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Effect of Emerging Soil Chemical Amendments on the Replant Soil Environment and Growth of *Malus hupehensis* Rehd. Seedlings

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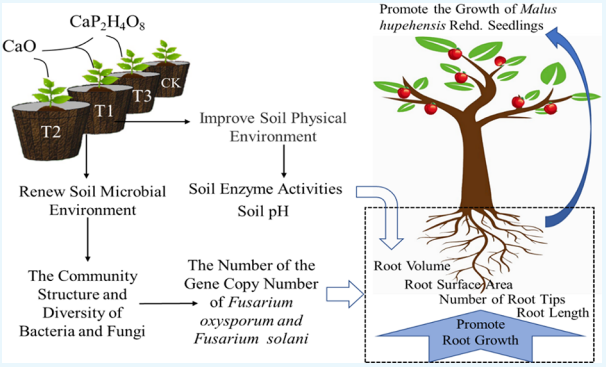
ABSTRACT: The effects of different soil chemical amendments (T1, 1‰ quicklime + 1‰ superphosphate; T2, 1‰ quicklime; T3, 1‰ superphosphate) on the soil environment and growth of *Malus hupehensis* Rehd. seedlings in aged apple orchard soil were studied to provide new insight into the prevention and control of apple replant disease. The amendments differed in their ability to ameliorate the soil environment; nevertheless, they all promoted the growth of *M. hupehensis* Rehd. seedlings, and the greatest enhancement of growth was observed in T1. On August 15, 2018, soil urease, sucrase, phosphatase, and catalase activities were 1.67 times, 1.32 times, 1.62 times, and 1.35 times higher in T1 compared with CK, respectively. The soil pH increased, which alleviated soil acidification. T1 also promoted the renewal of the community structure and the diversity of soil microorganisms. The copy numbers of *Fusarium solani* and *Fusarium oxysporum* were 71.96 and 70.30% lower in T1 compared with CK, respectively. The seedling height and root length of *M. hupehensis* Rehd. seedlings increased by 40.97 and 289.69% in T1 compared with CK, respectively. Therefore, soil replanting obstacles can be overcome with the application of quicklime and superphosphate; these soil chemical amendments also improve the soil microbial ecological environment and promote the growth of *M. hupehensis* Rehd. seedlings.



1. INTRODUCTION

China is one of the major producers and consumers of apples worldwide. Large areas of apple orchards need to be modified to accommodate the increase in the popularity of new apple varieties and reduce labor requirements, Apple replant disease is an inevitable consequence of the scarcity of land resources.[1] Replanting obstacles such as apple replant disease are common problems associated with apple replanting. Replanting obstacles result in the slow growth and development of replant apple saplings, reduce fruit yield and quality, and exacerbate the effects of diseases and pests.[2] Thus, finding ways to overcome replanting obstacles is key for promoting the sustainable development of the apple industry.

The causes of apple replanting obstacles are complex and include abiotic and biotic factors.[3] Long-term replanting leads to decreases in soil pH, the accumulation of toxic substances, and imbalances in soil nutrients.[4] The increase in harmful fungi associated with long-term replanting alters the soil microbial community structure.[5] The degree of soil acidification in apple orchards is severe, especially in the Bohai Bay area.[6] Soil acidification can alter the soil microbial community, reduce the activities of nitrifying bacteria, nitrogen-fixing bacteria, silicate bacteria, and phosphorus bacteria, and increase the abundance of pathogenic fungi, all of which hinder the transformation of soil nutrients.[7] Guo et al.[8] found that the application of quicklime to acid soil can alter soil pH and promote the growth of apples. Quicklime also plays a key role in eliminating soil pathogenic microorganisms. Li et al.[9] found that the application of quicklime to soil was effective for controlling *Rhizoctonia solani*, and Yang et al.[10] found that it was effective for controlling soilborne diseases such as black shank and bacterial wilt. Moharana et al.[11] found that phosphate fertilizer can improve the soil environment and increase soil enzyme activity. The application of a certain amount of phosphate fertilizer in orchards can effectively control the occurrence of apple replant disease or reduce the degree of damage that it induces.[12] Hao and Xiao[13] showed that adding superphosphate to replant soil can promote the growth of medicinal materials and alleviate replanting obstacles.

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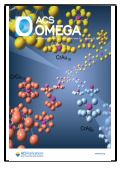

The application of quicklime has been shown to improve the stability of soil aggregates[14] and reduce the occurrence of soilborne diseases of strawberries grown in greenhouse conditions.[15] The application of superphosphate can increase the yield of rape, wheat, cotton, and other crops,[16,17] and both quicklime and superphosphate can ameliorate soil polluted with heavy metals and oil.[18,19] However, a few studies have shown that quicklime and superphosphate can ameliorate apple replant soil and improve its internal environment. There is thus a need to study the effects of quicklime, superphosphate, and quicklime−superphosphate mixtures on the replant soil environment and the growth of *Malus hupehensis* Rehd. seedlings. The results of this study provide new insight into the efficacy of quicklime, superphosphate, and quicklime−superphosphate mixtures for ameliorating the replant soil environment and alleviating apple replant disease. Our study also has implications for the chemical industry and agricultural production.

2. RESULTS

2.1. Effects of Different Soil Chemical Amendments on the pH of Replant Soil. In the two sampling periods (A and B), the degree to which each treatment altered the pH of replant soil varied (Figure 1). The greatest increase in replant soil pH was observed in T2; the replant soil pH increased in T1 and decreased in T3 relative to CK.

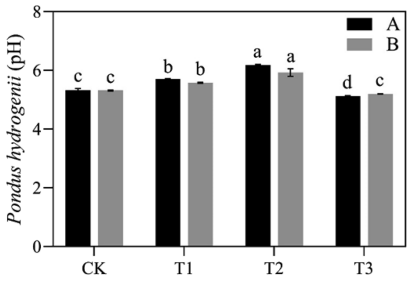

Figure 1. Effects of different soil chemical amendments on the pH of replant soil. A: August 15, 2018; B: August 15, 2019; CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. Data are means ± SE (n = 3); values marked with the same letter within a sampling date are not significantly different at $P < 0.05$ according to Duncan's new multiple-range test; ANOVA, analysis of variance.

