard still had a certain inhibitory effect on the growth of *M. hupehensis* seedlings.

When monocultures are grown on soil, the soil microbial community structure will deteriorate, and the species diversity will tend to become homogeneous. This will provide a favorable environment for pathogens, inhibit the propagation of beneficial bacteria, and ultimately reduce crop yields [49,50]. In addition, it is well known that *Mortierella* fungi have substantial potential for controlling soilborne diseases [51,52]; it is considered to be an indicator of soil health. In this study, high-throughput sequencing showed that the relative abundance of *Fusarium* in aged cherry orchard soil (pyz) fumigated with methyl bromide decreased compared with the aged cherry orchard soil control (pyl), and the relative abundance of *Mortierella* increased significantly. This showed that the soil of the aged cherry orchards was not suitable for the growth of *M. hupehensis* seedlings. The soil microbial community structure improved, and the soil environment was optimized after fumigation with methyl bromide.

4.3. Effects of Allelochemicals on the Seedlings

Studies have shown that allelochemicals in the soil can directly or indirectly affect plant growth [53]. Allelochemicals produced by some distantly related crops can reduce plant diseases, for example, the root exudates of lettuce can effectively inhibit the number of fusarium, reduce the occurrence of root and stem rot of cucumber, and increase the yield of cucumber [54]. But most allelopathic compounds have negative effects on plants, and the phenolic acids that cause continuous cropping obstacles of apple are among them [15]. They can also indirectly hinder the further growth and development of plants by affecting soil microorganisms, soil nutrients, and soil enzyme activities [55]. Rice et al. [56] showed that there may be a low concentration of allelopathic autotoxic compounds, such as phenolic acids, when quantified, and they may not be uniformly distributed in the soil. In addition, the content of allelopathic autotoxic compounds in the microecological environment close

to the root will be relatively high. Weir et al. [57] conducted an extensive amount of research that showed that the most fundamental mechanism of allelopathic autotoxicity to plant damage occurs by damaging the cell membrane system of plants. In this study, the types and contents of allelochemicals in the soil of aged apple orchard and aged cherry orchard were different, but gallic acid, benzoic acid, ferulic acid, and amygdalin were all not conducive to the growth of *M. hupehensis* seedlings, and the mixture of several allelochemicals substances inhibited the seedling growth more strongly; this indicates that different allelochemicals may have similar inhibitory effects on seedling growth. In addition, different types of allelochemicals have different degrees of inhibition on plants, which may be related to the different concentration thresholds of allelopathy of different types of allelochemicals; this result is consistent with those of Wang [58]. On this basis, the activities of SOD, POD, and CAT decreased significantly, and the root vitality of *M. hupehensis* seedlings was inhibited. Thus, these factors limit the growth potential of plants.

5. Conclusions

This study showed that, the biomass of *M. hupehensis*, root vitality and the activities of protective enzymes in seedlings that were directly planted in the soil of an aged cherry orchard were found to improve compared with those planted in the soil of an aged apple orchard. However, these parameters were significantly lower than those from an aged cherry orchard soil that had been fumigated with methyl bromide. Therefore, we further studied the factors affecting the growth of *M. hupehensis* seedlings. In terms of biological factors, we found that although the old cherry orchard soil and the old apple orchard soil had different fungal community structure, the abundance of *Fusarium* was still high, and the soil had been transformed into "fungal-type" soil, which is no longer suitable for the growth of *M. hupehensis* seedlings. In terms of abiotic factors, allelochemicals in the soil of aged cherry orchard also had a certain inhibitory effect on the growth of *M. hupehensis* seedlings, among which the concentration of amygdalin was low, but the inhibitory effect was the strongest. In summary, direct planting of *M. hupehensis* in aged cherry orchard soil inhibited seedling growth; this effect was mainly caused by the presence of harmful microorganisms and amygdalin in the soil, and some measures are needed to eliminate these factors. If methyl bromide is not approved in certain countries or regions, irradiation, heat treatments, solarization, or other chemical (organic) fumigation should be considered in the future (methyl bromide will be phased out worldwide).

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