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Variation of soil bacterial communities in a chronosequence of citrus orchard

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Abstract

Purpose: Soil microorganisms are vital for soil ecosystems through bioconversion of soil nutrients and maintenance of soil fertility to promoting the growth and development of citrus. However, understanding of how different planting years affect the soil bacterial community structures as related to nutrient availability in citrus orchards is limited.

Methods: Here, Illumina MiSeq technology was used to investigate changes in bacterial community structures with different ages of citrus orchards that were 2, 5, 10, 15, and 18 years old.

Results: The data showed that (1) soil bacterial community structures changed over the different growth stages of citrus orchards. With the extension of plantation age, the microbial diversity of citrus orchards increased gradually so that it was highest in 10-year-old citrus plantations but then decreased where the diversity of 18-year-old citrus ages was significantly lower than that of 10 and 15-year-old ones. *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* were the four dominant phyla in soil of citrus orchards, accounting for 30.85%, 24.89%, 14.27%, and 14.05% of the total soil bacterial communities, respectively. (2) Soil bacterial community structures in different succession stages were affected by soil pH and nutrients, in particular available potassium (AK).

Conclusion: This study advances the understanding of soil microbiota of orchards and their interactions related to environmental factors in citrus orchard, which will improve our ability to promote the function of soil bacteria, so as to improve soil pH and reduce potassium (K) fertilizer input and improve the fruit quality.

Keywords: Citrus, High-throughput sequencing technology, Soil bacterial community structures

Background

Citrus is one of the most widespread fruit crops, which has been planted in more than 140 countries and regions, mainly in China, Brazil, the USA, and India, with an annual citrus production of more than 100 million metric tons (Al-Rimawi et al. 2019). In 2018, China's citrus production area and yield reached 3.46 million hectares and 65 million tons, respectively, both ranking the first in the world (FAO 2019). On the other hand, to achieve higher citrus yield and economic benefits, excessive fertilization is often adopted where the averages of annual application rates of N, P_2O_5, K_2O fertilizers in China were reported to be 494, 364, and 397 kg hm^{-2}, respectively, with the ratio of 1:0.74: 0.80 (Lei et al. 2019). Excessive application of chemical fertilizers may bring about some changes in soil, such as soil acidification (Guo et al. 2010), decrease in microbial community structures (Wan et al. 2017), occurrence of soil borne diseases (Yang et al. 2001) and decline of citrus fruit quality (Li et al. 2019). Microorganisms are very sensitive to soil environmental changes and are reliable indicators of soil health and evaluation of changes in soil quality (Chen et al. 2012). Thus, abundant diversity is significant characteristic of soil microorganisms for promoting the sustainability and productivity of citrus orchard ecosystems. Most of the soil microorganisms of citrus orchards are beneficial microorganisms,

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which can maintain the balance of crop hormones, enhance crop stress resilience and support stable yield of citrus (Xu et al. 2018). Bacteria are also a main component of soil microbial and drive the circulation of soil nutrients, decomposition of organic matter, promotion of soil fertility, and suppression of plant diseases (Gao et al. 2021). The structure of soil bacterial communities can reflect the quality of soil ecological and directly affect crop yield and quality (Wang et al. 2018a). It has been reported that the factors affecting the diversity of soil bacteria in citrus orchards include soil parent material and pH (Joa et al. 2014), temperature (Luo et al. 2019), citrus fertilization methods (Hu et al. 2016), quality of irrigation water (Bastida et al. 2017), and the use of copper-containing fungicides (Zhou et al. 2011). However, the feature of the composition of soil bacterial community and their relationship with soil nutrient properties in citrus orchards of different ages remain unclear. To date, no reports have been published on variation of soil bacterial community diversity in different aged citrus plantations. Additionally, such report is very useful because soil microbes play a unique and indispensable role in the agricultural ecosystem balance.

Citrus is a perennial fruit tree, which is planted in the same place all year round, which is easy to produce continuous cropping obstacles. This study aims to comprehensively understand the variation in bacterial diversity and composition among different stages of citrus orchards using Illumina Miseq, and this technology can obtain more abundant microbial information and accurately reflect the nutritional status of soil (Mizrahi-Man et al. 2013). The main purpose was to reveal the diversity and composition of soil bacterial in citrus orchards at different ages, elucidate the environmental factors that influence the soil bacterial community, which can provide theoretical reference for the cultivation and management of citrus plantations.

Materials and methods

Site description and sample collection

The citrus orchards in this investigation are located in Fengjie County, Chongqing, China. In citrus orchards, the same application rates of N, P_2O_5, K_2O fertilizer and organic fertilizer is 0.50, 0.22, 0.40, and 10 kg/plant were performed, respectively. In that mature period of the citrus, the random sample was taken from around each tree. To avoid the wet area of fertilizer application hole and drip irrigation head, soil samples were collected near far 10 cm the tree crown drip waterline. The study was carried out in October 2018, ten soil sampling points were randomly collected according to the "Plum blossom" shape in each citrus orchard. Six citrus trees were randomly selected at each site and 10 collected soil samples were homogenized into one mixed sample. Each treatment included three sites, resulting in 15 soil composite samples were collected from 15 plantations at a depth of 0–40 cm. Subsequently, root and stones are removed from the soil sample. Soil samples were placed in sterile tubes and put in an ice box. The soil sample was carried to the laboratory, where each soil sample was air dried at room temperature in the laboratory and sieved at < 2 mm for chemical and physical properties analysis, other fraction was stored at − 80 °C until DNA extraction for high-throughput sequencing analysis.

The climatic conditions are subtropical humid monsoon with an annual average temperature is 16.4 °C in the area below 600 m, the area of 600–1000 m is 16.4–13.7 °C, the area of 1000–1400 m is 13.7–10.8 °C, and the temperature above 1400 m is lower than 10.8 °C, and an annual average precipitation of 1,132 mm. We selected citrus orchards with 2, 5, 10, 15, and 18 years of age planted in the same soil texture with a radius of about 2 km as the research object, and three citrus orchards with the same texture were selected for three repetitions. The sites are located latitude from 30°28′24″ to 30°53′53″, and longitude from 109°27′39″ to 109°56′30″, and at an altitude ranging from 379 to 534 m (Table 1). The soil types, topography, citrus cultivation, and management method of these citrus orchards were basically consistent. The cultivar of citrus was Neuer 72-1. The spacing in the rows and spacing between rows was 4 × 5 m.

Soil chemical properties analysis

Soil chemical properties were determined using standard methods. Soil pH value was measured by a pH-meter (soil to water ratio was 1:2.5) (Zhou et al. 2017). Soil organic carbon (SOM) was determined using the potassium dichromate titrimetric process. Soil total nitrogen (TN) was determined according to the Kjeldahl digestion method. Total phosphorus (TP) and total potassium (TK) was determined by ICP-AS after digestion with hydrofluoric acid (HF)- perchloric acid ($HClO_4$) (Bao 2000). Available nitrogen (AN), Available phosphorus (AP), Available potassium (AK) were measured via the alkaline

Table 1 Location parameters of citrus tree orchards with different ages

Ages	Logitude (N)	Latitude (E)	Altitude (m)
2	109°27′53″	30°52′24″	379
5	109°27′54″	30°53′28″	373
10	109°27′39″	30°53′27″	469
15	109°27′30″	30°53′42″	512
18	109°28′19″	30°53′53″	534

hydrolysable, molybdenum blue, and flame photometry methods, respectively (Lu 2000).