2.2. Effects of Different Soil Chemical Amendments on the Soil Enzyme Activities of Replant Soil. The degree to which soil enzyme activities were improved by different soil amendments varied. In period A, there were significant differences in the soil enzyme activities of all treatments and the control (Figure 2). The phosphatase activity, sucrase activity, and catalase activity of T1 were the highest, which were 1.62 times, 1.32 times, and 1.35 times higher compared with CK, respectively. The urease activity of T2 was the highest, which was 1.82 times higher compared with CK. In period B, the soil enzyme activities of all treatments were significantly increased in the treatments relative to the control.

2.3. Effects of Different Soil Chemical Amendments on the Gene Copy Number of *Fusarium solani* and *Fusarium oxysporum* in Replant Soil. The absolute quantitative analysis of the copy number of *F. solani* and *F. oxysporum* in soil was conducted using real-time polymerase chain reaction (PCR) (Figure 3). The gene copy numbers of *F. solani* and *F. oxysporum* were significantly reduced in all treatments. In period A, the copy numbers of *F. solani* in T1, T2, and T3 decreased by 71.96, 61.91, and 48.56%, respectively, compared with CK. The copy numbers of *F. oxysporum* decreased by 70.30, 64.88, and 55.56% in T1, T2, and T3, respectively. In period B, the gene copy numbers of the two *Fusarium* species recovered to varying degrees but were still lower compared with the control.

2.4. Different Soil Chemical Amendments on the Soil Microbial Diversity in Period B. PC1 and PC2 in the principal coordinate analysis (PCoA) explained 47.13 and 29.44% of the variation in bacterial species composition among different treatments. Soil treated with different soil chemical amendments was distant from the control (Figure 4**a**). PC1 and PC2 explained 40.50 and 37.99% of the variation in fungal species composition among treatments. Soil treated with different soil chemical amendments was distant from the control in PC1 (Figure 4**b**). The distances between different treatments from the same sampling period were statistically significant. The diversity of the microbial community significantly changed after the application of different soil chemical amendments. The change was the most pronounced in T1.

2.5. Effects of Different Soil Chemical Amendments on the Soil Microbial Community Structure in Period B. The microbial community structure of replant soil was significantly altered by the different soil chemical amendments. The relative abundances of *Proteobacteria, Gemmatimonadetes*, and *Bacteroidetes* were increased by 29.57, 44.08, and 194.52% in T1, 26.13, 41.05, and 85.44% in T2, and 19.77, 69.89, and 93.90% in T3, respectively, compared with CK (Figure 5a). The relative abundance of *Actinobacteria* was lower in the treatments compared with CK; the decrease was the most pronounced in T2, in which the abundance of *Actinobacteria* was 64.25% lower compared with CK. The relative abundance of *Chloroflexi* was also lower in the treatments compared with CK; the decrease was the most pronounced in T1, in which the abundance of *Chloroflexi* was 28.10% lower compared with CK. Compared with the bacterial community structure, the effect of different soil chemical amendments on the structure of the fungal community was more pronounced. The relative abundance of *Zygomycota* was increased in the three treatments compared with CK (Figure 5b); specifically, its relative abundances increased by 188.41, 1010.08, and 642.12% in T1, T2, and T3, respectively, and the increase in T1 was the largest. In addition, the relative abundance of *Basidiomycota* was increased by 860.61% in T1 compared with CK.

2.6. Effects of Mixed Soil Chemical Amendments on the Soil Microbial Community Structure in Period B. Because of the outstanding effect of T1, the differences between T1 and CK were analyzed at the genus level. The relative abundances of *Mizugakiibacter, Rhodanobacter, Luteibacter*, and *Lysobacter* increased significantly under T1 compared with CK (Figure 6a). The relative abundances of *Streptomyces, Pseudarthrobacter, Gaiella, Bacillus*, and *Amycolatopsis* were significantly decreased compared with CK. The relative abundances of *Guehomyces, Mortierella, Chaetomium*, and other fungi increased significantly after T1. The relative abundances of *Lecanicillium, Fusarium, Gibberella*, and *Clonostachys* decreased significantly relative to CK.

2.7. Effects of Different Soil Chemical Amendments on the Root Architecture of *M. hupehensis* Rehd. Seedlings. The root growth of *M. hupehensis* Rehd. seedlings

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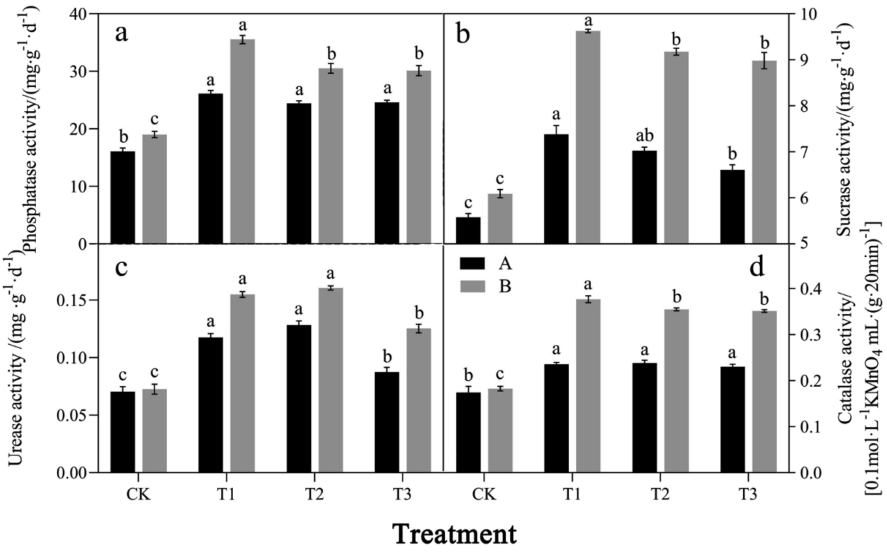

Figure 2. Effects of different soil chemical amendments on the soil enzyme activities of replant soil: (a) phosphatase activity, (b) sucrase activity, (c) urease activity, and (d) catalase activity. A: August 15, 2018; B: August 15, 2019; CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. Data are means ± SE (n = 3); values marked with the same letter within a sampling date are not significantly different at $P < 0.05$ according to Duncan's new multiple-range test; ANOVA, analysis of variance.

