Apple Scion Cultivars Regulate the Rhizosphere Microbiota of Scion/rootstock Combinations

Yi Wang (wangyi@cau.edu.cn)
China Agricultural University College of Horticulture https://orcid.org/0000-0002-6303-385X
Xiaofen Chai
China Agricultural University
Xiaona Wang
China Agricultural University
Hui Li
China Agricultural University
Xuefeng Xu
China Agricultural University
Ting Wu
China Agricultural University
Xinzhong Zhang
China Agricultural University
Zhenhai Han
China Agricultural University

Research

Keywords: Grafting, scion cultivar, rootstock genotype, rhizosphere bacteria community, sugar metabolism, plant growth-promoting traits

Posted Date: September 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-82653/v1

Abstract

Grafting is a useful technique in the production of horticultural products. In addition to providing root system, rootstocks can increase stress tolerance of plants, influence scion growth and fruit yield, and harbor rich microbial community. But whether the scion modify plant growth, rootstock phenotypes and rhizosphere bacterial community has not been clearly investigated. Here, 14 different combinations of two scion cultivars grafted on 7 rootstock genotypes were used as research materials, we analyzed the plant traits, rhizosphere bacterial community, and potential functionalities across these plants in the same orchard. We found that apple scion cultivars influenced the tree high and trunk circumference, and the sugar concentration in root varied significantly between scion cultivars, especially fructose and sucrose. Apple scion cultivars was the largest source of variation in the rhizosphere bacterial diversity and taxonomic composition of grafted combinations. The dominant rhizosphere bacterial taxa were correlated with the root sugar concentration, especially sucrose. And the PICRUSt showed that rhizosphere bacteria contained fructose and sucrose metabolism and with plant growth-promoting traits. Additionally, the scion cultivar significantly affected the predicted metabolism of the rhizosphere-associated bacterial communities. Our results showed that apple scion varieties could regulate the composition and structure of rhizosphere bacterial community in different scion / rootstock combinations, which may be achieved by controlling soluble sugar content, especially sucrose in roots.

Introduction

Plant root can release more than 20% of their photosynthetic products into the soil, which profoundly modified the rhizosphere soil environment [1]. The root exudates have different forms, including low molecular weight compounds, such as soluble sugars, organic acids, and secondary metabolites, such as polyphenols [2, 3], which can provide the energy and nutrients for microbes and selectively recruit microbiome to colonize the rhizosphere [4]. The rhizosphere microbes have been recognized as the second genome of plants, which together with their host plants form the symbiont [5]. Interactions between plants and their associated microbial communities are not unidirectional [6]. Microbiomes housed in the rhizosphere play a crucial role in promoting key functions such as pathogen defense, metabolism of plants [7]. In addition, rhizosphere microbiota modulate plant flowering time by producing phytohormones [8], and improve plant nutrient uptake by biogeochemical transformation of soil nutrients [8, 9]. In complex agroecosystems, the assembly of the rhizosphere microbiome is vulnerable and influenced by diverse abiotic factors, such as soil origins and properties, and agricultural practice [4, 10]. Additionally, plant species and genotype, age, and development stage also play an important role in recruiting the specific rhizosphere microbial community from soil [11-13]. Plant species and genotype have different composition and quantity of root exudates and root morphology, which are associated with differential microbial colonization [14, 15]. Rhizosphere microorganism is a research hotspot, especially field crops and model crops [13, 16, 17]. Much research in recent years has focused on horticultural crops rhizosphere microorganisms, particularly perennial crops [11, 18].

As the perennial crop, apple is most widely cultivated in the world. The total planting area has been estimated at 4.93 million ha, and more than 49% of the global apple production originates from China (http://www.fao.org). In China, 2.38 million ha soil is used for apple cultivation. In the production of commercial crops, including woody perennial crops (e.g., apple, grapevine, and citrus) and annual vegetables (e.g., tomato), grafting is widely used [19]. Grafting is an ancient agricultural practice that connecting the root system of a rootstock and the stem of a specific scion [20]. In the scion/rootstock combinations, moisture, nutrients, hormones and photosynthesis products can be transferred between the rootstock and scion through the xylem and phloem, and then effect the leaf morphology and root system [20]. In the grafted plant, rootstocks have the ability to control fruit yield and quality [21], to provide tolerance to biotic and abiotic stresses [22, 23] as well as to influence scion traits, such as flowering habits and dwarfing plants [20, 24]. Similarly, the characteristics of scion can affect root architecture and development by sugar metabolites, hormones, which is dependent on photosynthetic efficiency [25]. In recent years, high-throughput sequencing technologies have expedited research of microbial taxonomic diversity, especially in grafting combinations [26, 27]. Numerous experiments have established that rootstocks significantly shape the rhizosphere bacterial community [18, 28, 29], but we have little knowledge of how scion participate in the recruitment of microbiome in grafted plants [26].

China is the largest producer of apples in the world, most of the apple scion cultivars grown in China are *Malus × domestica* cultivars “Fuji” (approximately 72.72% of Chinese apple produce area), followed by “Delicious”, “Gala” [30]. Additionally, China, as a primary center of origin for *Malus*, has 17 wild species and 6 domesticated species [31]. The Chinese have utilized native apple species for millennia. Some native species, as rootstock, have graft affinity, nutrition absorption and disease resistance traits [20]. In the grafted plant, different aboveground scion cultivars have different photosynthetic efficiency, which has a direct influence on regulating the root architecture [25]. However, it remains unclear whether different apple scion cultivars assemble and modulate the rhizosphere bacterial composition.