DNA extraction, PCR amplification, and Illumina MiSeq sequencing

Total Microbial genomic DNA was extracted from 0.3 g homogenized soil sample using the soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's instructions, and the purified DNA was used for Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene. General primers were used for PCR amplification of the V3 + V4 regions of 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Bokulich et al. 2013). The PCR reactions were conducted according to the manufacturer's protocol (Jin et al. 2021).

Processing of sequencing data and statistical analysis

According to the direct overlapping relationship of PE (paired end) reads, the paired reads are spliced into a sequence. The reading quality and splicing effect are controlled and filtered, and the sequence direction is corrected according to the end of the box sequence. Finally, the filtered high-quality sequence was assigned to the sample according to the barcode.

An OTU-based analysis was used to calculate microbial richness and diversity with 97% sequence similarity. Taxonomic analysis of OTU sequences was tested using the RDP classifier Bayesian algorithm. Redundancy analysis (RDA) and mapping using the vegan package in R. Calculation of soil bacterial alpha diversity by Mothur, including Ace index, Chao index, Shannon index, and Simpson index (Wang et al. 2012). Statistical analysis was modeled with SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVAs were performed to assess the effects of different chronosequence of citrus orchard on soil physical and chemical properties and soil bacterial diversity. The Pearson correlation analysis was conducted to assess the correlations between soil properties and soil bacterial diversity and abundant phyla.

Results

Effects of different planting years on soil properties of citrus orchard

The study area of citrus orchards involved acidic soils with pH ranging from 4.81 to 5.47. With increasing the age of citrus trees, the soil pH decreased. In addition, the soil pH of the orchards that were more than 10 years old was less than 5.0 and there was serious soil acidification. The contents of total P, available N, available P and available K increased as the age of trees increased (Table 2).

Diversity of the soil bacterial community in citrus orchards

Fifteen soil samples were collected from five citrus orchards with different ages. A total of 2633 different OTUs (operational taxonomic units) was detected by clustering at 97% similarity level using *16S rRNA* high-throughput sequencing technology and were separated into 37 phyla, 90 classes, 183 orders, 346 families, 639 genera, and 1338 species.

The rarefaction curve mainly reflects the microbial diversity of each sample at different sequencing quantities. It can be used to compare the abundance, uniformity or diversity of species in samples with different sequencing data volumes as well as to verify the reasonability of the sequencing data volumes of samples. The term GJ2-18 means the citrus orchard soil samples with 2–18 years old, with increasing the number of samples, the rarefaction curve gradually leveled off and the number of OTUs gradually became saturated (Fig. 1). This indicated that sampling in this experiment was reasonable and sample sizes were large enough to reflect the statuses of soil bacterial communities comprehensively. Shannon curve shows the bacterial diversity in soil samples. With increasing the number of samples, Shannon index gradually stabilized, suggesting that the collected soil samples accurately reflected the diversity of soil bacteria (Fig. 2).

Shannon, Ace, Simpson, and Chao1 indexes of bacterial flora in citrus orchards with different planting years were obtained under the OTU similarity level of 0.97 through Mothur software analysis. Shannon and Simpson index can reflect the diversity of bacterial community, Ace and

Table 2 Soil chemical properties of the five citrus orchards

Citrus ages (years)	pH	SOM (%)	Total N (%)	Total P (mg/kg)	Total K (%)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
2	5.47 a	1.63 a	0.15 c	510.19 c	1.62 a	99.45 b	20.00 d	102.67 b
5	5.07 b	1.77 a	0.17 bc	533.11 c	1.70 a	88.96 b	21.25 d	133.33 ab
10	5.01 bc	1.69 a	0.17 bc	624.36 b	1.62 a	94.50 b	34.58 c	173.00 a
15	4.93 c	1.83 a	0.22 ab	811.88 a	1.45 a	154.44 a	43.33 a	163.33 a
18	4.81 d	1.51 a	0.23 a	836.64 a	1.62 a	155.15 a	45.67 b	173.33 a

Different letters in the same column indicate significant difference between citrus ages at 0.05 level. The same below

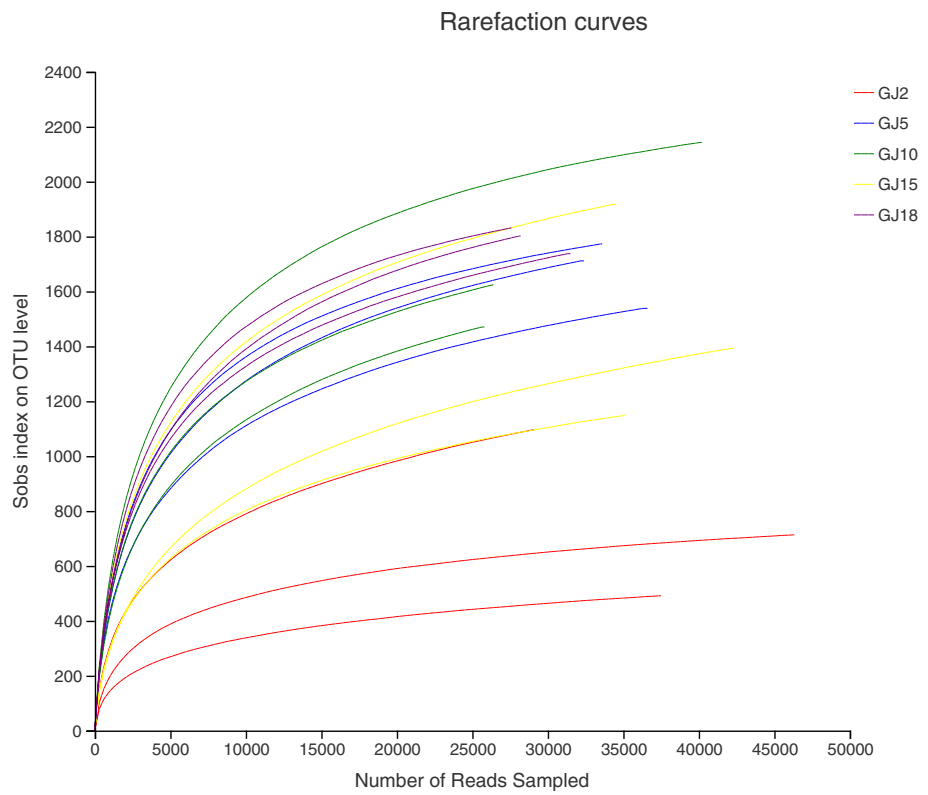

Fig. 1 Rarefaction curves. GJ2-18 were the 2–18 year-old citrus plantations

Chao1 index reflect the richness of the bacterial community. In this paper, Shannon index of soil planted for 2 years is significantly lower than that with other planting years, and Shannon index of soil planted for 5, 10, 15, and 18 years is 44.32%, 45.68%, 29.09%, and 50.68% higher than that planted for 2 years, respectively ($P < 0.05$). Soil bacterial communities show changes over the succession stages of citrus orchards. With the increase of planting years, the microbial diversity of citrus orchards increased gradually, that was highest in 10-year-old citrus plantations, and the diversity of 18-year-old citrus plantations was significantly lower than that of 10- and 15-year-old citrus plantations (Table 3).

