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Quicklime and Superphosphate Alleviating Apple Replant Disease by Improving Acidified Soil

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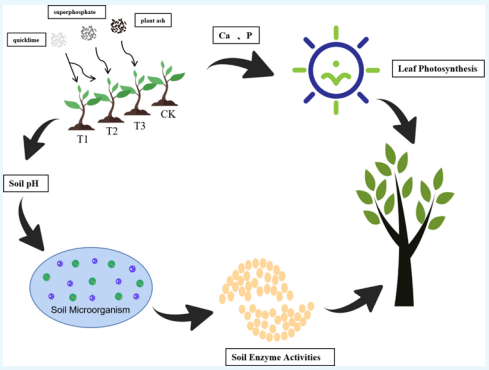
ABSTRACT: A two-year field experiment was carried out in order to study the effect of different soil modifiers on alleviating apple replant disease (ARD) in the apple orchards. Four treatments were as follows: replanted apple orchard soil (CK), replanted apple orchard soil treated with quicklime 1.0 g·kg^{-1} (T1), replanted apple orchard soil treated with 1.0 g·kg^{-1} quicklime and 1.0 g·kg^{-1} superphosphate (T2), and replanted apple orchard soil treated with 1.0 g·kg^{-1} plant ash (T3). Soil pH, plant biomass, soil bacteria, soil fungi, *Fusarium oxysporum*, soil enzymes, plant chlorophyll, and photosynthetic parameters were measured to detect the improvement effects of different soil amendments on acidified soil and to alleviate the ARD. The three treatments stably raised the pH of acidified soil and improved the conditions of the plant rhizosphere environment. Compared with the control, T1, T2, and T3 treatments significantly increased growth and plant biomass indexes, such as plant height and ground diameter, as well as photosynthetic parameters. Among the three treatments, T2 had the strongest effects. In July 2018 and July 2019, the number of bacteria was 151.3 and 190.5% higher in T2-treated soil than in control soil, and the number of soil fungi was 53.6 and 53.3% lower. In 2018 and 2019, the copy number of *Fusarium solani* was 63.6 and 58.6% lower and that of *F. oxysporum* was 51.8 and 55.7% lower. The T1, T2, and T3 treatments significantly increased soil enzyme activity and leaf chlorophyll content, and their effects were generally ranked T2 > T1 > T3. In conclusion, a combination of 1.0 g·kg^{-1} quicklime and 1.0 g·kg^{-1} superphosphate added to acidified replant soil increased the soil pH, improved the soil environment, increased the number of bacteria, reduced the number of fungi, increased soil enzyme activity, and improved plant photosynthetic capacity, thereby promoting the growth of replanted seedlings and effectively reducing ARD.



1. INTRODUCTION

China's apple production and cultivation area are ranked first in the world, but orchards planted in the 1980s have entered a period of senescence. Their yield and fruit quality have dropped sharply, not only reducing economic benefits but also hindering the continued development of China's apple industry. Solutions to the problems associated with old orchard replacement are therefore urgently needed. Because of limited land resources, most new orchards are planted in former orchard soil, and apple replant disease (ARD) is inevitable under these conditions. ARD is also referred to as apple continuous cropping obstacles or replant disease. It refers to the phenomenon in which fruit trees are replanted on the same land after the same or similar fruit tree species have been removed, causing growth of the replanted trees to be inhibited and promoting disease incidence.[1] Negative effects of ARD include aboveground and belowground growth retardation, symptoms of drought and nutritional stress, and reductions in yield.[2] In the apple root system, destruction of the cortical tissue and epidermal cells can be observed, lateral root numbers are reduced, and functional root hairs are almost entirely missing; root tip necrosis may also occur.[3−7] Judging from current research results, ARD cannot be explained by a single cause; instead, it reflects a combination of biotic and abiotic factors. Nonetheless, previous studies have shown that biotic factors are the main cause of the disease.[3] Reports suggest that soil microbial communities differ between ARD soils and soils that have not previously been planted with fruit trees,[8] consistent with the results of Manici et al. that ARD is primarily caused by an imbalance of the soil microbial structure and by the accumulation of harmful microorganisms. Soil fumigation can effectively reduce the biomass and activity of microorganisms and affect the composition of the microbial community; its positive effects can be attributed to biological factors.[9,10] Some

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researchers have isolated the same microorganisms from soils with replant problems, supporting the hypothesis that biological factors are the main cause of ARD.[11,12] Various abiotic factors such as orchard age, toxic substances in residual roots, and soil type, condition, and pH may also affect the occurrence and severity of ARD and thereby influence the growth of trees to varying degrees.[13−16] ARD is very common in apple-producing areas worldwide, and it is therefore very important to find an effective method for its prevention and control.