In this study, we used two apple scion varieties (Fuji and Golden Delicious) grafted on seven rootstocks (*Malus baccata*, *Malus hupehensis*, *Malus sieversii*, *Malus micromalus*, *Malus robusta*, *Malus prunifolia* and *Malus xiaojinensis*) to comprehensively evaluate the performances on tree growth, rhizosphere soil characters, and bacterial community in 14 scion-stock combinations. The purpose of this study is to confirm the contribution of apple scion or rootstock to the formation and function of rhizosphere bacterial community.

Materials And Methods

Experimental setup and sample collection

All samples were collected from 14 grafted combination, including two apple scion cultivars, named ‘Golden delicious’ and ‘Fuji’, grafted onto seven rootstocks, respectively. The information of the selected rootstocks was described in the additional file (Table S1). Among the 14 grafted combinations (Fig. S1), four trees of each combination were selected to collect samples. All the selected trees were cultivated in

the same orchard and subjected to the same disease control and soil management practices for fertilization, irrigation. The experimental orchard located in Changping District, Beijing in China (40º15'36" N, 116º13'19"E). The orchard was 8-year-old at moment of sampling.

The roots and rhizosphere samples were collected at 0-20 cm depth using sterile spade. The soil closely adhering to the sampled root were used to represent the rhizosphere. Then soil samples were placed in sterile bags on ice at the time of sampling, and brought to laboratory for further analysis. Each soil sample was sieved (< 2 mm) to remove plant tissues, roots, rocks, etc. and divided into two parts. One part of rhizosphere samples was used to extract DNA. The other was air-dried for soil physicochemical properties analysis. The roots were washed using sterile water and dried in a forced-air oven at 65 °C for 48 h for measuring the non-structural carbohydrates (NSC) concentration. In addition, for each plant, 20 healthy adult leaves were random selected from the annual branches of the in four directions to measure SPAD value and then the leaves were collected for measuring NSC content.

Determination of soil properties and plant traits

Soil organic matter (SOM) was evaluated by titration after mixing potassium dichromate and sulfuric acid [32]. Soil pH was assayed in a 1:2.5 (weight/volume) soil: water suspension with a digital pH meter (FE28, Mettler-Toledo Instruments (Shanghai) Co., Ltd., China). Soil alkali-hydrolysable nitrogen (N) was assayed using the diffusion absorption method. Soil available phosphorus (P) was extracted using 0.5 mol L^{-1} $NaHCO_3$ solution and were measured according to the method descried by Olsen et al. [33]. Soil available potassium (K) was extracted using 1 mol L^{-1} ammonium acetate (CH_3COONH_4) solution and then measured using flame atomic absorption spectrometry

Apple tree height was the distance from ground to the treetop point. Trunk circumference was measured at 15cm above the graft union using a tapeline. The selected leaves SPAD values were measured with a chlorophyll meter (SPAD 502; Minolta Co. Ltd, Osaka, Japan). The leaves were harvested and oven-dried for 30 min at 105°C and dried at 70°C for 24 h and the samples were then ground to a fine powder. The dried roots and leaves were pretreated using microwave oven (Mars, CEM, CA). The NSC concentrations (sucrose, fructose, glucose, and sorbitol) was measured using high performance liquid chromatography (HPLC) with a HewlettPackard 1100pump (PaloAlto, CA, USA) [34], and starch concentration was determined as described by Hoch et al. [35].

DNA extraction, PCR amplification and sequencing of bacterial 16S rRNA gene

Soil total genomic DNA was extracted from 0.5 g fresh soil using the Power Soil® DNA Isolation Kit (MoBio Laboratories Inc. Carlsbad, CA, USA) according to the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed using the NanoDrop ND-1000 UV-vis

spectrophotometer (ThermoScientific, Waltham, MA USA). The extracted DNA samples were stored at -80°C for further experiments.

The V3-V4 region of the bacterial 16S rRNA gene was amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [36]. For each sample, 10-bp barcode sequence was added to the 5’ end of both forward and reverse primers. Each sample was amplified in triplicate in 25 μL reaction system, containing 12.5 μL of 2× Taq PCR MasterMix, 3 μL of BSA (2 ng/μL), 1 μL of each primer (5 μM), 30 ng of template DNA, and ddH_2O filled to a total of 25 μL with the following condition: 94 °C for 5 min, followed by 28 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, with a final extension at 72 °C for 7 min. The PCR products were extracted from the gel using an AxyPrep DNA Gel Extraction Kit (AXYGEN, Union City, CA). Subsequently, purified amplicons were performed using the Illumina MiSeq PE300 platform at Allwegene Company, Beijing, China.

Bioinformatics analysis

The 16S rRNA gene sequences were processed using QIIME (v1.8.0) [37]. Raw forward and reverse reads for each sample were assembled into paired-end reads considering a minimum overlapping of 50 nucleotides and a maximum of one mismatch within the region using the fastq-join algorithm (https://expressionanalysis.github.io/ea-utils/). The paired reads were then quality filtered with a minimum of Q20. Sequences without either primer were discarded. The primer sequences have been removed and the individual sample files were merged in a single fasta file. Chimeras were then identified and filtered using the Usearch tool [38]. The high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the UPARSE [39], and created an OTU table. For each OTU, the representative sequence was picked. Taxonomic classification of the representative sequences for OTUs was performed in QIIME using the SILVA (SSU123) database based on the Ribosomal Database Project (RDP) Classifier tool [40] confidence threshold of 70%. To eliminate sample heterogeneity, the OTU table was normalized by rarefaction to an even sequencing depth. The rarefied OTU table was used to calculate alpha-diversity indexes including phylogenetic diversity, Chao1, and Shannon metrics [41]. Additionally, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was performed to predict the distribution of functional pathway genes of microbial community in the scion-rootstock combinations [42].