Soil bacterial community composition in citrus orchards

The 31 phylum in citrus orchard soil were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Bacteroidetes*, *Firmicutes*, *Nitrospirae*, *Planctomycetes*, *Saccharibacteria*, *Latescibacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Tectomicrobia*, *Aminicenantes*, *Deinococcus-Thermus*, *Parcubacteria*, *GAL15*, *Chlamydiae*, *SBR1093*, *Chlorobi*, *TM6__Dependentiae_*, *Ignavibacteriae*, *BRC1*, *Hydrogenedentes*, *FBP*, *Microgenomates*, *Elusimicrobia*, *Gracilibacteria*, *RBG-1__Zixibacteria_*, *Candidatus_Berkelbacteria* (Table 3).

The top 40 classes in citrus orchard soil were *Actinobacteria*, *Acidobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gemmatimonadetes, norank, Nitrospira, Anaerolineae, Ktedonobacteria, KD4-96, Sphingobacteriia*, *Thermomicrobia*, *Bacilli*, *TK10*, *Chloroflexia*, *Clostridia*, *Clostridia*, *S085*, *Planctomycetacia, unclassified, JG30-KF-CM66*, *Cytophagia*, *Cyanobacteria*, *Cyanobacteria*, *OPB35_soil_group*, *Spartobacteria*, *Gitt-GS-136*, *Flavobacteriia*, *Caldilineae*, *OM190*, *Ardenticatenia*, *Phycisphaerae*, *SBR2076*, *Deinococci*, *Pla3_lineage*, *Chlamydiae*, *Chlorobia*, *Opitutae* (Table 4).

The top 40 orders in citrus orchard soil were *norank, Xanthomonadales, Rhizobiales, Acidimicrobiales, Frankiales, Gaiellales, Rhodospirillales, Planctomycetales, Sphingobacteriales, Solirubrobacterales, Gemmatimonadales, Anaerolineales, Nitrosomonadales, Micrococcales, Micromonosporales, Solibacterales, Bacillales, Blastocatellales, Myxococcales, JG30-KF-AS9, Burkholderiales, Propionibacteriales, Desulfurellales, Chloroflexales, unclassified, JG30-KF-CM45, Pseudomonadales, SC-I-84, Streptomycetales, Cytophagales, Corynebacteriales, Caulobacterales, Sphaerobacterales, Clostridiales, Ktedonobacterales, Halanaerobiales, NB1-j, TRA3-20, Chthoniobacterales, Streptosporangiales* (Table 4).

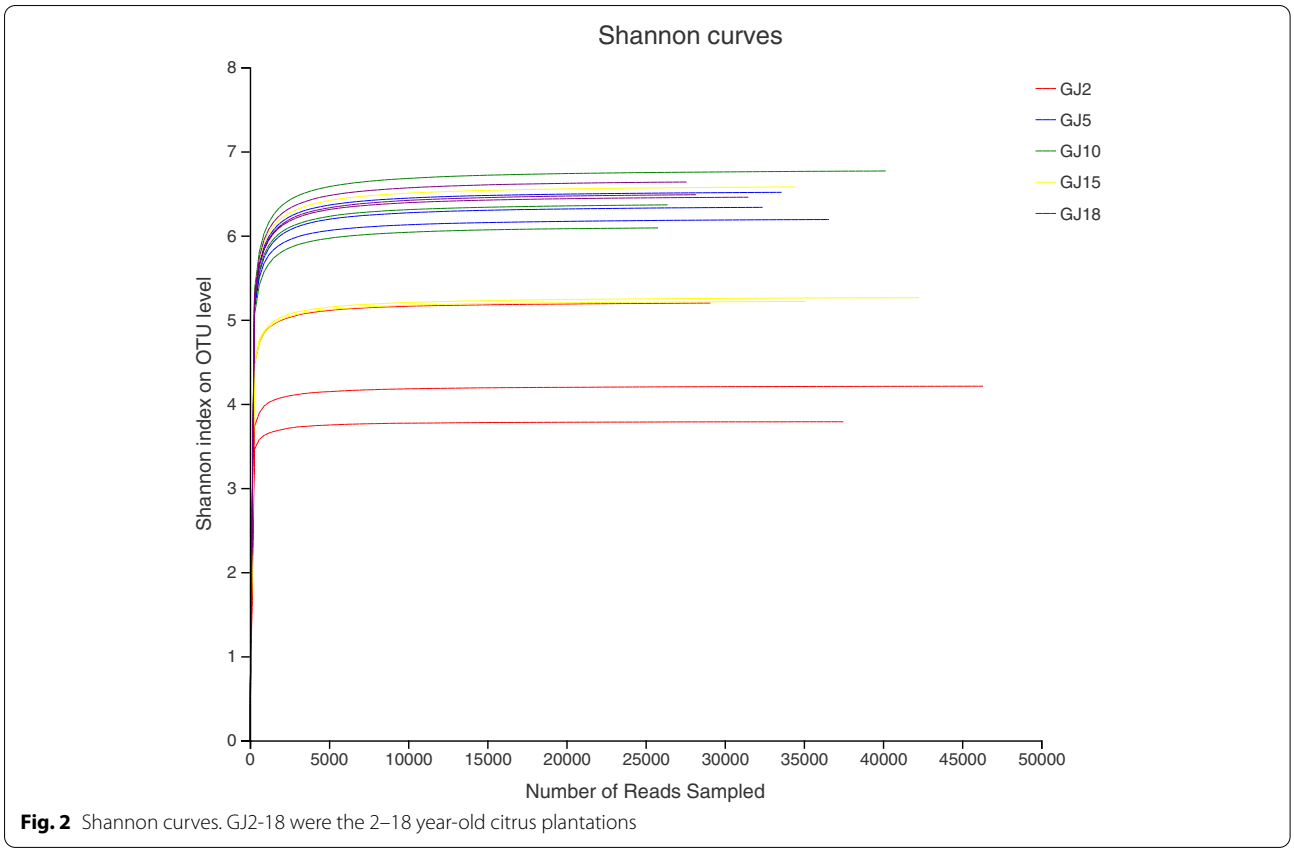

Fig. 2 Shannon curves. GJ2-18 were the 2–18 year-old citrus plantations

The top 40 families in citrus orchard soil were *Norank, Xanthomonadales, Rhizobiales, Acidimicrobiales, Frankiales, Gaiellales, Rhodospirillales, Planctomycetales, Sphingobacteriales, Solirubrobacterales, Gemmatimonadales, Anaerolineales, Nitrosomonadales, Micrococcales, Micromonosporales, Solibacterales, Bacillales, Blastocatellales, Myxococcales, JG30-KF-AS9, Burkholderiales, Propionibacteriales, Desulfurellales, Chloroflexales, Unclassified, JG30-KF-CM45, Pseudomonadales, SC-I-84, Streptomycetales, Cytophagales, Corynebacteriales, Caulobacterales, Sphaerobacterales, Clostridiales, Ktedonobacterales, Halanaerobiales, NB1-j, TRA3-20, Chthoniobacterales, Streptosporangiales* (Table 4).