Studies have found that a very high multiple cropping index, continuous cropping throughout the year, and continuous cropping in general lead to a decrease in soil organic matter content and buffering capacity, causing a significant decrease in soil pH (i.e., soil acidification). Soil acidification is a relatively obvious feature of replanted soil. By improving soil acidification, we may be able to optimize the rhizosphere microbial community structure, thus promoting root system growth, chlorophyll content, and photosynthesis, all of which are important for alleviating ARD. Studies have shown that the application of lime can slow down soil acidification. Lime increases the concentration and ionic strength of Ca^{2+} in the soil solution and causes clay flocculation, thereby improving soil structure and hydraulic conductivity.[17] In arid and semi-arid countries, lime and other acid-neutralizing materials are used to improve degraded soil.[18] In traditional tillage and no-till systems, mixing lime and black liquor may not only increase soil pH but also accelerate the downward movement of lime to correct the pH of the soil below the soil surface.[19] Lime materials, such as quicklime (CaO) and limestone ($CaCO_3$), are widely used in China and Western countries to increase soil pH and the content of alkaline cations (such as Ca^{2+} and Mg^{2+}) and reduce the possible toxicity of Mn^{2+} and Al^{3+} (ref 19). Superphosphate, a commercial phosphate fertilizer that has been widely used as an additive to improve the quality of compost products, can also reduce heavy metal toxicity[20,21] and delay the biodegradation of organic matter.[22] Studies have shown that adding superphosphate to dairy cow manure increases the pH of the system and thus promotes the degradation of refractory substances such as cellulose and lignin, which are finally converted into humus to increase soil nutrients.[23,24] Plant ash is the alkali residue produced by the burning of plant materials and is widely produced by straw power plants. Because plant ash contains a large amount of potassium, it can be used as a high-quality potassium fertilizer for agricultural production.[25] In addition, plant ash also contains a variety of alkaline components that, when applied to the soil, can alleviate soil acidification and improve the soil environment. Earlier pot experiments indicated that appropriate concentrations of quicklime, 1:1 quicklime and superphosphate, and plant ash could raise the pH of acidified soil, improve the soil environment and microbial community structure, and thus alleviate apple continuous cropping obstacles. The three treatments that showed the best effects in the pot experiment were then used for a field experiment. The aims of the present study were to (i) study the effects of selected concentrations of quicklime, 1:1 quicklime and superphosphate, and plant ash on the growth of biennial grafted apple trees for 2 years under field conditions; (ii) analyze the changes in the fungal community structure in the soil after 2 consecutive years of experiments; and (iii) clarify the mechanism(s) by which quicklime, superphosphate, and plant ash ameliorate ARD. Our results have important practical significance and provide a new approach for the renewal of old apple orchards.

2. RESULTS

2.1. Effects of Different Soil Amendments on the pH of Replanted Soil. During the two sampling periods (A and B), each treatment altered the pH of the replanted soil to a different degree (Figure 1). Compared with the control treatment, all

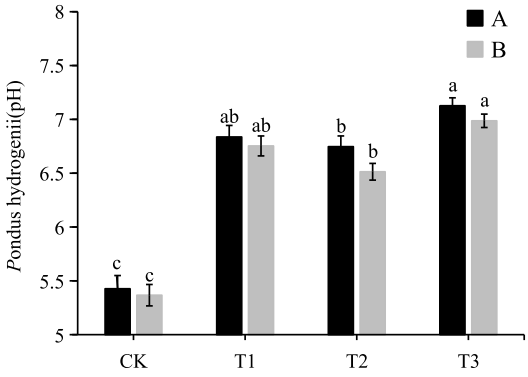

Figure 1. Effects of different soil amendments on the pH of replanted soil. A = July 15, 2018; B = July 15, 2019; CK = untreated control (replant soil); T1 = 1.0 g·kg^{-1} quicklime; T2 = 1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate; T3 = 1.0 g·kg^{-1} plant ash. Data are means ± SE (n = 3); 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.

three treatments significantly increased the soil pH and T3 had the best effect, followed by T1 and T2, but the differences among the three treatments were not significant. Overall, the pH of the replanted soil was slightly lower in 2019 than in 2018.

2.2. Effects of Different Soil Amendments on the Plant Phenotypic Parameters of Grafted Seedlings. Compared with the control, quicklime, plant ash, and the 1:1 mixture of quicklime and superphosphate all promoted the growth of grafted seedlings and differed significantly from the control (Table 1). The T2 treatment showed the strongest effects: in 2018, plant height, ground diameter, number of branches, and branch length were 26.6, 31.7, 95.3, and 42.0% higher, respectively, in T2 than in the continuous cropping control. In 2019, these indicators were 37.7, 31.0, 94.7, and 55.0% higher in T2 than in the control.

2.3. Effects of Different Soil Amendments on the Number of Microorganisms in Replanted Soil. In 2018 and 2019, the number of bacteria was significantly higher in each amended treatment than in the control, and the number of fungi was significantly lower (Table 2). Again, the T2 treatment had the strongest effect. In 2018 and 2019, the number of soil bacteria was 151.3 and 190.5% higher in T2 than in the control, and the number of soil fungi was 53.6 and 53.3% lower. The T1 treatment had the second strongest effect. In 2018 and 2019, the number of bacteria was 122.3 and 115.1% higher in T1 relative to the control, and the number of fungi was 52.4 and 51.8% lower. There were no significant differences between the T1 and T2 treatments.

2.4. Effects of Different Soil Amendments on the Gene Copy Numbers of *Fusarium oxysporum* and *Fusarium solani* in Replanted Soil. Changes in the gene copy numbers of *F. solani* and *F. oxysporum* in the soil over time were determined by real-time fluorescence qPCR (Figure 2). The gene copy numbers of *Fusarium* species were reduced by the soil amendments. In 2018, the copy numbers of *F. oxysporum* were reduced by 51.4, 51.8, and 41.8% in T1, T2, and T3, respectively,

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Table 1. Effects of Different Soil Amendments on the Plant Phenotypic Parameters of Grafted Seedlings[a]

date	treatment	height/cm	ground diameter/mm	amount of hair branch	branch length/cm
July 15, 2018	CK	164.64 ± 4.25c	20.04 ± 0.68d	9.51 ± 0.83c	44.64 ± 2.41b
	T1	195.01 ± 4.39ab	24.94 ± 0.30b	17.05 ± 1.08ab	58.11 ± 3.50a
	T2	208.46 ± 6.84a	26.40 ± 0.36a	18.57 ± 1.25a	63.41 ± 3.54a
	T3	184.91 ± 8.16b	22.37 ± 0.31c	13.70 ± 1.23b	57.22 ± 2.02a
July 15, 2019	CK	190.21 ± 6.71c	29.86 ± 0.45d	15.41 ± 1.42b	70.66 ± 5.11c
	T1	238.52 ± 3.49b	35.76 ± 0.31b	25.33 ± 2.04a	96.71 ± 6.18ab
	T2	261.94 ± 2.92a	39.11 ± 0.49a	30.01 ± 4.11a	109.51 ± 4.58a
	T3	224.36 ± 7.38b	33.41 ± 0.34c	24.25 ± 2.00a	92.59 ± 1.64b