Statistical analysis

The figures of plant traits, soil properties, and bacterial alpha-diversity and composition were created using GraphPad Prism v8.0.2 (GraphPad Software, Inc., La Jolla, CA, USA). A two-way ANOVA model was performed for apple tree traits (plant height, trunk circumference, and SPAD value), soil properties (SOM, pH, and nutrient), and sugar in roots and leaves with apple scion cultivar and rootstock genotype as explanatory variables. If scion cultivar was a statistically significant predictor, an independent-samples t-

test was performed. Statistical analysis was performed using SPSS software v20 (IBM Corp., Armonk, NY, USA).

A two-way ANOVA was performed with scion cultivar and rootstock as explanatory variables to analyze rhizosphere bacterial alpha-diversity. The alpha-diversity indexes were logit-transformed prior to ANOVAs. To visualize bacterial community compositional variation between scion cultivars and rootstock genotypes, a principal coordinates analysis (PCoA) based on Bray-Curtis similarity matrices was performed using R v3.6.0. In order to calculate the amount of variation attributed to scion cultivars (2 levels), rootstock genotypes (7 levels), and their interactions, permutational analyses of variance (PERMANOVA) [43] was implemented using adonis function in vegan package of R with 999 permutations. Manhattan plots were used to observed bacterial community shifts by arraying OTUs according to their taxonomy and show their difference in the rhizosphere between 'Fuji' and 'Golden Delicious'. Additionally, Circos graphs for bacterial community compositions, respectively at phylum and genus, were developed using OmicShare tools (http://www.omicshare.com/tools). We also performed two-way ANOVA to analyze how scion cultivar, rootstock genotype, and their interaction influenced the relative abundance of bacteria. Taxon abundances were arcsine-transformed prior to ANOVAs. In addition, Spearman's rank correlation coefficient was performed to investigate the correlation between bacteria and root sugar concentration.

Results

Apple scion cultivar influence plant growth and soil property

Among apple scion-rootstock combinations, the plant height ranged from 3.22 m to 5.26 m, trunk circumference ranged from 23.48 cm to 42.85 cm, and leaf SPAD value ranged from 55.72 to 60.78, but did not vary among rootstock genotypes when grafted 'Fuji' and 'Golden Delicious', respectively (Table S2). On average, apple scion cultivar was the main effect factors of plant growth: plant height (P = 0.002), trunk circumference (P < 0.001), and SPAD value (P < 0.001) were differentially produced by scion cultivars (Table S3). In leaves, all non-structural carbohydrates, except sorbitol, varied between apple scion cultivars: starch (P < 0.001), fructose (P < 0.001), glucose (P < 0.001), and sucrose (P < 0.001) (Table S5). Starch, fructose and glucose concentrations were lower for 'Fuji' compared to 'Golden Delicious', but 'Fuji' had higher sorbitol and sucrose concentrations versus 'Golden Delicious' (Fig.1a). Starch (P < 0.001) and fructose (P < 0.001) differed among rootstock genotypes, especially 'Fuji' as scion (Table S4, 5). Scion/rootstock interactions were significant for starch and sucrose (Table S5). In roots, fructose, sorbitol, and sucrose were differed among rootstock genotypes and between scion cultivars and had significant scion/rootstock interactions (Table S4, S5). All the various parameters expressed greater concentrations in the 'Fuji' versus 'Golden Delicious' (Fig.1b).

A range of soil characteristics were determined for four replicates rhizosphere soil per scion-rootstock combinations and bulk soil samples. As expected, lower levels of pH were detected in rhizosphere soil than that in bulk soil (deduced by 3.12-4.93%), whereas rhizosphere soil had higher levels of soil organic

matter, AN, AP and AK compared to bulk soil, increased by 81-131.9%, 29.92-68.20%, 19.30-66.83% and 73.01-148.05%, respectively (Fig. S2). Furthermore, all soil traits, except AN, were significantly influenced by scion cultivars (Table S6), of which, rhizosphere soil pH of ‘Fuji’ was significantly lower compared to ‘Golden Delicious’ (Fig.2a), contrary, ‘Fuji’ rhizosphere soil nutrition was higher as compared to ‘Golden Delicious’, especially AN (Fig.2). Additionally, all soil properties, except AK, were varied among rootstock genotypes and their interactions (Table S6).

Diversity of rhizosphere bacterial community are modified by the apple scion cultivar

To investigate the microbial composition and diversity of apple grafted combinations rhizosphere, the V3-V4 regions of the bacterial 16S rRNA gene were sequenced on the Illumina MiSeq PE300 platform. In total, we obtained 3 758 432 high-quality sequence from 56 samples (average, 67 114, range, 26 334 to 149 423 per sample) and 9 311 operational taxonomic units (OTUs) were identified (Table S7).

We found that the rhizosphere bacterial evenness (designed as Shannon index and phylogenetic diversity) and richness (represented by Chao1) did not significant differ among rootstock genotypes when grafted with ‘Fuji’ or ‘Golden Delicious’, But rhizosphere bacterial diversity differed between scion cultivars (Shannon: $P < 0.01$, phylogenetic diversity: $P < 0.001$, and Chao1: $P < 0.01$, Fig. 3). In addition, there was a significant scion/rootstock interaction for Shannon index ($P = 0.038$). All these diversity indices were significantly higher in the ‘Golden Delicious’ rhizosphere soils than those in ‘Fuji’ (Fig. 3).

