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Effect of Hydrogen Peroxide on the Soil Microbial Community Structure and Growth of *Malus hupehensis* Rehd. Seedlings under Replant Conditions

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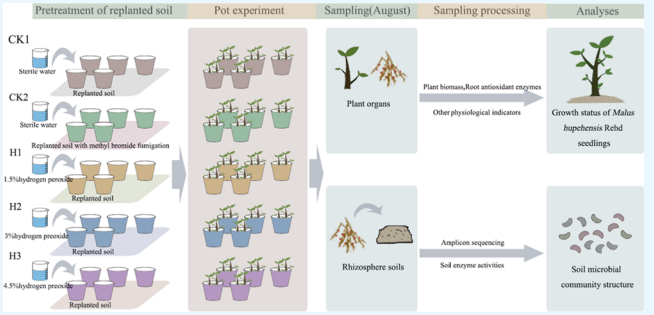
ABSTRACT: Apple replant disease (ARD) is common in apple production, which seriously affects the growth and development of apples. In this study, hydrogen peroxide with a bactericidal effect was used to treat the replanted soil, and the effects of different concentrations of hydrogen peroxide on replanted seedlings and soil microbiology were investigated in order to seek a green, clean way to control ARD. Five treatments were set up in this study: replanted soil (CK1), replanted soil with methyl bromide fumigation (CK2), replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3). The results showed that hydrogen peroxide treatment improved replanted seedling growth and also inactivated a certain number of *Fusarium*, while the *Bacillus*, *Mortierella*, and *Guehomyces* also became more abundant in relative terms. The best results were obtained with replanted soil + 4.5% hydrogen peroxide (H3). Consequently, hydrogen peroxide applied to the soil can effectively prevent and control ARD.



1. INTRODUCTION

Apple replant disease (ARD) commonly refers to a situation where the old trees were removed, and they become weak or even die when young trees are replanted in the same orchard soil.[1] Due to frequent tree species optimization, but limited land resources, it is difficult to avoid replanting cultivation in the original site during orchard renovation, which in turn leads to the widespread occurrence of ARD, and significantly restricts the sustainable development of the apple industry.[2] Replanted young apple trees usually exhibit such phenomena as show delayed root extension, slow plant metabolism, poor resistance to stress, and even death of the whole plant.[3] ARD causes serious economic losses throughout the whole life cycle of an orchard.[4] Thus, it is urgent to develop alternative clean and green measures to control ARD.

ARD was caused by a combination of reasons,[5,6] for example, nematodes, oomycetes, and chemosensitive autotoxic substance imbalance in the structure of soil microbiology,[7−9] among which soil microbiological imbalance is known to be the main reason for ARD.[10,11] When fruit trees are planted in orchards for successive years, beneficial bacteria decrease and the number of soil pathogenic fungi increases, which eventually results in a decrease in the yield of fruit trees.[12] In South Africa, Washington, Italy, and other apple producing countries, *Fusarium*, *Pythium*, *Phytophthora*, and *Cryptococcus* were considered to be harmful pathogens that cause ARD.[13,14] Liu[15] found that the frequency of *Fusarium* was high when isolating and identifying harmful fungi in the soil of replanted orchards. *Fusarium* showed high pathogenicity to the *M. hupehensis* Rehd. seedlings.[16]

In the United States, the use of brassicaceae seed meal to mitigate ARD has achieved better results.[4,17] However, in China, due to various reasons, soil fumigation is still a common way to control ARD.[18,19] However, although traditional soil chemical fumigants have obvious effects, they also have disadvantages such as easy residue, pollution of the natural environment, and potential hazards to human health.[20,21] Therefore, it is very important to seek a green, efficient, and reliable measure to solve ARD. Hydrogen peroxide is a simple, safe, economical, and environmentally friendly oxidizing agent, and the by-product of hydrogen peroxide is water, which is often called the "Green Oxidant". Therefore, the use of hydrogen peroxide has been concerned by researchers for a long time.[22] It is widely used in medicine, agriculture, food safety, and clinical and environ-

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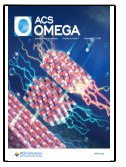

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mental applications.[23,24] For example, in the medical field, hydrogen peroxide was often used as a bactericide or disinfectant.[25] In the food processing industry, hydrogen peroxide was often used to kill microorganisms on food packaging bags, containers, and disinfectants such as drinking water, which could effectively inhibit the growth of microorganisms.[26] In the agricultural field, Kyeong-Hwan et al[27] used hydrogen peroxide vapor that interfered with the growth of disease-causing microorganisms. This was because hydrogen peroxide could react with lipid double bonds in the microbial cell wall and enter the microbial interior, acting on proteins and lipids and polysaccharides, altering cell permeability, and leading to cell lysis and death.[28] In addition, traditional chemical fumigation was time-consuming and requires film covering. In contrast, hydrogen peroxide to kill harmful microorganism in replanted soils may be a clean, green measure. However, there was little literature describing the application of hydrogen peroxide in affecting the severity of ARD by killing soil microorganisms.

In the present experiment, we evaluated the feasibility of hydrogen peroxide to mitigate ARD under replanting conditions. We aimed to (1) determine the optimal concentration of hydrogen peroxide; (2) how different concentrations of hydrogen peroxide affect the soil microbial community structure; (3) response of replanted seedlings to various concentrations of hydrogen peroxide, thus providing new insights into the ARD mitigation.

2. MATERIALS AND METHODS

2.1. Experimental Materials. The replanted soil was obtained from an old orchard in Manzhuang Town where apple trees have been planted for 34 years. The soil type was sandy loam. After the top soil was cleaned off, multiple points soil was randomly selected within a depth of 20−40 cm and mixed well. Supplementary Table 1 describes soil basic physicochemical properties.

The experiment material was *M. hupehensis* Rehd. seedlings, and it is a very widely planted rootstock of apple in China. *M. hupehensis* Rehd. seeds were stratificated for roughly 40 days at 4 °C. The seeds were planted in seedling cups contained with seedling substrates when white radicles were seen. When the seedling grew to 5−6 true leaves, the seedlings with similar growth, complete leaves, and no pests and diseases were transplanted into tile pots with different soil treatments (pot diameter 24 cm, height 18 cm, and soil weight 7 kg).

Methyl bromide fumigation products are provided by Jiangsu Lianyungang Dead Sea Bromide Co.

Hydrogen peroxide was purchased from Jinan Kunfeng Chemical Co., Ltd., with a concentration of 27.5%.

2.2. Experimental Design. The experiment was performed from April to October 2021 at the Science and Technology Innovation Park of Shandong Agricultural University (36.16°N, 117.1 6°E). A total of 5 treatments were examined: replanted soil (CK1), replanted soil with methyl bromide fumigation (CK2), replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3).

Methyl bromide fumigation treatment and hydrogen peroxide treatment were performed 15 days before the planting of *M. hupehensis* Rehd. seedlings (performed in mid-April 2021). Methyl bromide fumigant was mixed with replanting soil and put into the sealed trellis film for sealing, the soil layer is controlled about 20 cm, and 50 g of methyl bromide fumigation per square meter of soil is applied. If converted to pot, 0.125 g/kg is applied to each pot, and the temperature is controlled at 15 °C.[19] Hydrogen peroxide was diluted in water in three different proportions (1.5, 3.0, and 4.5%). The soil was irrigated until the upper-middle layer of soil reached a saturated state (518 mL). Equal amounts of sterile water were added to CK1 and CK2 treatments as control. Five repetitions were set up for each treatment.

