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Full Length Article

Effects of Root Restriction Cultivation of *Prunus persica* on Field Soil Microorganisms and Properties

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Abstract

Root restriction cultivation is a new cultivation method. In this study, we determined the relationships between the microbial composition and properties of the rhizospheric soil after cultivating peach (*Prunus persica* (L.) Batsch) for three years under root restriction and normal cultivation methods. The dynamic changes in the microbial compositions were analyzed by high throughput sequencing of the V3–V4 regions of bacterial 16S rRNA to explore the responses of rhizosphere bacteria to the different cultivation patterns. The soil properties were determined using experimental methods. The results showed that the abundance of *Proteobacteria* increased significantly in the soil under root restriction and *Bacteroidetes* and *Actinobacteria* were more sensitive than other phyla. The soil organic matter (SOM) content, pH, available nitrogen (AN), available potassium (AK), and available phosphorus (AP) affected rhizospheric microorganisms. In addition, the available nitrogen, available phosphorus, and available potassium concentrations were lower in the root restriction cultivation groups compared with the normal cultivation group. The pH decreased by about 1 unit in the normal control soil after cultivation for 3 years. Our results suggest that the composition of the soil bacteria in the rhizosphere of *P. persica* responded differently to the two-cultivation pattern. The findings obtained in this study may provide insights to facilitate improvements in the soil quality during peach tree cultivation. © 2020 Friends Science Publishers

Keywords: Peach; Fruit quality; Soil bacteria; Rhizosphere soil; Available nutrients

Introduction

The peach [*Prunus persica* (L.) Batsch] is one of the most widely cultivated fruit trees throughout the world (Allison 1976) and it has high economic value. Peach trees are cultivated using a variety of methods. Many experimental studies have shown that root-limited cultivation can promote the production of lateral roots by fruit trees (Peren 2020), inhibit the growth of new shoots, promote flower formation, increase the fruit set rate, enhance the yield, and improve the seedling quality (Smith and Graber 1948). Root restriction cultivation is a new cultivation technique where physical or ecological methods are used to constrain the plant root system within a certain volume, and to control plant root growth in order to regulate the vegetative and reproductive growth of the aerial parts (Mukti and Ashikeen 2005). Root growth and the external environment can vary under different root-restricted cultivation methods, thereby resulting in corresponding root function changes that can affect the external growth of plants as well as internal physiological and biochemical reactions (Guo *et al.* 2017). This method can promote root regeneration and new root growth, and inhibit the growth of shoots above ground.

The high salt content of saline–alkali land can cause water deficiencies in plants. Saline–alkali soil is readily degraded and the water-holding capacity and water permeability are poor. The soil enzyme activities are inhibited in saline–alkali land, which affects the soil organic matter conversion process and the fertility declines as a consequence (Sun *et al.* 2012). Soil salinization has become an important factor that limits the development of agricultural production. Soil salinization directly affects the physiology of plants to reduce crop yields. The development of new salt-tolerant crop varieties is a promising strategy but the productivity of plants is still hindered by salinization. Thus, the increasing salinity of soils means that it is necessary to find new solutions for improving the plant growth conditions (Paul and Lade 2014).

The microorganisms in farmland soil play important roles in matter cycling and they can affect the soil biochemistry (Jacoby *et al.* 2017). These microorganisms are able to adapt to abiotic stress conditions, such as soil salinization and osmotic stress (Xu and Fujiyama 2013). Previous studies have shown that these microbes contribute significantly to plant growth by maintaining the cycling of

soil nutrients and promoting soil–plant interactions (Zhang *et al.* 2016). Bacterial and fungal communities have been applied as indicators to evaluate the effects of various cultivation methods on the soil ecological environment (Zornoza *et al.* 2009). The diversities of bacteria and fungi are closely related to the complexity and variability of the soil ecological environment. Thus, the microbial complexity can greatly influence the sustainable development of farmland soil (Parausic *et al.* 2013), and high microbial diversity will have beneficial effects on the quality and fertility of the soil. However, degradation of the soil ecosystem will reduce the microbial diversity (Teketay 2001). Therefore, high bacterial and fungal diversities may facilitate the development of saline–alkali land and improve the physical and chemical properties of the soil. Soil salinization is an important issue that has affected agricultural production and the soil quality for many years in northern China. Root restriction cultivation has been applied widely in recent years for fruit tree cultivation in this area. However, the effects of different root cultivation modes on diversities and abundances of soil bacterial and fungal in land remain unclear.

Due to the development of next generation sequencing, the compositions of the bacterial and fungal communities in soil can be determined readily by 16S rRNA and internal transcribed spacer (ITS) sequencing. Some of these organisms can cause plant diseases. However, few studies have investigated the effects of different cultivation methods on the composition and function of the microbial communities in soil. Mechanistic details of the effects of soil microorganisms and different cultivation patterns on plant growth are still lacking. In the present study, we determined the changes in the microbial community in response to cultivation for several years under root-limited or normal conditions, and the microbial populations that exhibited significant increases or decreases. In order to study the response of the soil microbial community to soil salinization under root-limited cultivation conditions, the bacterial V4–V5 16S rRNA and fungal ITS gene regions were sequenced, and the physical and chemical properties of the soils were determined using experimental methods.

Materials and Methods

Soil sampling and experimental treatments

The field test site was established in 2015 at Xiaobotouzhen, Wudi County, Binzhou City, China (117.12°E, 37.15°N). The 3 years old peach trees were in the same growth condition. Soil samples were collected from January 18, 2016 to August 7, 2019. The experiment tested two treatments: (1) root restriction cultivation (XG group) and (2) normal cultivation without root restriction (BXG group), as well as a normal field without any trees as the control (NC group). Each field was treated with the same amount of fertilizer (urea: 30 kg 667 m^{-2}, K_2SO_4: 12 kg 667 m^{-2} and $Ca(H_2PO_4)_2 \cdot H_2O$: 1 kg/667 m^2). Three replicates were conducted for each treatment. Table 1 shows the detailed location information. In total, 21 rhizospheric samples were collected from seven points with three replicates for each treatment. The samples were sieved through a 2-mm mesh, before removing any impurities, roots, and stones. Each sample was then divided into two portions, where one was used for the chemical analyses the other one was stored at −80°C for DNA extraction.

