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Amygdalin and Benzoic Acid on the Influences of the Soil Environment and Growth of *Malus hupehensis* Rehd. Seedlings

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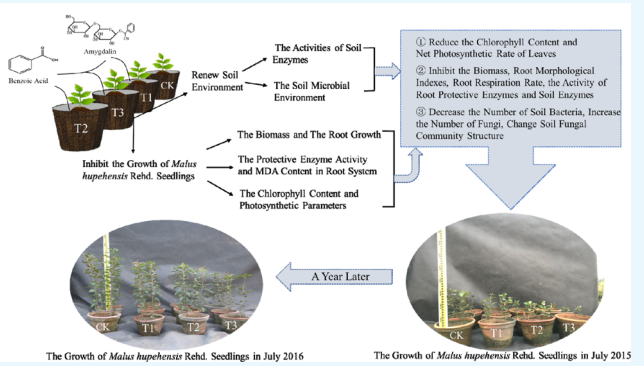
ABSTRACT: Crop rotation in fruit trees is an effective approach for addressing some of the problems of continuous cropping. To determine whether aged peach orchard soil is suitable for planting apple trees, we studied the effects of two substances abundant in aged peach orchard soil—amygdalin and benzoic acid—on the soil microbial community structure, soil enzyme activity, and the growth of *Malus hupehensis* Rehd. seedlings. Soils treated with amygdalin (T1), benzoic acid (T2), and a mixed solution of amygdalin and benzoic acid (T3) were used to plant *M. hupehensis* Rehd. seedlings. Compared with fallow (control) soil, the soil microbial community structure, soil enzyme activities, and root protective enzyme activities, leaf chlorophyll content, and net photosynthetic rate decreased in the three treatments. The biomass and root index of *M. hupehensis* Rehd. seedlings significantly decreased. Compared with T3, the plant height, ground diameter, fresh weight, dry weight, root length, root surface area, root volume, and root respiration rate of *M. hupehensis* Rehd. seedlings in T2 in 2015 (2016 in parentheses) decreased by 19.3% (12.6%), 8.7% (7.1%), 21.2% (13.3%), 9.1% (19.6%), 7.9% (25.3%), 40.7% (28.8%), 46.2% (21.1%), and 44.2% (27.5%), respectively. Compared with T3, the same variables in T1 in 2015 (2016 in parentheses) decreased by 34.9% (16.7%), 27.6% (9.8%), 53.6% (19.4%), and 50% (20.5%), 24.1% (31.4%), 55.1% (37.6%), 63.2% (28.2%), and 47.0% (28.7%), respectively. Thus, the inhibitory effect of T3 was the strongest, followed by T2 and T1. In sum, amygdalin and benzoic acid are harmful substances in aged peach orchard soil that inhibit the growth of *M. hupehensis* Rehd. seedlings.



1. INTRODUCTION

The replanting of new peach varieties has become essential in the face of limited land resources.[1,2] One of the major challenges of replanting is the inhibition of plant growth caused by the continuous planting of the same crop or closely related crops in the same plot.[3] Replant obstacles are universal and have become a major global problem now. At present, crop rotation is the most effective and commonly used method to overcome this problem.[4]

Previous studies have shown that allelopathic substances secreted by roots are the cause of the inhibition of plant growth in replant soil,[5] and allelopathic effects are widespread in ecosystems.[6] Phenolic acids, which are formed by the combination of a hydroxyl group (−OH) with an aromatic hydrocarbon group, are the most important and common plant allelopathic substances and have received increased attention in recent years.[7,8] Previous studies have shown that some phenolic acids found in plant root exudates may be active allelopathic substances that negatively affect the growth of related plants.[9,10] Lodhi[11] found that the undergrowth of red oak and white oak forests was often stunted or bare because of phenolic acids, and the degradation of soil fertility caused by artificial fast-growing forests of Chinese fir and eucalyptus trees greatly contributed to the decline in productivity, which was related to the accumulation of phenolic acids in soil. Wei et al.[12] found that the leaf extracts of *Solidago canadensis* L. and *Conyza canadensis* L. significantly inhibited the germination and seedling growth of lettuce seeds. Hu and Zhang[13] found that different tissue extracts of *Nitraria* significantly inhibited the growth of the three weeds, and the allelopathic compounds produced by their tissues were one of the reasons for their successful invasion in South China. The root systems of eggplant, peanut, and soybean have been shown to secrete a