Table 3 Soil bacterial community diversity index of citrus tree orchards with different ages

Ages	Chao1 index	Ace	Shannon-Wiener index	Shannonever index
2	1072 c	1028 c	4.40 c	0.66 c
5	1840 b	1725 b	5.28 b	0.75 b
10	2058 a	2012 a	6.52 a	0.87 a
15	2035 a	2014 a	6.41 a	0.86 a
18	1950 b	1923 b	5.68 b	0.78 b

The top 40 genera in citrus orchard soil were *norank_c__Acidobacteria, Mizugakiibacter, Acidothermus, norank_o__Gaiellales, Nitrospira, norank_f__Anaerolineaceae, norank_c__KD4-96, norank_f__Gemmatimonadaceae, norank_f__Nitrosomonadaceae, norank_c__TK10, Gaiella, Acidobacterium, norank_o__JG30-KF-AS9, norank_o__Acidimicrobiales, norank_c__Actinobacteria, norank_p__Saccharibacteria, norank_f__Elev-16S-1332, Sphingomonas, Roseiflexus, H16, norank_o__JG30-KF-CM45, norank_f__DA111, RB41, Bryobacter, Bradyrhizobium, norank_f__Xanthobacteraceae, Acidibacter, norank_c__S085, norank_p__Latescibacteria, unclassified_f__Acetobacteraceae, Nocardioides, Bacillus, norank_f__Rhodospirillaceae, norank_o__SC-I-84, Streptomyces, Candidatus_Solibacter, norank_f__Acidimicrobiaceae, norank_f__Planctomycetaceae, orank_c__JG30-KF-CM66, unclassified_f__Micromonosporaceae* (Table 4).

There were differences in abundance of the soil bacterial community phyla and genus in different ages of citrus orchards (Figs. 3 and 4). *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* are the four dominant bacterial community in soil samples, accounting for 30.85%, 24.89%, 14.27%, and 14.05% of soil bacteria community respectively. The abundance of

Table 4 Relative abundance of the dominant bacterial phylum, classes, orders, families, and genera in the citrus orchards

Phylum(%)	Classes(%)	Orders(%)	Families(%)	Genera(%)
Proteobacteria (30.85)	Actinobacteria (24.81)	Norank (21.57)	Norank (32.01)	Norank_c__Acidobacteria (7.73)
Actinobacteria (24.89)	Acidobacteria (14.27)	Xanthomonadales (8.02)	Xanthomonadaceae (5.80)	Mizugakiibacter (4.45)
Acidobacteria (14.27)	Alphaproteobacteria (13.63)	Rhizobiales (6.81)	Acidothermaceae (4.23)	Acidothermus (4.23)
Chloroflexi (14.05)	Gammaproteobacteria (8.83)	Acidimicrobiales (5.57)	Acidobacteriaceae__Subgroup_1_(2.74)	norank_o__Gaiellales (3.09)
Gemmatimonadetes (2.78)	Betaproteobacteria (4.91)	Frankiales (5.40)	Acetobacteraceae (2.49)	Nitrospira (2.52)
Bacteroidetes (2.68)	Deltaproteobacteria (3.39)	Gaiellales (4.87)	Gemmatimonadaceae (2.41)	norank_f__Anaerolineaceae (2.41)
Firmicutes (2.62)	Gemmatimonadetes (2.78)	Rhodospirillales (4.	Anaerolineaceae (2.41)	norank_c__KD4-96 (1.96)
Nitrospirae (2.53)	Norank (2.60)	Planctomycetales (3.42)	Nitrosomonadaceae (1.98)	norank_f__Gemmatimonadaceae (1.95)
Planctomycetes (1.34)	Nitrospira (2.52)	Sphingobacteriales (3.33)	Xanthobacteraceae (1.75)	norank_f__Nitrosomonadaceae (1.91)
Saccharibacteria (1.18)	Anaerolineae (2.41)	Solirubrobacterales (2.93)	Solibacteraceae__Subgroup_3_(1.67)	norank_c__TK10 (1.60)
Latescibacteria (0.85)	Ktedonobacteria (2.22)	Gemmatimonadales (2.41)	Xanthomonadales_Incertae_Sedis (1.60)	Gaiella (1.58)
Verrucomicrobia (0.62)	KD4-96 (1.96)	Anaerolineales (2.41)	Gaiellaceae (1.58)	Acidobacterium (1.49)
Cyanobacteria (0.55)	Sphingobacteriia (1.80)	Nitrosomonadales (1.98)	Blastocatellaceae__Subgroup_4_(1.55)	norank_o__JG30-KF-AS9 (1.49)
Tectomicrobia (0.32)	Thermomicrobia (1.66)	Micrococcales (1.88)	Chitinophagaceae (1.38)	norank_o__Acidimicrobiales (1.49)
Aminicenantes (0.084)	Bacilli (1.62)	Micromonosporales (1.74)	Sphingomonadaceae (1.33)	norank_c__Actinobacteria (1.30)
Deinococcus-Thermus (0.082)	TK10 (1.60)	Solibacterales (1.67)	Nocardioidaceae (1.29)	norank_p__Saccharibacteria (1.17)
Parcubacteria (0.065)	Chloroflexia (1.08)	Bacillales (1.59)	Desulfurellaceae (1.22)	norank_f__Elev-16S-1332 (1.11)
GAL15 (0.046)	Clostridia (0.94)	Blastocatellales (1.55)	Hyphomicrobiaceae (1.12)	Sphingomonas (1.05)
Chlamydiae (0.045)	Clostridia (0.94)	Myxococcales (1.55)	Elev-16S-1332 (1.11)	Roseiflexus (1.03)
SBR1093 (0.043)	S085 (0.85)	JG30-KF-AS9 (1.49)	Bradyrhizobiaceae (1.00)	H16 (1.02)
Chlorobi (0.042)	Planctomycetacia (0.80)	Burkholderiales (1.45)	Rhodospirillaceae (0.923)	norank_o__JG30-KF-CM45 (0.96)
TM6__Dependentiae_ (0.022)	Unclassified (0.63)	Propionibacteriales (1.37)	Microbacteriaceae (0.88)	norank_f__DA111 (0.95)
Ignavibacteriae (0.015)	JG30-KF-CM66 (0.59)	Desulfurellales (1.22	Bacillaceae (0.87)	RB41 (0.93)
BRC1 (0.0102)	Cytophagia (0.58)	Chloroflexales (1.06)	Planctomycetaceae (0.80)	Bryobacter (0.92)
Hydrogenedentes (0.0056)	Cyanobacteria (0.54)	Unclassified (0.97)	Rhizobiales_Incertae_Sedis (0.923)	Bradyrhizobium (0.91)
FBP (0.0039)	Cyanobacteria (0.54)	JG30-KF-CM45 (0.96)	Pseudomonadaceae (0.71)	norank_f__Xanthobacteraceae (0.90)
Microgenomates (0.0022)	OPB35_soil_group (0.35)	Pseudomonadales (0.72)	Streptomycetaceae (0.71)	Acidibacter (0.90)
Elusimicrobia (0.0015)	Spartobacteria (0.33)	SC-I-84 (0.72)	Comamonadaceae (0.65)	norank_c__S085 (0.85)
Gracilibacteria (0.0011)	Gitt-GS-136 (0.32)	Streptomycetales (0.72)	Cytophagaceae (0.57)	norank_p__Latescibacteria (0.84)
RBG-1__Zixibacteria_ (0.0011)	Flavobacteriia (0.29)	Cytophagales (0.57)	Rhodobiaceae (0.56)	unclassified_f__Acetobacteraceae (0.83)
Candidatus_Berkelbacteria (0.0007)	Caldilineae (0.26)	Corynebacteriales (0.51)	Rhodobiaceae (0.56)	Nocardioides (0.77)
	OM190 (0.25)	Caulobacterales (0.49)	Intrasporangiaceae (0.54)	Bacillus (0.76)
	Ardenticatenia (0.24)	Sphaerobacterales (0.49)	Solirubrobacteraceae (0.51)	norank_f__Rhodospirillaceae (0.74)
	Phycisphaerae (0.23)	Clostridiales (0.48)	Haliangiaceae (0.50)	norank_o__SC-I-84 (0.72)
	SBR2076 (0.10)	Ktedonobacterales (0.45)	Sphaerobacteraceae (0.49)	Streptomyces (0.71)