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

Table 2. Effects of Different Soil Amendments on the Number of Microorganisms of Replanted Soil[a]

date	treatment	bacteria ($\times 10^5$ cfu/g)	fungi ($\times 10^3$ cfu/g)	bacteria/fungi ($\times 10^2$)
July 15, 2018	CK	19.38 ± 1.15c	50.64 ± 1.23a	0.38 ± 0.01c
	T1	43.08 ± 2.57ab	24.12 ± 1.28c	1.84 ± 0.08a
	T2	48.70 ± 1.22a	23.52 ± 0.88c	1.99 ± 0.11a
	T3	38.27 ± 3.78b	30.21 ± 0.74b	1.26 ± 0.10b
July 15, 2019	CK	25.21 ± 2.92c	63.07 ± 5.24a	0.41 ± 0.09c
	T1	54.22 ± 2.90b	30.40 ± 3.20b	1.82 ± 0.22ab
	T2	73.24 ± 4.21a	29.46 ± 1.42b	2.46 ± 0.40a
	T3	47.51 ± 3.32b	35.04 ± 2.02b	1.36 ± 0.12b

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

and the copy numbers of *F. solani* were reduced by 62.1, 63.6, and 49.3%. In 2019, the copy numbers of *F. oxysporum* were reduced by 53.4, 55.7, and 48.0% in T1, T2, and T3, respectively, and the copy numbers of *F. solani* were reduced by 57.6, 58.6, and 52.3%, respectively.

2.5. Effects of Different Soil Amendments on the Microbial Community Structure of Replanted Soil. The application of different soil amendments significantly affected the soil microbial community structure of replanted soil. Two years after the application of the soil amendments, the relative abundance of *Acidobacteria* in the soil bacterial community was significantly higher. T2 showed the greatest increase in *Acidobacteria* abundance: 395.5% compared with the control. *Acidobacteria* abundance also increased by 267.1 and 65.2% in T1 and T3 relative to the control, respectively (Figure 3a). By contrast, the relative abundance of *Firmicutes* decreased significantly after soil amendment treatment. T2 showed the strongest effect: its relative abundance of *Firmicutes* decreased by 76.8% compared with the control, whereas that of T1 and T3 decreased by 75.1 and 53.3%, respectively. The relative abundance of *Ascomycota* in the soil fungal community decreased significantly by 10.0, 15.6, and 8.6% in T1, T2, and T3, respectively (Figure 3b). In T1, the relative abundance of *Basidiomycota* increased. Compared with the control, the relative abundance of *Basidiomycota* increased by 81.0 and 89.1% in T2 and T3, respectively.

2.6. PCA of Different Treatments. In a principal component analysis (PCA) of the different treatments, the PC1 axis explained 90.47% of the variation in the bacterial community structure and 74.44% of the variation in the fungal community structure (Figure 4). CK was mainly concentrated in the third quadrant, T1 and T2 were concentrated in the first quadrant, and T2 was concentrated in the fourth quadrant. For both the bacterial and fungal PCA, T2 was most distant from the CK, indicating that their microbial community structures were most different.

2.7. Effects of Different Soil Amendments on Enzyme Activities of Replanted Soil. The application of quicklime, plant ash, and a 1:1 quicklime and superphosphate mixture to the replanted soil increased the activity of multiple soil enzymes (Figure 5). In 2018, compared with the control, soil urease activity increased by 100.5, 92.2, and 72.2% in T1, T2, and T3, respectively; invertase activity increased by 58.8, 79.3, and 47.8%; phosphatase activity increased by 38.7, 57.3, and 32.4%; and catalase activity increased by 36.0, 65.5, and 38.5%. In 2019,

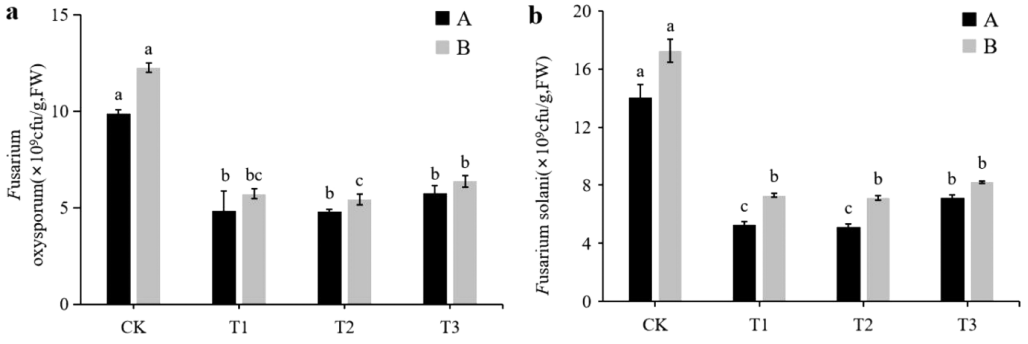

Figure 2. Effects of different soil amendments on the gene copy numbers of *F. oxysporum* and *F. solani* in replanted soil. (a) *F. solani*; (b) *F. oxysporum*. A = July 15, 2018; B = July 15, 2019; CK = untreated control (replanted soil); T1 = 1.0 g·kg^{-1} quicklime; T2 = 1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate; T3 = 1.0 g·kg^{-1} plant ash. 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.