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variety of phenolic acids that can have toxic effects and reduce the incidence of crop diseases.[14−16] The accumulation of phenolic acid self-toxic substances in soil has been identified to be an important cause of apple replant disease.[17] Zhang et al.[18] found that six types of phenolic acids in apple orchard soil could inhibit the growth of *Malus hupehensis* Rehd. seedlings, and benzoic acid had the greatest inhibitory effect. Previous studies showed that amygdalin, a typical secondary metabolite of peach, was abundant in peach trees and could be degraded by residues to form hydrocyanic acid (HCN), which seriously damaged the next crop.[19] Another study found that peach orchard soil contains large amounts of phenolic acids, such as amygdalin and benzoic acid, which adversely affects the replanting of peach trees and thus reduces the production of new peach orchards.[20,21] Sotomayor[22] research also showed that amygdalin is a very important factor in the process of peach replantation. Yang[23] identified benzoic acid, cinnamic acid, caffeic acid, syringic acid, and ferulic acid in peach orchard soil and found that benzoic acid had the highest content.

Although allelochemicals have been shown to have a strong inhibitory effect on growth and soil enzyme activity under continuous monoculture,[24] few studies have assessed whether the main allelochemicals in the aged soil of peach orchard affect the growth of apple seedlings and whether it is feasible to remove peach trees and plant apple seedlings directly. In this study, we used *M. hupehensis* (Pamp.) Rehd. var. *pingyiensis* (hereafter referred to as *M. hupehensis* Rehd. seedlings), a common rootstock of apple, as the test material.[25] Studies have shown that *M. hupehensis* Rehd. seedlings have the problem of replanting obstacles.[26−30] In this study, amygdalin and benzoic acid in peach orchard soil were applied to fallow land to explore the effects of amygdalin and benzoic acid on fallow soil and *M. hupehensis* Rehd. seedlings. The feasibility of removing peach trees and directly planting apple trees was explored. Generally, our study provides new insights into how aged peach orchards can be transformed into apple orchards.

2. RESULTS AND DISCUSSION

2.1. Effect of Amygdalin and Benzoic Acid in Peach Orchard Soil on the Biomass of *M. hupehensis* Rehd. Seedlings. In both years of data, the biomass of *M. hupehensis* Rehd. seedlings treated with amygdalin (T1), benzoic acid (T2), and both amygdalin and benzoic acid (T3) were significantly lower compared with the control group (Table 1); specifically, the biomass of seedlings was lowest in T3, followed by T2, T1, and CK. In 2015, compared with CK, the plant height, ground diameter, fresh weight, and dry weight were reduced by 27.4, 28.9, 50.9, and 46.9% in T1; 41.3, 44.0, 71.6, and 70.1% in T2; and 52.6, 48.2, 77.7, and 73.6% in T3, respectively. Wang et al.[31] found that five phenolic acids in the soil of the aged apple orchard substantially inhibited the growth, biomass, and development of the subsequent crop of *M. hupehensis* Rehd. seedlings, and the toxic effect of phloridzin was the strongest. Zhu et al.[21] found that the physiological activities of peach seedlings were severely inhibited after the addition of root extract, and the root exudates of aged peach orchard soil were thought to contain a large amount of amygdalin and benzoic acid. In this experiment, the concentrations of amygdalin, benzoic acid, and a solution containing both amygdalin and benzoic acid in aged peach orchard soil were applied to fallow soil. The biomass of *M. hupehensis* Rehd. seedlings was significantly reduced in all three treatments. The inhibitory effect of amygdalin was slightly weaker than that of benzoic acid, and the inhibitory effect of the mixed treatment was the strongest, indicating that the concentrations of amygdalin and benzoic acid in aged peach orchard soil could inhibit the growth of *M. hupehensis* Rehd. seedlings.