Principal coordinate analysis (PCoA) of pairwise Bray-Curtis similarity matrices were performed to investigate rhizosphere bacterial beta diversity. The results revealed that rhizosphere bacteria of scion-rootstock combinations formed two distinct clusters, which separated along the second coordinate axis (Fig. 4a). Additionally, we found that scion cultivar explained 21.1% of total variance ($p < 0.001$, PERMANOVA), and a significantly interaction between scion cultivar and rootstock genotype, explained 16.6% of total variance ($P = 0.002$, PERMANOVA), in addition to the rootstock genotype (12.0%, $P = 0.321$, PERMANOVA, Table 1), suggesting that the largest source of variation in the rhizosphere bacteria was the different scion cultivars. Subsequently, we divided all samples into two groups according to scion cultivar and analyzed separately. The results showed that the apple rootstock genotype explained 37.6% in combinations grafted with ‘Fuji’ ($P = 0.013$, PERMANOVA, Fig. 4b) and 32.7% in combinations grafted with ‘Golden Delicious’ ($P = 0.093$, PERMANOVA, Fig. 4c). Pair-wise post hoc test comparison using PERMANOVA on the Bray Curtis similarity matrices, we found that the rhizobacterial community were similar among the rootstocks (grafted with ‘Fuji’), except *M. xiaojinensis* and *M.hupehensis*, and rhizobacterial structure did not differ among rootstock genotypes when grafted with ‘Golden Delicious’ (Fig. S3). We also used weighted UniFrac distances to measure the effect of scion cultivar on the bacterial community. The results observed with Bray-Curtis metrics, rhizosphere bacterial diversity revealed a significant difference between ‘Fuji’ and ‘Golden Delicious’ (Fig. S4).

Rhizosphere bacterial assembly is regulated by grafting different scion cultivars

Next, we examined variation in the rhizosphere bacteria of grafted combinations. According to the classification of OTUs, the apple scion-rootstock combinations rhizosphere harbored 49 bacterial phyla. The most dominated bacterial phyla were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, and Firmicutes (relative abundance added up to 79-88%) in all samples (Fig. S5a). Manhattan plots showed that 1,309 OTUs were dramatically different between two scion cultivars, mostly belonging to a wide range of bacterial phyla, including Proteobacteria, Acidobacteria, and Actinobacteria (Fig. 5a). The relative abundance of Proteobacteria, Acidobacteria, Gemmatimonadetes, and Planctomycetes significantly differed between scion cultivars, but did not show strikingly difference among rootstock genotypes (Fig. 5b, Fig. S5a). Conversely, Saccharibacteria has differently abundance among rootstock genotypes grafting the same scion cultivar (Fig. S5a, Table S8). While Actinobacteria and Verrucomicrobia relative abundance differed between scion cultivars and among rootstock genotypes, and there was a significant interaction between scion cultivar and rootstock genotype (Table S8). To further dissect the difference bacterial composition between scion cultivars, we found that among of the dominant bacterial phyla, Proteobacteria, Actinobacteria, and Firmicutes showed greater relative abundance in ‘Fuji’ than that in ‘Golden Delicious’ (Fig. 5b). On the contrary, the abundance of Acidobacteria were found specifically enhanced in rhizosphere of rootstocks grafted ‘Golden Delicious’ (Fig. 5b).

To acquire the best discriminant performance of taxa across grafted combinations, we classified the relative abundances of bacterial taxa in the genus level, the most abundant bacteria (relative abundance > 1.0%) were *Pseudomonas*, *Bacillus*, *Steroidobacter*, *Pseudarthrobacter*, *Streptomyces*, *RB41*, *Sphingopyxis*, *Rhizobium*, *Acidibacter*, and *Bradyrhizobium* (Fig.S5b). Out of ten dominant bacterial genera, all differed between two scion cultivars, except *Pseudomonas* (Fig. 5c). Out of the dominant bacteria genera, *Bacillus*, *RB41*, and *Rhizobium* only varied between two scion cultivars, *Steroidobacter*, *Streptomyces*, *Sphingopyxis*, and *Bradyrhizobium* differed between scion cultivars and among rootstock genotypes, and there was a significant interaction between scion cultivar and rootstock genotype (Table S9). Additionally, all except RB41 in the rootstock rhizosphere were remarkably increased from grafted ‘Fuji’ to grafted ‘Golden Delicious’, especially *Bacillus*, *Streptomyces*, *Sphingopyxis*, and *Rhizobium* (Fig. 5c). For instance, ‘Fuji’ had a high relative abundance of *Bacillus*, *Streptomyces*, *Sphingopyxis*, and *Rhizobium* (RA 4.2%, 2.85%, 1.90%, and 1.53%, respectively), which were approximately twice as much as that in ‘Golden Delicious’ (RA 1.98%, 1.21%, 0.84%, and 0.89%, respectively) (Fig. 5c). The above results indicated that apple scion cultivars as above the ground parts of scion-rootstock combinations had a significant regulatory impact on the rhizosphere bacterial diversity and taxonomic composition (Table 1).

Table 1 PERMANOVA on the Bray-Curtis similarity matrices estimated the components of factor affect rhizosphere bacterial beta diversity in apple scion-rootstock combinations.