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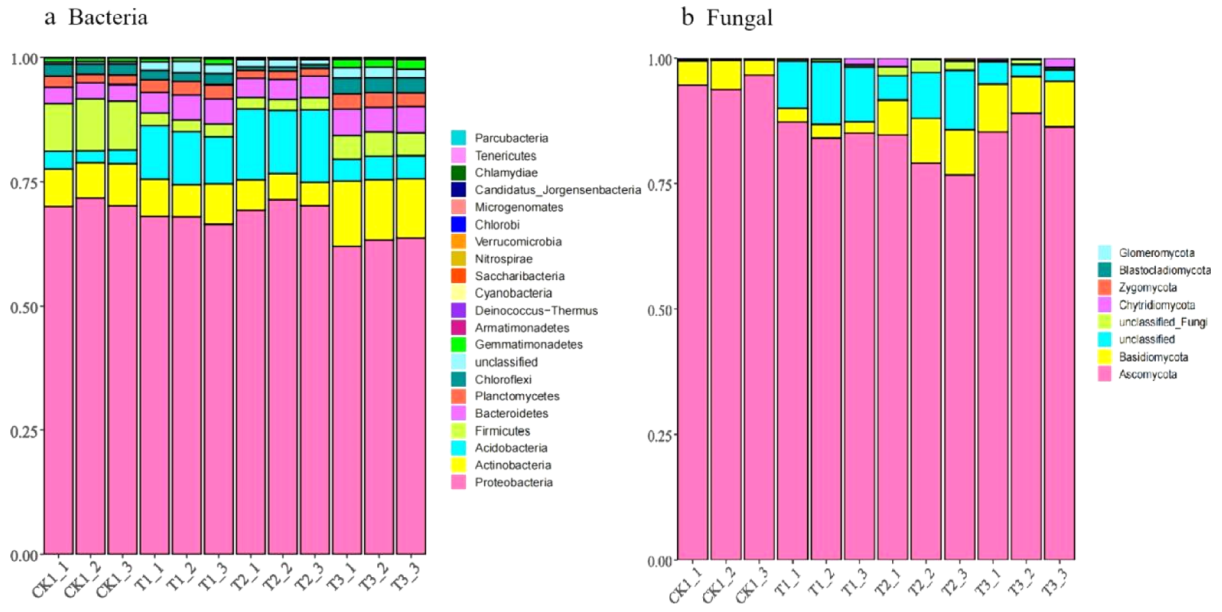

Figure 3. Changes in the relative abundance of bacterial (a) and fungal (b) species at the phylum level in different treatments. (a) Bacterial; (b) fungal; CK = untreated control (replanted soil); T1 = 1.0 g·kg^{-1} quicklime; T2 = 1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate; T3 = 1.0 g·kg^{-1} plant ash. The horizontal axis represents the proportion of species in the sample; the columns of different colors represent different species.

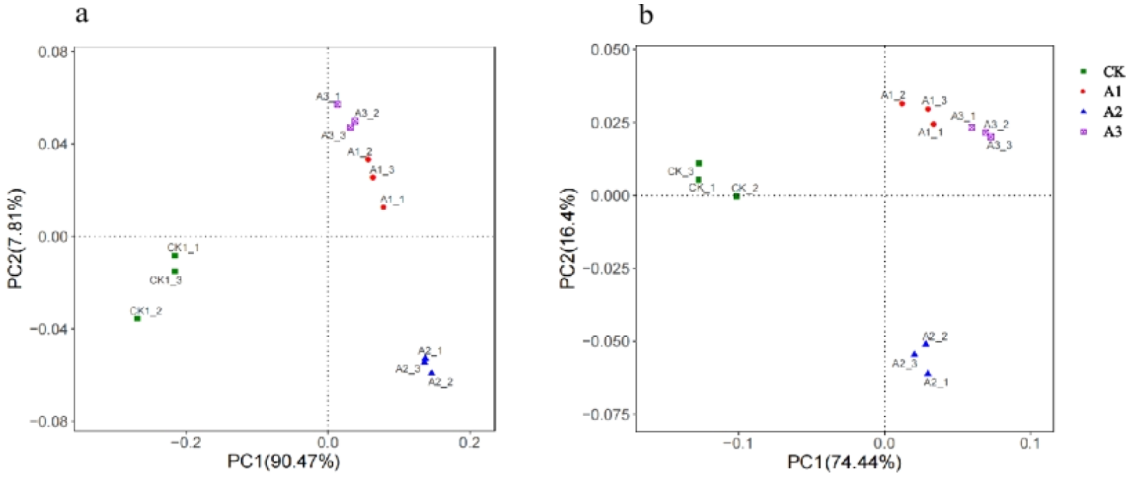

Figure 4. PCA of different treatments. The scales of the abscissa and ordinate axes are relative distances. (a) Bacterial; (b) fungal; A1 = untreated control (replanted soil); A2 = 1.0 g·kg^{-1} quicklime; A3 = 1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate; A4 = 1.0 g·kg^{-1} plant ash. The PC1/2 value represents the percentage that can explain the results of a comprehensive analysis.

urease activity increased by 104.5, 94.4, and 75.8% in T1, T2, and T3, respectively; invertase activity increased by 103.7, 141.3, and 83.4%; phosphatase activity increased by 49.6, 78.8, and 42.1%; and catalase activity increased by 67.2, 81.3, and 57.1%. In general, T2 (1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate) had the greatest effect.

2.8. Effects of Different Soil Amendments on Photosynthetic Parameters of Grafted Seedlings. In 2018, T1, T2, and T3 increased the net photosynthetic rate (P_n), intercellular carbon dioxide concentration (C_i), stomatal conductance (G_s), and transpiration rate (T_r) of grafted seedlings to varying degrees relative to the control treatment (Figure 6). T2 again had the greatest effect. P_n, C_i, G_s, and T_r of grafted seedlings were 1.67, 1.29, 1.25, and 1.20 times higher in the T2 than in the control. In 2019, these parameters were 1.58, 1.40, 1.27, and 1.20 times higher in T2. The four photosynthetic parameters were slightly higher in T3 than in the control, but these differences were not significant. The effect of T1 was intermediate between that of T2 and T3, and the difference between T1 and the control was also more significant.

2.9. Effects of Different Soil Amendments on Leaf Chlorophyll Content of Grafted Seedlings. Applying quicklime, a 1:1 quicklime and superphosphate mixture, and plant ash to replant soil increased the chlorophyll content of grafted seedlings (Table 3). In 2018, the leaf chlorophyll *a* content was 32.5, 48.9, and 24.6% higher in T1, T2, and T3 than in the control; the chlorophyll *b* content was 45.0, 68.9, and 26.2% higher; and the carotenoid content was 21.0, 30.0, and 16.9% higher. In 2019, the leaf chlorophyll *a* content was 35.6, 49.9, and 30.2% higher in T1, T2, and T3 than in the control; the chlorophyll *b* content was 53.7, 79.3, and 37.3% higher; and the carotenoid content was 25.3, 34.4, and 16.3% higher.