Table 4 (continued)

Phylum(%)	Classes(%)	Orders(%)	Families(%)	Genera(%)
	Deinococci (0.08)	Halanaerobiales (0.45)	Rhodospirillales_Incertae_Sedis (0.47)	Candidatus_Solibacter (0.68)
	Pla3_lineage (0.04)	NB1-j (0.42)	unclassified_o__Acidimicrobiales (0.47)	norank_f__Acidimicrobiaceae (0.63)
	Chlamydiae (0.04)	TRA3-20 (0.37)	ODP1230B8.23 (0.45)	norank_f__Planctomycetaceae (0.61)
	Chlorobia (0.04)	Chthoniobacterales (0.35)	Planococcaceae (0.42)	norank_c__JG30-KF-CM66 (0.59)
	Opitutae (0.03)	Streptosporangiales (0.32)	Methylobacteriaceae (0.41)	unclassified_f__Micromonosporaceae (0.59)

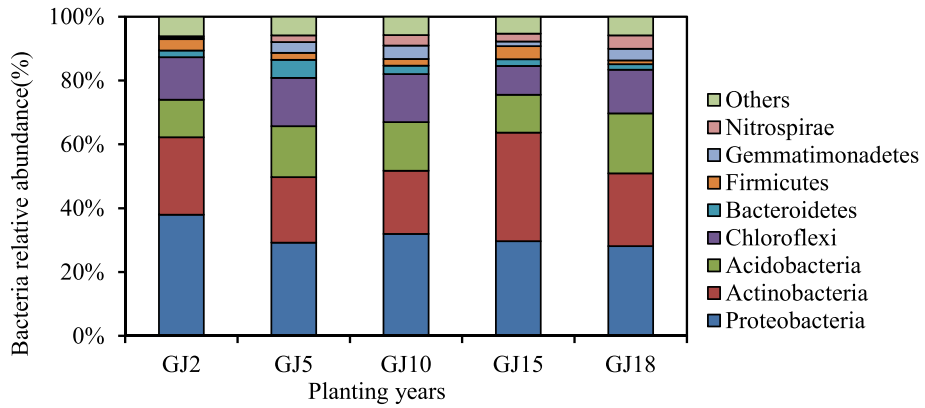

Fig. 3 Soil bacterial phylum levels distribution of citrus tree orchards with different ages

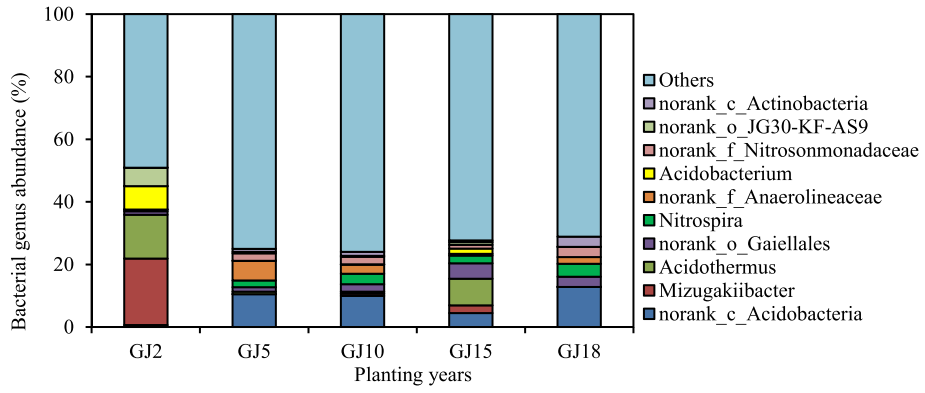

Fig. 4 Soil bacterial genus levels distribution of citrus tree orchards with different ages

Proteobacteria is the highest in 2-year-old citrus tree plantations (37.92%), and the abundance in 5–18-year-old citrus tree plantations is 29.17%~31.93%. The abundance of *Actinobacteria* changed significantly with the increase of planting years, its abundance gradually decreased to 19.8% in 2–10th years, and reached the highest abundance (34%) in 15-year-old citrus tree plantations. The abundance of *Acidobacteria* and *Chloroflexi* reached the maximum in 18 years (18.8%) and 5-year-old citrus tree plantations (15.12%) respectively, and the lowest in 2-year-old citrus tree plantations (11.77%) and 15 year-old citrus tree plantations (9.04%). At the genus level, the species composition of Citrus soil microorganisms planted in 2-year-old citrus

