Soil Microbial Community Development in a Cherry Replant Site

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Abstract

Introduction: Replant disease has been studied extensively, but it has not been accurately defined and the causal mechanisms have not been elucidated. Understanding the microbial community changes in cherry (*Prunus spp. L.*) replant soil is important, as soil microbial communities could be affected by planting cherry seedlings and, in turn, they could impact growth of the seedlings.

Methods: Biolog Eco Plates and Denaturing Gradient Gel Electrophoresis (DGGE) were used to analyse the genomic and metabolic soil microbial communities of cherry replants.

Results: Community Level Physiological Profile (CLPP) results showed that soil microbial diversity and metabolic activity were enhanced by replanting cherry, and Average Well Colour Development (AWCD), substrate evenness (*E*), and the utilisation of phenols were positively correlated with cherry continuous replanting number ($r=0.7107, 0.6055$ and 0.6443 , respectively; all $P < 0.05$). Additionally, DGGE results revealed that there was no obvious difference in soil bacterial community composition, but significant differences in fungal communities, related to the continuous number of cherry plantings, were observed. Analysis of the band sequence indicated that *Fusarium oxysporum*, *Fusarium falciforme*, *Fusarium solani* and *Verticillium nigriscens* stimulated in RP.

Conclusion: Our results, evidence that utilisation of phenol and the pathogens in soil play key role in cherry replant systems.

Keywords: Cherry replant site; Soil microbial community; CLPP; DGGE; Soil borne pathogenic fungi

Introduction

Replant problems, which included poor growth of trees and occurred after replanting the same or closely related species, was caused by biotic and abiotic factors and resulted in severe stunting, shortened internodes, rosetted leaves, small root systems, decayed or discoloured roots, and even death [1-3]. Several lines of evidence indicate that plant-associated microbial communities (biotic factors) are crucial for fruit tree health [3,4]. Further research revealed that fungal communities in the rhizosphere as a factor can affect many species of plants growth and yield at replant sites [5-9]. Especially, the soil borne

pathogenic fungi in replanted soil such like *Fusarium oxysporum*, causing vascular wilt and root diseases on a broad range of agricultural plants worldwide, and leading to considerable yield and economic losses [10-12]. However, there is few published paper on whether the soil borne pathogenic fungi was the most critical factors leading to cherry replant disease at present. In addition, replant disease is often related to the plant species, and in terms of fruit trees, even pome and stone fruits have distinct replant problems [13]. Therefore, it is very necessary to study microbial community in cherry replant site.

In assessing functional diversity of the microbial communities in replanting cherry soil, Biolog Eco Plates has been used to acquire microbial CLPPs [14]. Genomic and metabolic analysis of the soil microbial communities would provide an exhaustive approach to characterise microbial communities and identify the factors causing cherry replant disease [15]. Therefore, the objectives of this study were to determine the soil microbial community changes in cherry replanted soil, to explore the relationship between cherry replant disease and certain microbial species, and to clarify the pathogenesis of cherry replantation disease.

Material and Methods

Site description

The experiment was performed in a cherry cultivating garden (34°45'5"N, 114°1'34"E) in Henan province, China. The average elevation of the study area was 78 m above sea level. The climate was a typical temperate continental monsoon climate, with a mean annual temperature of 14.2°C. Mean annual precipitation was approximately 616 mm. The top soil contained 58.1 mg/ kg available phosphorus, 145.9 mg/ kg available potassium, 4.78 mg/ kg ammonia nitrogen, and 76.99 mg/kg nitrate nitrogen, with a pH-H₂O of 6.8.

Experiment design

The experiment was performed as described in Table 1. All cherry rootstocks were 'ZY-1'. The cherry seedlings were first planted in Replanted Plot (RP) January, 2010 and removed in February, 2013. Then cherry seedlings were planted in RP and

Table 1: The soil sample codes.

Sample code	Layer		Treatments
	0–20cm	21–40cm	
NP	NP1	NP2	Never-cherry-planted plot as control
FP	FP1	FP2	First planted cherry plot
RP	RP1	RP2	Replanted cherry plot

first planted plots (FP) in July, 2013. Cherry seedlings in RP were affected by replant disease, and inhibition rate of new shoots was higher than 30%. We used a randomized complete block design with three replication. There were five random sampling sites in each plot. Soil samples were collected from each sampling site, and five samples from each site were grouped, and mixed together. A total of 375 kg/ha compound fertilizer (N, P₂O₅, K₂O, all at percentages of 15%), and 9,000 kg/ha organic fertilizer, were applied every September in all plots. In addition, no chemicals were used in all plots.