Determination of soil properties

The soil pH was measured with a glass electrode using a soil to water mixture at a ratio of 1:2.5 (Islam and Weil 2000). The SOM contents were measured using Chinese Standard Method GB9834-88. The Kjeldahl method was used to measure the soil available nitrogen. The available phosphorus contents were determined with the Olsen method (Li *et al.* 2004). Flame atomic absorption spectrophotometry was conducted to determine the soil available potassium contents. Soil mineral elements (sodium, Na; calcium, Ca; magnesium, Mg) were determined by the methods of nitric acid (HNO_3) digestion and then measured by inductively coupled plasma atomic emission spectroscopy (Thermo Electron ICP-6000) (Shaheen *et al.* 2016).

Soil extracellular enzyme activities

The activities of α-glucosidase, β-glucosidase, chitinase, cellobiase, urease and phosphatase were determined as described previously (Corbel and Hendry 1985) using 10 g of each fresh soil sample. Each 10 g soil sample was suspended in 25 mL of MilliQ water and the mixture was incubated for 10 min at 25°C with stirring at 250 rpm. Next, 100 mL of sterile Milli-Q water was added to dilute the enzymes.

The α-glucosidase and β-glucosidase activities were measured using Soil α-Glucosidase (5-α-GC) and Soil β-Glucosidase (s-β-GC) Activity Detection Kits (Solarbio Life Science Co. Ltd., Beijing, China). The urease activity was determined by toluene titration. The chitinase activity was determined according to Chinese Standard GB T 34799-2017. Cellobiase was determined by 3,5-dinitrosalicylic acid titration.

Soil DNA extraction and high-throughput sequencing

DNA was extracted from 1.0 g of each fresh soil sample in triplicate using a Tiangen DP336-02 Soil DNA Extraction Kit (Tiangen Biotech Co. Ltd., Beijing, China) according to the manufacturer's instructions. The quality and quantity of the DNA were determined with a Nano Drop™2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). 16S rRNA and ITS1 were amplified for high-throughput sequencing as described previously (Shen *et al.*

2013). Primers F515 (5′-GTGCCAGCMGCCGCGG-3′)/R907 (5′-CCGTCAATTCMTTTRAGTTT-3′) and ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′)/ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the V4–V5 regions of the bacterial 16S rRNA and fungal ITS1, respectively. PCR was then performed as described previously (Sun *et al.* 2015). The PCR products were purified and analyzed with an Illumina HiSeqXten sequencer (Illumina Inc., CA, USA) to obtain the raw sequencing reads.

Bioinformatics analysis

In order to integrate the original double-end sequencing data, we first used the sliding window method to perform quality screening for the double-end sequences in FASTQ format, where the window size was 10 bp, the step size was 1 bp, and the movement started from the first base position at the 5′ end. The average quality of bases in the window was required to be ≥ Q20 (the average sequencing accuracy of the bases was over 99%). The sequence was truncated from the first window when the average quality value was lower than Q20, while the length of the sequence after truncation was required to be ≥ 150 bp, and an ambiguous base N was not allowed. Subsequently, the double-ended sequences that passed the initial quality screening were paired and connected according to the overlapping bases with FLASH software (v. 1.2.7, http://ccb.jhu.edu/software/FLASH/) (Magoc and Salzberg 2011). The overlapping base length for the two sequences in Read 1 and Read 2 had to be ≥ 10 bp, and the number of base mismatches was less than 10% of the overlapped base length. Finally, according to the index information (the barcode sequence comprising a small base sequence used to identify the sample at the beginning of the sequence) corresponding to each sample, the connected sequence identification was assigned to the corresponding sample to obtain the valid sequence for each sample.

The UCLUST sequence comparison tool (Edgar *et al.* 2015) in QIIME software was used to merge and divide the previously obtained sequences according to sequence similarity of 97% in order to obtain operational taxonomic units (OTUs). The most abundant sequence for each OTU was selected as the representative OTU sequence. According to the number of OTU sequences present in each sample, we constructed a matrix file for the OTU abundances in each sample (OTU table). The QIIME OTU Classifier was used to identify the taxonomy of each 16S rRNA and ITS1 gene sequence against the RDP (Release 11.1, http://rdp.cme.msu.edu/) (Maidak *et al.* 2001) and Silva (Release132, http://www.arb-silva.de) (Pruesse *et al.* 2007) databases, respectively. Alpha-diversity analyses based on the Chao1, ACE, Shannon, and Simpson indices were conducted using Mothur software (Schloss *et al.* 2009). Redundancy analysis (RDA) was performed to determine the variations in the microbial communities with potential effects on the soil properties (Zuur *et al.* 2010).

Table 1: Soil samples cultivation years and pattern

Sample name	Cultivation years	Cultivation pattern
XG11	1	root limited cultivation
XG12	1	root limited
XG13	1	root limited
XG21	2	root limited
XG22	2	root limited
XG23	2	root limited
XG01	3	root limited
XG02	3	root limited
XG03	3	root limited
BXG11	1	not limited
BXG12	1	not limited
BXG13	1	not limited
BXG21	2	not limited
BXG22	2	not limited
BXG23	3	not limited
BXG31	3	not limited
BXG32	3	not limited
BXG33	3	not limited
NC1	0	Soil without plant
NC2	0	Soil without plant
NC3	0	Soil without plant

Results

Soil properties

The SOM, AN, AP, and AK contents in each test group are shown in Table 2. The pH of the soil decreased by 0.5 units compared with the NC group after peach cultivation for 3 years. The pH of the soil in each field decreased gradually as the number of years under cultivation increased. The SOM contents gradually decreased in all samples with the number of years under cultivation. After root-restricted cultivation for 3 years, the SOM content decreased more significantly than that under the normal cultivation pattern, thereby suggesting that root restriction may be beneficial for SOM utilization.