Table 1. Effect of Amygdalin and Benzoic Acid on the Plant Biomass of *Malus hupehensis* Rehd. Seedlings[a,b]

date	treatment	height (cm)	ground diameter (mm)	fresh weight (g)	dry weight (g)
July 15, 2015	CK	29.2a	4.1a	11.4a	3.7a
	T1	21.2b	2.9b	5.6b	2.0b
	T2	17.1c	2.3c	3.3c	1.1c
	T3	13.8d	2.1c	2.6c	1.0c
July 15, 2016	CK	75.6a	12.8a	76.9a	34.5a
	T1	64.0b	10.2b	62.3b	27.3b
	T2	61.0b	9.9b	57.9b	27.0b
	T3	53.3c	9.2c	50.2b	21.7c

[a]Means followed by the same lowercase letter within each column in the same year are not significantly different ($p > 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. [b]Ground diameter: stem diameter near the ground, and the weight average of three *M. hupehensis* Rehd. seedlings (three repeats).

2.2. Effect of Amygdalin and Benzoic Acid in Peach Orchard Soil on the Root Growth of *M. hupehensis* Rehd. Seedlings. Amygdalin and benzoic acid significantly inhibited seedling root growth (Table 2); the strongest inhibitory effect was observed in the mixed treatment (T3). In 2015, compared with the control (CK), seedling root length, root surface area, and root volume were significantly reduced by 63.7, 77.2, and 80.2% in T3; 52.1, 49.2, and 47.7% in the amygdalin treatment (T1); and 60.5, 61.5, and 64.7% in the benzoic acid treatment (T2), respectively. The root morphological index and the root respiration rate of *M. hupehensis* Rehd. seedlings were significantly inhibited by the application of amygdalin, benzoic acid, and the mixed solution, indicating that amygdalin and benzoic acid could inhibit the root growth of *M. hupehensis* Rehd. seedlings.

The root respiration rate is an important physiological index for measuring the degree of stress experienced by the root system, and root respiration can directly or indirectly affect the absorption and transportation of nutrient elements by root cells.[32] When the root respiration rate is inhibited, the absorption, transport, accumulation, and regulation of water and nutrients in plants are inhibited.[33] Compared with CK, the root respiration rate of *M. hupehensis* Rehd. seedlings under all treatments was significantly inhibited; specifically, the root respiration rate was the lowest in T3, followed by T2, T1, and CK (Table 2). In 2015, the root respiration rate significantly decreased by 47.0, 49.7, and 71.9% in T1, T2, and T3, respectively, compared with CK.

2.3. Effect of Amygdalin and Benzoic Acid in Peach Orchard Soil on Protective Enzyme Activity and Malondialdehyde (MDA) Content in the Root System of *M. hupehensis* Rehd. Seedlings. Under normal growth conditions, there is a dynamic balance between the generation and elimination of free radicals in plant tissue cells. Under adverse conditions, the activity of antioxidant enzymes is reduced, and the dynamic balance in plants is broken, which results in the excessive accumulation of free radicals in the root system and an increase in the MDA content, thereby harming

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Table 2. Effect of Amygdalin and Benzoic Acid on the Root Growth of *Malus hupehensis* Rehd. Seedling[a,b]

date	treatment	root length (cm)	root surface area (cm^2)	root volume (cm^3)	root respiration rate (nmol min^{-1} g^{-1} FW)
July 15, 2015	CK	1005.7a	204.2a	3.5a	857.7a
	T1	481.9b	103.8b	1.9b	454.7b
	T2	396.9b	78.6bc	1.3bc	431.7b
	T3	365.6b	46.6c	0.7c	240.9c
July 15, 2016	CK	6412.7a	1875.8a	85.6a	910.4a
	T1	4914.9b	1255.8b	61.8b	561.2b
	T2	4510.7b	1101.1b	55.6b	552.0b
	T3	3371.6c	784.1c	44.4c	400.0c

[a]Means followed by the same lowercase letter within each column in the same year are not significantly different ($p > 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. [b]FW: fresh weight.