Analysis of the Community Level Physiological Profile (CLPP)

The community level physiological profile was performed using Biolog Eco Plate™ (Biolog Inc., CA, USA). Each Biolog Eco Plate contained 31 carbon sources in triplicate and three negative controls in a 96-well-plate format [16]. Briefly, 1 g of soil was placed in an autoclaved triangular flask with 99 ml of 0.85 % sterilised NaCl solution. The soil suspensions were shaken on a reciprocal shaker for 30 min at a speed of 200r/min, and then stored at 4°C for 30 min. A total of 150 µL of solution was placed in each well, and all plates were cultivated at 25°C for 192 h. The optical density at both 590 nm (colour development plus turbidity) and 750 nm (turbidity only) was read every 24 h by the Ultra Micro-plated Reader (Elx 808, BIO TEK Instruments Inc., Winooski, VT, USA).

Substrate-related diversity indices were based on Carbon Source Utilisation Patterns (CSUPs). Biolog Eco-plates could also be used to calculate diversity indices. To compare functional diversity within the never-cherry-planted plot (NP), FP, and RP, the 96 h data, which were in the exponential phase, were used for statistical analysis of the CLPPs [17]. According to Zak et al. [18], the Shannon diversity (*H*), substrate richness (*S*), substrate evenness (*E*), and Simpson dominance (*D*) on the different carbon substrate guilds were calculated.

Extraction of DNA from soil

DNA was extracted in triplicate from each sample using the FastDNA® SPIN Kit for Soil (BIO101, Carlsbad, CA, USA).

Bacterial community analysis: PCR was performed with 5 µL 10 × PCR buffer (Applied Bio systems), 3.2 µL dNTP mixture 2.5 mM, 0.4 µL ExTaq (5U/µL), and 1 µL GC-338F (CGCCCGGGCGCGCCCCGGGGCGGGGCGGGGG CGCGGGGGCCTACGGGAG-GCAGCAG) as a forward primer, with GC-clamp and 1 µL 518R (ATTACC GCGGCTGCTGG) as reverse primers (20µM each), and 50 ng purified DNA extracts diluted to 50 µL with ddH₂O. Amplification was performed at 94°C for 5 min, followed by 30 cycles

of 94°C for 1 min, 55°C for 45 s, 72°C for 1 min, and finally 72°C for 10 min [19]. A 10 µL aliquot of the PCR products was loaded into 7% (w/v) acryl amide gel containing a linear chemical gradient ranging from 35–55% denaturant. The gels were run for 5 h at 150 V. After electrophoresis, the gels were soaked in Gel Red solution (GelRed™ Nucleic Acid Gel Stain; Biotium, Hayward, CA, USA) for 30 min and photographed with a Gel-Doc2000 (Bio-Rad Laboratories, Hercules, CA, USA). The different DGGE bands were excised and re-amplified for sequencing with a 338f/518r primer pair.

Fungi community analysis: The forward primers ITS1f 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS1f-gc 5'-CGC CCG CCGCGCGCGGGCGGG CGGGGCGGGGCACGGGGGCTTGGTCATT-TAGAGGAAGTAA-3', and the reverse primers ITS2 5'-GCTGCGT-TCTTCATCGATGC-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3', were used in this study. PCR was used to amplify the ITS1 region of the fungal rDNA. A fragment comprising both ITS1 and ITS2 was amplified in the first PCR reaction, using the primer pair ITS1f/ITS4. After purification of the PCR product, the ITS1 region was amplified in the second PCR reaction using the ITS1f-gc/ITS2 primers. The first PCR reaction consisted of 2.5 µL 10 × Ex Tap buffer, 2 µL dNTPs (2.5 µM), 0.25 µL ExTaq Polymerase (5 U/µL), 0.5 µL ITS1f primer (20 pmol/µL), 0.5 µL ITS4 primer (20 pmol/µL), 1.5 U DNA polymerase, 1 µL DNA template (50 ng/µL), and 0.5 H₂O to bring the volume to 25 µL. Cycling conditions were 94°C for 5 min, 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and, finally, 72°C for 10 min.