On May 01, 2021, replanted seedlings with consistent growth were planted in each treatment (two seedlings per pot). Each treatment received uniform pruning, irrigation, and management. Plant and soil samples were taken from the 5 treatments on August 15. Each treatment group was sampled by randomly chosen three seedlings. Rhizosphere soil was collected from the pot by removing soil at the top and around the pot, and it was mixed thoroughly. The rhizosphere soil samples were sealed in a resealable bag and divided into three parts after sieving through 20 mesh; one part was air-dried under natural conditions and performed for the measurement of soil enzyme activity; one part was put into a 4 °C refrigerator for the determination of soil microorganisms; and the other was placed at −80 °C for Illumina MiSeq sequencing. When the plant samples were collected, the *M. hupehensis* Rehd. seedlings were washed. Vigorous white roots were excised and stored in liquid nitrogen for the determination of root enzymes and viability. Fresh and intact leaves were selected from the plant samples and stored at −20 °C to determine the chlorophyll content.

2.3. Indicators for Plant and Soil Measurements. The plant heights and ground diameters were measured with conventional methods such as a pylon ruler and dial calipers. The soil on the seedlings was washed off by water, excess water was wiped off, and the fresh seedlings were weighed with an electronic balance. After the determination, it was quenched at 105 °C for 30 min and dried at 65 °C, and the dry mass was weighed. The determination of the chlorophyll content was carried out by extraction with ethanol[29] (Supplementary Material 1.1). The determination of photosynthetic parameters was carried out on August 14 (sunny day, no wind) from 9:00 am to 11:00 am (Supplementary Material 1.2). The TTC method was used to determine the root respiration rate[30] (Supplementary Material 1.3). The determination of root antioxidant enzyme activity refers to the method of Singh et al.[31] (Supplementary Material 1.4).

The number of culturable microorganisms in the soil was measured by the dilution plate counting method,[32] and the culturable bacteria and fungi were measured by Luria-Bertani, potato dextrose agar (Supplementary Material 1.5). Soil enzyme activity was measured by referring to the method determined by Chen et al.[33] Soil sucrase activity was determined by the 3-amino-5-nitrosalicylic acid colorimetric method. Soil urease activity was determined by the sodium phenol−sodium hypochlorite colorimetric method. Soil neutral phosphatase activity was performed with the phenyl disodium phosphate colorimetric method (Supplementary Material 1.6). DNA was obtained from 0.5 g of fresh soil by the E.Z.N.A. soil DNA extraction kit.[34] The gene copy number of *F. oxysporum* was determined by real-time fluorescence quantitative polymerase chain reaction using a Bio-Rad CFX96 quantitative PCR instrument (Supplementary Material 1.7). JR (5′-GGCCTGAGGG TTGTAATG-3′) and JF (5′-CG AGTTA-TACAACTCATCAACC-3′) were the primers employed for the reaction. Soil microbial communities in Illumina MiSeq sequencing: 338F (5′-ACTCCTACGGGAGGCAGCAG-3′)

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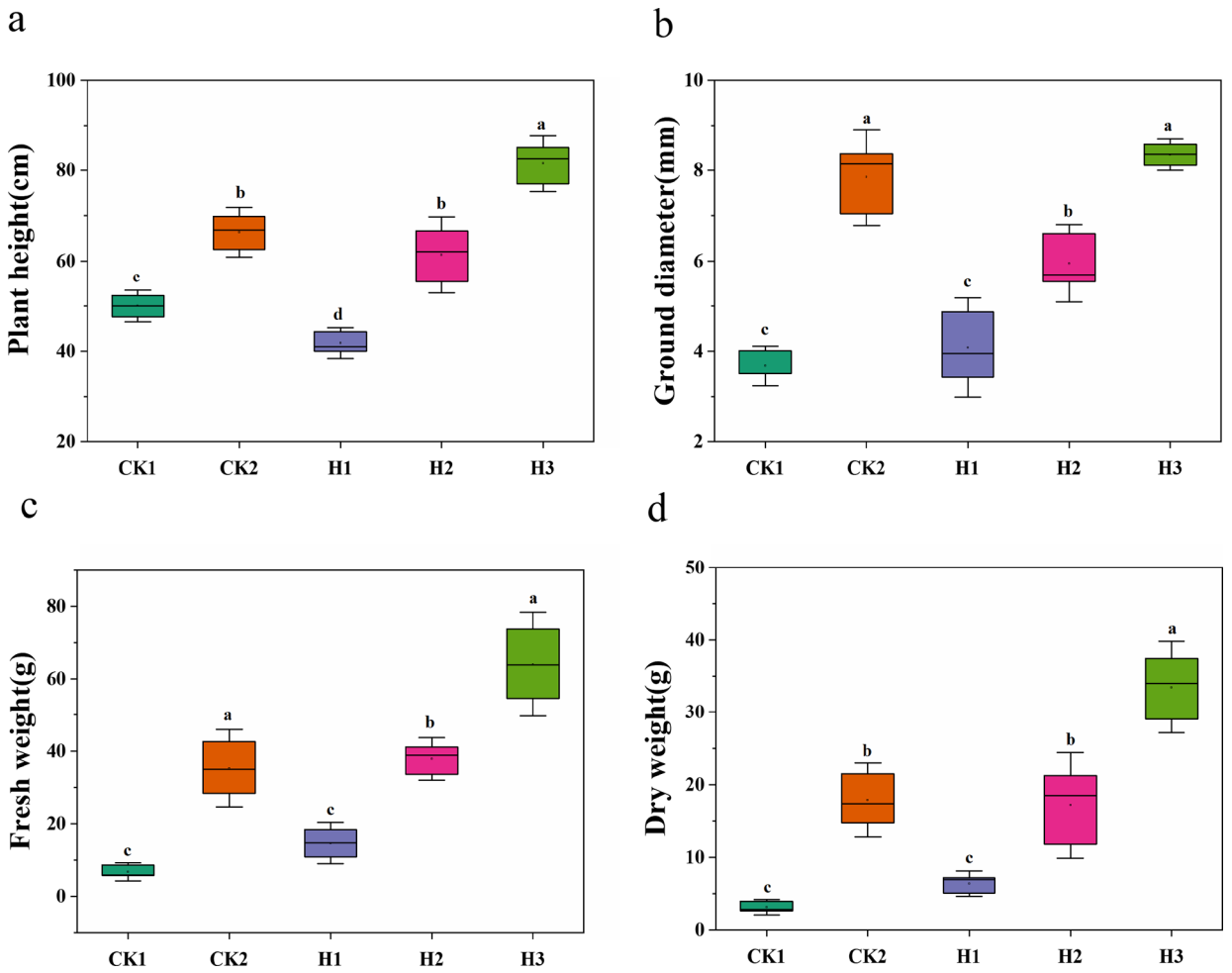

Figure 1. Growth status of replanted seedlings. (a) Plant height; (b) ground diameter; (c) fresh weight; (d) dry weight; CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide; letters reflect different degrees of treatment variation ($P < 0.05$).

and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were 16S rRNA primer sequences,[35] ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were ITS primer sequences.[36]