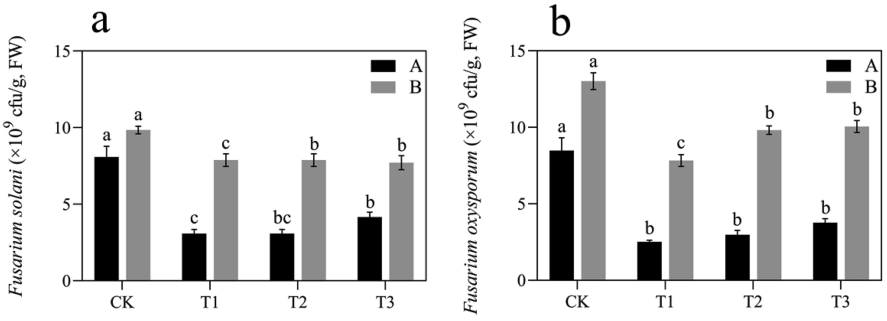

Figure 3. Effects of different soil chemical amendments on the gene copy numbers of *F. solani* and *F. oxysporum* in replant soil: (a) *F. solani* and (b) *F. oxysporum*. A: August 15, 2018; B: August 15, 2019; CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. Data are means ± SE (n = 3); values marked with the same letter within a sampling date are not significantly different at $P < 0.05$ according to Duncan's new multiple-range test; ANOVA, analysis of variance.

was significantly increased in all treatments compared with CK, and the increase in T1 was the largest (Figure 7). Compared with CK, the root length, number of root tips, root volume, and root surface area were increased by 289.69, 98.70, 303.04, and 219.30% in T1, 218.99, 70.94, 227.62, and 174.43% in T2, and 172.55, 71.89, 196.96, and 161.07% in T3, respectively. The patterns in periods B and A were the same.

2.8. Effects of Different Soil Amendments on the Biomass of *M. hupehensis* Rehd. Seedlings. The growth of *M. hupehensis* Rehd. seedlings was significantly increased in all treatments compared with CK, and the increase was the most pronounced in T1 (Table 1). In period A, the plant height, ground diameter, fresh weight of aboveground and underground parts, and dry weight of aboveground and underground biomass increased by 40.97, 50.03, 86.82, 171.92, 81.43, and 161.20%, respectively, compared with CK. The patterns in periods B and A were the same.

3. DISCUSSION

Soil chemical amendments can improve the physical and chemical properties of soil, regulate the soil microbial community structure, and promote plant growth. They are of low cost and have the potential to be more widely used.[20] Soil chemical amendments regulate the soil microenvironment and thus can aid in the remediation of the replant soil environment.

3.1. Effects of Different Soil Chemical Amendments on the pH and Soil Enzyme Activities of Replant Soil. In our experiment, the pH of replant soil was significantly increased in T1 and T2 compared with CK, and the pH in T1 and T2 significantly differed. This pattern may stem from the fact that quicklime is alkaline and superphosphate is weakly acidic. They both can effectively modify soil pH, and the mixture of quicklime and superphosphate can alleviate soil acidification. Soil enzymes are an important component of soil biological activity, as they are directly involved in the processes of soil material transformation, nutrient release, and fixation. Changes in soil enzyme activities can alter the availability of nutrients absorbed by crops; the activities of soil enzymes are thus often used as an indicator of microbial activity and soil fertility.[21] Soil urease is mainly derived from plants and microorganisms and is the key enzyme for determining N transformation in soil. Its activity reflects the direction and intensity of various biochemical processes.[22] Sucrase and phosphatase activities are closely related to soil nutrient metabolism, and catalase activity indicates the total biological activity and fertility status of soil.[23] The activities of urease,

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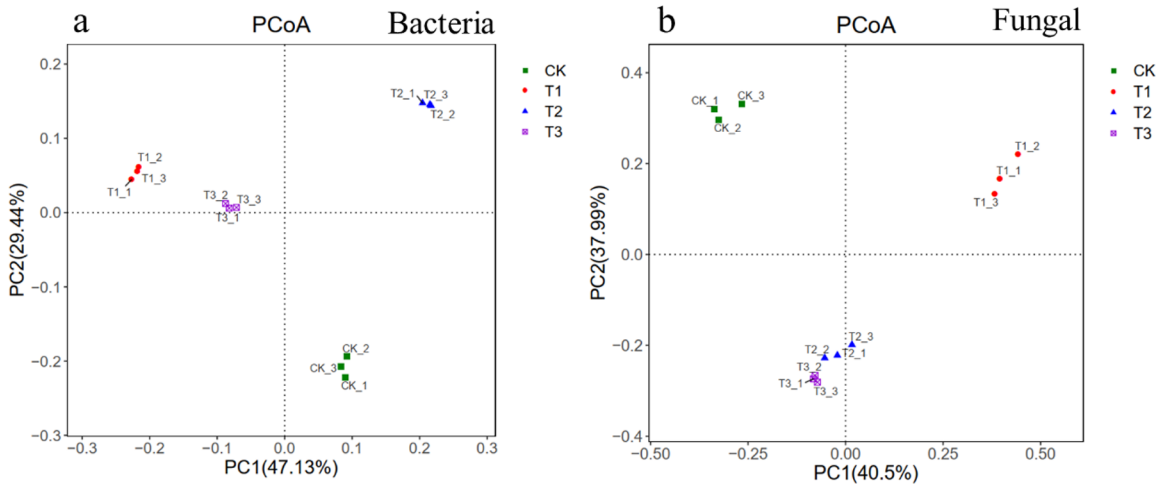