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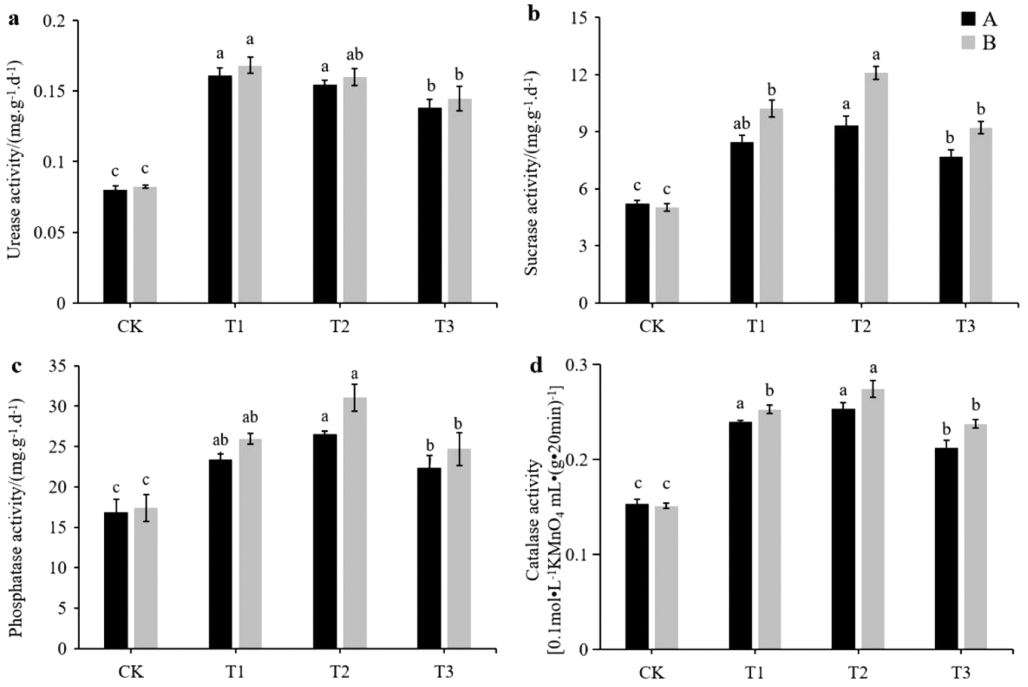

Figure 5. Effects of different soil amendments on the soil enzyme activities of replanted soil: (a) urease activity; (b) sucrase activity; (c) phosphatase activity; and (d) catalase activity. A = July 15, 2018; B = July 15, 2019; CK = untreated control (replant soil); T1 = 1.0 g·kg^{-1} quicklime; T2 = 1.0 g·kg^{-1} quicklime + 1.0 g·kg^{-1} superphosphate; T3 = 1.0 g·kg^{-1} plant ash. 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.

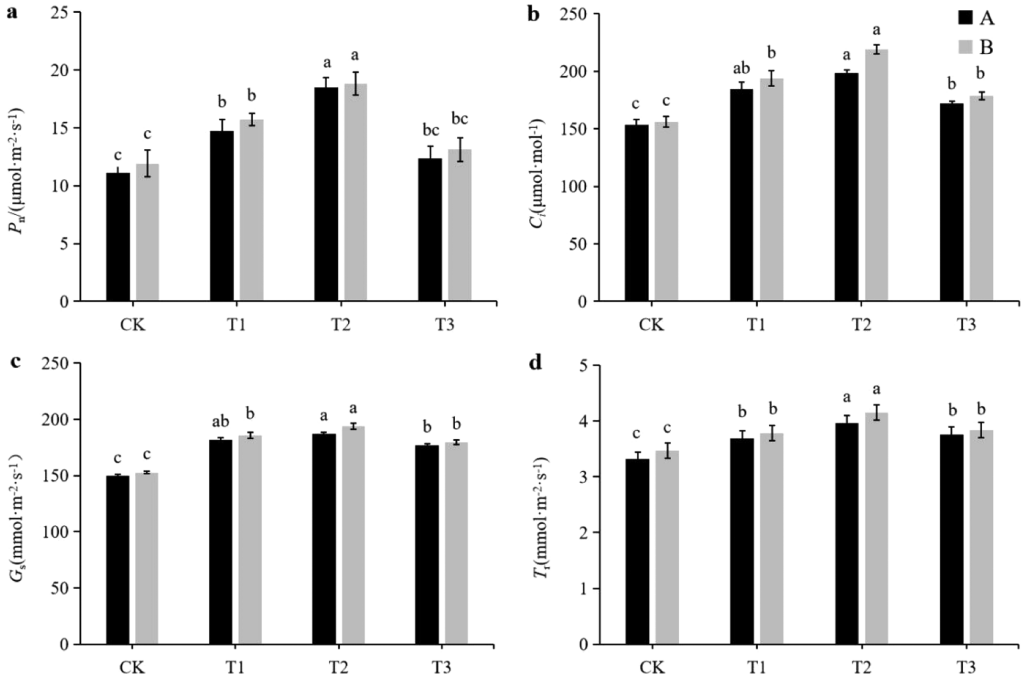

Figure 6. Effects of different soil amendments on photosynthetic parameters of grafted seedlings. (a) Net photosynthetic rate (P_n); (b) intercellular CO_2 concentration (C_i); (c) stomatal conductance (G_s); and (d) transpiration rate (T_r). A = July 15, 2018; B = July 15, 2019; CK = untreated control (replant soil); T1 = 1.0 g·kg^{-1} quicklime; T2 = 1.0 g·kg^{-1} quicklime + 1.0g·kg^{-1} superphosphate; T3 = 1.0 g·kg^{-1} plant ash. 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.