Factor	Sum Sq	R^2	F	Pr (>F)
Scion cultivar	0.50841	0.2106	17.5876	< 0.001
Rootstock genotype	0.2907	0.12	1.1181	0.321
Interaction	0.40085	0.166	2.3111	0.002
Residual	1.21411	0.503		
Total	2.41407	1		

Spearman correlations between the root sugar and rhizosphere bacterial communities

Spearman's rank correlation analysis was conducted to assess the effect of root sugars in shaping the rhizosphere bacterial assembly (Fig. 6). As showed in the heatmap, fructose in root was positively correlated with Actinobacteria and negatively correlated with Acidobacteria, Elusimicrobia, and Gemmatimonadetes. Root sucrose showed the greatest positive correlation with the abundance of Actinobacteria, Saccharibacteria, and Cyanbacteria, and was negatively correlated with the abundance of Acidobacteria, Gemmatimonadetes, Nitrospirae, Elusimicrobia, and Latescibacteria. Sorbitol showed the positive correlation with the abundance of Actinobacteria. Moreover, Saccharibacteria and Nitrospirae presented, respectively, the positive and negative correlation with starch in root (Fig. 6a). At bacterial genus level, all except *Preudarthrobacter* showed the positive correlation with sugars in root, being especially positively correlated with sucrose (Fig. 6b). The abundance of Streptomyces, Sphingopyxis, and Sphingomonas were positively correlated with root fructose and sorbitol. Moreover, *Rhizobium* and *Acidibacter* were significantly positively correlated with Fructose and starch, respectively (Fig. 6b).

Apple scion cultivars drive the rhizosphere bacterial functional metabolism

Using PICRUSt as a predictive exploratory tool to establish if the expression of rhizosphere bacterial functions were correlated with the apple scion cultivars. We found that 41 level 2 KEGG Orthology groups (KOs) were represented in the rhizosphere data set (Fig. S6), and 22 KEGG pathways were significantly different abundance between ‘Fuji’ and ‘Golden Delicious’ (Fig. 7a). Nine KEGG pathways were significantly greater in rhizosphere of ‘Fuji’; by contrast, ‘Golden Delicious’ rhizosphere exhibited enrichment for 13 KEGG pathways (Fig. 7a). Importantly, the pathways of carbohydrate metabolism and energy metabolism represented about 16% of all the results, and ‘Fuji’ exhibited a greater abundance of carbohydrate metabolism and lower abundance of Energy metabolism than those of ‘Golden Delicious’ (Fig. 7a). A sophisticated analysis of the functional groups revealed that the most enriched functional

subfunctions of Carbohydrate metabolism were influenced by apple scion cultivars, which including fructose and mannose metabolism, starch and sucrose metabolism, and galactose metabolism (Fig. 7b). Additionally, bacterial community plant growth-promoting (PGP) traits (such as nitrogen metabolism, sulfur metabolism, and inositol phosphate metabolism) were spread throughout the bacterial communities associated with the apple scion cultivars (Fig. 7b, c).

Discussion

Scion cultivars alter growth traits and sugar content of graft combinations

Rootstocks are known to modify scion resistance and phenotypes, such as abiotic stress tolerance (drought, cold, and salt tolerance), disease resistance, plant height, fruit quality and yield [20], and vice versa. Swarbrick [44] found that scion influenced rootstock traits, especially root development, but less attention has been paid to the fact. In the present study, we performed an investigation in the plant traits of common apple grafted combinations, the result showed that scion strongly affected the plant height, which is a major scion trait in plant families [21]. Our results are in general agreement with previous reports that the scion cultivar has a major effect on shoot development and root parameters in grafted grapevines [45]. Additionally, the non-structural carbohydrate contents (such as sucrose, fructose, and glucose) in leaf and root were significantly higher when ‘Fuji’ grafted on the rootstocks. It is generally accepted that the supply of sugar, hormones, and nucleic acids from the shoot play a significant role in root development [25, 46]. As the only source of sugar and the basis of plant growth, photosynthesis play an important role to control plant development and increase yield [45]. The previous study indicated that apple cultivar ‘Honeycrisp’ and ‘Yanfu 3’ showed significantly difference in photosynthetic capacity [47]. Li et al. [25] found soluble sugar contents were dramatically different in grafted MB relative to WT, especially sucrose and fructose. Leaf age, leaf morphology and chlorophylls content are the crucial physiological indicators of the plant's photosynthetic potential [48, 49]. Here, we also found that the leaf SPAD value of ‘Fuji’ was significantly higher than that of ‘Golden Delicious’, indicating that ‘Fuji’ as a scion have higher light capture and energy conservation capabilities [48]. Several studies have shown that the distribution of carbohydrates from shoot to root can be a trophic role to control root elongation and be a signaling pathway to trigger bud outgrowth [50], which possibly contributed to the maintenance of plant hight [45].

Above-ground of graft combinations affect rhizosphere bacterial communities

The roots are assumed to play a directly role in the establishment and maintenance of the interaction between host plants and soil microorganisms, which mainly though the root structure and exudates [12, 16, 51]. The relationships between roots and microbial communities may not always be causal, Lopez-Angulo et al. [52] found that aboveground plant composition affected rhizosphere microbial composition

and richness. Several studies showed that the plant species and genotypes regulated the particular microbial taxa recruited to the rhizosphere from the soil [6, 12]. Most of these studies have focused on the plant entire [13, 53, 54], we still have limited knowledge of how microbiome structures are determined in/on grafted crop plants, whose above-ground (scion) and below-ground (rootstock) genotypes are different with each other [52]. In the prensent study, we used two scion cultivars grafted onto seven tootstock genotypes, and the grafting combinations cultivated in the same orchard and subjected to the same agricultural management practices. Using grafted apple as experimental materials, our study provides a new perspective on the effects of host above-ground and under-ground genotype on microbial communities.