Table 3. Effects of Different Treatments on SOD, POD, CAT, and MDA Activities in the Roots of *Malus hupehensis* Rehd. Seedlings[a,b,c]

date	treatment	SOD (U g^{-1} FW)	POD (U min^{-1} g^{-1} FW)	CAT (U min^{-1} g^{-1} FW)	MDA (mmol g^{-1} FW)
July 15, 2015	CK	247.1a	22.9a	28.8a	3.2a
	T1	174.4b	12.9b	10.1b	5.1b
	T2	142.2b	10.2c	8.5b	5.6c
	T3	103.6c	5.3d	7.9b	6.6d
July 15, 2016	CK	254.6a	23.7a	32.6a	4.6a
	T1	194.0b	16.4b	14.8b	5.5b
	T2	156.3c	14.4c	14.1b	6.1b
	T3	122.2d	10.5d	11.4c	7.1c

[a]Means followed by the same lowercase letter within each column in the same year are not significantly different ($p > 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. [b]SOD: superoxide dismutase, POD: peroxidase, CAT: catalase, and MDA: malondialdehyde. [c]FW: fresh weight.

Table 4. Effects of Amygdalin and Benzoic Acid on the Chlorophyll Content and Photosynthetic Parameters of *Malus hupehensis* Rehd. Seedlings[a,b,c]

date	treatment	chlorophyll *a* (mg g^{-1} FW)	chlorophyll *b* (mg g^{-1} FW)	carotenoid (mg g^{-1} FW)	P_n (μmol m^{-2} s^{-1})	G_s (μmol m^{-2} s^{-1})	C_i (μmol mol^{-1})	T_r (μmol m^{-2} s^{-1})
July 15, 2015	CK	19.0a	15.4a	4.0a	11.6a	187.7a	4.8a	286.0a
	T1	17.9b	14.8b	3.9b	8.0b	136.3b	3.8b	293.3a
	T2	17.4b	14.3c	3.8b	7.3bc	125.0b	3.6bc	290.3a
	T3	16.1c	12.8d	3.7c	6.0c	114.0b	3.1c	288.0a
July 15, 2016	CK	19.8a	16.5a	4.2a	13.3a	201.7a	5.6a	311.7a
	T1	18.8b	15.8b	4.1b	9.3b	164.0b	4.5b	293.7a
	T2	18.4b	15.4c	4.0c	8.6b	157.3b	4.3b	298.7a
	T3	17.3c	14.3d	3.8d	7.6c	133.7c	3.5c	289.3a

[a]Means followed by the same lowercase letter within each column in the same year are not significantly different ($p > 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. [b]P_n: net photosynthetic rate, G_s: stomatal conductance, C_i: internal CO_2 concentration, and T_r: transpiration rate. [c]FW: fresh weight.

the root system.[34] Antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are critically important for alleviating free radical damage; the amount of antioxidant enzyme activity indicates the degree of stress injury sustained by the roots.[35] In 2015, compared with the control (CK), the protective enzyme activities were significantly lower in all three treatments (Table 3); specifically, the SOD, POD, and CAT enzyme activities decreased by 43.6, 29.4, and 64.9% in the amygdalin treatment (T1); 42.6, 55.4, and 70.7% in the benzoic acid treatment (T2); and 58.1, 76.9, and 72.5% in the mixed treatment (T3), respectively. However, the MDA content in the root system significantly increased, which was 1.59, 1.74, and 2.05 times higher in T1, T2, and T3, respectively, compared with the control. Previous research has shown that benzoic acid can alter the content of active oxygen and inhibit the growth of cucumber roots,[36] suggesting that amygdalin and benzoic acid can inhibit root protective enzyme activities, lead to the accumulation of free radicals in the root system of seedlings, and increase the MDA content. Benzoic acid had a greater inhibitory effect than amygdalin, but the inhibitory effect of the mixed treatment was the strongest.

2.4. Effect of Amygdalin and Benzoic Acid in Peach Orchard Soil on the Chlorophyll Content and Photosynthetic Parameters of *M. hupehensis* Rehd. Seedlings.

Under stress, the membranes of plant cells are destroyed, which results in a decrease in chlorophyll synthesis.[37] The chlorophyll content of seedling leaves was significantly reduced in all treatments in July 2015 (Table 4). Compared with the control (CK), the content of chlorophyll *a*, chlorophyll *b*, and carotenoid in seedling leaves were reduced by 5.8, 4.1, and 2.5% in the amygdalin treatment (T1); 8.4, 7.1, and 5.0% in the benzoic acid treatment (T2); and 15.3, 16.9, and 7.5% in the mixed treatment (T3), respectively. These patterns were