3. DISCUSSION

3.1. Effects of Different Soil Amendments on the Physical and Chemical Properties of Replanted Soil. Long-term continuous cropping had significantly reduced the soil pH in the apple orchard, and the acidification of the soil was obvious. Soil acidification alters biogeochemical cycling and damages ecosystem function. The results of this study showed that appropriate amounts of quicklime, superphosphate, and plant ash could improve the acidified soil in a replant orchard, and all three treatments significantly increased the replanted soil

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Table 3. Effects of Different Soil Amendments on Leaf Chlorophyll Content of Grafted Seedlings[a]

date	treatment	chlorophyll a/(mg·g^{-1} FW)	chlorophyll b/(mg·g^{-1} FW)	carotene/(mg·g^{-1} FW)
July 15, 2018	CK	14.67 ± 0.65c	7.43 ± 0.54c	2.9 ± 0.02c
	T1	19.44 ± 0.50ab	10.77 ± 0.62b	3.51 ± 0.07ab
	T2	21.84 ± 0.84a	12.55 ± 0.43a	3.77 ± 0.07a
	T3	18.28 ± 1.30b	9.38 ± 0.22b	3.39 ± 0.19b
July 15, 2019	CK	14.88 ± 0.61c	7.39 ± 0.22c	2.88 ± 0.13c
	T1	20.18 ± 0.65ab	11.36 ± 0.49b	3.61 ± 0.12ab
	T2	22.31 ± 0.73a	13.25 ± 0.63a	3.87 ± 0.07a
	T3	19.38 ± 0.87b	10.15 ± 0.25b	3.35 ± 0.21b

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

pH. T3 had the greatest effect on soil pH, and there are several potential explanations for this result: the main chemical component of quicklime is CaO. CaO can neutralize H^+, and quicklime adds exchangeable Ca^{2+} to the soil, reduces the cation exchange capacity, limits the toxicity of heavy metals (such as Al, Cu, and Cd), and gradually increases the acidic buffering performance and pH of the soil.[26−32] At the same time, the flocculation of Ca^{2+} and the cementation of lime itself are considered to be important short-term mechanisms. In the long term, increases in crop yield induced by lime increase the input of organic matter to the soil, ultimately increasing soil organic matter content and soil biological activity, both of which can improve soil stability and porosity.[17,33] Studies have shown that lime treatment significantly increases the pH of the 0−10 cm soil layer and has little effect on the pH of the subsoil.[34] In addition, a high concentration of quicklime is thought to impair plant growth and development because it can cause soil compaction and reduce soil permeability.[35] After superphosphate is applied to acidic soil, its main component is monocalcium phosphate, which will undergo exchange reactions with free iron and aluminum ions in the soil, then neutralize acidic soil substances, and increase the organic matter content, carbon to nitrogen ratio, and availability of nutrients.[36] The calcium provided by limestone, superphosphate, and plant ash may also be beneficial to plant defense responses because calcium strengthens the cell wall by cross-linking pectin and participates in defense signal transduction.[37] However, some studies have reported that calcium added to soil in the soil conditioner has little effect on the incidence of diseases caused by *F. oxysporum* relative to the effect of increasing soil pH to 7.0 or more under acidic conditions.[38,39]

3.2. Effects of Different Soil Amendments on Microbial Community Structure and Diversity in Replanted Soil. Soil microbial biomass can directly or indirectly reflect changes in the soil fertility and soil environmental changes, as it is a very sensitive biological indicator. Long-term continuous cropping reduces the number of beneficial microorganisms in the soil and increases the number of soil-borne microbial pathogens, changing the soil microbial community structure from a "bacterial" type to a "fungal" type. This can eventually lead to a breakdown in the microecological balance of the plant rhizosphere.[3,39−43] In many regions, harmful fungi in continuously cropped soil are considered to be the main cause of replant disease. By sampling and analysis of replanted apple orchards around Bohai Bay, researchers found that *Fusarium* fungi was the main pathogenic fungi in the replanted apple orchards of this region.[44] In light of this finding, we also measured the copy numbers of *F. solani* and *F. oxysporum* in the replanted soil.

Soil microbial communities can respond to ecological factors such as soil pH and soil conditions. Soil microorganisms are more sensitive to changes in environmental pH because their cells are in direct contact with the environment.[45] The results of previous studies indicate that pH strongly influences the radial growth of fungi. Differences in soil pH may change the growth rate of *Fusarium* spp., the amount of spores attached to plant roots, and the amount of inoculum.[46] The application of superphosphate increases the phosphorus content of the soil, and the phosphate fertilizer is a key factor that controls the total number and diversity of soil microbial colonies.[47] Phosphorus may also directly promote the growth of culturable bacteria. Applying phosphorus to the soil during rice production promotes the accumulation of soil organic carbon, which stimulates the growth of microorganisms[48] and improves the soil community structure.

Here, we found that three soil amendment treatments increased the number of bacteria and reduced the number of fungi in replanted soil, and the copy numbers of *F. solani* and *F. oxysporum* in the replanted soil were also significantly reduced. T2 had the strongest effect. These results show that quicklime, superphosphate, and plant ash can directly or indirectly inhibit the growth of some harmful fungi by adjusting the soil pH or increasing the availability of nutrient elements.[49] These materials may also promote the growth of soil bacteria and optimize the structure of the soil microbial community. The application of quicklime, calcium superphosphate, and plant ash to the replanted soil changed the microbial community structure under the original replanted environmental conditions, accelerated the transformation and decomposition rate of soil nutrients, and increased the number of bacteria, thereby alleviating ARD to some extent.