2.4. Data Analysis. The original sequencing was filtered out using fastp software to eliminate the ones smaller than 50 bp. According to their overlapped sequences, sequences over 10 bp were assembled using flash software. Operational taxonomic unit (OTU) clustering analysis was usually conducted with 97% similarity. Based on OTU results, the alpha diversity index was calculated in different treatments by using mothur (https://mothur.org/wiki/calculators/.version 1.30.2) and plotted with origin 2018 software (Origin Lab Corporation, USA). The rarefaction curves were plotted by R language, and the R language (version 3.3.1) vegan package was employed for Cluster heat map analysis. Principal coordinate analysis (PCoA) was calculated for each sample at the genus level. The significance of the difference between multiple groups was tested by the Kruskal−Wallis *H* test. Functional annotation and prediction of bacterial and fungal communities were performed using PICRUSt1 and FUNGuild prediction analysis, respectively. SPSS 26.0 (IBM SPSS Statistics, IBM Corporation, USA) was used for mean comparison and single-factor analysis of variance. The Duncan-style new multiple range method was used for significant difference comparison, and data were composed of mean ± standard error. Origin 2018 software was used to construct the figures.

3. RESULTS

3.1. Influences of Hydrogen Peroxide on the Growth of Replanted Seedlings. There were significant differences in the growth status of replanted seedlings under different concentrations of hydrogen peroxide treatment (Figure 1). H2 and H3 treatments obviously improved the growth of replanted seedlings; among them, H3 treatment had the best effect. The plant height, ground diameter, fresh weight, and dry weight of the H3 treatment increased 0.63, 1.27, 8.47, and 9.69 times, respectively, compared to CK1. Compared with CK2, H3 treatment increased 0.23, 0.06, 0.82, and 0.87 times in the plant height, ground diameter, fresh weight, and dry weight, respectively (Figure 1).

3.2. Influences of Hydrogen Peroxide on the Chlorophyll Content and Photosynthetic Parameters of Replanted Seedlings. Replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) all significantly increased the chlorophyll a and b contents (Table 1). Among them, the treatment with replanted soil + 4.5% hydrogen peroxide (H3) had the best effect, followed by CK2.

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Table 1. Effects of Different Treatments on Chlorophyll a and Chlorophyll b in the Leaves of Replanted Seedlings[a]

treatment	chlorophyll a ($mg \cdot g^{-1} \cdot FW$)	chlorophyll b ($mg \cdot g^{-1} \cdot FW$)
CK1	18.45 ± 0.77d	5.46 ± 0.54d
CK2	23.44 ± 0.20b	8.23 ± 0.45ab
H1	21.37 ± 0.68c	6.06 ± 0.35d
H2	22.81 ± 0.56bc	6.80 ± 0.49cd
H3	25.83 ± 0.68a	9.79 ± 0.74a

[a]Note: letters reflect different degrees of treatment variation ($P < 0.05$).

Compared with CK1, chlorophyll a, b increased by 15.8 and 11.0% in the replanted soil + 1.5% hydrogen peroxide (H1) treatment; 23.6 and 24.5% in the replanted soil + 3.0% hydrogen peroxide (H2) treatment; and 40.0 and 79.3% in replanted soil + 4.5% hydrogen peroxide (H3) treatment, respectively. The differences in chlorophyll a, b between H1 and H2 were not significant.

Replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments all significantly improved replanted seedling photosynthetic parameters (Figure 2). The application of replanted soil + 4.5% hydrogen peroxide (H3) was shown to be significantly different from all other treatments. Compared with CK1, intercellular carbon dioxide concentration, net photosynthetic rate, stomatal conductance, and transpiration rate of replanted soil + 4.5% hydrogen peroxide (H3) treatment increased by 14.7, 106.3, 81.8, and 56.7%, respectively. The difference in the intercellular carbon dioxide concentration between replanted soil + 1.5% hydrogen peroxide (H1) and replanted soil + 3.0% hydrogen peroxide (H2) treatments was not significant.

3.3. Influences of Hydrogen Peroxide on Root Antioxidant Enzyme Activities and the Root Respiration Rate of Replanted Seedlings. Replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments all significantly improved root antioxidant enzyme activity (Figure 3). Compared with CK2, the superoxide dismutase (SOD) and catalase (CAT) activities of H3 increased by 21.55 and 4.93%, respectively. Significant differences were observed in the root antioxidant enzyme activities of replanted soil (CK1) and hydrogen peroxide treatment. The H1, H2, and H3 treatments also significantly increased the root respiration rates by 26.1, 51.3, and 96.4%, respectively, compared to replanted soil (CK1).

3.4. Influences of Hydrogen Peroxide on Soil Enzyme Activity. Replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted