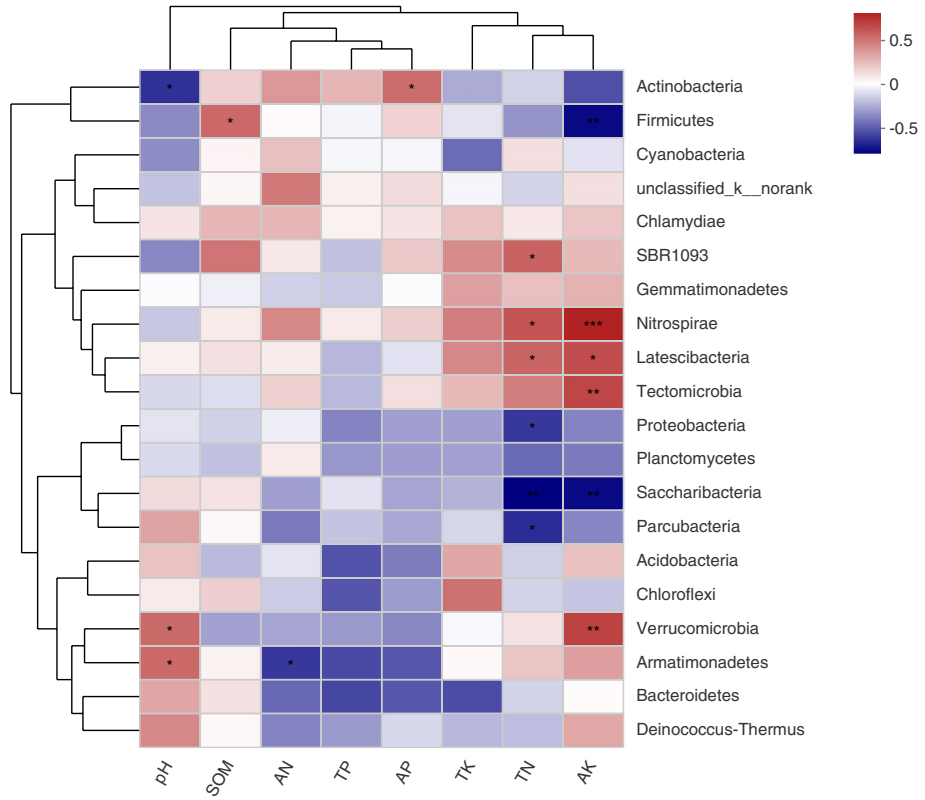

Fig. 5 Correlation heat map of the top twenty phyla and soil properties

tree plantations was significantly different from that in other year-old citrus tree plantations. *Mizugakiibacter, Acidothermus, Acidobacterium, norank_ o__ Jg30-kf-as9* has high abundance in 2-year-old citrus tree plantations. In 5–18 year-old citrus tree plantations of soil, *norank_ c__ Acidobacteria, norank_ o__ Gaiellales, norank_ f__ Anaerolineaceae* is dominant (Fig. 4).

The heat map shows the correlation between the top 20 dominant bacterial phyla in citrus soil and the soil properties. *Actinobacteria* showed a significant negative correlation with AK and pH, and significant positive correlation with AP. *Firmicutes* showed significant positive correlation with SOM and significant negative correlation with AK. *SBR1093* showed significant positive correlation with TN, *Nitrospirae* showed significant positive correlation with TN, and showed significant positive correlation with AK. *Latescibacteria* showed significant positive correlation with AK and TN. *Tectomicrobia* showed significant positive correlation with AK. *Proteobacteria* showed significant negative correlation with TN. *Saccharibacteria* showed significant negative correlation with TN and AK. *Parcubacteria* showed significant negative correlation with TN, *Verrucomicrobia* showed significant positive correlation with pH, and showed significant positive correlation with AK, *Armatimonadetes* showed significant positive correlation with pH and showed significant negative correlation with AN (Fig. 5).

The heat map shows the change of soil bacterial community composition of the top 10 bacterial at genus level of the citrus orchards in different planting years. The relative abundance of *Mizugakiibacter* was the highest (20.78%) in 2 years, which was significantly higher than that in other years ($P < 0.05$). The relative abundances of *Nitrospira*, *norank_f__Anaerolineaceae*, *norank_f__Nitrosomonadaceae*, *norank_f__Gemmatimonadaceae* were the lowest in 2 years of planting, followed by 15 years of planting, while the other years were relatively high, and the difference reached a significant level ($P < 0.05$). Whereas, the bacterial relative abundance of *Acidobacterium* was the highest in 2 years (6.97%), followed by 15 years (1.45), and almost 0 in the rest years (Fig. 6).

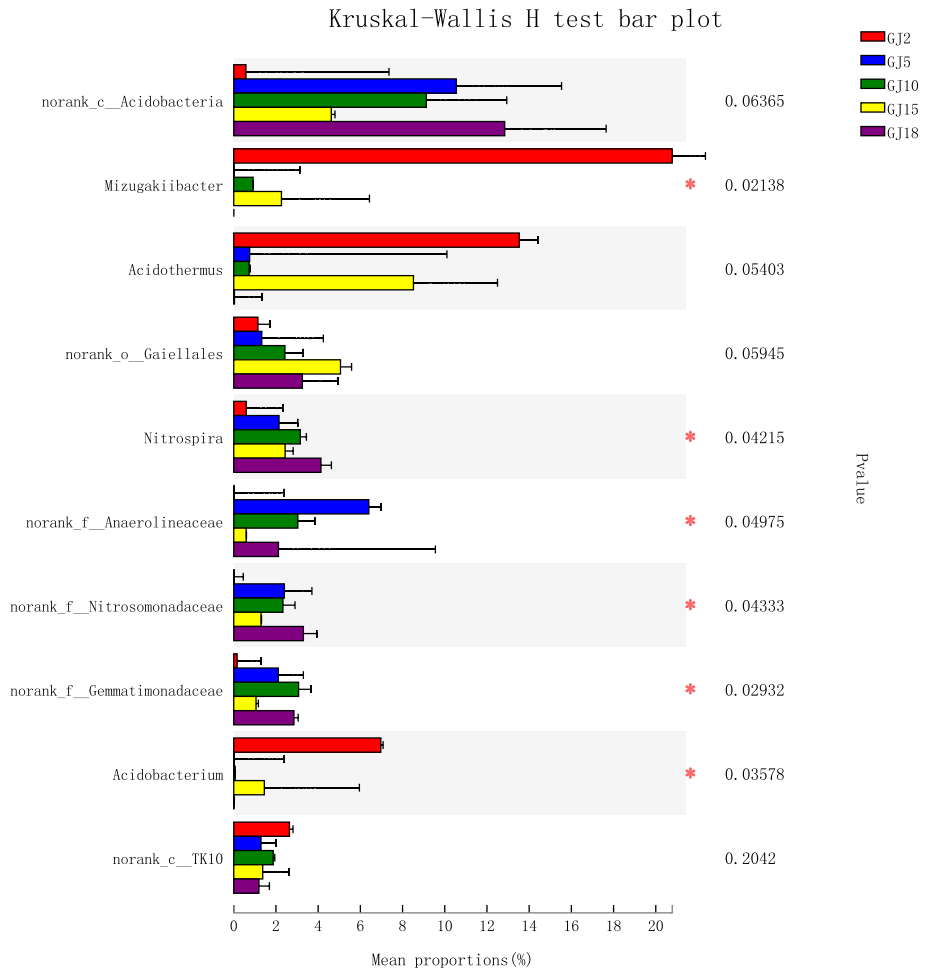

Fig. 6 Variation of soil bacterial community composition of the top 10 bacterial at genus level in citrus orchards with different ages. Date represent average of three replicates and error bars represent standard deviations. The right side is the p value,* indicate $P \leq 0.05$