3.3. Effects of Different Soil Amendments on Soil Enzyme Activities in Replanted Soil. Soil enzymes are produced by soil microorganisms and are an important part of the soil ecosystem. Microorganisms respond rapidly to soil changes caused by natural processes and human activities, and changes in microbial activities can change the availability of nutrients absorbed by crops. For this reason, microorganisms are generally considered to be biological indicators of soil quality and biosensors[50−52] that can often be used to assess environmental status.[53,54] Microorganisms also have a direct impact on crop growth, development, and yield.[55] Phosphatase is one of the enzymes that convert phosphorus from unusable, organically bound forms into phosphate ions that can be absorbed by microorganisms and plants. Phosphatase is a good indicator of soil organic phosphorus mineralization potential and biological activity, and its activity is related to soil and vegetation conditions.[56,57] Most soil ureases come from microorganisms

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and plants.[58,59] Urease is an enzyme that catalyzes the hydrolysis of urea and is widely used to evaluate changes in soil quality in response to soil management.[60] Soil invertase is closely related to the metabolism of soil organic matter and the content of soil nitrogen and phosphorus; its activity can reflect the level of soil fertility and biological activity. The enzymatic reaction products of soil invertase can directly affect crop growth. Catalase activity may be related to the metabolic activities of aerobic organisms, and it has been used as an indicator of soil fertility.[61] Soil pH affects the activity of soil enzymes by controlling microbial enzyme production, conformational changes in the enzymes themselves induced by ionization, and the availability of substrates and enzyme cofactors.[62] Previous studies have found that some enzymatic reactions are very sensitive to changes in soil pH and can only be performed in a narrow pH range.[63] Our research showed that T1, T2, and T3 significantly increased the activities of urease, invertase, phosphatase, and catalase to different degrees compared with the replanted soil control treatment. Soil urease activity increased most significantly after the application of 1.0 $g{\cdot}kg^{-1}$ quicklime to the replanted soil, perhaps because quicklime stimulates a rapid increase in soil pH, which can promote soil microbial activity and bacterial abundance. Other studies have found that quicklime application can also increase the nitrogen content and available phosphorus in acidic soils and can promote soil enzyme activity.[64−69] The mixed application of 1.0 $g{\cdot}kg^{-1}$g quicklime and 1.0 $g{\cdot}kg^{-1}$ calcium superphosphate had a marked effect on the activities of sucrase, phosphatase, and catalase. After the soil pH was raised with quicklime, the application of calcium superphosphate may have stimulated the release of root exudates and the activities of rhizosphere microorganisms and may have increased soil calcium and phosphorus availability. It may thus have increased soil fertility and improved the rhizosphere environment to enhance soil enzyme activity.[70] In summary, lower pH may cause changes in the composition and size of the microbial community,[71] which in turn affects soil enzyme kinetics. The addition of quicklime, superphosphate, or plant ash to the replanted soil raises the soil pH and improves the environment of the soil microbial community, helping to alleviate ARD.

3.4. Effects of Different Soil Amendments on Photosynthesis and Biomass of Grafted Seedlings. Photosynthesis is one of the most important metabolic processes for plant growth. Under stress conditions, the inner membrane structure of plant thylakoids is damaged, chlorophyll synthesis is reduced, and the net photosynthetic rate and transpiration rate of leaves decrease, eventually limiting plant growth.[72] In addition, harmful fungi such as *F. solani* infect apple seedlings in replanted soil, causing leaf water deficit and stomatal closure; the resulting stomatal limitation leads to a decrease in CO_2 assimilation.[73,74] Feedback inhibition of photosynthetic electron transfer can then lead to an increase in chloroplast reactive oxygen species (ROS), which induce oxidative stress in the chloroplast and slow or stop photosynthesis.[75,76] The addition of quicklime and superphosphate increases the calcium and phosphorus content of the soil. Calcium ions (Ca^{2+}) are essential nutrients for plant growth and development and participate in multiple developmental processes, such as flower induction, flower bud differentiation, and flowering time regulation. Calcium serves as a signaling molecule that participates in photosynthetic electron transfer and photosynthetic phosphorylation, as well as other physiological and biochemical processes. Calcium plays an important role in plant photosynthesis: the application of calcium can alleviate the attenuation of the net photosynthetic rate, increase leaf chlorophyll content, and improve photosynthetic metabolism, enabling leaves to maintain high photosynthetic performance.[77−79] The phosphorus in superphosphate also plays an important role in photosynthesis. Phosphorus deficiency reduces plant photosynthesis by reducing the efficiency of the Calvin cycle and the regeneration of 1,5-ribulose diphosphate ribulose(RuBP). Phosphorus deficiency reduces photosynthesis through its effects on carbon assimilation, electron transport between PSII and PSI, and carbohydrate relocation.[80−82] Therefore, the addition of different soil amendments directly or indirectly promotes plant growth through various metabolic pathways in plant development. Here, the net photosynthetic rate, transpiration rate, intercellular carbon dioxide concentration, transpiration rate, and chlorophyll content were lowest in 2-year-old grafted seedlings under control replanted conditions. Photosynthetic parameters and chlorophyll content were significantly higher when soil was amended with quicklime or with the 1:1 quicklime and calcium superphosphate mixture, but the plant ash treatment had no significant effect. This result may reflect the fact that quicklime and calcium superphosphate can improve the soil environment, increase soil calcium and phosphorus content, promote plant growth, reduce ROS damage to chlorophyll molecules, improve photosynthetic electron transfer and photosynthetic capacity, and increase the proportion of captured light energy used for photosynthesis.

4. CONCLUSIONS

Soil acidification is a relatively obvious feature of replanted soil. It is essential to mitigate ARD by improving soil acidification. The application of quicklime, calcium superphosphate, and plant ash can increase the pH of acidified soil, improve the soil environment, and promote apple sapling growth. Among all the experimental treatments, the application of 1.0 $g{\cdot}kg^{-1}$ quicklime with 1.0 $g{\cdot}kg^{-1}$ superphosphate (T2) produced the best results.

5. MATERIALS AND METHODS

5.1. Experimental Materials. The experiment was conducted in a replanted apple orchard in Fengmaozhai (37.39°N, 120.09°E), Laizhou City, Yantai, Shandong Province from March 2018 to October 2019. The area has a temperate monsoon climate, with an annual precipitation of about 600 mm and an annual average temperature of about 12.6 °C.