The second PCR reaction consisted of 10 × PCR buffer, 3.2 µL dNTPs (2.5 mM), 0.4 µL ExTaq Polymerase (5 U/µL), 0.5 µL ITS1f-gc primer (20 pmol/µL), 0.5 µL ITS2 primer (20 pmol/µL), 1 µL DNA polymerase, and 1 µL of PCR product obtained from the first PCR as a template to bring the volume to 50 µL with ddH₂O. Cycling conditions were 94°C for 5 min, 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and, finally, 72°C for 10 min.

A 10 µL aliquot of the PCR products was loaded into a 7% (w/v) acryl amide gel containing a linear chemical gradient ranging from 30–65% denaturant. The gels were run for 8 h at 120 V. After electrophoresis, the gels were soaked in Gel Red solution (GelRed™ Nucleic Acid Gel Stain, Biotium) for 30 min and photographed with a Gel-Doc2000 (Bio-Rad Laboratories). The different DGGE bands were excised and re-amplified for sequencing with a ITS1f/ITS2 primer pair.

Statistical analysis: Digitised DGGE images were analysed with Quantity One software. The dice similarity matrix was constructed for all lanes, and Redundancy Analysis (RDA) was performed using Canoco software (ver. 4.5; Microcomputer Power, Ithaca, NY, USA). Data were subjected to one-way analysis of variance (ANOVA) (Dunnett's; *P* ≤ 0.05). The values in the figures and tables correspond to the average of triplicate replication ± standard error (SE).

Sequencing and phylogenetic analysis: The 16S rRNA gene sequences of the bacterial and ITS gene sequences of fungi isolates were aligned and a phylogenetic tree was obtained using MEGA 4. The maximum similarity of sequences was checked on

the web at the National Center for Biotechnology Information (NCBI) by comparing sequences with the nucleotide collection database, using the nucleotide BLAST program.

Results

Community level physiological profiles (CLPP)

Community level physiological profiles of the six soil samples were analysed using BiologEcoplate™. Assays were performed over a 96 h incubation period (Figure 1; Tables 2, 3). Substrate richness (S) of RP was obviously greater than NP and FP (Table 2) in both soil layers (0–20 and 20–40 cm). Additionally, S was found to be slightly lower at the first cherry planting compared to the replanted cherry. Evenness (E) was found to be non-significantly different among NP, FP, and RP, while FP and RP were a little greater than NP, revealing that E of substrates could be affected by cherry planting. From the 0–20 cm soil layer, Simpson dominance (D) of FP1 was significantly higher than NP1 and RP1, indicating that D was markedly increased by the first

cherry planting, and decreased by replanting cherry. However, D of RP2 was higher than NP2 and FP2, showing that D of microbial communities could be increased by replanting cherry from the 20–40 cm soil layer. Shannon's diversity index (H') of RP was obviously greater than NP and FP; otherwise, FP1 was lower than RP1 and NP1, indicating that the microbial communities were affected by the cherry planting. H' of samples from the 0–20 cm soil layer revealed that H' increased with the increasing number of cherry plantings. Overall, the diversity indices of samples from the 0–20 cm soil layer were obviously greater than those from the 20–40 cm layer.

Utilisation of the six carbon guild (polymer, carbohydrates, phenols, carboxylic acid, amino acids, amines) is listed in Table 3. The utilisation of carbon guild profiles varied among NP, FP, and RP. It was illustrated that the average optical density of the carbohydrates and amino acids was much higher than other carbon guilds. The maximum utilisation of carbohydrates, phenol, amino acids, and amines was RP, while the lowest was FP. At the 20–40 cm soil layer, the maximum utilisation of carboxylic acids was NP1, but NP2 was the lowest at the 20–40 cm soil layer. Overall, RP samples were capable of utilising more carbon guild than NP and FP.

The RDA ordination plot (Figure 1) displayed two axes that accounted for 70.9 %, and an object at the right side on a response (microbial) or an explanatory (continuous number of cherry plantings and soil depth) variable approximated the value of the object along that variable. Soil microbial diversity and metabolic activity were enhanced by replanting cherry, and AWCD, E , and the utilisation of phenols were positively correlated with a continuous number of cherry plantings ($r = 0.7107, 0.6055$ and 0.6443 , respectively; all $P < 0.05$). Additionally, microbial diversity and metabolic activity were weakened by a deep soil layer, and H' and S were significantly and negatively correlated with soil depth ($r = -0.8141$ and -0.9245 , respectively; both $P < 0.05$).