The AN, AP, and AK concentrations tended to decrease significantly in the soil under root-restricted cultivation (XG group) compared with the normal cultivation pattern. No clear patterns were found in the concentrations of sodium, calcium, and magnesium ions due to their low variation.

Soil enzyme activities

As shown in Table 2, the α-glucosidase activity decreased significantly in the NC soil (79.24 mg g^{-1}) and it was significantly lower under XG compared with NC. The α-glucosidase activity increased gradually to 85.25, 112.67, and 148.65 μmol g^{-1} after cultivation for 1, 2, and 3 years under XG, respectively. The α-glucosidase activity was significantly higher under XG after 3 years (148.65 mg g^{-1}) compared with BXG, and the α-glucosidase activity did not increase significantly in BXG. The β-glucosidase activity was significantly higher under XG compared with those in BXG and NC after the same number of years of cultivation.

Table 2: Soil chemical properties

	pH	SOM (g/kg)	AN (g/kg)	AP (g/kg)	AK (g/kg)	Na (g/kg)	Ca (g/kg)	Mg (g/kg)
XG1	8.85 ± 0.09ab	9.59 ± 0.19	168 ± 3a	7.29 ± 0.06ab	124.00 ± 3ab	14.56 ± 1.56ab	11.46 ± 1.71a	7.09 ± 0.76a
XG2	8.61 ± 0.2bc	7.11 ± 0.34	150.67 ± 5.69ab	6.08 ± 0.05b	95.33 ± 2.08b	13.67 ± 2.06b	10.34 ± 1.41a	6.09 ± 0.76a
XG3	8.16 ± 0.08d	5.26 ± 0.17	113.33 ± 9.45c	5.29 ± 0.16c	69.67 ± 1.53c	14.37 ± 0.87ab	11.41 ± 0.96a	6.09 ± 0.76a
BXG1	8.82 ± 0.06ab	9.63 ± 0.11	165.67 ± 5.13a	7.49 ± 0.16a	127.33 ± 1.53ab	15.96 ± 1.34ab	12.19 ± 2.36a	6.09 ± 0.76a
BXG2	8.71 ± 0.06bc	8.02 ± 0.07	147 ± 6.08ab	6.61 ± 0.22ab	101.67 ± 2.08bc	16.22 ± 1.43ab	10.86 ± 1.74a	6.09 ± 0.76a
BXG3	8.52 ± 0.03c	7.46 ± 0.15	116 ± 4c	5.95 ± 0.07bc	84.67 ± 3.06c	13.85 ± 2.06b	9.92 ± 0.76a	7.09 ± 0.76a
NC	8.86 ± 0.07a	8.79 ± 0.22	170.33 ± 10.6a	7.69 ± 0.15a	135.33 ± 3.51a	17.32 ± 1.76a	10.32 ± 0.95a	6.43 ± 0.76a

SOM, Soil organic matter; AN, Active nitrogen; AP, Active phosphorus; AK, Active potassium; Values are presented as mean ± SD (n ≥ 3)

The cellobiase and chitinase activities were 4.19 and 73.05 in XG, respectively, and they were significantly higher than those in BXG (46.50%) and NC (55.49%).

The phosphatase activity decreased gradually over the years under each cultivation pattern. The highest phosphatase activity was determined in NC (364.49 μg g^{-1}). The urease activities were significantly higher under XG and BXG than NC (709.29 μg g^{-1}). The highest urease activity was found in XG2 (1393.79 μg g^{-1}).

Diversities of bacterial and fungal communities

Alpha diversity analysis was conducted to determine the effects of XG cultivation on the richness and diversity of the soil bacteria and fungi. In total, 1,482,439 paired raw reads of V3–V4 16S rRNA sequences were obtained to assess the soil bacterial communities. After quality filtering and merging, 1,365,030 clean tags were used for subsequent analyses. On average, we obtained at least 34,843 high-quality bacterial 16S rRNA gene reads from each soil sample in seven groups from the total of 21 samples. In total, 2,566,235 paired raw reads and 2,460,227 valid reads of ITS1 sequences were obtained after Illumina sequencing for assessing the fungal diversity.

The numbers of OTUs in each group based on sequence similarity greater than 97% are shown in Table 3. All of the high-quality sequences were clustered into 4270 OTUs, with 230 to 534 in each sample. The species richness at the OTU level was represented using Shannon, Simpson, ACE, and Chao1 indices (Table 4). In terms of the soil bacteria, the Shannon indices ranged from 4.11 to 6.05, with the highest in XG1 and the lowest in NC. The community richness and diversity values were higher in XG2 than those in NC. In term of the fungal diversity, the Shannon indices ranged from 2.20 for NC to 3.61 for XG2, where the trends were similar to the bacterial diversity indices (Table 4). The Shannon and Chao1 values for fungi were lower in NC and BXG1 compared with XG2 and XG3. The richness values for the bacterial and fungal communities were significantly lower in NC. The highest fungal diversity values were found in BXG2 and XG, and the lowest in BXG3 and XG3.