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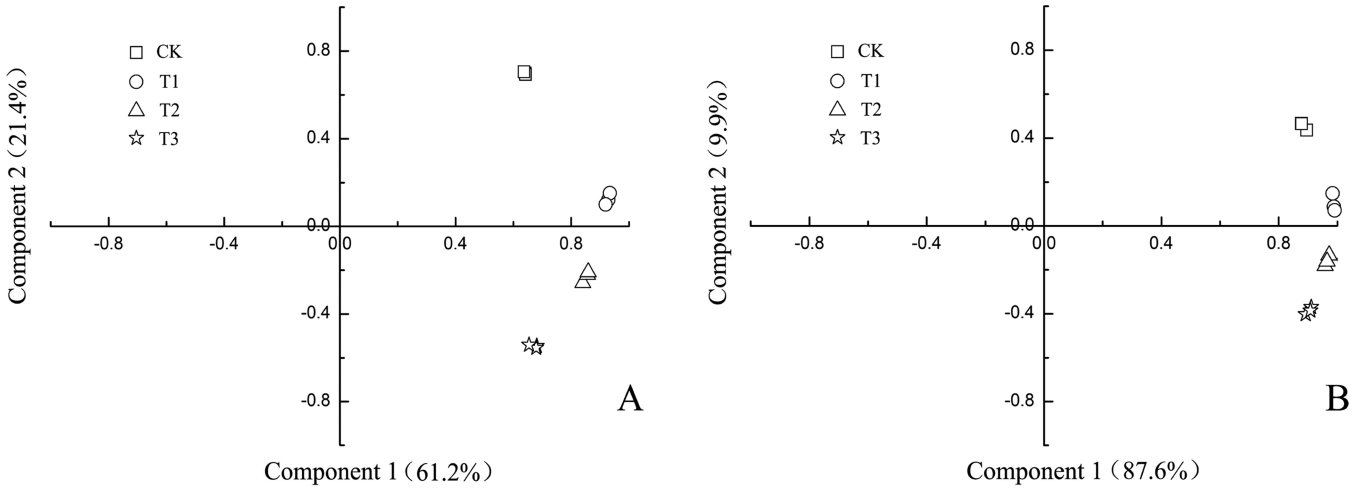

Figure 1. Principal component analysis (PCA) of the T-RFLP profiles of fungi among different treatments. Period A: July 15, 2015, PC1 and PC2 accounted for 61.20 and 21.40% of the variance, respectively. Period B: July 15, 2016, PC1 and PC2 accounted for 87.60 and 9.90% of the variance, respectively. The samples treated with different treatments were amygdalin (T1), benzoic acid (T2), and a mixed solution of amygdalin and benzoic acid (T3).

Table 5. Effects of Amygdalin and Benzoic Acid on the Soil Microbial Community Structure and Soil Enzyme Activity[a,b]

date	treatment	number of soil bacteria ($\times 10^5$ CFU g^{-1})	number of soil fungi ($\times 10^3$ CFU g^{-1})	neutral phosphatase (mg g^{-1} d^{-1})	urease (mg g^{-1} d^{-1})	sucrase (mg g^{-1} d^{-1})	catalase (mL g^{-1})
July 15, 2015	CK	78.0a	54.3c	38.9a	0.14a	10.4a	0.66a
	T1	58.0b	65.7b	26.3b	0.10b	7.5b	0.56b
	T2	55.7b	66.3b	25.3b	0.10b	7.3b	0.55b
	T3	41.0c	84.0a	20.9c	0.06c	5.7c	0.43c
July 15, 2016	CK	70.0a	62.3c	45.3a	0.15a	11.6a	0.75a
	T1	58.3b	74.3b	28.1b	0.11b	8.8b	0.65b
	T2	55.3b	77.7b	27.5b	0.10b	8.4b	0.64b
	T3	41.7c	91.0a	21.8c	0.07c	6.2c	0.52c

[a]Means followed by the same lowercase letter within each column in the same year are not significantly different ($p > 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. [b]CFU: colony-forming unit.

consistent with the results of Sunaina and Singh[38] in tomatoes. In addition, the inhibitory effect of the mixed treatment on the chlorophyll synthesis of leaves was greater compared with amygdalin and benzoic acid alone, which indicated that allelopathy was enhanced by the joint action of multiple substances.