The basic physical and chemical properties of the test soil were as follows: loam soil type, pH = 5.32, 33.89 $mg{\cdot}kg^{-1}$ nitrate nitrogen (NO^{3-}−N), 22.25 $mg{\cdot}kg^{-1}$ ammonium nitrogen (NH^{4+}−N) 9.79 $mg{\cdot}kg^{-1}$available phosphorus, 21.71 $mg{\cdot}kg^{-1}$ available potassium, and 5.09 $g{\cdot}kg^{-1}$ organic matter. Quicklime and superphosphate were purchased from Shanghai Guangnuo Chemical Technology Co., Ltd., and plant ash was purchased from Yizhan Experimental Equipment Co., Ltd. The experimental plant materials were biennial apple grafted seedlings where the root stock was T337 and the scion was Yanfu 3.

5.2. Experimental Design and Treatments. In March 2018, we selected a plot of land (40 m long × 20 m wide) in the old apple orchard (37.39°N, 120.09°E) in Fengmaozhai, Yantai City in Shandong Province. The old apple trees and residual roots were removed, and the soil was turned deeply by rotary tillage. Four treatments were set up: the three treatments that demonstrated the best effects in a previous pot experiment and an untreated control. Specifically, the four treatments were

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replanted apple orchard soil (CK), replanted soil treated with 1.0 g·kg^{-1} quicklime (T1), replanted soil treated with 1.0 g·kg^{-1} quicklime and 1.0 g·kg^{-1} superphosphate (T2), and replanted soil treated with 1.0 g·kg^{-1} plant ash (T3).

Saplings were planted in April 2018. We chose 4 rows as 4 treatments and 1 row as 1 treatment and dug out 40 cm square tree pits in the row. The spacing between the same row of tree pits was 1 m, and the row spacing was 4 m. The excavated soil was placed in the next tree pit and mixed with different test treatments. The required quicklime, superphosphate, plant ash, and replanting soil were planted at the same time in 4 treatments, and 20 saplings per treatment were then planted at the same time and grown with identical water and fertilizer management.

Sampling was performed in July 2018 and 2019. Three plants of similar size were selected for each measurement. Biomass was measured directly in the field, and the data were recorded. A five point sampling method was used for soil samples. When taking soil samples, the topsoil was removed first, and then, the rhizosphere soil was obtained with a ring knife. The soil was mixed, screened through a 2 mm sieve, and quickly stored in liquid nitrogen. The sieved soil samples were divided into three parts: one part was frozen at −80 °C for soil microbial analysis, the sample for DNA extraction was frozen at −20 °C, and the other was naturally air-dried to estimate the test indicators such as soil enzymes.

5.3. Measurement Indexes. *5.3.1. Plant Index.* Plant height, stem diameter, branch length, and dry and fresh weights were measured using a ruler, vernier caliper, tape, and electronic scale.

5.3.2. Photosynthetic Parameters. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of sapling leaves were measured with a Ciras-3 portable photosynthetic instrument (PP Systems, UK) from 9:00 a.m. to 11:00 a.m. on a sunny day in mid-July of 2018 and 2019. Three plants were randomly selected for these measurements, and three mature leaves (3rd to 5th leaves from the top) were measured per plant. Photosynthetic measurements were made at a light intensity of 1000 ± 50 μmol m^{-2} s^{-1}, a CO_2 concentration of 360 ± 20 μL L^{-1}, and a temperature of 26 ± 1 °C.[83]

5.3.3. pH Measurements. A PHS-2F pH meter (Shanghai INESA Scientific Instrument Co., Ltd.) was used to measure soil pH.

5.3.4. Soil Microbial Determination. Bacteria, fungi, and actinomycetes were determined by the plate coating method. Beef extract peptone medium was used for bacteria, PDA selective medium was used for fungi, and No. 1 medium was used for actinomycetes.[84]

5.3.5. Real-Time Quantitative Polymerase Chain Reaction (qPCR). qPCR was performed as described by Wang.[85] DNA was extracted according to the instructions of the E.Z.N.A. Soil DNA Extraction Kit (Omega Bio-Tek, Norcross, GA, USA), and qPCR was performed on a CFX96 Thermal Cycler (Bio-Rad) to quantify the gene copy number of *F. oxysporum* and *F. solani* in the soil. The primer pairs were FR (5′-GGCCTGAGGGTTG-TAATG-3′) and FF (5′-CGAGTTATACAACTCATCAACC-3′) and JR (5′-GAACGCGAATTAACGCGAGTC-3′) and JF (5′-CATACCACTTGTTGTCTCGGC-3′).

5.3.6. Soil Enzyme Determination. Soil enzyme activities were measured as described by Guan.[86] Colorimetric methods were used for urease, invertase, and phosphatase, and the potassium permanganate titration method was used for catalase. Soil urease activity was expressed as the mass of NH_3−N in 1 g soil after 24 h (mg/[g·d]). Soil invertase activity was expressed as the mass of glucose in 1 g soil after 24 h (mg/[g·d]). Soil phosphatase activity was expressed as the mass of phenol in 1 g soil after 24 h (mg/[g·d]). Soil catalase activity was expressed as the volume of 0.1 M potassium permanganate in 1 g soil (mL/g).

5.3.7. DNA Extraction and High-Throughput Sequencing. The E.Z.N.A. soil DNA extraction kit (Omega Bio-Tek) was used to extract DNA. The primers for fungi were ITS1 (F: 5′-AACCTGCGGAAGGATCATT-3′ and R: 5′-GARCCAAGA-GATCCRTTG-3′). The adapters were merged, PCR amplification was performed, and the final product was purified. A sequencing library was constructed after quantification and homogenization. After passing the quality inspection, the library was mixed, denatured, and sequenced on the Illumina MiSeq platform (Beijing Yuanyi Biotechnology Co., Ltd., Beijing, China), followed by bioinformatics analysis.

5.4. Data Analysis. All data are expressed as the mean ± standard deviation of three replicates. Microsoft Excel 2003 was used for data processing and graphing, SPSS19.0 was used for variance analysis, and *t*-tests or one-way analysis of variance was used to evaluate significant differences between the samples. $P < 0.05$ was considered to be statistically significant. Duncan's new complex range method and *t*-test were used to assess the significance of differences. Based on the OTU abundance table, the R language tools were used to obtain the relative abundance of bacterial and fungal species and conduct PCA.

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Notes

The authors declare no competing financial interest.

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