Compositions of bacterial and fungal communities

The relative abundances of the main bacterial and fungal taxa in the different soil samples at the phylum and genus levels are shown in Fig. 1. The bacterial community composition changed significantly in NC, thereby demonstrating that cultivating plants could help to improve the soil bacterial community. At the phylum level, *Proteobacteria* dominated the communities in all of the samples, with the highest proportion of 54.3% in NC and the lowest of 21.62% in BXG1, followed by *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Bacteroidetes* in decreasing order. These six common phyla dominated comprised more than 90% of all of the bacterial communities under NC, XG, and BXG. *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Gemmatimonadetes* comprised 82.4–89.1% and 82.3–87.5% of the total bacterial taxa in XG and BXG, respectively (Fig. 1). Within *Proteobacteria*, the class *Gammaproteobacteria* was most abundant in NC (47.2%) but the relative abundance of this class was lowest in BXG1 (0.09%). *Actinobacteria* comprised the second most dominant phylum in all samples, with 14.9% in BXG2.

The least abundant of the top 15 classes was *Deltaproteobacteria* with a highest proportion of 2.8% in BXG2 (data not shown). At the genus level, the most abundant members of the bacterial community were *Arthrobacter*, *Lysobacter*, uncultured bacterium Subgroup 6, and uncultured bacterium *Gemmatimonadaceae*, which comprised 7.3, 6.8, 5.6 and 5.4%, respectively, of the genera in XG1, XG2, and XG3 (Fig. 2). By contrast, in BXG1, BXG2, and BXG3, *Lysobacter*, uncultured bacterium Subgroup 6, *Arthrobacter*, uncultured bacterium *Gemmatimonadaceae*, and RB41 were the dominant genera, where they comprised 22.50, 25.90, 14.90, 18.50 and 12.20% of the total genera, respectively. In addition, *Lysobacter* (0.34%) and *Cryobacterium* (8.7%) were the dominant genera in NC (Fig. 2).

The fungal community compositions were significantly affected by cultivation with plants (Fig. 3 and 4). *Ascomycota* was the dominant phylum and it accounted for 54.78% of the total sequences on average, while *Mortierellomycota* and *Basidiomycota* comprised 36.55 and 3.16%, respectively (Fig. 3). The proportions of *Zoopagomycota* and *Glomeromycota* were less than 0.1% in each treatment. BXG2, BXG3, XG2, and XG3 contained lower relative abundances of *Ascomycota* than BXG1, XG1, and NC. Compared with the other treatments and times, the relative abundances of the phylum *Mortierellomycota* were higher in BXG2, BXG3, XG2, and XG3, whereas the

Table 3: Activity of five extracellular soil enzymes

	α-Glucosidase	β-Glucosidase	Cellobiase	Chitinase	Phosphatase	Urease
XG1	76.08±6.82c	100.51±8.22c	3.68±0.84b	37.06±4.83c	340.81±98.41a	963.91±183.59c
XG2	93.34±8.96bc	130.03±12.65b	3.75±0.37ab	49.02±6.09b	325.57±80.01bc	1393.79±277.86a
XG3	148.65±7.97a	143.79±15.72a	4.19±0.41a	73.05±5.37a	288.87±37.44d	1226.91±222.42ab
BXG1	72.34±9.21bc	125.68±8.9b	3.00±0.11c	51.81±7.88b	319.55±59.41b	1131.86±219.33b
BXG2	85.25±8.34b	130±8.35a	3.6±0.43b	54.70±4.98b	313.99±36.39c	817.32±43.55cd
BXG3	112.67±5.7b	113.65±10.66b	3.43±0.2b	57.94±7.53b	231.31±56.47e	958.34±67.23c
NC	59.24±9.17d	83.48±6.94c	2.86±0.42d	46.98±4.63b	364.49±26.07a	709.29±106.84d

XG1,2,3 and BXG1,2,3 were soils from root restriction cultivation method and normal cultivation pattern for 1,2,3 years respectively. NC was soils samples without plant.Values are the means ±standard deviations (n ≥ 3).

Table 4: Comparisons of OTUs and alpha-diversity index in each group

		shannon	simpson	ace	chao
NC	bacteria	4.1147±0.0479	0.095±0.0041	970.0997±38.3115	996.6788±38.9847
	fungi	3.606±0.2204	0.1348±0.0334	258.7147±11.7913	265.6667±14.6072
BXG1	bacteria	5.8626±0.0181	0.0067±0.0004	1084.2928±2.7284	1117.0611±8.4919
	fungi	2.2007±0.0929	0.2148±0.02	172.6625±3.5284	173.0129±5.4133
BXG2	bacteria	5.3637±0.0634	0.0245±0.002	1418.5527±6.928	1431.6483±8.186
	fungi	2.2168±0.0249	0.1938±0.0148	107.4197±6.0566	108.4375±5.9432
BXG3	bacteria	5.8457±0.0078	0.0128±0.0005	1455.3623±0.4069	1466.6698±5.6031
	fungi	3.398±0.0178	0.0945±0.0035	240.3863±3.4438	245.1667±4.4729
XG1	bacteria	6.0535±0.0554	0.009±0.0005	1436.0238±6.6797	1473.9288±4.0093
	fungi	3.1857±0.3171	0.1585±0.0532	164.348±7.434	174.0667±2.0342
XG2	bacteria	5.9367±0.0459	0.0141±0.0019	1521.5527±13.2345	1546.3528±19.6163
	fungi	2.9283±0.0424	0.0864±0.0043	153.9547±5.0252	156.183±5.3236
XG3	bacteria	5.6952±0.0554	0.0153±0.0022	1421.7694±18.4288	1443.4586±18.3478
	fungi	3.3082±0.3974	0.132±0.0328	191.0006±25.5467	192.8±23.4813