We performed PCoA based on Bray-Curtis community dissimilarity matrices revealed that the rhizosphere bacterial communities were significantly different between 'Fuji' and 'Golden Delicious', and scion cultivar explained roughly 21% of the compositional variation in the bacterial community ($P < 0.001$, PERMANOVA). However, rootstock genotypes have no significantly influence in the bacterial community ($P = 0.321$, PERMANOVA). Interestingly, a significantly interaction between scion cultivar and rootstock genotype, which is largely ignored, could explain 16.6% of total variance ($P = 0.002$, PERMANOVA). Additionally, apple grafted combinations rhizosphere recruited complex bacterial communities that were largely including Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, and Firmicutes which were also the dominant communities in the rhizosphere of non-grafted apple rootstocks [29]. The relative abundance of the major bacteria strikingly altered between scion cultivars, which suggest that for grafted perennial crops, scion cultivar also has an important effect on the microbiome assembly [26]. The results are inconsistent with several published studies that investigating tomato and grape grafted onto different rootstock genotypes [18, 28]. Our study investigated the rhizosphere microbial community composition across 14 kinds of scion-rootstock combinations, two scion cultivars grafted on seven rootstock genotypes, and 'Fuji' and 'Golden Delicious' have little common in their pedigree [31]. Apple scion via their leaf photosynthetic capacity, root excretion, and leaf litter decomposition to influenced the rhizosphere soil matters, all of that effect the rhizosphere bacterial structure and specialized rhizobiomes colonization [25, 55].

Potential functionalities of rhizosphere bacterial associated with grafted apple plants

The functional potential of grafted apple rhizosphere bacterial community is mainly carbohydrate metabolism, especially fructose, mannose, starch, and sucrose metabolism. It has been reported that carbohydrate utilization systems are of particularly importance to provide carbon and energy to bacteria, which affect cellular processes such as biofilm formation [56]. Additionally, grafted apple rhizosphere bacteria potential functionalities contained nitrogen metabolism, sulfur metabolism, and inositol phosphate metabolism. It is well known that soil microorganisms play key roles in soil nutrient cycling [57, 58], produce auxins, cytokinins and other hormones that directly promote plant growth [59], and provide defense against abiotic stresses [60]. We analyzed the relative abundance of bacterial genera

assembled rhizosphere of grafted plants, which revealed that dominant bacteria comprising *Pseudomonas*, *Bacillus*, *Steroidobacter*, *Pseudarthrobacter*, *Streptomyces, Sphingopyxis, Rhizobium, Acidibacter*, and *Bradyrhizobium*. The relative abundance of the majority bacteria is higher in 'Fuji' than that in 'Golden Delicious', but no significant effect of rootstock genotypes on these bacterial taxa. Previous studies have reported that *Pseudomonas*, *Bacillus*, and *Streptomyces* can help host plant to resist pathogens [61]. *Rhizobium* and *Bradyrhizobium* are well-known symbiotic nitrogen fixers, which can increase the biological nitrogen fixation for host plants [62]. In the present study, we found that bacterial communities were relatively more responsive to root sugar, especially to sucrose and fructose, in rhizosphere combinations-associated microbial communities. For the bacteria, sugar (such as fructose) not only is a carbon source, but also plays a role as a signal molecule stimulating the expression of phosphatase genes to mediate organic phosphorus mineralization processes [63], and improving soil available nutrients for plant capture [64, 65]. In addition, potential functionalities of rhizosphere bacterial associated with grafted apple plants were significant difference between 'Fuji' and 'Golden Delicious', indicating that the aboveground part of the grafted plant play a significant role in the screening and potential functionalities of rhizosphere bacteria.

Conclusion

In the complex grafting combinations system, plant growth traits are modified by scion cultivars, and scion also regulated the sugar concentration in root, affecting the rhizosphere soil matter and nutrient concentration. In addition, the dominant bacterial genus includes *Pseudomonas*, *Bacillus*, *Steroidobacter*, *Pseudarthrobacte, Streptomyces*, *Sphingopyxis*, *Rhizobium*, *Acidibacter*, and *Bradyrhizobium*. The scion cultivars of the grafting combinations strongly influenced the selection and recruitment of bacterial components. Interesting, scion cultivars and root stocks have a minor but significant interaction effect on the rhizosphere bacteria recruitment. We also found that there were significant Spearman correlations between the root sugar and rhizosphere bacterial communities, especially sucrose, which can stimulate the potential functionalities of rhizosphere. Based on the present results, we proposed a hypothetical model of the scion cultivar and rootstock genotype on the rhizosphere bacterial community and functions (Fig. 8). In the future, we will study the mechanism of how the scion to modify the root and to alter the rhizosphere bacterial taxa assembly. Understanding the relationship among scion, rootstock, and bacteria in the rhizosphere will provide useful information for orchard management and production, and provide a research basis for the improvement the potential functionalities and the development of biological fertilizer.

Declarations

Acknowledgement

Not applicable

Authors' contributions

YW and ZHH conceived and designed the study. XFC wrote the manuscript. XFC, XNW, and HL collected all the samples and plant trait data. XFC and XNW performed soil nutrient, non-structural carbohydrates of roots and leaves. XFC, XFX, TW, and XZZ performed the bioinformatics analysis and analyzed the data. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key Research and Development Program of China (2016YFD0201103), the earmarked fund for China Agriculture Research System (CARS-27), and the key Laboratory of Beijing Municipality of Stress Physiology and Molecular Biology for Fruit Trees.

Availability of data and materials

The sequences obtained in this study were deposited in the National Center for Biotechnology Information Sequence Reads Archive (SRA) under the BioProject number PRJNA540414.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflict of Interest

There are not conflict of interest declared.