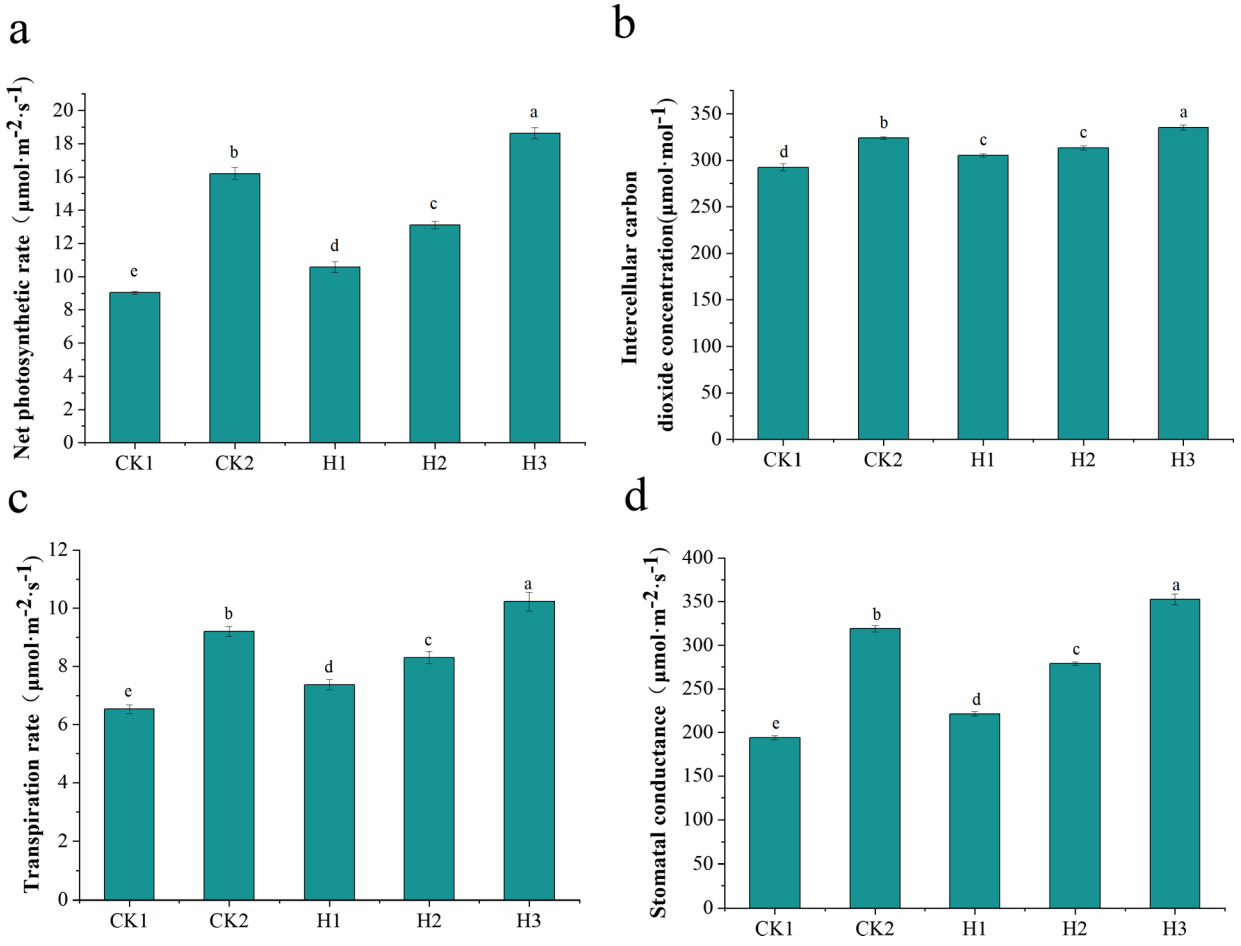

Figure 2. Effect of hydrogen peroxide treatment on photosynthetic parameters of replanted seedlings. (a) Net photosynthetic rate; (b) intercellular carbon dioxide concentration; (c) transpiration rate; (d) stomatal conductance; CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide; letters reflect different degrees of treatment variation ($P < 0.05$).

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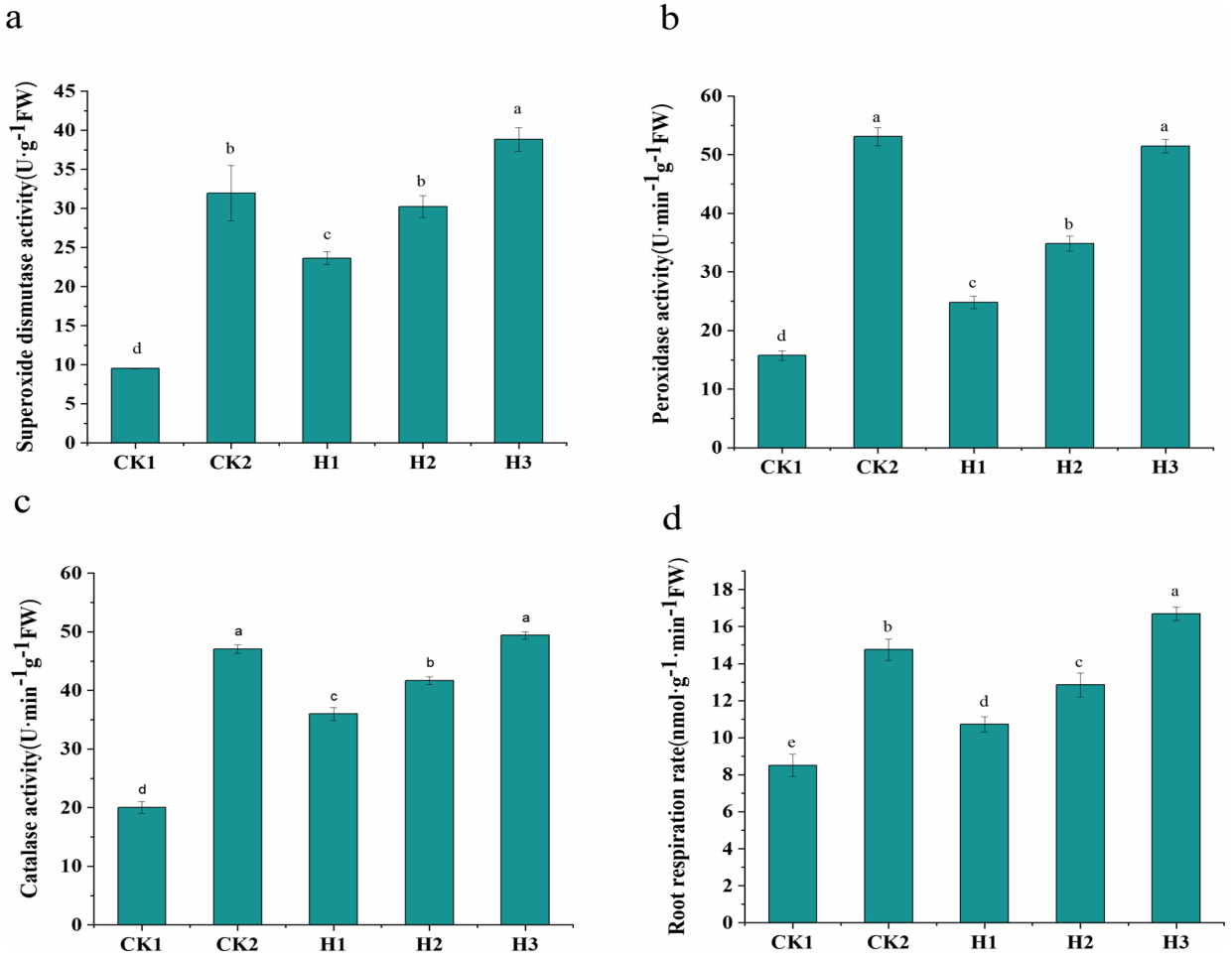

Figure 3. Effects of root antioxidant enzymes and the root respiration rate of replanted seedlings under hydrogen peroxide treatment. (a) Superoxide dismutase activity; (b) catalase activity; (c) peroxidase activity; (d) root respiration rate; letters reflect different degrees of treatment variation ($P < 0.05$).

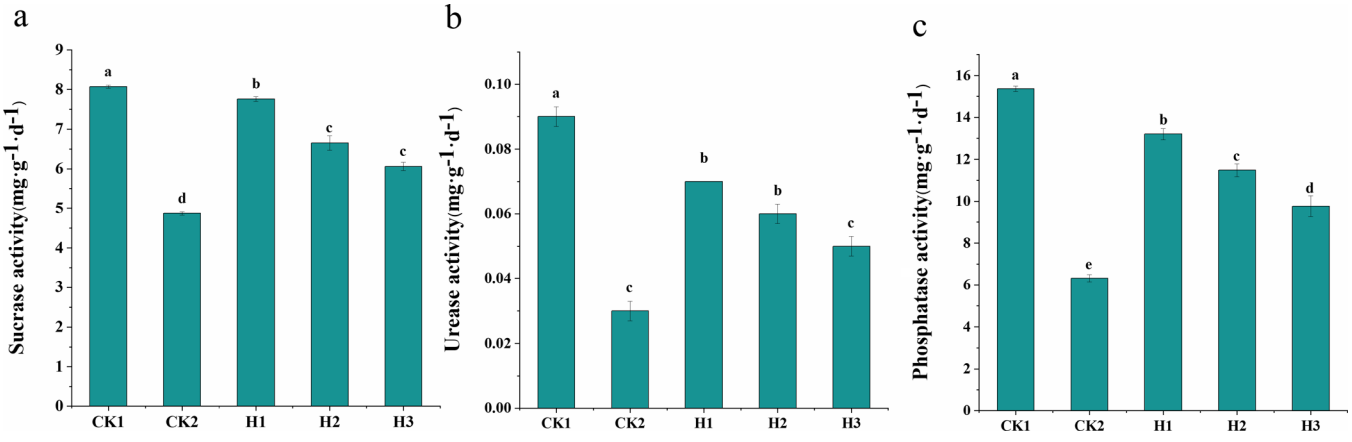

Figure 4. Effect of hydrogen peroxide treatment on soil enzyme activity. (a) Sucrase activity; (b) urease activity; (c) phosphatase activity. CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide; letters reflect different degrees of treatment variations ($P < 0.05$).

soil + 4.5% hydrogen peroxide (H3) treatments all significantly reduced the activity of soil enzymes (Figure 4). Among them, replanted soil with CK2 had the largest decrease, followed by replanted soil + 4.5% hydrogen peroxide (H3) treatment. Compared to CK1, sucrase, urease, and phosphatase activity were reduced by 3.80, 22.2, and 14.1%, in replanted soil + 1.5% hydrogen peroxide (H1); 17.5, 33.3, and 25.3%, in replanted soil + 3.0% hydrogen peroxide (H2); and 24.9, 44.4, and 36.5% in replanted soil + 4.5% hydrogen peroxide (H3), respectively.