Factors driving variability of bacterial community in citrus orchards

A redundancy analysis (RDA) showed that the soil variables together explained 50.45% of the variation in the structure of bacterial communities, with the first two axes explaining 30.54% and 19.91% of variation, respectively (Fig. 7). The bacterial communities in the second year were separated from those of the other treatments along the first axis. The proteobacteria was closely correlated with soil pH. The included angles of pH, AK and AN with the primary shaft were acute, suggesting a great correlation of these factors with the primary shaft, and in this case, a markedly positive correlation. The included angles of SOM and TN with the secondary shaft were obtuse, indicating a close negative correlation of these environmental factors with the diversity of soil bacterial communities. The correlation coefficient between the environmental factors and bacterial diversity in citrus soil is shown in Table 5. The data shows that the contents of soil available nutrients, in particular potassium ($r^2 = 0.7207$; $P = 0.002$), had a great influence on the changes in bacterial diversity in citrus orchards, whereas other environmental factors had weak influence (Table 5).

Factors structuring soil bacterial community diversity in citrus orchards

There is a significant positive correlation between the content of AK, pH, SOM and the Chao1 index, Shannon, and Shannonever index of citrus soil bacteria, that is, the abundance of citrus soil bacteria increases with the increase of available potassium content (Table 5).

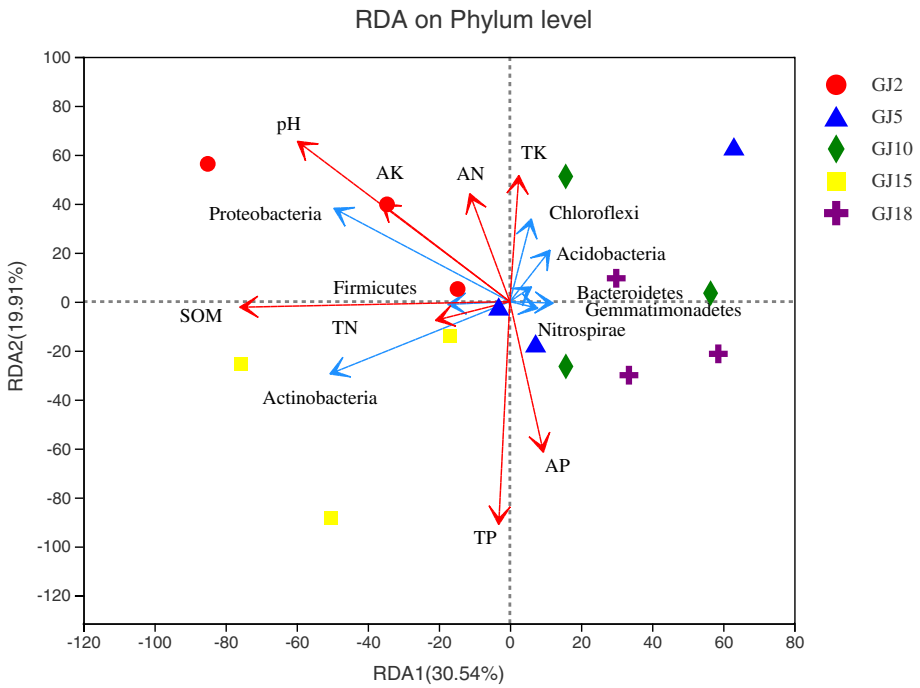

Fig. 7 Soil bacterial community and soil factors in citrus orchards with different ages. Red arrows indicate the direction and impact degree of environmental factors; the angle between them represents positive and negative correlation (acute angle indicate positive correlation; obtuse angle indicate negative correlation; straight angle indicate no correlation)

Table 5 Correlation between soil bacterial diversity index and soil properties

Index	pH	SOM	TN	TP	TK	AN	AP	AK
Chao1	− 0.791**	− 0.759**	− 0.527*	0.527*	− 0.430	0.077	0.596*	0.777**
Shannon-Wiener	− 0.678*	− 0.687**	− 0.461	0.616*	− 0.473	− 0.240	0.641*	0.747**
Shannonever	− 0.659*	− 0.705**	− 0.498	0.590*	− 0.501	− 0.215	0.607*	0.724**

* indicate $P < 0.05$

** indicate $P < 0.01$

Discussion

Soil chemical properties of citrus plantations

The suitable soil pH for citrus growth was reported to be 4.5–8.5 and the range of optimal root system development and nutrient absorption was 6.0–6.5 (Hakanson 1980). In this investigation, the soil pH ranged from 4.64 to 5.47. In orchards older than 15 years, the soil pH was lower than 5.0. Despite it was within the adaptive range of citrus planting, it was not conducive to the absorption of nutrients by citrus roots. The pH values of soils of younger orchards (2–10 years) were higher than those of older ones (15–20 years). Obviously, soil pH is rapidly decreasing and Total N, P, K and available N, P, K is rapidly increasing with increasing the planting years (Table 1). The reason for the decrease in soil pH may be that over longer planting periods, the application of overuse compound fertilizer containing 15% N, 15% P_2O_5, and 15% K_2O and physiological acidic fertilizers such as $(NH_4)_2SO_4$, $CO(NH_2)_2$, and seldom apply basic fertilizers such as lime. Overuse fertilizers led to poor soil buffering performance and exchangeable cation leaching (Barak et al. 1997). Soil acidification affected the availability of soil nutrition, cation exchange capacity (CEC), thus inducing soil and leaf nutrient imbalance, which suppressed the growth and development of fruit trees by increasing the solubility of some toxic metal elements such as Al and Mn in the soil and reducing the fruit quality (Ross et al. 1985). Soil acidification has become a principal factor restricting the production of fruit trees in China and soil quality became worse after longer planting years (Li et al. 2015).