References

1. Paul GD, Anthony JM, Penny RH. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol. 2010;72:313-27. https://doi.org/10.1111/j.1574-6941.2010.00860.x.
2. Huang XF, Chaparro JM, Reardon KF, Zhang R, Shen Q, Vivanco JM. Rhizosphere interactions: root exudates, microbes, and microbial communities. Botany. 2014;92:267-75. http://doi.org/10.1139/cjb-2013-0225.

3. Kuzyakov Y, Domanski G. Carbon input by plants into the soil. J Plant Nutr Soil Sci. 2000;163:421-31. https://doi.org/10.1002/1522-2624(200008)163:4<421::AID-JPLN421>3.0.CO;2-R.
4. Sasse J, Martinoia E, Northen T. Feed your friends: do plant exudates shape the root microbiome? Trends Plant Sci. 2018;23:25-41. http://doi.org/10.1016/j.tplants.2017.09.003.
5. Rosenberg E, Zilber-Rosenberg I. Microbes drive evolution of animals and plants: the hologenome concept. Mbio. 2016;7. http://doi.org/10.1128/mBio.01395-15.
6. Veach AM, Morris R, Yip DZ, Yang ZK, Engle NL, Cregger MA, et al. Rhizosphere microbiomes diverge among *Populus trichocarpa* plant-host genotypes and chemotypes, but it depends on soil origin. Microbiome. 2019;7:76. http://doi.org/10.1186/s40168-019-0668-8.
7. Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, et al. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. Nat Commun. 2018;9:2738. http://doi.org/10.1038/s41467-018-05122-7.
8. Lu T, Ke M, Lavoie M, Jin Y, Fan X, Zhang Z, et al. Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome. 2018;6:231. http://doi.org/10.1186/s40168-018-0615-0.
9. Wei X, Hu Y, Razavi BS, Zhou J, Shen J, Nannipieri P, et al. Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. Soil Biol Biochem. 2019;131:62-70. http://doi.org/10.1016/j.soilbio.2018.12.025.
10. Zheng W, Gong Q, Zhao Z, Liu J, Zhai B, Wang Z, et al. Changes in the soil bacterial community structure and enzyme activities after intercrop mulch with cover crop for eight years in an orchard. Eur J Soil Biol. 2018;86:34-41. http://doi.org/10.1016/j.ejsobi.2018.01.009.
11. D'Amico F, Candela M, Turroni S, Biagi E, Brigidi P, Bega A, et al. The rootstock regulates microbiome diversity in root and rhizosphere compartments of *Vitis vinifera* cultivar Lambrusco. Front Microbiol. 2018;9:2240. http://doi.org/10.3389/fmicb.2018.02240.
12. Mahoney AK, Yin C, Hulbert SH. Community structure, species variation, and potential functions of rhizosphere-associated bacteria of different winter wheat (*Triticum aestivum*) cultivars. Front Plant Sci. 2017;8:132. http://doi.org/10.3389/fpls.2017.00132.
13. Edwards J, Johnson C, Santos-Medellin C, Lurie E, Podishetty NK, Bhatnagar S, et al. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci USA. 2015;112:E911-E20. http://doi.org/10.1073/pnas.1414592112.
14. Haichar FeZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, et al. Plant host habitat and root exudates shape soil bacterial community structure. ISME J. 2008;2:1221-30. http://doi.org/10.1038/ismej.2008.80.
15. Zhalnina K, Louie KB, Hao Z, Mansoori N, da Rocha UN, Shi S, et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol. 2018;3:470-80. http://doi.org/10.1038/s41564-018-0129-3.
16. Peiffera JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, et al. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc Natl Acad Sci USA. 2013;110:6548-53. https://doi.org/10.1073/pnas.1302837110.

17. Bulgarelli D, Rott M, Schlaeppi K, van Themaat EVL, Ahmadinejad N, Assenza F, et al. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature. 2012;488:91-5. http://doi.org/10.1038/nature11336.
18. Marasco R, Rolli E, Fusi M, Michoud G, Daffonchio D. Grapevine rootstocks shape underground bacterial microbiome and networking but not potential functionality. Microbiome. 2018;6:3. http://doi.org/10.1186/s40168-017-0391-2.
19. Gautier AT, Chambaud C, Brocard L, Ollat N, Gambetta GA, Delrot S, et al. Merging genotypes: graft union formation and scion-rootstock interactions. J Exp Bot. 2019;70:747-55. http://doi.org/10.1093/jxb/ery422.
20. Warschefsky EJ, Klein LL, Frank MH, Chitwood DH, Londo JP, von Wettberg EJB, et al. Rootstocks: diversity, domestication, and impacts on shoot phenotypes. Trends Plant Sci. 2016;21:418-37. http://doi.org/10.1016/j.tplants.2015.11.008.
21. Koepke T, Dhingra A. Rootstock scion somatogenetic interactions in perennial composite plants. Plant Cell Rep. 2013;32:1321-37. http://doi.org/10.1007/s00299-013-1471-9.
22. Goldschmidt EE. Plant grafting: new mechanisms, evolutionary implications. Front Plant Sci. 2014;5. http://doi.org/10.3389/fpls.2014.00727.
23. Han Q, Guo Q, Korpelainen H, Niinemets U, Li C. Rootstock determines the drought resistance of poplar grafting combinations. Tree Physiol. 2019;39:1855-66. http://doi.org/10.1093/treephys/tpz102.
24. Li H, Zhang H, Yu C, Ma L, Wang Y, Zhang X, et al. Possible roles of auxin and zeatin for initiating the dwarfing effect of M9 used as apple rootstock or interstock. Acta Physiol Plant. 2011;34:235-44. http://doi.org/10.1007/s11738-011-0822-9.
25. Li G, Ma J, Tan M, Mao J, An N, Sha G, et al. Transcriptome analysis reveals the effects of sugar metabolism and auxin and cytokinin signaling pathways on root growth and development of grafted apple. BMC Genomics. 2016;17:150. http://doi.org/10.1186/s12864-016-2484-x.
26. Liu J, Abdelfattah A, Norelli J, Burchard E, Schena L, Droby S, et al. Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. Microbiome. 2018;6:18. http://doi.org/10.1186/s40168-018-0403-x.
27. Bai Y, Ren P, Feng P, Yan H, Li W. Shift in rhizospheric and endophytic bacterial communities of tomato caused by salinity and grafting. Sci Total Environt. 2020;734:139388. http://doi.org/10.1016/j.scitotenv.2020.139388.
28. Poudel R, Jumpponen A, Kennelly MM, Rivard CL, Gomez-Montano L, Garrett KA. Rootstocks shape the rhizobiome: rhizosphere and endosphere bacterial communities in the grafted tomato system. Appl Environ Microb. 2019;85. http://doi.org/10.1128/aem.01765-18.
29. Chai X, Wang L, Yang Y, Xie L, Zhang J, Wu T, et al. Apple rootstocks of different nitrogen tolerance affect the rhizosphere bacterial community composition. J Appl Microbiol. 2019;126:595-607. http://doi.org/10.1111/jam.14121.