3.5. Analysis of Culturable Microorganisms and RT-qPCR Analysis of *Fusarium oxysporum*. The addition of different concentrations of hydrogen peroxide can significantly

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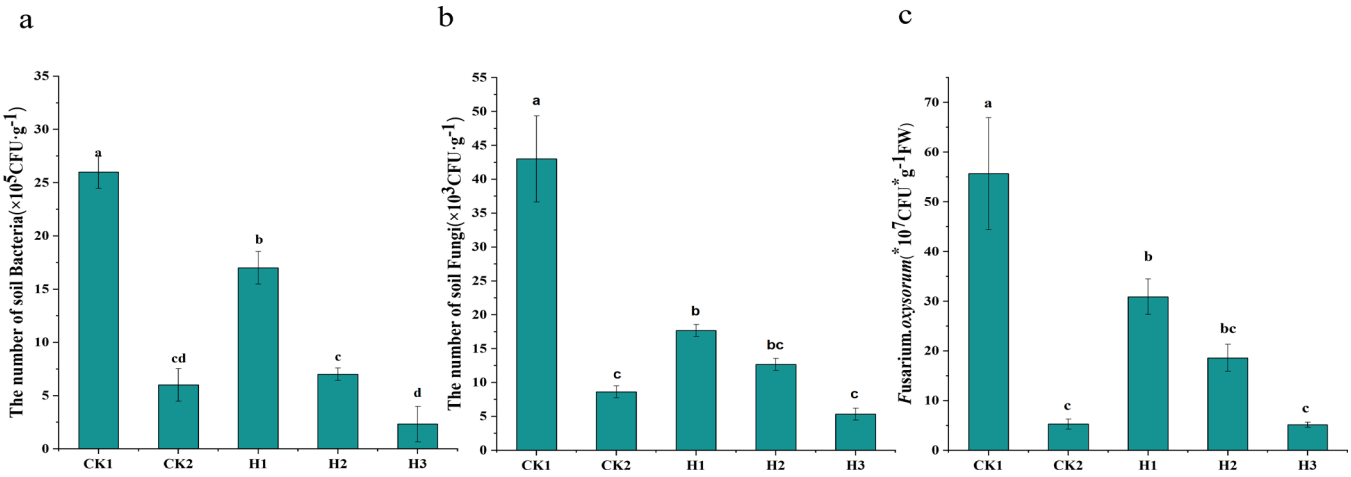

Figure 5. Changes in soil microbial population under different treatments. (a) Number of soil bacteria; (b) number of soil fungi; (c) gene copy number of *F. oxysporum*; CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide; letters reflect different degrees of treatment variations ($P < 0.05$).

improve the environment of replanted soil (Figure 5). In particular, replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments significantly reduced the bacteria, fungi, and *Fusarium oxysporum* numbers in the soil. Replanted soil + 4.5% hydrogen peroxide (H3) treatment had the largest decrease, followed by replanted soil + 3.0% hydrogen peroxide (H2) and finally replanted soil + 1.5% hydrogen peroxide (H1). Compared with the replanted soil (CK1), soil bacteria and fungi were decreased by 34.6 and 58.9% in the replanted soil + 1.5% hydrogen peroxide (H1) treatment; 73.1 and 70.5% in the replanted soil + 3.0% hydrogen peroxide (H2) treatment; and 91.0 and 87.6% in the replanted soil + 4.5% hydrogen peroxide (H3) treatment, respectively. In addition, compared with replanted soil (CK1), *Fusarium oxysporum* of replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments decreased by 44.5, 66.6, and 90.8%, respectively. The gene copy number of *F. oxysporum* in the other treatments was lower than that in replanted soil (CK1).

3.6. Analysis of Soil Microbial Community Composition at the Genus Level. By sequencing, the dilution curve of fungi and bacteria tend to be flat, which indicated that the data volume of microbial communities of soil environmental samples was close to saturation, and it indicated that the current sequencing quantities could reflect the majority of microbial diversity information in the samples (Figure S1). The abundance heatmap for bacteria and fungi genera was constructed from the top 10 dominant species in the sample. *Arthrobacter*, *Sphingomona*, *RB41*, *Bacillus*, and *Gaiella* were the main bacterial species, except for unclassified bacteria (Figure 6a). *Mortierella*, *Pseudallescheria*, *Fusarium*, *Lophiostoma*, *Guehomyces*, *Humicota*, *Trichoderma*, and *Kermia* were the dominant fungal species except for the unclassified fungi (Figure 6b). The species of bacterial and fungal genera were basically the same between treatments, but their relative abundance differed significantly. The comparative abundance of *Bacillus* increased by 14.03% for the H3 treatment compared to CK1; the comparative abundance of *Fusarium* decreased by 40.29% for the H3 treatment compared to CK1. In addition, compared with

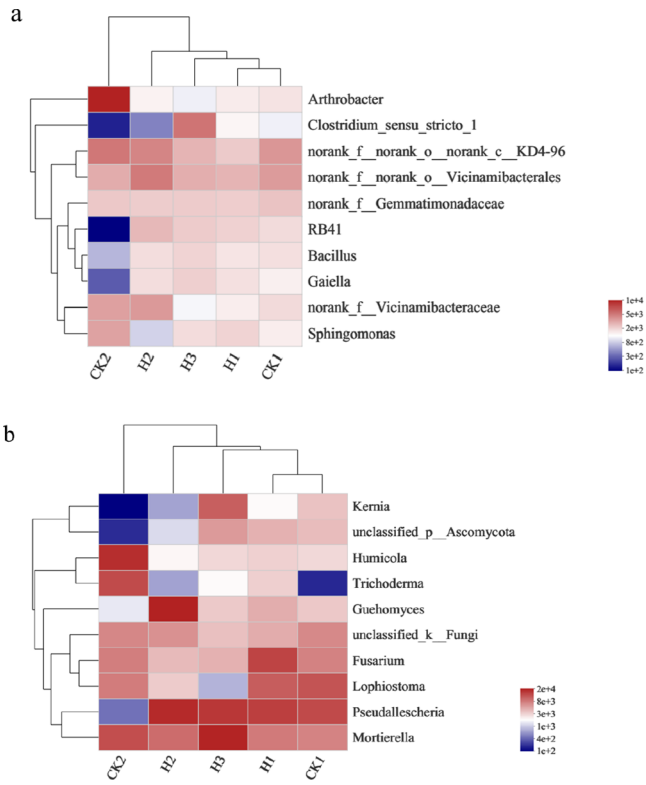

Figure 6. Differences between species of bacterial genus (a) and fungal genus (b) for different treatments. CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide.