Figure 4. (a, b) Beta diversity of soil bacteria and fungi at the genus level following the application of different soil amendments to replant soil: (a) PCoA of bacteria and (b) PCoA of fungi. CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. Student's *t*-test was used to assess differences in beta diversity between different soil chemical amendments and untreated replant soil (CK). Principal coordinate analysis (PCoA) was based on the Bray−Curtis distance metric at the genus level, and the results are displayed as a scatter diagram. The *x*-axis and *y*-axis represent the first two principal coordinate axes, and the percentage represents the degree of variation explained by the principal axis. The scale of the *x*-axis and *y*-axis is a relative distance measure with no practical significance; different colors and shapes of points indicate different sample groups. The proximity of two sample points is positively related to the similarity of the species compositions of the two samples.

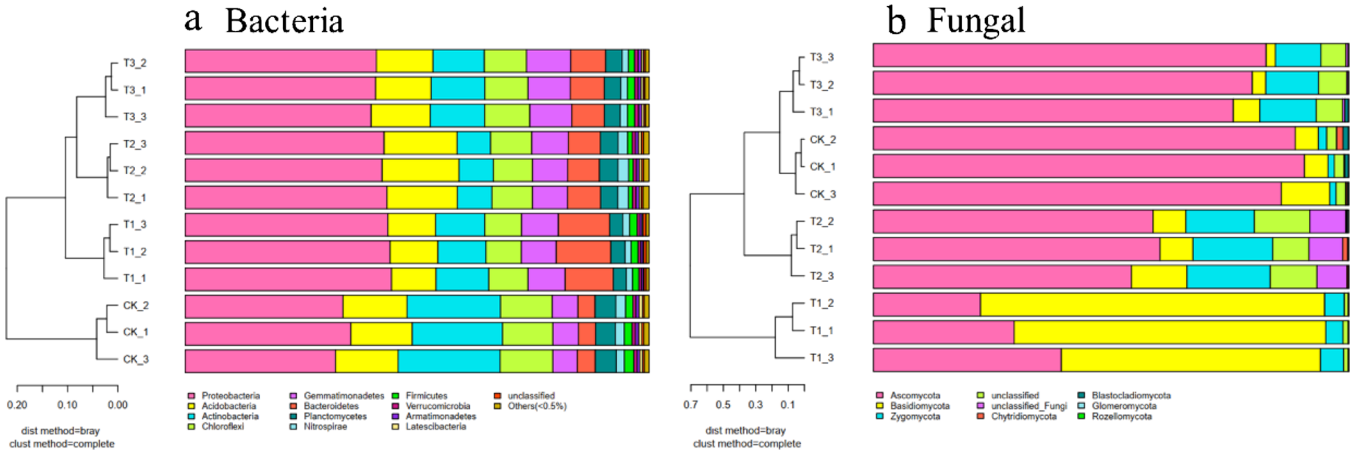

Figure 5. (a, b) Hierarchical clustering and annotated community bar chart of the bacterial and fungal communities in different replant soil samples at the phylum level following the application of different soil chemical amendments to replant soil. CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. The vertical axis is the sample name, the left part is the sample hierarchy cluster analysis, the length between branches corresponds to the distance between samples, the horizontal axis represents the proportion of species in the sample, the columns of different colors represent different species, and the length of the columns represents the proportion of species.

sucrase, phosphatase, and catalase increased to different degrees in the treatments compared with CK. The increase in soil urease activity in T2 was the most pronounced, which may stem from the rapid increase in soil pH, the increase in soil microbial activity, and the increase in the adsorption and utilization of soil nitrogen by plants. The activities of sucrase, catalase, and phosphatase were increased in T1. This might stem from the fact that the mixture of superphosphate and quicklime can cause the root system to secrete enzymes into the soil and promote the release of root exudates, thereby stimulating the activities of rhizosphere microorganisms and leading to an increase in soil enzyme activities.[24] Thus, the addition of quicklime and superphosphate can improve the replant soil environment and alleviate replanting obstacles.

3.2. Effects of Different Soil Chemical Amendments on the Structure and Diversity of the Soil Microbial Community of Replant Soil. Soil microorganisms underlie many biological aspects of soil health in terrestrial ecosystems, and disturbances of microbial ecology affect soil biological processes.[25] Long-term soil replanting obstacles can seriously impair the health of the soil environment by altering the microbial community structure, damaging the microecological balance, and transforming the soil environment from a "bacterial" type to a "fungal" type.[26] Numerous studies have shown that *Fusarium*, *Trichosporon*, *Cylindrocarpon*, and *Pythium* are the main causes of replanting obstacles in apple-producing countries such as the United States, Italy, and South Africa.[27,28] Wang et al.[29] found that *Fusarium* fungi were the main pathogens responsible for apple replanting obstacles in