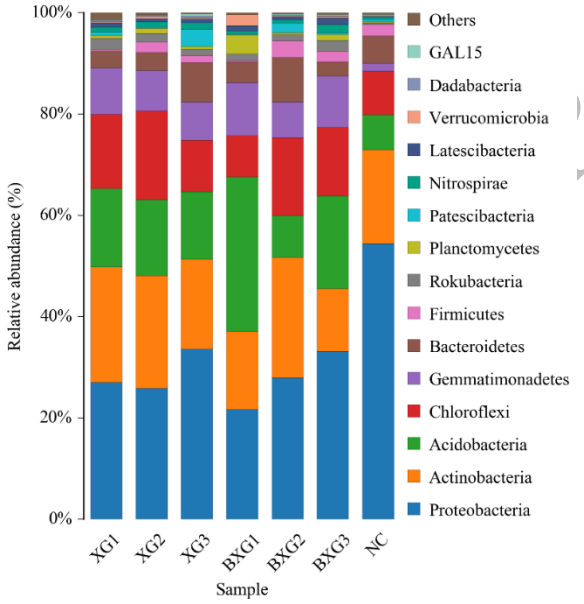

Fig. 1: Microbial community bar plot of relative abundances of bacterial on phyla

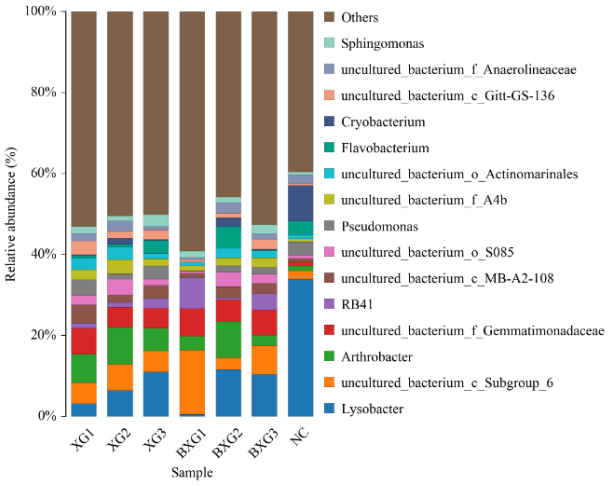

Fig. 2: Microbial community bar plot of relative abundances of bacterial on genus

relative abundances of *Ascomycota* were lower. The abundances of *Basidiomycota* increased significantly after 2 and 3 years under XG and BXG compared with those after 1 year and NC. The dominant genera comprised *Mortierella*, *Pseudogymnoascus*, and *Botryotrichum* with average relative abundances of 36.37, 11.70 and 7.65%, respectively (Fig. 4).

Multivariate analysis of microbial community compositions and environmental factors

RDA was performed to evaluate the correlations between the microbial communities at the phylum level and the enzyme activities (Fig. 5). The correlations between the 10 most dominant phyla and environmental parameters were determined by RDA. RDA1 and RDA2 explained 29.59% of the total variance in the data. XG1, XG2, and XG3 were positively correlated with the activities of α-glucosidase, β-glucosidase, cellobiase, phosphatase, and urease,