Previous studies have shown that photosynthesis is inhibited, and the net photosynthetic rate decreases under stress.[39] Yu et al.[40] found that benzoic acid and its derivatives had the greatest inhibitory effect on the net photosynthesis of cucumber leaves. In July 2015, the net photosynthetic rate of seedling leaves under each treatment significantly decreased (Table 4); specifically, the net photosynthetic rate was lowest in T3, followed by T2, T1, and CK. Compared with the control (CK), the net photosynthetic rate was reduced by 31.0, 37.0, and 48.7% in T1, T2, and T3, respectively. The same pattern was observed for the stomatal conductance and transpiration rate of leaves, but there was no significant difference in the intercellular CO_2 concentration between treatments. This may stem from the fact that allelopathic substances affect the stomatal closure of plant leaves, which reduces the photosynthesis and transpiration of plants. In addition, phenolic acids might destroy the structure of the chloroplasts of seedling leaves, reducing the chloroplast volume, chlorophyll content, and thus the photosynthetic rate.[41] The same pattern was observed in 2016, indicating that the concentrations of amygdalin and benzoic acid in aged peach orchard soil could inhibit the growth of *M. hupehensis* Rehd. seedlings.

2.5. Effect of Amygdalin and Benzoic Acid in Peach Orchard Soil on the Soil Microbial Community Structure and Soil Enzyme Activity. Principal component analysis (PCA) was performed based on the distribution and abundance of terminal restriction fragments (T-RFs) in the different treatments. Amygdalin (T1), benzoic acid (T2), and the mixed treatment (T3) altered the structure of the soil fungal community to different degrees in July 2015 and July 2016 (Figure 1); the T1−CK distance was the lowest, followed by T2-CK and T3-CK.

T1, T2, and T3 inhibited the growth of bacteria and promoted the growth of fungi in soil (Table 5). In July 2015, the number of soil bacteria in T1, T2, and T3 was 25.6, 28.6, and 47.4% lower and the number of fungi was 20.9, 22.1, and 54.6% higher compared with CK, respectively.

T1, T2, and T3 varied in the degrees to which they inhibited soil enzyme activity; soil enzyme activity was the lowest in T3, followed by T2, T1, and CK. In July 2015, compared with CK, the neutral phosphatase activity was decreased by 32.6, 35.1, and 46.6%; the urease activity was decreased by 26.8, 30.8, and 59.6%; the sucrase activity was decreased by 27.8, 29.2, and 44.9%; and the catalase activity was decreased by 15.8, 16.5, and 34.9% in T1, T2, and T3, respectively. The patterns of soil

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enzyme activity and the number of microorganisms in July 2016 were consistent with those in July 2015.

Soil microorganisms are living organisms in soil that participate in a series of biochemical reactions and thus affect soil enzyme activity. Soil enzyme activity can directly reflect soil biological activity; thus, it is an important indicator for measuring soil physical and chemical properties as well as soil fertility.[42] Blum et al.[43] found that the accumulation of phenolic acids in soil was closely related to changes in soil microorganisms. Bais et al.[44] found that root exudates and allelochemicals could affect the soil microbial community. Yin et al.[45] conducted an in vitro experiment of silicon carbide quantum dot-labeled *Fusarium oxysporum* and found that phloridzin promoted the growth of *F. oxysporum* hyphae. The results of our experiment showed that the addition of amygdalin and benzoic acid resulted in a decrease in the number of bacteria in soil, an increase in the number of fungi, a decrease in soil enzyme activity, and changes in the soil fungal community structure. Li et al.[46] found that the content of *p*-hydroxybenzoic acid and coumaric acid in the soil increased with the number of continuous cropping years of peanuts, which led to a significant decrease in the number of bacteria and actinomycetes in rhizosphere soil and an increase in the number of fungi. The addition of amygdalin and benzoic acid inhibits the growth of soil bacteria, promotes the growth of some pathogenic fungi, alters the original soil microecological balance, and thus reduces soil enzyme activity.

3. CONCLUSIONS

The effects of amygdalin, benzoic acid, and the mixed solution on the soil environment and plant growth were studied through an experiment. Our results showed that adding 4.44 mg kg^{-1} amygdalin, 1.17 mg kg^{-1} benzoic acid, and a mixture of these two compounds to the soil significantly reduced the *M. hupehensis* Rehd. seedling root biomass, root morphology index, root activity, protective enzyme activity, net photosynthetic rate, bacteria, and soil enzyme activity; increased the content of MDA and fungi; and had toxic effects on *M. hupehensis* Rehd. seedlings. Therefore, amygdalin and benzoic acid are harmful substances that should be removed during soil remediation. Generally, the results of this study provide useful insights into soil management in crop rotation.