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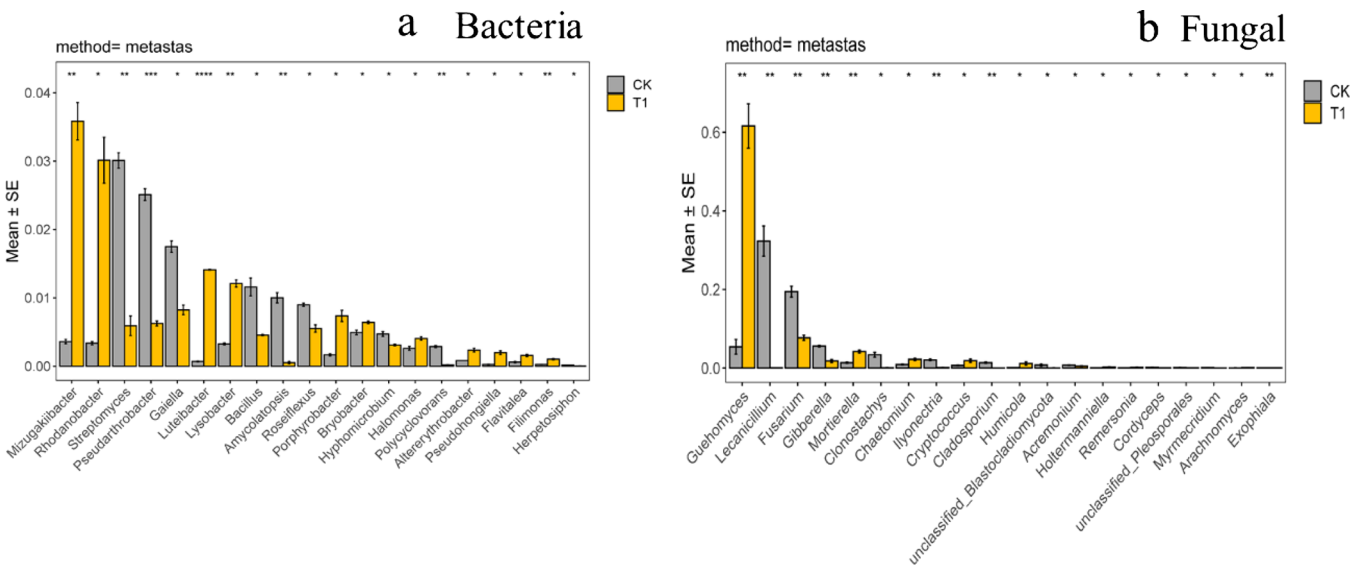

Figure 6. Effects of mixed soil chemical amendments on the soil microbial community structure in period B: (a) bacteria and (b) fungi. CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate. The *x*-axis represents the different generic groupings; the gray boxes represent CK (untreated soil), and the yellow boxes represent T1 (a mixture of quicklime and superphosphate). The *y*-axis represents the average relative abundance of a species in different groups. The significance of differences between two samples in a given period was estimated using a *t*-test. *$0.01 < P \leq 0.05$, **$0.001 < P \leq 0.01$, ***$P \leq 0.001$. Bars represent the mean ± SE of three replicates.

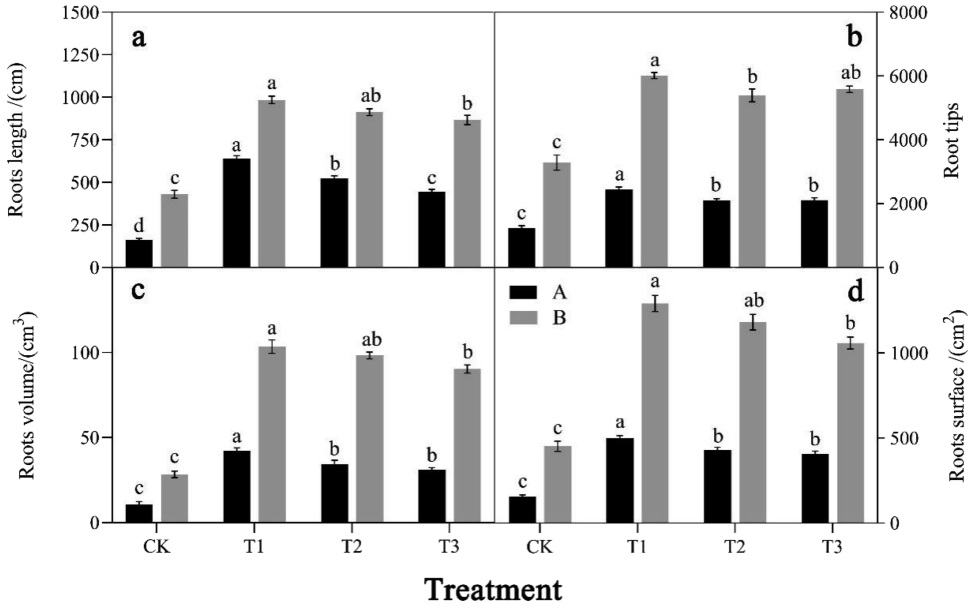

Figure 7. Effects of different soil chemical amendments on the root architecture of *M. hupehensis* Rehd. seedlings: (a) root length, (b) root tips, (c) root volume, and (d) root surface area. A: August 15, 2018; B: August 15, 2019; CK: untreated control (replant soil); T1: 1‰ quicklime + 1‰ superphosphate; T2: 1‰ quicklime; T3: 1‰ superphosphate. Data are means ± SE ($n = 3$); values marked with the same letter within a sampling date are not significantly different at $P < 0.05$ according to Duncan's new multiple-range test; ANOVA, analysis of variance.

the Bohai Bay area in China. In this experiment, the gene copy numbers of *F. oxysporum* and *F. solani* were significantly reduced in the treatments compared with CK. The gene copy numbers of the two *Fusarium* species recovered continuously, but the differences were still significant compared with the control. This is consistent with research on banana by Fan and Li[30] showing that banana *Fusarium* wilt was negatively correlated with soil pH. This stems from the ability of *F. oxysporum* to quickly infect plants under acidic soil conditions; an acidic environment is also not conducive to the growth and reproduction of beneficial bacteria. On the one hand, quicklime can promote the growth of rhizosphere bacteria and inhibit the growth of fungi by increasing soil pH;[31] on the other hand, it can directly inhibit the mycelial growth and sporulation of *F. oxysporum*.[32] The addition of superphosphate increased the content of phosphorus in replant soil. Phosphorus in soil is a key nutrient for phosphate-solubilizing bacteria and other microorganisms, as it can enhance their activity, contribute to the formation of new microflora, and optimize the structure of the soil microbial community.[33] The application of phosphate fertilizer in orchards can also be used to control the occurrence of apple replant disease or reduce the damage induced by *Fusarium*.[34] The above series of studies have shown that applying different soil chemical amendments