DGGE profiles

Clustering of the microbial communities revealed the existence of two groups with a similarity of 24%, indicating relatively large differences among the samples. Although the similarity of replicates was not so high, the difference among samples was obvious. Two principal components (PCAs)

Figure 1: Redundancy Analysis (RDA) of soil microbiological properties with soil depth and the continuous number of cherry plantings.

Table 2: Shannon's diversity index (H'), substrate evenness (E), substrate richness (S), and Simpson dominance (D) for the microbial communities in six soil samples at 96 h.

Treatment	AWCD	H'	E	S	D
NP1	0.384 ± 0.004 ^{bc}	3.010 ± 0.0336 ^{ab}	1.049 ± 0.016 ^a	17.667 ± 0.667 ^b	0.943 ± 0.002 ^{ab}
FP1	0.4567 ± 0.034 ^a	3.004 ± 0.029 ^b	1.028 ± 0.024 ^a	18.667 ± 0.882 ^{ab}	0.945 ± 0.001 ^a
RP1	0.490 ± 0.01V2 ^a	3.217 ± 0.182 ^a	1.067 ± 0.045 ^a	20.333 ± 0.882 ^a	0.931 ± 0.014 ^{ab}
NP2	0.346 ± 0.012 ^c	2.699 ± 0.014 ^c	1.014 ± 0.004 ^a	14.333 ± 0.333 ^c	0.923 ± 0.001 ^b
FP2	0.348 ± 0.021 ^c	2.832 ± 0.0753 ^{bc}	1.084 ± 0.10 ^a	13.667 ± 0.667 ^c	0.927 ± 0.006 ^b
RP2	0.428 ± 0.026 ^{ab}	2.912 ± 0.0123 ^{bc}	1.067 ± 0.01 ^a	15.333 ± 0.577 ^c	0.937 ± 0.001 ^{ab}

AWCD, average well colour development; NP, never-cherry-planted plot; FP, first planted cherry plot; RP, cherry replanted plot. Mean values ($n = 3$) with the Duncan test ($P \leq 0.05$).

Table 3: Average optical density of six types of six soil sample substrates at 96 h.

Treatment	Polymers	Carbohydrates	Phenols	Carboxylic acids	Amino acids	Amines
NP1	1.745 ± 0.144 ^a	4.768 ± 0.181 ^b	0.806 ± 0.096 ^{bc}	1.259 ± 0.071 ^a	2.765 ± 0.071 ^c	1.016 ± 0.039 ^{ab}
FP1	1.117 ± 0.085 ^d	2.455 ± 0.070 ^d	0.449 ± 0.047 ^c	0.895 ± 0.050 ^b	1.795 ± 0.037 ^d	0.614 ± 0.037 ^b
RP1	1.686 ± 0.071 ^{ab}	6.078 ± 0.135 ^a	1.191 ± 0.028 ^a	0.846 ± 0.134 ^b	3.496 ± 0.049 ^{ab}	1.217 ± 0.057 ^a
NP2	1.398 ± 0.081 ^{bcd}	3.577 ± 0.181 ^c	0.588 ± 0.049 ^c	0.651 ± 0.068 ^b	3.154 ± 0.053 ^{bc}	1.039 ± 0.039 ^{ab}
FP2	1.338 ± 0.048 ^{cd}	4.433 ± 0.459 ^b	0.526 ± 0.245 ^c	0.861 ± 0.086 ^b	2.702 ± 0.220 ^c	0.929 ± 0.136 ^{ab}
RP2	1.465 ± 0.096 ^{abc}	4.423 ± 0.007 ^b	1.100 ± 0.054 ^{ab}	1.270 ± 0.167 ^a	3.958 ± 0.343 ^a	1.371 ± 0.291 ^a

Mean values (n = 3) with the Duncan test ($P \leq 0.05$)

accounted for 59.7% of the total variation and showed that each soil sample formed its own cluster. In general, the soil sample (soil layer 0–20 cm) clusters exhibited a negative PCA2. On the other hand, NP clusters had a positive correlation with PCA1, while FPs were negative with both PCA1 and PCA2 (Figure 2a).