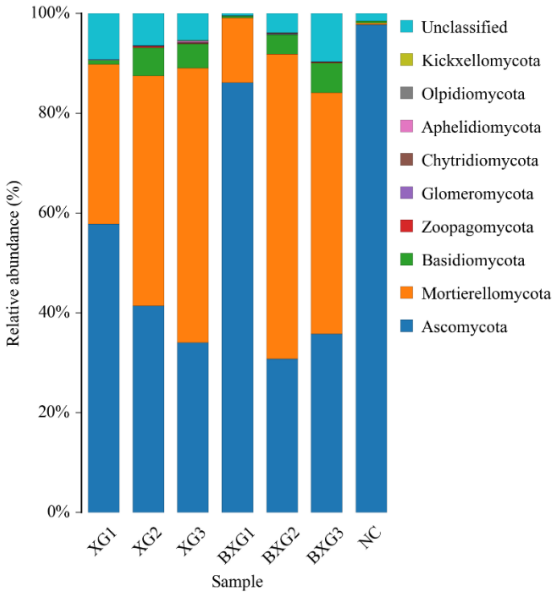

Fig. 3: Microbial community bar plot of relative abundances of fungal phyla

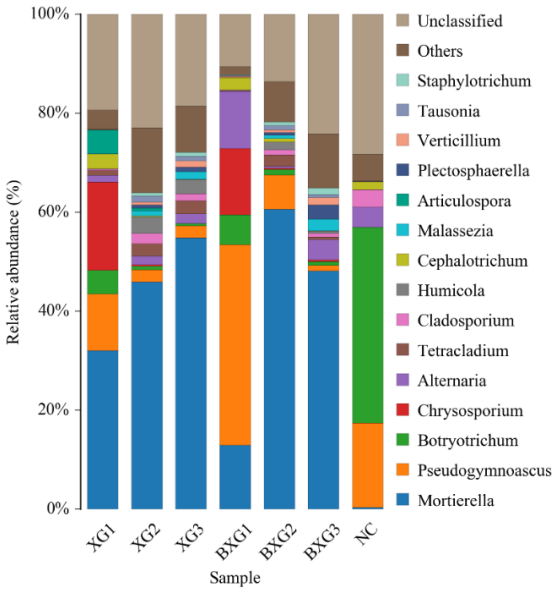

Fig. 4: Microbial community bar plot of relative abundances of fungal genus

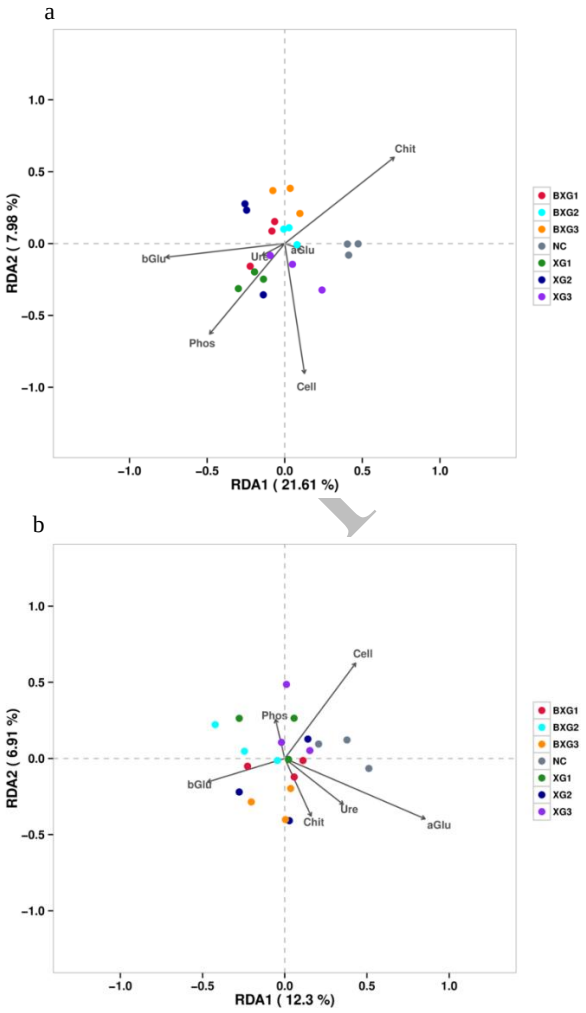

Fig. 5: Redundancy analysis of the microbial communities and enzyme activity

The RDA of bacterial communities and enzyme activity

The RDA of fungal communities and enzyme activity

The relationship between a parameter and the microbial communities and samples was provided by the length of the physicochemical parameter arrow in the ordination plot

aGlu, α-glucosidase; bGlu, β-glucosidase; Cell, cellobiase; Phos, phosphatase; Chit, Chitinase; Ure, urease

whereas BXG1, BXG2 and BXG3 had negative correlations (Fig. 5a). The fungal enzyme activities exhibited different patterns in the cultivation groups. XG3 was positively correlated with the phosphatase and cellobiase activities, whereas BXG3 had negative correlations (Fig. 5b). SOM, pH, AP, AN, and AK were strongly negatively linked with *Nitrospirae*, whereas *Proteobacteria* had positive correlations with pH, AK and AN.

Among the five soil chemical parameters, AN, AK, and pH were correlated with specific bacterial phyla (Fig. 6a). *Chloroflexi* was negatively correlated with SOM, pH, AK, and AN but positively correlated with AP. Among the fungal phyla, *Ascomycota* had the strongest positive relationships with SOM, AN, AK, pH, and AP. *Basidiomycota* had negative relationships with SOM, AK, pH, and AP (Fig. 6b).

KEGG functional predictions based on 16S rRNA

The metabolic processes related to the soil microbiota were predicted based on comparisons with previously published 16S rRNA data using the PICRUSt method. Carbohydrate metabolism, amino acid metabolism, energy metabolism, and metabolism of cofactors and vitamins were affected mainly by soil bacteria (Fig. 7). Changes in the metabolic

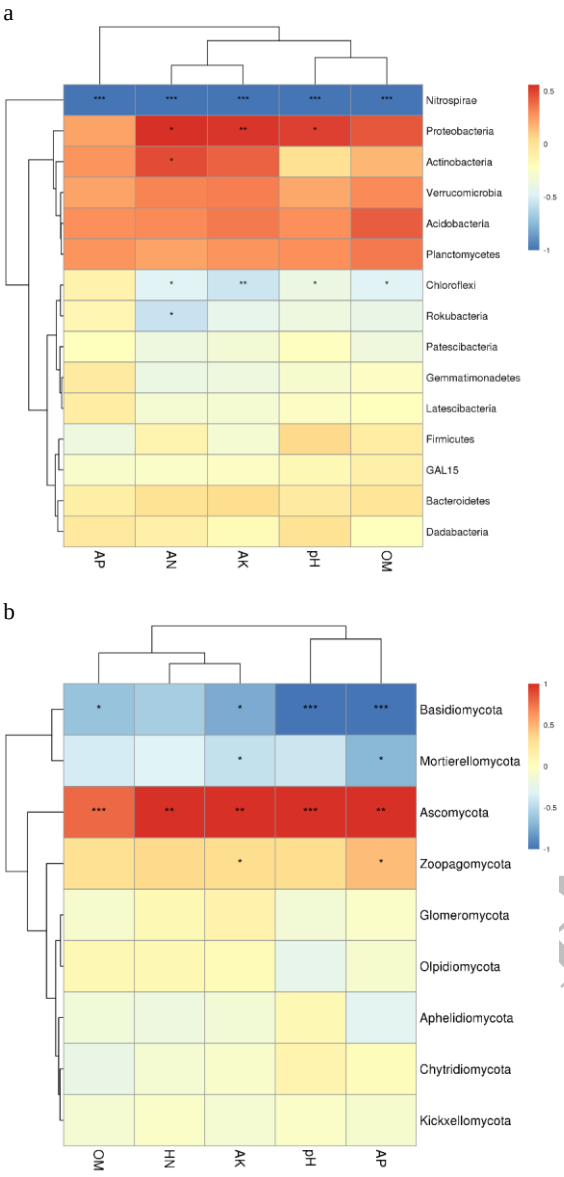

Fig. 6: Relationship of microbial composition and soil chemical property

Relationship of bacterial composition and soil chemical property
Relationship of fungal composition and soil chemical property
OM, organic materials; AN, available nitrogen; AP, available phosphorus; AK, available potassium

processes were found in BXG compared with XG, including an increased carbohydrate metabolism rate and decreased nucleotide metabolism rate.