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Table 1. Effects of Different Soil Amendments on the Biomass of *M. hupehensis* Rehd. Seedlings[a]

				fresh weight/g		dry weight/g	
date	treatment	height/cm	ground diameter/mm	aboveground	underground	aboveground	underground
August 15, 2018	CK	48.43 ± 4.10c	5.26 ± 0.15c	15.40 ± 0.29c	5.69 ± 0.20d	7.79 ± 0.13c	2.89 ± 0.08d
	T1	68.27 ± 1.69a	7.89 ± 0.13a	28.78 ± 1.29a	15.46 ± 0.31a	14.13 ± 0.59a	7.54 ± 0.23a
	T2	64.87 ± 2.18ab	6.60 ± 0.33b	23.75 ± 0.69b	13.70 ± 0.31b	12.22 ± 0.46b	6.78 ± 0.12b
	T3	58.76 ± 1.80b	6.83 ± 0.14b	22.67 ± 0.66b	10.76 ± 0.45c	11.41 ± 0.33b	5.32 ± 0.18c
August 15, 2019	CK	101.07 ± 1.96d	10.53 ± 0.58c	28.47 ± 0.47c	10.58 ± 0.78c	14.51 ± 0.61c	7.08 ± 0.50c
	T1	136.93 ± 1.65a	18.48 ± 0.33a	53.87 ± 2.85a	31.16 ± 1.16a	26.51 ± 1.19a	15.78 ± 0.67a
	T2	125.80 ± 1.93b	15.13 ± 1.05b	43.81 ± 1.27b	23.55 ± 1.04b	21.77 ± 0.76b	12.33 ± 0.47b
	T3	116.13 ± 1.44c	14.21 ± 0.43b	39.68 ± 0.98b	21.26 ± 0.79b	19.51 ± 0.79b	11.10 ± 0.88b

[a]Note: Data in the table are mean ± SE; different lowercase letters in the same column and the same period indicate significant differences between different treatments ($P < 0.05$).

can not only alleviate soil acidification but can also optimize the soil microbial environment. In this study, the single application of quicklime can alleviate soil acidification, and the application of quicklime and superphosphate mixture can not only alleviate soil acidification but also change the structure of the soil microbial community so as to better alleviate replanting obstacles.

3.3. Effects of Different Soil Chemical Amendments on the Growth of *M. hupehensis* Rehd. Seedlings. A healthy soil environment is conducive to the growth of plants.[35] The seedling biomass and root system configuration of *M. hupehensis* Rehd. seedlings directly reflect the growth environment. As the center of plant underground metabolism, the root system is not only an important place for plants to absorb water and nutrients, but it is also an important site for the synthesis of organic compounds and physiologically active substances.[36] In this experiment, the biomass and root architecture parameters of replanted *M. hupehensis* Rehd. seedlings were increased by different soil additives, and significant differences were observed between the treatments and the control. This may stem from the fact that quicklime (an alkaline substance) increases soil pH, alleviates the stress of acidified soil on plant roots, and promotes plant growth.[37] The application of superphosphate can provide phosphorus to plants. The addition of an appropriate amount of phosphorus in replant orchard soil can improve seedling biomass, promote seedling root growth, enhance seedling disease resistance, and prevent replant diseases.[38] The mixed application of quicklime and superphosphate promoted plant growth to the greatest extent, which may stem from the fact that these two amendments have a synergistic effect in improving the replant soil environment.[39,40]

4. CONCLUSIONS

Adding a mixture of the emerging soil chemical amendments quicklime and superphosphate to apple replant soil can significantly improve soil enzyme activity, alleviate soil acidification, and improve the soil microbial community structure and diversity. It can also reduce the probability of apple replant disease and promote the growth of aboveground *M. hupehensis* Rehd. seedlings. The results of this research indicate that these emerging chemicals could be used to improve agricultural production. There is a need for more work to promote the multidisciplinary integration of these two fields.

5. MATERIALS AND METHODS

5.1. Experimental Materials. The experiment was conducted at the College of Horticulture Science and Engineering, Shandong Agricultural University, National Apple Engineering Technology Research Center, in 2019 (36.16°N, 117.15°E).

The soil was collected from Fengmaozhai, Laizhou City, Yantai City, Shandong Province (37.41°N, 120.07°E), from a 32-year-old apple orchard; the *Malus* rootstock was *Malus* × *robusta* Rehder. In April 2018, the topsoil was removed, and multiple points were randomly sampled in an area with a depth of 10−40 cm and a distance of approximately 80 cm from the trunk; the samples were then mixed. The basic physical and chemical properties of the soil were as follows: NO_3^--N, 33.89 mg·kg^{-1}; NH_4^+-N, 22.25 mg·kg^{-1}; available phosphorus, 9.79 mg·kg^{-1}; available potassium, 21.71 mg·kg^{-1}; organic matter, 5.09 g·kg^{-1}; pH, 5.23. Quicklime and superphosphate were purchased from Shanghai Guangnuo Chemical Technology Co., Ltd. The seedling substrate was purchased from Jiangsu Huai'an Hongyang Agricultural Technology Development Co., Ltd. The main components of the seedling substrate were peat, cassava residue, decomposed plant straw, vermiculite, perlite, and edible fungus stick, and the pH was maintained between 6.5 and 7.0.

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. The seeds of *M. hupehensis* Rehd. seedlings were layered at 4 °C for approximately 30 days until the seeds became white. The seeds were sown in the seedling tray at the end of March 2018 until the seedlings had six true leaves.