CK1, CK2, H1, H2, and H3 treatments obviously increased the comparative abundance of *Mortierella*, and the largest increase was observed in the treatment of replant soil + 4.5% hydrogen peroxide (H3), followed by CK2 treatment. Compared to CK1, replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments significantly increased the comparative abundance of *Trichoderma*, with CK2 showing the largest increase.

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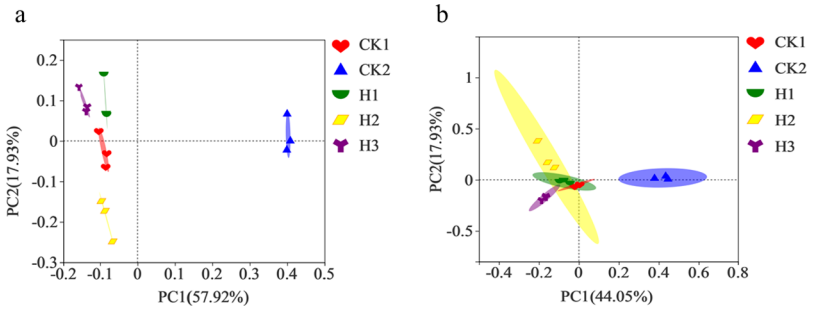

Figure 7. PCoA was calculated for each sample on the basis of the Bray−Curtis distance matrix at the bacterial genus (a) and fungal genus (b) level. CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide.

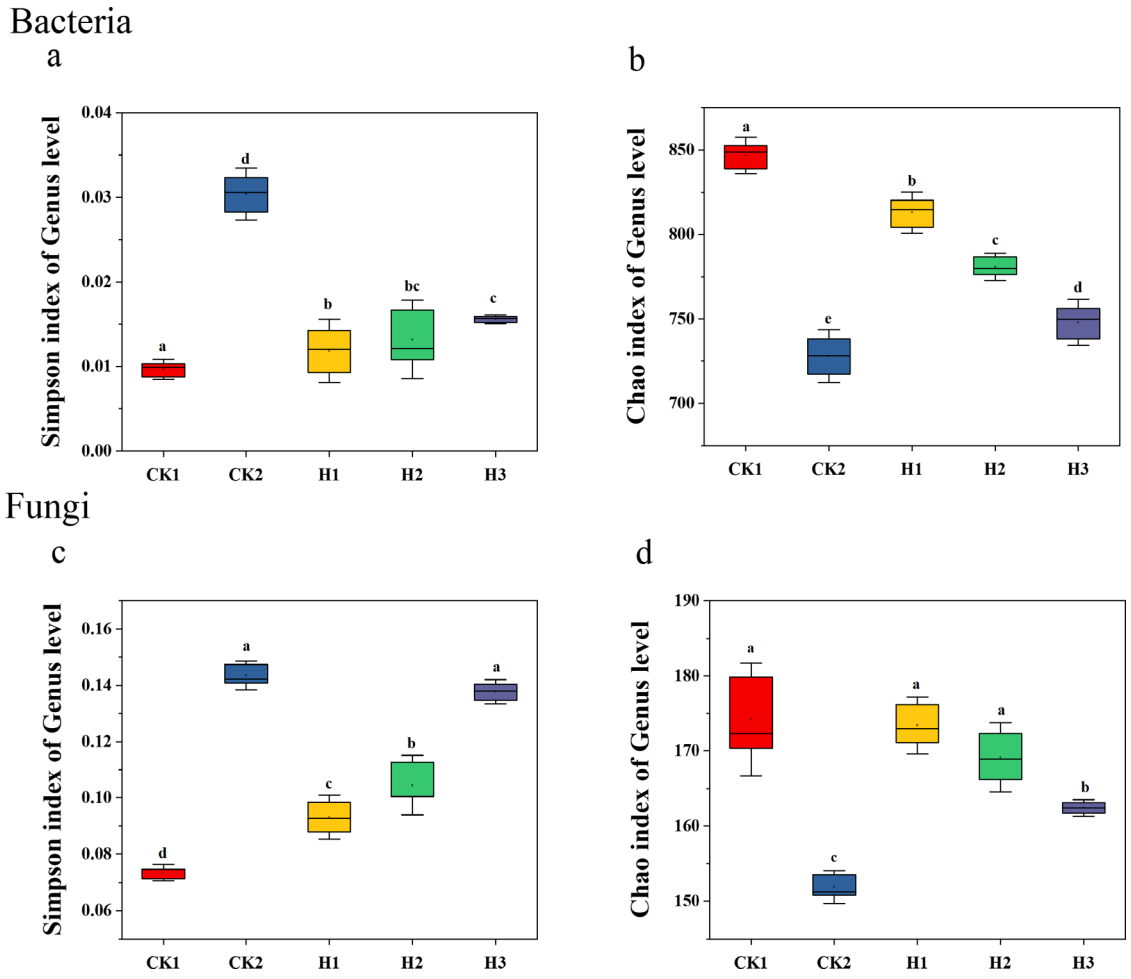

Figure 8. Diversity of microorganisms under different treatments. Simpson index for species of the genera bacteria (a) and fungi (c); Chao index for species of the genera bacteria (b) and fungi (d). CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide; letters reflect different degrees of treatment variations ($P < 0.05$).

3.7. Differences in the Microbial Community Composition in Different Treatments. The PCoA plot showed that PC1 and PC2 explained 75.85% of the changes in bacterial communities and 61.98% of the changes in fungal communities, respectively (Figure 7). Microbial communities were significantly different between treatments, CK2 being the most distant from CK1, meaning that the differences were the greatest. The three different hydrogen peroxide concentration treatments also had a certain distance from the CK1, and the application of hydrogen peroxide in the replanted soil might have an impact on the change of the microbial community structure. The treatments were subjected to the Kruskal−Wallis *H* test based on the different microbial community structures, at the level of bacterial genera, *Gaiella*, *Pedomicrobium*, *Romboutsia*, and *Turicibacter* that were more abundant in the hydrogen peroxide-treated soils, with CK2 treatment being the most pronounced (Figure S2). At the fungal genus level, the comparative abundance of *Mortierella*, *Guehomyces* in the soil treated with hydrogen peroxide was significantly higher, while