4. MATERIALS AND METHODS

4.1. Study Site. This experiment was conducted in the experimental station of the National Apple Engineering Technology Research Center on the south campus of Shandong Agricultural University from March 2015 to October 2016. It was located in Huangjiazhuang village, Tai'an city, Shandong Province, China (36°09′32″ N, 117°09′30″ E). *M. hupehensis* Rehd. seedlings were used in experiments, which have the problem of replanting obstacles.[26−30] The seeds of *M. hupehensis* Rehd. were stratified at 4 °C for approximately 40 days. After the seeds became white, they were seeded in the seedling tray in March 2015. In May 2015, the seedlings grew six true leaves, and seedlings showing the same growth trend were transplanted to clay tile pots (upper inner diameter of 25 cm, lower inner diameter of 17 cm, and 18 cm in height) with 6.5 kg of fallow soil in each basin. Fallow soil in which wheat had previously been planted but not fruit trees was collected from farmland soil near the peach orchard. The available potassium content (44.54 mg kg^{-1}) was extracted by 1 M CH_3COONH_4 and analyzed by a flame photometer (model 410, Sherwood Co., England).[47] The available phosphorus content (47.38 mg kg^{-1}) was extracted by 0.5 M $NaHCO_3$ and analyzed by a Discrete Autoanalyzer (Smart Chem 200, Alliance Co., France).[48] The available ammonium nitrogen content (4.19 mg kg^{-1}) and the nitrate-nitrogen content (7.72 mg kg^{-1}) were extracted by 0.01 M $CaCl_2$[49] and analyzed by an AA3 Autoanalyzer (model AA3-A001-02E, Bran-Luebbe, Germany). The organic matter content (5.60 g kg^{-1}) was determined by the potassium dichromate method.[50] The amygdalin content (0 mg kg^{-1}) was determined by high-performance liquid chromatography (HPLC) after extraction by a Soxhlet extractor and ultrasonic degassing.[51] Benzoic acid was extracted from the soil by an accelerated solvent extraction method, and the content of benzoic acid (1.17 mg kg^{-1}) was determined by HPLC.[52]

4.2. Experimental Design. In Chezhuang village, Tai'an city, Shandong Province, China (36°11′38″ N, 116°38′53″ E), the soil was randomly collected from several points in a 15-year-old peach garden (Okubao/Wild peach), mixed well, air-dried at room temperature, and sifted through a 12-mesh sieve.

In the process of experiment preparation, we detected the following six typical phenolic acids in peach orchard soil: benzoic acid, caffeic acid, syringic acid, amygdalin, ferulic acid, and cinnamic acid. Following the method of Yin,[52] benzoic acid, caffeic acid, syringic acid, ferulic acid, and cinnamic acid was extracted from the soil by an accelerated solvent extraction method, and the content of them was determined by HPLC. Following the method of Zhang,[51] amygdalin was extracted by a Soxhlet extractor and ultrasonic degassing, and the content of amygdalin was determined by HPLC. The contents of benzoic acid, caffeic acid, syringic acid, amygdalin, ferulic acid, and cinnamic acid were 10.35, 2.58, 0.86, 4.44, 0.25, and 0.18 mg kg^{-1}, respectively.