5.2. Experimental Design and Treatment. A preliminary experiment was conducted in which different concentrations of quicklime (0.5, 1, and 2 g/kg), superphosphate (0.5, 1, and 2 g/kg), and mixtures of quicklime and superphosphate (0.5 g/kg + 0.5 g/kg, 1 g/kg + 1 g/kg, and 2 g/kg + 2 g/kg) were applied to determine suitable concentrations for seedling growth. The phenotypes of the seedlings were optimal under the following treatments: 1 g/kg quicklime, 1 g/kg superphosphate, and the mixture of 1 g/kg superphosphate and 1 g/kg quicklime. Among these three treatments, seedling growth was maximized under the mixture of 1 g/kg superphosphate and 1 g/kg quicklime. Thus, four treatments were used in this experiment: replant soil (CK), replant soil with 1‰ quicklime (T1), replant soil with 1‰ superphosphate (T2), and replant soil with 1‰ quicklime + 1‰ superphosphate (T3).

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On April 3, 2018, the quicklime and superphosphate required for the different experimental treatments were mixed with replant soil and then placed into clay pots with an upper diameter of 25 cm, lower diameter of 17 cm, and height of 18 cm; each pot contained approximately 6.5 kg of soil. On May 1, 2018, *M. hupehensis* Rehd. seedlings were planted. *M. hupehensis* Rehd. seedlings with similar growth status were used for transplanting. Two seedlings were planted in each basin, and there were 20 pots in each treatment. Plants were watered via drip irrigation.

Soil samples were collected and measured on August 15, 2018 and August 15, 2019. At each sampling date, three replicate pots were randomly selected from each treatment for measurement. During sampling, 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 immediately placed into liquid nitrogen and stored at −80 °C for subsequent analysis of the soil microbial community structure, DNA extraction, and real-time fluorescence quantitative PCR. The other bag was naturally air-dried for the measurements of soil enzyme activity and pH. Three seedlings of *M. hupehensis* were collected at each sampling event. After washing, the plant height, diameter at ground height, fresh weight, dry weight, and root system architecture were measured.

5.3. Soil pH and Enzyme Activity. The pH of soil was determined using a PHS-2f pH meter (Shanghai Yidian Scientific Instrument Co., Ltd.). First, 10 g of air-dried soil was placed through a 2 mm sieve. The filtered soil was then placed into a 50 mL triangular flask; 25 mL of boiled and cooled distilled water was added, mixed thoroughly, and left to stand for 20−30 min before measurements were taken with a pH meter. Before measurements, the instrument was calibrated using two standard buffer solutions of different pH values: one at pH 4 and the other at pH 7. During measurements, the glass probe ball was immersed into the suspension layer, ensuring that the probe completely made contact with the suspension. After the reading, the probe was removed slowly and rinsed with deionized water. The position of the glass probe in contact with the suspension was the same in each sample.

Soil urease activity was determined using a colorimetric assay with sodium phenate−sodium hypochlorite. First, 5 g of air-dried soil samples was weighed in a 50 mL triangulated flask, and 1 mL of toluene was added, followed by shaking until the contents were evenly mixed. After 15 min, 10 mL of 10% urea solution and citrate buffer solution was added, followed by shaking and incubation at 37 °C for 24 h. After culture, the filtrate was filtered, and 1 mL of filtrate was added into a 50 mL volumetric flask. Next, 4 mL of sodium phenol solution and 3 mL of sodium hypochlorite solution were added and shaken well. After 20 min, the mixture was diluted to a 50 mL mark, and the spectrophotometer was colorimetric at 578 nm (the blue color of indophenol remained stable). Urease activity was calculated by subtracting the absorbance value of the sample from the difference in the absorbance value of the control sample, and the ammonia nitrogen content was calculated according to the standard curve.

The activity of urease (Ure) was represented by the ammonia-nitrogen content (mg) in 1 g of soil after 24 h. The formula for determining soil urease activity was as follows:

$$\text{Ure} = a \times V \times n/m$$

where a is the concentration of ammonium-nitrogen obtained from the standard curve (mg/mL), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil phosphatase activity was determined using a colorimetric assay with disodium phenyl phosphate. First, 5 g of air-dried soil samples was placed in a 200 mL triangulation flask, and 2.5 mL of toluene was added. After shaking for 15 min, 20 mL of 0.5% benzene-disodium phosphate was added. After shaking, the samples were placed in an incubator and cultured at 37 °C for 24 h. Next, 100 mL of 0.3% aluminum sulfate solution was added to the culture medium and filtered. Three milliliters of filtrate was then absorbed into 50 mL volumetric bottles, and 5 mL of buffer solution and four drops of chlorodibromo-*p*-benzoquinone imine reagent were added to each bottle. After color development, the solution was diluted to the scale, and the colorimetric determination was conducted 30 min later. The boric acid buffer was blue and colorimetric at 660 nm on the spectrophotometer. To draw the standard curves, 1, 3, 5, 7, 9, 11, and 13 mL of phenolic working fluids were taken for color development and volume determination. After color stability was achieved, the standard curve was drawn with the colorimetric method. Phosphatase activity was expressed in phenolic micrograms per gram of soil.

The activity of phosphatase (Pho) was represented by the phenol content (mg) in 1 g of soil after 24 h. The formula for determining soil phosphatase activity was as follows:

$$\text{Pho} = a \times V \times n/m$$

where a is the concentration of phenol obtained from the standard curve (mg/mL), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil sucrase activity was determined using a colorimetric assay with 3,5-dinitrosalicylic acid. First, 5 g of air-dried soil samples was placed in a 50 mL triangulated flask, and 10 mL of 1% starch solution was injected. This was followed by the addition of 10 mL of pH 5.6 phosphate buffer solution and five drops of toluene, shaking, and storage in an incubator. The samples were then cultured at 37 °C for 24 h. After culture, the suspension was filtered. Next, 1 mL of filtrate was poured into a 50 mL volumetric flask. Two milliliters of 3,5-dinitrosalicylic acid solution was added and heated in a boiling water bath for 5 min; the solution was then moved to the volumetric flask to the running water to cool. After a constant volume of 50 mL was achieved, colorimetry was performed at 508 nm on a spectrophotometer. Glucose solution was used as the standard.