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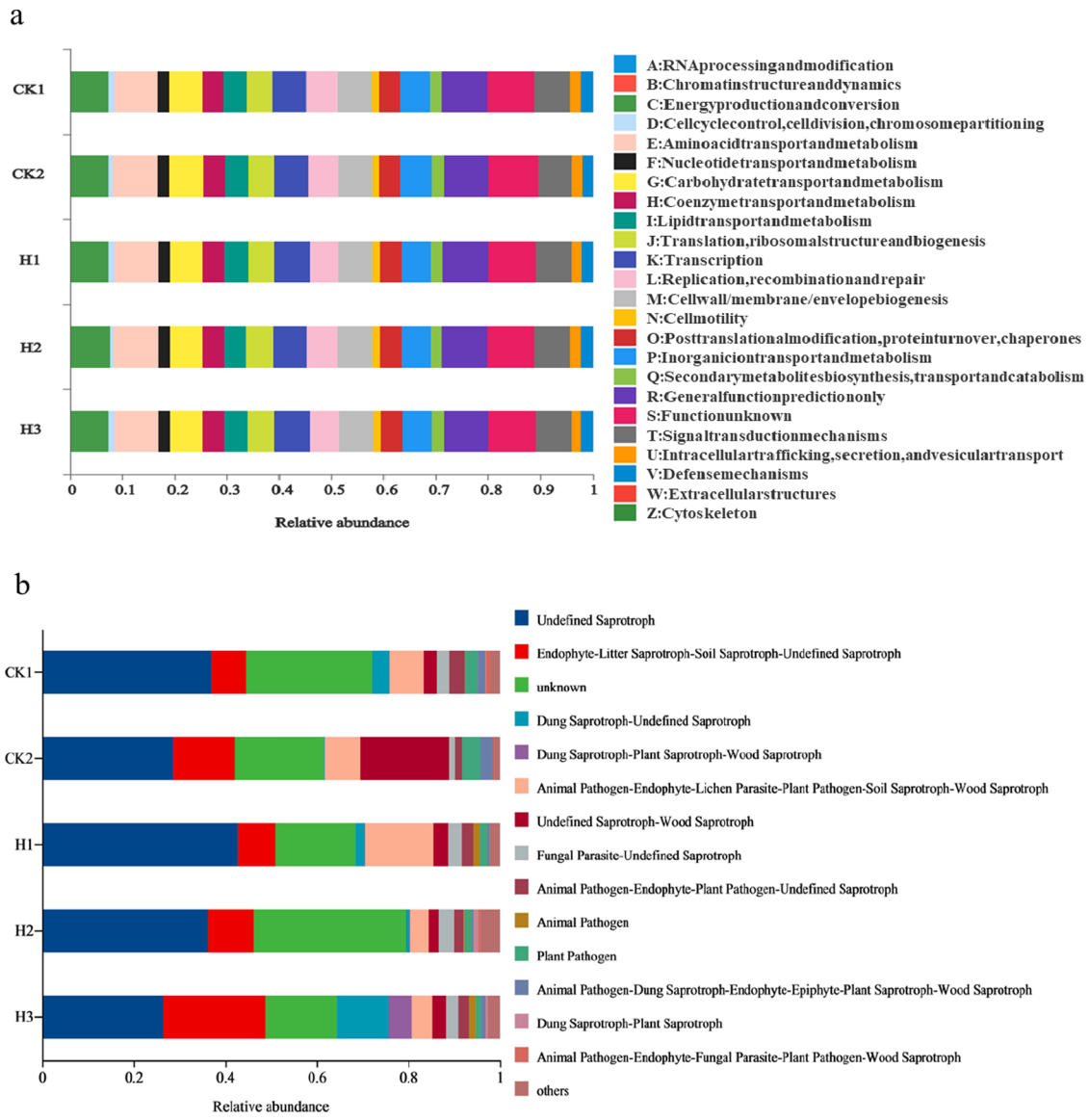

Figure 9. Predicted function of bacterial (a) community and fungal (b) community. CK1: replanted soil; CK2: replanted soil with methyl bromide fumigation; H1: replanted soil + 1.5% hydrogen peroxide; H2: replanted soil + 3.0% hydrogen peroxide; H3: replanted soil + 4.5% hydrogen peroxide.

the comparative abundance of *Fusarium* and *Lophiostoma* was significantly lower (Figure S2).

3.8. Analysis of Microbial Alpha Diversity under Different Concentrations of Hydrogen Peroxide Treatment. The Simpson and Chao indices of bacteria and fungi in soil treatments with different concentrations of hydrogen peroxide were significantly different. In general, the Simpson index often estimated microbial diversity in samples, and the smaller the Simpson index, the higher the community diversity. The Simpson indices of replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments were all higher than those of CK1, indicating that the microbial diversity of soil treated with hydrogen peroxide was lower (Figure 8a,c). The Chao index was used to reflect the abundance of microorganisms, and the comparative abundance of bacteria and fungi in the hydrogen peroxide-treated soil showed a decreasing trend, with significant differences in the Chao index of bacterial genera between treatments. The Chao index of fungi genera in CK2 and H3 treatments showed some differences (Figure 8b,d).

3.9. Microbial Community Function Prediction. PICRUSt1 and FUNGuild prediction analyses were made to analyze the possible functions of bacterial and fungal communities in the rhizosphere soil, respectively. PICRUSt1 results indicated that a total of 24 taxon functions were obtained in the bacterial communities. Except for the unclassified functions and prediction-only functions, the top three functions were amino acid transport and metabolism, energy production and conversion, and signal transduction mechanisms, and their relative abundance in the bacterial community was 4.68, 4.01, and 3.72%, respectively (Figure 9a). Among the fungal communities, undefined saprotroph, endophyte-litter saprotroph-soil saprotroph-undefined saprotroph, animal pathogen-endophyte-lichen parasite-plant pathogen-soil saprotroph-wood saprotroph were the three function taxa with higher abundance (Figure 9b). Among them, the only functional group that corresponds to the genus *Mortierella* belongs to is endophyte-litter saprotroph-soil saprotroph-undefined saprotroph, and it

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had the highest abundance in the H3 treatment. The only fungal genera corresponding to animal pathogen-endophyte-lichen parasite-plant pathogen-soil saprotroph-wood saprotroph was *Fusarium*, and the abundance of *Fusarium* was significantly lower in the replanted soil + 3.0% hydrogen peroxide (H2) and replanted soil + 4.5% hydrogen peroxide (H3) treatments than in CK1(Table S2).

4. DISCUSSION

ARD can lead to stunted tree growth, root rot, reduced fruit production, and then result in tree mortality, ultimately shortening the life span of replanted apple orchards.[37,38] The main causes of ARD include increased numbers of harmful fungi and changes in the microbial community structure in the soil under replanting conditions.[19,39] This study showed that different concentrations of hydrogen peroxide could promote the replanted seedling growth from the physiological indicators, and both the plant biomass of replanted soil + 3.0% hydrogen peroxide (H2) and replanted soil + 4.5% hydrogen peroxide (H3) treatments were obviously higher than that of CK1. This status was likely due to hydrogen peroxide killing pathogenic fungi in the soil, improving the soil environment for replanting and promoting plant growth.[40] Plant growth cannot be achieved without photosynthesis, and chlorophyll is the main photosensitive pigment for photosynthesis to absorb light energy, which has an important impact on plant growth.[41] Therefore, the chlorophyll content affects photosynthetic parameters, which in turn affects the growth of *M. hupehensis* Rehd. seedlings.[42] Ozaki et al.[43] found that the photosynthetic rate of melon leaves was also enhanced when treated with hydrogen peroxide. Compared with CK1, the chlorophyll content and photosynthetic parameters were obviously varied among different hydrogen peroxide treatments, with replanted soil + 4.5% hydrogen peroxide (H3) treatment being the most effective. Hydrogen peroxide impacts the structure of soil microbial communities and changes the microbial-mediated soil nutrient conversion process to facilitate nutrient uptake by crops. Therefore, the growth of the plants was enhanced and their chlorophyll content and photosynthetic parameters were also enhanced.[44]