The relationship of DGGE patterns to fungal communities is shown in Figure 2b. DGGE patterns representing the fungal communities in the soil samples showed low similarity indices of 19%, indicating that the treatments NP, FP, and RP were separated according to the cherry planting times. Furthermore, PCA analysis showed three relatively distinct groupings, with clusters of interstitial communities from FP to the group on the left side, the RP on the upper right side, and the NP below. Otherwise, two PCAs accounted for 56.1% of the variation. In the DGGE (Table 4, S1; Figure 3, 4), bands 2, 3, 4, 7, 8, and 9 appeared in the FP and RP. Analysis of the band sequence indicated that bands 2, 3, 4, 8 and 9 were closely related to *Fusarium oxysporum* f. sp. *Fusarium falciforme*, *Fusarium oxysporum*, *Fusarium solani*, and bands 7 was closely related *Gibellulopsis nigrescens*.

Discussion

Various factors, such as soil microbial communities, pests in the soil, physico-chemical soil properties, autotoxicity, and other unknown factors, might contribute to replant problems. The study on soil mineral elements and enzyme activity demonstrated that there was no significant difference in soil nutrient content and enzyme activity between RP and NP (the data is not listed). Hence, it was revealed that soil mineral elements and enzyme activity were not the key factors of cherry replant disease. To reveal the effects of replanting cherry on soil microbes, molecular microbial diversity, and the CLPP of RP were analysed. Soil microbial communities with the greatest reservoir of biological diversity were crucial for plant health [20]. Hence, the changes of microbial and metabolic structure might be the main factors resulting in cherry replant disease. It was revealed that RP has high CLPP, compared to NP and FP. Utilisation of phenols is positively correlated with the frequency of cherry plantings. It has been suggested that phenols acting as allelochemicals compounds reduce the growth of seedlings [21], and autotoxicity is an important cause of replant disease [22,23]. In many cases, allelochemicals could not only negatively affect plants, but also stimulate the growth of soilborne pathogens [24,25]. Yu et al. found that *Fusarium flocciferum* and *Cephalosporium*

acremonium could alleviate the autotoxicity induced by phenolic acids in cucumber [26]. These observations suggested that there is a close relationship between utilisation of phenols and soil fungi in replanted soil. In this paper, the genomic and metabolic soil microbial communities of cherry replants were analysed, and the study on phenols content and microbial community is in progress.

DGGE analysis was adopted to detect the change of fungi and bacteria with continuous cherry plantings. In this study, significant differences in fungal communities, in relation to continuous cherry planting, were observed (Figure 2c). No obvious differences in rhizosphere bacterial community composition were observed between RP and FP (Figure 2d). Interestingly, *Fusarium oxysporum*, *Fusarium falciforme*, *Fusarium solani* and *Verticillium nigrescens* stimulated in RP. It is generally known that traditional crop rotations are an effective strategy to control many soil-borne diseases, but King et al. supposed that, due to a long period of time, pathogens such as *F. oxysporum* survived, and the effectiveness of crop rotation is weakened once a disease has occurred [27]. Accordingly, soil-borne diseases has become one of the important restricted reasons for crop yield. Numerous micro conidia produced by *F. Solani* on the cut surfaces of healthy wax apple trees led to diseased twigs under moist conditions, even resulting in twig blight [28]. *F. oxysporum* is a destructive vascular pathogen, causing vascular wilt and root diseases on a broad range of agricultural plants worldwide, leading to considerable yield and economic losses [10–12]. However, *F. oxysporum* have a specific relationship with plants causing wilting, for instance *F. oxysporum* formae speciales lycopersici causing with in tomato [29], *F. oxysporum* f.sp. cubense causing wilt in banana [30], *F. oxysporum* f. sp. vasinfectum causing wilt in cotton [31]. *Fusarium* species as pathogens could result in wilt disease in *Pisum sativum* and roselle plants, with damping-off and root rot wilt diseases [32,33]. Moreover, recent findings indicated *F. oxysporum* caused root and crown rot on sweet cherry (*Prunus avium*) in British Columbia. *Gibellulopsis nigrescens* (Synonymy: *Verticillium nigrescens*) as a highly controversial *Verticillium* spp., is pathogenic on many crops (such like potato, tomato, antirrhinum, eggplant, soybeans and *M. sativa* [34–38]. However, the recent report revealed that *G. nigrescens* was a promising biocontrol agent for *Verticillium* wilt of cotton [39].

Obviously, the results of CLPP and molecular diversity reveal that pathogens and utilisation of phenols in soil play keys roles

Figure 2: Cluster and Principal Component Analysis (PCA) of the Denaturing Gradient Gel Electrophoresis (DGGE) profiles of bacterial (a and b) and fungal (c and d) amplicons. The DGGE bands from treatments were denoted as never-cherry-planted plot (NP) 1, NP2, first planted cherry plot (FP) 1, FP2, cherry replanted plot (RP) 1, and RP2.