There were four treatments in the experiment: (1) fallow soil (CK), (2) amygdalin treatment (T1), (3) benzoic acid treatment (T2), and (4) mixed treatment of amygdalin and benzoic acid (T3). The benzoic acid and amygdalin used were analytically pure. The concentration of benzoic acid and amygdalin in peach orchard soil was determined to be 10.35 and 4.44 mg kg^{-1}, respectively. Benzoic acid and amygdalin reagents were prepared in an equal volume of less than 0.2% (v/v) absolute ethanol solution. The amygdalin solution concentration was 57.72 mg L^{-1}, and the benzoic acid solution concentration was 134.55 mg L^{-1}. Either 500 mL of the amygdalin solution, 500 mL of the benzoic acid solution, or a mixed solution of 500 mL of the amygdalin solution and 500 mL of the benzoic acid solution was applied to each pot; the same volume of less than 0.2% (v/v) anhydrous ethanol solution was added to the control soil. Each solution was applied to the seedlings before planting; there were 20 pots for each treatment, and fertilizer and water management were the same for all treatments after planting *M. hupehensis* Rehd. seedlings and soil samples were collected in July 2015 and 2016, respectively. Especially in July 2016, the seedlings was 14 month old, which is the indicator for the second year. In general, the 2-year indicator is more persuasive. During sampling, three ports were randomly selected as three replicates; the soil was removed around the basin and the surface layer, sifted through a 2 mm sieve, and placed into three sealed bags. One bag was stored in a refrigerator at 4 °C to measure the quantity of soil microorganisms. Another bag was quickly placed into liquid nitrogen and stored in a −80 °C

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refrigerator for the soil microbial community structure. The last bag was naturally air-dried for the determination of soil enzyme activity. Three *M. hupehensis* Rehd. seedlings were randomly selected as three repeats for growth index determination. The final result was the average of three repeats.

4.3. Measurements. *4.3.1. Biomass.* Measurements of seedling height, ground diameter, and fresh mass were taken by a ruler, vernier caliper, and electronic scale, respectively. After measurements, *M. hupehensis* Rehd. seedlings were wrapped tightly in paper bags and placed in a constant temperature oven. After drying, the seedlings were carefully removed, and dry mass was measured with an electronic scale.

4.3.2. Root System Configuration Parameters. At each sampling event, three *M. hupehensis* Rehd. seedlings were collected from each treatment. Roots were then washed in clean water, laid flat on a hard plastic container, and spread out in water. WinRHIZO (2007 version) was used to measure the root length, root surface area, and root volume number of seedlings from the sample images.

4.3.3. Root Respiration Rate. At each sampling event, three *M. hupehensis* Rehd. seedlings were collected for each treatment. Roots were then washed in clean water, and 0.5 g of fresh white roots were evenly divided into 0.1 cm segments. An Oxytherm oxygen electrode (Hansatech, U.K.) was used to determine the root respiration rate.

4.3.4. Antioxidant Enzyme Activity. Antioxidant enzymes (SOD, POD, CAT, and MDA) were extracted.[53] The activity of superoxide dismutase (SOD) was determined by the nitrogen blue tetrazole (NBT) method.[54] The activity of peroxidase (POD) was determined by the guaiacol method.[55] The catalase (CAT) activity was determined by the decomposition hydrogen peroxide content rate method.[56] The content of malondialdehyde (MDA) was determined by the thiobarbituric acid method.[57]

4.3.5. Photosynthetic Parameters in Leaves. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of functional leaves of *M. hupehensis* Rehd. seedlings were determined from 9 to 11 am using a CIRAS-3 portable photosynthetic apparatus (PP Systems, U.K.).[58]

4.3.6. Soil Microorganisms. Soil bacteria and fungi were determined using the dilution plate counting method. The bacteria were cultured under a beef extract peptone medium at 37 °C, and the fungi were cultured under a PDA selective medium at 28 °C.[59]

4.3.7. Soil Microbial Community Structure. The extraction and purification of the total DNA of sample genomes were performed per the instructions of the E.Z.N.A. Soil DNA Kit, which was used for T-RFLP analysis. The common primers of the fungal ITS region ITS1-F (5′-CTTGGTCATTTAGAC-GAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGAT-GATGC-3′) were used to amplify these sequences, and the PCR products were digested with the restriction enzyme *Hha*I. The enzyme digestion products were sent to Sangon Bioengineering for sequencing, and SPSS 19.0 software was used for fungal community cluster analysis and PCA.

4.3.8. Soil Enzyme Activity. Phosphatase was determined by the phenyl disodium phosphate colorimetric method. The colorimetric method was used for urease and sucrase. The volumetric flask method was used for catalase.[60]

4.4. Data Analysis. The experimental data were managed in Microsoft Excel 2003 and Origin 8.5. ANOVA was performed using SPSS 19.0, and significant differences were determined using Duncan's multiple range test ($p < 0.05$).

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Notes

The authors declare no competing financial interest.

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