Structure of soil bacterial communities in different aged citrus plantations

Soil bacteria have been increasingly recognized as crucial elements for sustainable agricultural development and are involved in promoting soil health and citrus growth. Soil microorganisms constitute a sensitive index reflecting the quality of soil (Kaurin et al. 2018). In this study, a detailed analysis of soil bacterial structure in citrus orchards was investigated. The diversity in soil bacteria and composition varied over the successive stages of citrus orchards. As the planting period increased, the microbial diversity in soils of citrus orchards increased gradually, where it was highest in 10-year-old plantations and then decreased where the diversity of 18-year-old plantations was significantly lower than that of 10- and 15-year-old ones. *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* were the four dominant bacterial communities in the soil samples, respectively. This is consistent with data on the composition of bacterial communities in citrus soils in other studies (Bastida et al. 2017; Trivedi et al. 2012; Joa et al. 2014). Numerous reports have shown that the richness and diversity of the soil microbial communities will guarantee plant normal growth and health (Luan et al. 2015). In this experiment, the abundance of *Proteobacteria* was highest in 2-year-old citrus orchards but lower in 5–18-year-old ones. Some studies have shown that *Proteobacteria* can participate to the process of soil nitrogen fixation and phosphorus dissolution, degradation of lignin, and aromatic compounds (Weller 2007), and has a positive correlation with soil carbon content (Fig. 5). *Actinobacteria* was the main phyla of the soil bacterial community in citrus orchard, as well as forest ecosystem and agriculture soil (Tajik et al. 2020). *Actinobacteria* showed a significant negative correlation with AK and pH (Fig. 5), which may be related to the acidity in citrus orchard soil. The changes in the abundance of *Acidobacteria* and *Chloroflexi* were consistent with those of the soil bacterial diversity index, where both increased initially but then decreased, being lower in 15-year-old citrus plantations. Some studies have shown that *Acidobacteria* and *Chloroflexi* were Oligotrophic Bacteria, which were dominant when the substrate concentration was low (Fierer et al. 2007), and could decompose soil organic matter in extreme environments (Wang et al. 2018b). As the main phylum of bacteria, *Acidobacteria* and *Proteobacteria* showed positive relationships with soil nutrition and they affect plant growth productivity and fruit quality (Chai et al. 2021). The presence of specific *Actimobacteria* can affect the titratable acids, which may determine the fruit flavor and quality of citrus fruit.

The diversity and richness of soil microbial play a vital role in maintaining the soil quality, the function, and sustainability of soil ecosystem (Garbeva et al. 2004) and fruit quality. Bacterial diversity and richness were decreased in the 15- and 18-year orchard soils compared with that in the 10-year soil, indicating that citrus continuous cropping may have decreased the bacterial diversity through the 15 or 18 years of plantation. In summary, soil nutrient (K) and pH can affect the soil environment and shape the communities and metabolic activity of soil microbes with the extension of plantation age. Consequently, then affect fruit quality (Chai et al. 2021). Therefore, some measures must adjust the soil microecological environment of citrus orchards with long planting periods, the application of organic fertilizers, green manure, and bio-charcoal (Yasutaka et al. 2002) has been reported to significantly improve the microbial diversity and the quality of soil.

Relationship between microbial characteristics and soil chemical properties

Previous studies have found that soil physic-chemical properties played important roles in controlling the diversity of microbial communities in terms of species richness and vegetative biomass (Zhou et al. 2017), soil pH (Fierer and Jackson 2006), forms of land utilization (Suleiman et al. 2013), seasonal differences (Thoms and Gleixner 2013), and altitude (Zhang et al. 2019). Moreover, it has been documented that soil pH mainly influenced the soil bacterial communities (Nacke et al. 2011). Soil bacteria were significant negative correlated with soil pH in rubber ecosystem or agricultural soil (Zhou et al. 2017). In this study, we also found that the overall diversity indices of soil bacteria were significant negative correlated with soil pH in citrus plantations. It is a general conclusion. Long-term large-scale fertilization led to excessive accumulation of N, P, K, and decrease of pH in orange orchard soil. Decreases in soil pH caused by fertilizer application especially NH_4^+-N fertilizer are likely to reduce soil microbial activities and their roles in nutrient transformation in the older orange orchards (Wan et al. 2017). Soil pH had a strong impact on the composition of soil microbial communities. Two assumptions were put forward to explain the relationship between soil pH and diversity of soil bacterial communities. Zhou et al. (2017) proposed that the pH could affect soil bacteria communities and reduces the net growth of individual taxa in a certain soil pH range. The other assumption stated that bacterial communities were indirectly altered by soil pH, presumably as a result of changing soil characteristics, such as nutrient availability, chemical form and land use. These factors are often directly or indirectly related to soil pH where they may drive the observed changes in community composition (Nacke et al. 2011).

A significant positive correlation was found between soil bacterial abundance and available potassium (AK). *Actinobacteria* showed a significant negative correlation with AK and pH and significant positive correlation with AP. *Firmicutes* showed significant positive correlation with SOM and significant negative correlation with AK (Fig. 5). The values of pH and K were the most important factors correlated with bacterial phyla. This result is remarkable because in recent years, most studies have focused on N and P, but overlooked the importance of K. However, the specific influencing mechanism remains unclear. Potassium is a quality element having a significant relationship with the yield and quality of citrus fruits. The decrease in K fertilizer application decreases the diameter, weight and yield of citrus fruits (Han et al. 2008; Ben Mimoun et al. 2018). Therefore, further research is needed to understand the relationship between soil bacteria and K content, and to clarify the optimal range of K content in soil, so as to improve the productivity of citrus and the proportion of marketable fruits. Our results showed soil bacterial community structure changes due to soil nutrients status as affected by long-term chemical fertilizers. The factors affecting the diversity and structure of citrus soil bacteria are complex and multifaceted. Therefore, it may not be simple to pin point the mechanism of changing the citrus soil bacteria. Future agricultural practice in citrus production should involve tight control of soil pH as well as potassium fertilizer input to reduce the negative effects on soil bacterial community structures. To maintain the diversity and abundance of soil bacteria, tighter control over soil nutrients would reduce soil acidification caused by human disturbance and establish more productive citrus planting systems.

Conclusion

(1) The richness and diversity of soil microbial communities in citrus orchards increased firstly and then significantly decreased with the extension of plantation age. The diversity of microbial communities was highest in 10-year-old citrus orchards. *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* were the four main bacterial communities in citrus orchards.

(2) The pH and available nutrients (K) were the major factors affecting the structure of microbial communities in orchard. Future citrus production should pay more attention to appropriate K input to maintain the sustainable development of the orchard micro-ecosystems.

(3) More than 10 years old citrus orchards should receive more attention to the improving soil acidity and nutrient management measures to continuously maintain the micro-ecological health of citrus orchard soil, which may contribute to micro-ecosystem preservation and restoration of orchard soils. These findings could advance the understanding of soil microbial ecology of orchards in China. Results can provide basis for future studies on sustainable agricultural measures to solve the problem of long-term continuous cropping soils of citrus.

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Authors' contributions
Yabo Jin contributed to the experiments and data analysis, and completed the first draft of the paper. Zheng Fang contributed to the experiments, record data, and data analysis. Xinbin Zhou guided the completion of this experiment and provided critical reading and revision suggestions. The authors read and approved the final manuscript.

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N/A

Declarations

Ethics approval and consent to participate
The study did not violate ethics, and all participants agreed to publish the paper.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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