Figure 3: DGGE profile of ITS rDNA fragments of soil fungi.

Figure 4: Neighbour-joining phylogenetic tree showing the relationship between cherry replanted soil fungi and related fungi based on the sequences of ITS regions of nuclear rDNA.

Table S1: The ITS sequences of the dominant strains fungi.

Number	Sequence
1	TTTCGCTGTGGCTGATTACGATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTGCTGCGTTCT TCATCGATGCGAGAACCCTAGAGATCCGTTGTTGAAAGTTTAACTTTATTACAATAGTTATTTCAG ACACTACATGCAACTAAACAAGCGTTCAAACCTACACCCGTGGAAGGTAACCCGACCTGCGGAACA GGCGGAGCACCCGTAACCTCCAGGAAGCAACAGGTACAAGTAAACAAGTGGTTGAATACAGCCA CCAGCAGTCAGCGTTTACACCTTAGCTGGAGCGTACTTTTAAATGATCCTTCCGAGGTTTAC CTACGGAATCGTCGACTCGAGGATGCAAGCTTGGCACTGGCCGTCGTTTACAACGTCGTGA CTGGGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
2	TATTGCGGTGAGTGATACGATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTTCGCTAGGTGA ACCTGCGGAGGGATCATTACCGAGTTTACAACCTCCAAACCCCTGTGAACATACCTATACGTTGC CTCGGCGGATCAGCCCGCGCCCGTAAAAAGGGACGCGCCGCGGAGGACCCCTAAACTCTGTTT TTAGTGAACCTTCGAGTAAACAACAATAAATCAAACCTTCAACAACGGATCTCTTGGTT CTGGCATCGATGAAGAACGAGCAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTC GTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCTTGCAGA
3	ATTCGATCTGGATGATTACGATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTTCGCTAGGTG AACCTGCGGAGGGATCATTACCGAGTTATAACAACCTCATCAACCCCTGTGAACATACCTATAACGTT GCCTCGCGGGAAACAGACGGCCCGTAACACGGGCGCCCGCTCAGAGGACCCCTAACTCTGT TTCTATAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAACTTTCAACAACGGATCTCTTGG GCTCTGGCATCGATGAAGAACGCCCAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCC GTGCTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
4	AACTTCGTGTTTCATGTGAGCTGATACGATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTGCT GCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTAACTATTATATAGTACT CAGACAGTATAAACAACAGAGTTTAGGTCTCTGGCAAGCGCTCGCCGGCGGAGCCAGCAGC CCGAGGGCAGGCTTGCCAAAGCAACAAGTGTATAAACAAGGGTGGTAGGTTACCCGGGAGG CCTTGGCGCAACCCGGGCGACTACTGTAATGATCCTTCCGAGGTTCACTACGGAACCTGTTG ACGACTGTTACTTCTCTAAATGACCAAGATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGG CCGTCGTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCTTGCAGA
5	GTAGCATTGCACATGATTACGAATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTGCTGCGTT CTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTAAAGTTTATGTATAACAATAAT TCAGACAAAATTCAAAAAAACAGAGTTTATAATTACCCCGCGTCCCGCCGCGCCGCGGGGG CGCCCGGGCGTTACTTCTCTAAATGACCAAGAATCGTCGACCTGCAGGCATGCAAGCTTGGCA CTGGCCGTCGTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCTTGCAGA
6	AAAAGCGTAGCTCAGGCAAACCGAATTTCGAAGCTTCGGTACCCGGGGATCCTCTAGAGATTCC GTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCTA TCGTTGCCCTCGGCGGTGCGCGCTCCGCGGCGCCGAGAGGACCCCAAACTCTGTTTATACATA GTATCTTCTGAGTAACAGGATTAATAAATCAAACCTTCAACAACGGATCTCTTGGTTCTGGCA TCGATGAAGAACGAGCAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTAA CAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
7	ATACAGCGAGAACATGATCGAATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTTCGCTAGGT GAACCTGCGGAGGGATCATTACCGAGTACTATAACTCATAAACCCCTTGTGAACCTTCATACCTGT TGCTTCGGCGGCGCGCTCCGCGGCGTGCCTCGGCGGACATTATCAGAACTCTGTGTCGAACCCGA CGATACTTCTGAGTGTCTAAGCGAAGTGTAAAACTTCAACAACGGATCTCTTGGTTCCAGCA CGATGAAGAACGAGCAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTAA CAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