Principal component analysis (PCA) and linear discriminant analysis

PCA showed that the bacterial community compositions were separated mainly by the cultivation pattern, but not significantly by the cultivation time (Fig. 6a). The bacterial samples were clustered under the XG cultivation pattern

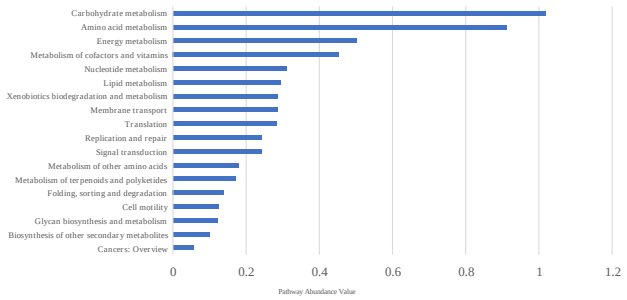

Fig. 7: PICRUSt analysis results of predicted functional pathways in soil microbiota

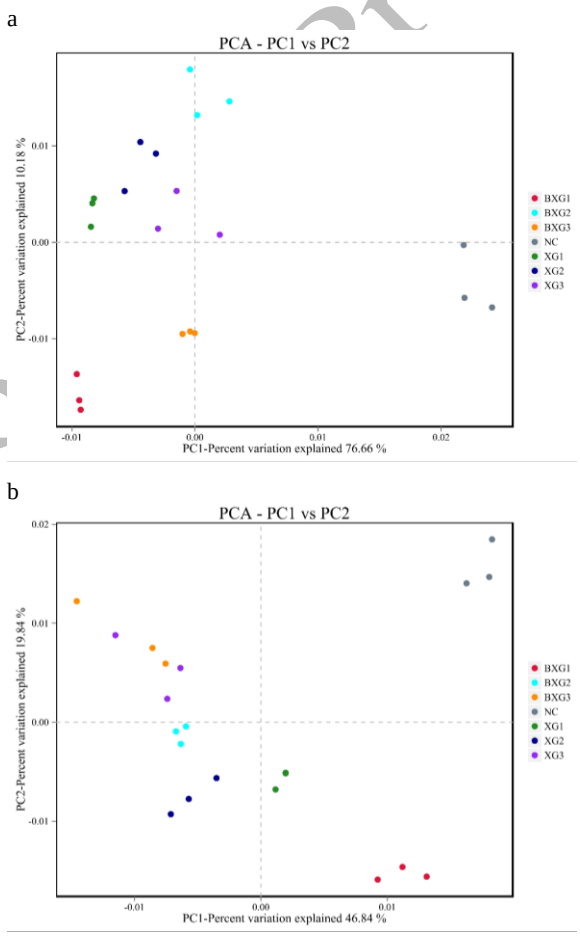

Fig. 8: Multiple-sample PCA analysis of bacteria (a) and fungi (b) in different cultivation patterns and years

(Fig. 8a). The fungal communities were clustered by both the cultivation year and method (Fig. 8b).

Linear discriminant analysis effect size (LEfSe) analysis was conducted to further discriminate the bacterial and fungal taxa in different groups at the phylum to genus levels (Fig. 9). The results obtained by LEfSe analysis showed that the bacterial phyla comprising *Chloroflexi*, *Proteobacteria*, *Actinobacteria*, and *Gemmatimonadetes*

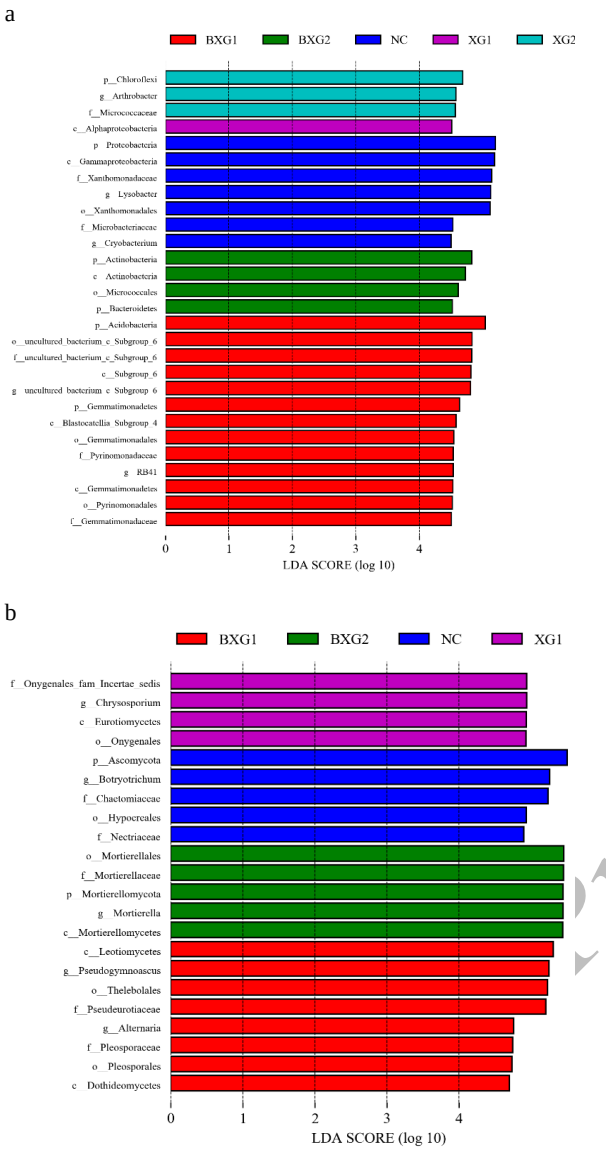

Fig. 9: The LDA scores (log 10) of the significantly differential soil groups provided by LEfSe in bacteria (a) and fungi (b). The color of each bar corresponds to the group with the highest averaged abundance

were affected most by the treatments (Fig. 9a), where *Ascomycota* and *Mortierellomycota* varied most significantly in NC and BXG, respectively (Fig. 9b).

Discussion

After cultivation for 3 years, the pH decreased by about 1 unit under NC. In addition, cultivation with plants (XG and BXG) gradually decreased the soil pH over time, as also shown in a previous study (Sun *et al*. 2015). The AK, AN, and AP levels decreased gradually over time under peach cultivation. The N, P, and K concentrations were lower under XG than BXG, possibly due to the greater fertilizer utilization efficiency. Similar studies showed that root restriction cultivation increased the nutrient utilization efficiency (Xue *et al.* 2011).