Most studies have shown that in general, free radical production and elimination in plants often remain in relative balance. When confronted with adversity conditions, the production rate of free radicals was far greater than the elimination rate, and the plant was damaged.[45] Under the condition of long-term continuous cropping, the deteriorating soil environment could create a threat of adversity in apple seedlings. The relative balance of free radicals was broken, and seedling growth was threatened. Superoxide dismutase, peroxidase, and catalase were the three important factors in the protection of plants from excessive free radicals. The three work synergistically to protect the inner membrane structure of the plant body and reduce the damage to the membrane structure by free radicals.[46] In this study, it was found that hydrogen peroxide treatment enhanced the level of antioxidant enzymes in plants. The main reason is that hydrogen peroxide has a strong oxidizing and sterilizing effect, which purifies the soil environment and reduces microbial damage to the root system, thus promoting the growth of plants.[47] The second may have a small amount of hydrogen peroxide surviving in the soil that can directly or indirectly activate antioxidant enzymes during stress, thus increasing the induced resistance of the plant.[48] Studies have demonstrated that hydrogen peroxide provides a more vigorous root system in wheat.[49] Hydrogen peroxide can also promote rooting and root growth of ground cover chrysanthemum plugs.[50]

A good rhizosphere microbial community structure could maintain the balance of soil microhabitats and ensure normal growth of plants.[51] Soil microorganisms were important for plant growth as an important indicator for assessing soil ecosystems.[52] Generally speaking, beneficial soil microorganisms have a positive effect on plant development, while harmful microorganisms can hinder plant development, and even result in death. For example, *Fusarium wilt* in many important crops worldwide is caused by *Fusarium*.[32] RT-PCR analysis illustrated that that CK2, H1, H2, H3 treatments obviously decreased the gene copy number of *F. oxysporium*. The methyl bromide fumigant has great potential to kill pests and pathogens in the soil and also reduces the population of *Fusarium*.[32] Hydrogen peroxide possesses microbicidal and sporicidal activity.[53]

Research shows that *Mortierella* has a symbiotic or reciprocal relationship with plants.[54] *Mortierella* converts insoluble phosphorus and potassium in the soil into available phosphorus and potassium for plant uptake and utilization, thus improving plant resistance.[55] *Mortierella* was shown to have a negative relationship with the occurrence of ARD and could play a vital role in suppressing ARD by competing for nutrients or resisting nutrients.[56] *Fusarium* was severely positively interrelated with ARD in China,[57,58] and it exhibits strong pathogenicity.[56] *Bacillus* not only inhibited the reproduction of pathogenic fungi, improved microbial community structure, and enhanced plant disease resistance but also secreted catabolic phytase to increase the amount of free phosphorus in the soil, promoted phosphorus uptake by plants, and improved the crop yield.[59] Making fungi fertilizer from *Trichoderma asperellum* strain both promoted replanted seeding growth and development, optimized the soil microhabitats, and effectively alleviated ARD.[60] In aquatic and terrestrial habitats, *Lophiostom* commonly occurs as sapwood on branches, stems, or bark in woody and herbaceous plants.[61] Intaraudom et al.[62] found that the secondary metabolites of *Lophiostoma bipolare* BCC25910 had no obvious inhibitory effect on disease-causing bacteria. In this experiment, the soil microbial community after hydrogen peroxide treatment was studied using amplicon sequencing technology. We found that soil treatment with hydrogen peroxide significantly improved the comparative abundance of *Bacillus*, *Pedomicrobium*, *Mortierella*, *Guehomyces*, and *Trichoderma* and significantly reduced the comparative abundance of *Fusarium* and *Lophiostoma*, with the most obvious effect of replanted soil + 4.5% hydrogen peroxide (H3) treatment. Hydrogen peroxide treatment significantly altered the soil microhabitats and distinctly reduced the number of *Fusarium*. Li[63] found that the accumulation of hydrogen peroxide has the effect of directly poisoning and killing pathogenic bacteria.

The diversity and richness of species were commonly expressed by Alpha diversity. In this study, the simpson index of replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments was higher than that of CK1, indicating that the microbial diversity of replanted soil + 1.5% hydrogen peroxide (H1), replanted soil + 3.0% hydrogen peroxide (H2), and replanted soil + 4.5% hydrogen peroxide (H3) treatments was lower than that of CK1. Li[64] found a decreased microbial community diversity after soil fumigant was applied, and this is the same result as that in our study. Hydrogen peroxide is a broad-spectrum disinfectant that inhibits harmful

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microorganisms while also having an effect on beneficial microorganisms, causing a "vacuum" in the soil.[65] Soil enzymes are the results of soil microbial metabolism and decomposition of plant and animal residues,[66] and it is involved in a series of biochemical reactions in the soil.[67] In this study, hydrogen peroxide treatment reduced the soil enzyme activity, and the higher the application concentration, the more obvious the reduction in soil enzyme activity. At the same time, the soil microbial population also decreased to different degrees. This is probably due to the application of hydrogen peroxide that killed some of the microorganisms associated with soil enzyme activity in the soil, which in turn led to a decrease in soil enzyme activity. This is in agreement with the findings of Klose et al.[68] who used bromomethane fumigation of soil to cause a decrease in soil microbial population and soil enzyme activity.

In the present study, 24 bacterial taxa functions were obtained by PICRUSt1 for bacteria community prediction analysis, among which amino acid transport and metabolism functions were more abundant, which was consistent with the results of Yang et al.[69] FUNGuild prediction analysis showed that animal pathogen-endophyte-lichen parasite-plant pathogen-soil saprotroph-wood saprotroph corresponded to the fungi genus was only *Fusarium*, and the higher functional abundance of *Fusarium* corresponded to its higher abundance in the functional group composition. The high functional abundance of *Fusarium* was in line with the higher abundance of its functional taxa. Correspondingly, the *Fusarium* abundance in replanted soil (CK1) was higher. FUNGuild suggests that *Fusarium* was the key causal agent of ARD. The abundance of *Mortierella* in soil after hydrogen peroxide treatment was obviously high, compared to replanted soil, and the most obvious treatment was with replanted soil + 4.5% hydrogen peroxide (H3) treatment. Free radicals produced by hydrogen peroxide are known to damage DNA of spores in one of the species.[70] This indicates that hydrogen peroxide reduced the abundance of *Fusarium* and increased the number of beneficial microorganisms, which effectively improved the soil microhabitat and alleviated ARD.

5. CONCLUSIONS

In this experiment, we found that the hydrogen peroxide treatment promoted the growth and development of replanted seedlings and improved the chlorophyll content, photosynthetic parameters, and root antioxidant enzyme activities of seedlings. Soil microhabitat also changed to different degrees, especially, increased the comparative abundance of *Bacillus*, *Mortierella*, decreased the comparative abundance of *Fusarium*. In summary, the effect of replanted soil + 4.5% hydrogen peroxide (H3) treatment was the most significant, which effectively alleviated ARD.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06665.

Description of methods for the determination of indicators such as root antioxidant enzyme activity and soil culturable microorganisms; information about soil physicochemical properties; dilution curves; the Kruskal−Wallis test used to statistically compare the relative abundance of bacterial and fungal genera on different treatments; and distribution of OTUs of functional taxa (PDF)

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Notes

The authors declare no competing financial interest.

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