8	GGATCGTCAGATGATTACGATTTCGAGCTCGGTACCCGGGGATCCTCTAGAGATTGCTGCGTTCTT CATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTGTATTATTTATGTTTACTCAGAA GTTACATATAGAAACAGAGTTTAGGGTCTCTGGCGGGCGTCCCGTTTACCGGGAGCGGGCT GATCCGCGGAGGCAACAAGTGGTATGTTACAGGGGTTGGGAGTTGTAACTCGGTAATGATC CCTCCGAGGTTTACCTACGGAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGT TTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCTTGCAGA
9	CACAAACGTAGAATGGAAACCGAATTTCGAGCTTCGGTACCCGGGGATCCTCTAGAGATTGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTAAATTTATTGCTTGTTCAC TCAGAAAAACATTAAAAAACAGAGTTAGGGTCTCTGGCGGGGCGGCCCGTTGTTACAGGG CCGTCTGTTCCCGCGGAAGCAACGTTTAGGTATGTTACAGGGTTGATGAGTTGATATAACTCCG TAATGATCCCTCCGAGGTTACCTACGGAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTG GCCGTCGTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
10	AAAGCGTAGACCCAGCTAAGAATTTCGAGCTTCGGTACCCGGGGATCCTCTAGAGATTCTTGGT ATTTAGAGGAAGTAACAGTCGTAACAAGTCTCCGTTGGTGAACAGCGGAGGGATCATTACAG AGTTGTAACCTCCCAAACCTATGTGAACATACCTGTTGCCCTCGGCGGCTACCCGCGAGCTACC CTGTAGTACCTGTAGTCCGCGGCGGAATACAAAACCTTGTGTTTTCAGTTGTCATCTCTGAGA ATAAAACAAATAAATCAAACCTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAAGCG AGCAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTACAACGTCGTGACTG GGAAAACCTGGCGTTACCAACTTAACGCCCTTGCAGA
11	TAAGGCGCAGACCATGCTACCGAATTTCGAGCTTCGGTACCCGGGGATCCTCTAGAGATTGCTGC GTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTGTATTATTTATCGGTTTAA CTCAGAAGACTTCTTAAATGTAATCAGGGTTTGGGGTCTCTGGCGGGCTGTCGGAACAGGCAC CGCCGAGGCAACAATAGGTATGTTACAGGGGTTGGGAGTTGTAACTCGGTAATGATCCCTC CGCAGGTTACCTACGGAATCGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTAA CAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTTAATCGCCCTTGCAGA
12	ATTAACGATAGACCATGATAAGAATTTCGAGCTTCGGTACCCGGGGATCCTCTAGAGATTGCTGCGT TCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTGTAAATTTATTTGTTACTGAC GCTGATTGCAATTACAAAAGTTTATGTTTGTCTAGTGGTGGCGGAACCCACCAAGGAACAA GAAGTACGCAAAAGACAAGGGTGAATAATTACGCAAGGCTGTAAACCCGAGAGGTTCCAGCCCG CCTTCATATTGTGTAATGATCCCTCCGAGGTTACCTACGGAATCGTCGACCTGCAGGCATG CAAGCTTGGCACTGGCCGTCGTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCAACTT AACGCCCTTGCAGA

Table 4: Sequences and sources used to construct phylogenetic trees.

Taxon	GenBank accession No.
<i>Ascomycete sp.</i>	DQ683976
<i>Fusarium oxysporum</i> f. sp.	JN400714
<i>Fusarium falciforme</i>	KP132214
<i>Geomyces sp.</i>	JX270512
<i>Pyrenomataceae sp.</i>	HQ533849
<i>Neonectria candida</i>	AJ608955
<i>Gibellulopsis nigrescens</i>	JN187977
<i>Fusarium oxysporum</i>	HQ694500
<i>Fusarium solani</i>	HQ696879
<i>fungus sp.</i>	HM439595
<i>Neonectria sp.</i>	JF429683
uncultured fungus	FJ761637

in cherry replant systems. It was indicated that pphenols in the soil content is closely associated with stimulated microbes in the RP. Therefore, in order to verify the relationship between them, further research is in progre.

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