The SOM content was significantly higher under NC than XG and the SOM content decreased with the cultivation time. Previous studies have demonstrated that the nutrients required for plant growth are mainly stored as SOM. The availability of nutrients to the plant roots also depends partly on their transformation by microbial communities (Walker and Bernal 2006). Our results showed that the SOM contents clearly increased with the number of years of cultivation, but there were no significant differences under the different cultivation patterns in the same year of cultivation, thereby indicating that the different cultivation patterns had no significant effects on the soil nutrient levels. The soil microbial community has close relationships with the soil enzyme activity levels (Zhang *et al.* 2013). In the present study, the α-glucosidase activity was significantly higher in XG than BXG. The results also indicated that the α-glucosidase activity increased with the number of years of cultivation. The results obtained by RDA and the correlation heatmap showed that α-glucosidase had important links with the bacterial communities. A previous study showed that the soil pH decreased significantly with the α-glucosidase level (Acosta-Martínez and Tabatabai 2000), which agrees with our results. The cellulase activity in the same field increased gradually with number of years of cultivation and the cellulase activity was higher in XG3 compared with BXG3. The mineralization of organic phosphorus is usually assessed based on the phosphatase activity (Krishnamoorthy 1990). We found that the phosphatase activity in the same field decreased gradually with the number of years of cultivation and the highest phosphatase activity was found in NC. The chitinase and cellulase activities exhibited similar trends with the highest levels in XG3 and the lowest in NC. Urea is hydrolyzed into carbon dioxide and ammonia is transformed by urease (Corbel and Hendry 1985). The urease activities were significantly lower in NC and BXG than XG2. The soil enzyme activities are stimulated by plant roots (Touceda *et al.* 2017; Benbi *et al.* 2017). Our results indicated that the urease activities were significantly lower under BXG and NC than XG, thereby suggesting that XG could improve the urease status. The soil enzyme activities and root structure are also related. The effects of the cultivation time on the enzyme activities were determined based on heatmaps and RDA in our study. Major differences in the bacterial compositions were found compared with NC, where XG and BXG had different effects on the bacterial communities. Open root and root-limited cultivation are both applied as common cultivation methods in China, but few previous studies have investigated the bacterial and fungal communities under normal cultivation (root unlimited) and root-limited

cultivation patterns. The variations in the bacterial communities under different fertilizer conditions were explored in previous studies, which indicated that *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Chloroflexi*, and *Proteobacteria* were the dominant phyla. Members of *Proteobacteria* play significant roles in the biotransformation and biodegradation of SOM, especially the genus *Pseudomonas.* Previous studies have shown that carbon, nitrogen, and phosphorus cycling are mediated partly by members of the genus *Pseudomonas.* Thus, *Proteobacteria* might be highly involved in the utilization of SOM under root-limited planting. We found that the abundance of *Proteobacteria* decreased after cultivation for 1–3 years, whereas that of *Gemmatimonadetes* increased. *Betaproteobacteria* also participate in nitrogen fixation and the oxidization of ammonium to produce nitrite, as found in waste water and farmland soil.

Reducing the accumulation of N, P, and K in planted soil could alleviate the acidification and salinization of soil. A previous study found that increased soil acidity was caused by excess nitrogen fertilization (Han *et al.* 2015), and we obtained similar findings in the present study. The soil bacteria and fungi compositions in XG3 had the most negative associations with the high pH, P, N, K and SOM levels. The communities in BXG1, BXG2, XG1 and XG2 were positively correlated with all five soil parameters, whereas negative relationships were found under BXG3 and XG3. The changes in the patterns were similar for both fungi and bacteria. The bacterial and fungal community compositions were separated by the cultivation pattern and number of years of cultivation by PCA. The bacterial and fungal samples were clustered by the same cultivation method, but the NC samples were clustered separately from XG and BXG.

To elucidate the distinctions among the bacterial and fungal communities under different soil treatments, LEfSe was conducted to analyze the differences in the bacterial and fungal taxa at the phylum to genus levels. *Chloroflexi*, *Proteobacteria*, *Actinobacteria*, and *Gemmatimonadetes* were identified as phyla that changed greatly in abundance by LEfSe analysis. The abundances of *Ascomycota* and *Mortierellomycota* differed significantly in NC and BXG. *Ascomycota* and *Mortierellomycota* are common and abundant in planted soils. In the present study, the abundance of *Mortierellomycota* increased in the fields cultivated with *Prunus persica*, and it may have improved the soil's alkalinity.

The genus *Lysobacter* in the phylum *Proteobacteria* contains important biocontrol bacteria, which have beneficial antagonistic effects against nematodes. The increased abundance of *Lysobacter* in XG3 may have been beneficial for the growth of the fruit trees. Finally, the results obtained based on the correlation heatmaps and RDA showed that pH, AN, and AP contributed to the different effects of the microbial community under XG compared with BXG.

Conclusion

In this study, specific bacterial and fungal community compositions were obtained under different cultivation patterns. The bacterial and fungal genera mainly belonged to the phyla *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*, and *Ascomycota*, *Mortierellomycota*, and *Basidiomycota*. The environmental parameters and abundances of various phyla were affected by the different cultivation methods according to multivariate analysis. The result suggested that XG had beneficial effects on improving the soil's alkalinity and changing the microbial populations. The results obtained in this study provide fundamental insights into the microbial ecology in fields cultivated under root restriction.

Author Contributions

Wu Chong planned the experiments. Han Zhen and Zhang Qingtian interpreted the results. Wu Chong, Tao Jihan, and Zhang Qingtian wrote the manuscript. Wu Chong conducted statistical analyses of the data and prepared the illustrations.

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