The activity of sucrase (Suc) was represented by the glucose content (mg) in 1 g of soil after 24 h. The formula for determining soil sucrase activity was as follows:

$$\text{Suc} = a \times V \times n/m$$

where a is the concentration of glucose obtained from the standard curve (mg/mL), V is the volume of the chromatic liquid (50 mL), n is the separation multiple, and m is the weight of the drying soil (g).

Soil catalase activity was determined using potassium permanganate titration. First, 2 g of air-dried soil samples was placed in a 100 mL trigonometric bottle and injected with 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution. A control (40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution) was injected into a triangular bottle without the addition of soil samples. The triangular bottle was shaken on a shaking machine for 20 min, and 5 mL of 3 N sulfuric acid was added to stabilize the undecomposed

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hydrogen peroxide. The suspension in the bottle was then filtered with a slow filter paper, followed by absorption of 25 mL of filtrate and titration with 0.1 N potassium permanganate to the light pink terminal point.

The activity of catalase (Cat) was represented by a volume (mL) of 0.1 N potassium permanganate in 1 g of soil after 20 min. The formula for determining soil catalase activity was as follows:

$$\text{Cat} = (A - B) \times T$$

where B is the amount of potassium permanganate (mL) consumed for titrating the soil filtrate (mL), A is the amount of potassium permanganate (mL) consumed for titrating 25 mL of the original hydrogen peroxide mixture (mL), and T is the correction value for potassium permanganate titration.

5.4. DNA Extraction and Real-Time Quantitative Analysis of *F. solani* and *F. oxysporum*. The extraction and purification of total genomic DNA from the sampled soil were performed per the instructions of the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The CFX Connect system (Bio-Rad, Hercules, CA, USA) was used to analyze the copy number of *F. solani* genes in the soil by real-time quantitative PCR. The primers were FR (5′-GGCCTGAGGGTTGTAATG-3′), FF (5′-CGAGTTATA-CAACTCATCAACC-3′), JR (5′-GAACGCGAATTAACGC-GAGTC-3′), and JF (5′-CATACCACTTGTTGTCTCGGC-3′). The reactions were performed per the instructions of the SYBR Premix Ex Taq Kit (TaKaRa Biotech Co., Ltd., Dalian, China). Each reaction in the 25 μL PCR system included 1.5 μL of DNA template, 12.5 μL of SYBR Premix Ex Taq II (TaKaRa), 1 μL of each primer, and 9 μL of double-distilled water. The thermal cycling parameters were as follows: predenaturation at 95 °C for 30 s, denaturation at 95 °C for 5 s, and annealing at 60 °C for 30 s for a total of 40 cycles.

5.5. Root Morphological Index. First, three *M. hupehensis* Rehd. seedlings were taken from each treatment on August 15, 2018 for measurements of the root morphological index. Roots were then washed in clean water, laid flat on a hard plastic container, and spread out in water. The WinRHIZO (Seiko Epson Corporation, Nagano Prefecture, Japan, version 2007) root analysis system was used to measure the root length, total volume, total surface area, and root tip number of seedlings from the sample images. Second, three *M. hupehensis* Rehd. seedlings were taken from each treatment on August 15, 2019. The measured indexes and their methods are as above.

5.6. Plant Biomass. First, three *M. hupehensis* Rehd. seedlings were taken from each treatment on August 15, 2018 and washed in clean water. Measurements of the seedling height, ground diameter, and fresh weight were taken using a ruler, vernier calipers, and an electronic scale, respectively. After measurements, *M. hupehensis* Rehd. seedlings were wrapped tightly in paper bags and placed in a constant temperature oven at 80 °C. After drying, seedlings were removed carefully, and the dry mass was measured with an electronic scale. Second, three *M. hupehensis* Rehd. seedlings were taken from each treatment on August 15, 2019. The measured indexes and their methods are as above.

5.7. DNA Extraction and High-Throughput Sequencing Analysis. DNA was extracted using the MO BIO Power Soil DNA Extraction kit (MO BIO Laboratories, Carlsbad, USA) and quantified using a NanoDrop spectrophotometer. PCR amplification of the 16S rRNA gene was conducted using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGTCAATTCMTTTGAGTTT-3′), and that for the ITS1 gene was conducted using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′). The PCR reaction was performed in a 25 μL reaction volume containing 1 μL of DNA template, 250 mmol L^{-1} dNTPs, 1 μL of each primer, 1× reaction buffer, and 0.5 units of Phusion DNA Polymerase (New England Biolabs, USA). Each sample was amplified in triplicate under the following cycling parameters: initial denaturation at 94 °C for 2 min, 25 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, and a final extension of 72 °C for 5 min. The libraries were sequenced using 2 × 300 bp paired-end sequencing on the MiSeq platform using the MiSeq v3 Reagent Kit (Illumina) at Tiny Gene Bio-Tech Co., Ltd. (Shanghai, China).

5.8. Bioinformatics and Statistical Analysis. Data were presented as mean ± standard deviation of triplicate cultures. Analysis of variance was performed using SPSS19.0 software (version 19.0, SPSS Inc., Chicago, USA), and significant differences were detected by Duncan's new complex range method. Statistical analyses were performed in Microsoft Excel Office 2019 and GraphPad Prism 8.0 (Origin Lab Corporation, San Diego, CA 92108, USA). A one-way analysis of variance with a *t*-test was used to assess significant differences between two samples; $P < 0.05$ was considered statistically significant.

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Notes

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