30. Wang Y, Li W, Xu X, Qiu C, Wu T, Wei Q, et al. Progress of apple rootstock breeding and its use. Hortic Plant J. 2019;5:183-91. http://doi.org/10.1016/j.hpj.2019.06.001.
31. Gao Y, Liu F, Wang K, Wang D, Gong X, Liu L, et al. Genetic diversity of *Malus* cultivars and wild relatives in the Chinese National Repository of Apple Germplasm Resources. Tree Genet Genomes. 2015;11. http://doi.org/10.1007/s11295-015-0913-7.
32. Yeomans JC, Bremner JM. A rapid and precise method for routine determination of organic carbon in soil. Commun Soil Sci Plant. 1988;19:1467-76. http://doi.org/10.1080/00103628809368027.
33. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U S Dept Agric Circ. 1954;939:1-19.
34. Vemmos SN. Carbohydrate changes in flowers, leaves, shoots and spurs of 'Cox's Orange Pippin' apple during flowering and fruit setting periods. J Horti Sci. 1995;70:889-900. http://doi.org/10.1080/14620316.1995.11515364.
35. Hoch G, Richter A, Korner C. Non-structural carbon compounds in temperate forest trees. Plant Cell Environ. 2003;26:1067-81. http://doi.org/10.1046/j.0016-8025.2003.01032.x.
36. Mori H, Maruyama F, Kato H, Toyoda A, Dozono A, Ohtsubo Y, et al. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. DNA Res. 2014;21:217-27. http://doi.org/10.1093/dnares/dst052.
37. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-6. http://doi.org/10.1038/nmeth.f.303.
38. Dupont PY, Eaton CJ, Wargent JJ, Fechtner S, Solomon P, Schmid J, et al. Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. New Phytol. 2015;208:1227-40. http://doi.org/10.1111/nph.13614.
39. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10:996-8. http://doi.org/10.1038/nmeth.2604.
40. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 2009;37:D141-5. http://doi.org/10.1093/nar/gkn879.
41. Schloss PD, Gevers D, Westcott SL. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One. 2011;6:E27310. http://doi.org/10.1371/journal.pone.0027310.
42. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31:814-21. http://doi.org/10.1038/nbt.2676.
43. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001;26:32-46. http://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

44. Swarbrick T. Rootstock and scion relationship. Some effects of scion variety upon the rootstock. J Pomol Hort Sci. 1930;8:210-28. http://doi.org/10.1080/03683621.1930.11513361.
45. Tandonnet JP, Cookson SJ, Vivin P, Ollat N. Scion genotype controls biomass allocation and root development in grafted grapevine. Aust J Grape and Wine R. 2009;16:290-300. http://doi.org/10.1111/j.1755-0238.2009.00090.x.
46. Ljung K, Nemhauser JL, Perata P. New mechanistic links between sugar and hormone signalling networks. Curr Opin Plant Biol. 2015;25:130-7. http://doi.org/10.1016/j.pbi.2015.05.022.
47. Zhu Y, Wu Y, Hu Y, Jia X, Zhao T, Cheng L, et al. Tolerance of two apple rootstocks to short-term salt stress: focus on chlorophyll degradation, photosynthesis, hormone and leaf ultrastructures. Acta Physiol Plant. 2019;41. http://doi.org/10.1007/s11738-019-2877-y.
48. Evans JR. Improving photosynthesis. Plant Physiol. 2013;162:1780-93. http://doi.org/10.1104/pp.113.219006.
49. Boughalleb F, Hajlaoui H. Physiological and anatomical changes induced by drought in two olive cultivars (cv Zalmati and Chemlali). Acta Physiol Plant. 2010;33:53-65. http://doi.org/10.1007/s11738-010-0516-8.
50. Barbier F, Peron T, Lecerf M, Perez-Garcia MD, Barriere Q, Rolcik J, et al. Sucrose is an early modulator of the key hormonal mechanisms controlling bud outgrowth in *Rosa hybrida*. J Exp Bot. 2015;66:2569-82. http://doi.org/10.1093/jxb/erv047.
51. Leisso R, Rudell D, Mazzola M. Targeted metabolic profiling indicates apple rootstock genotype-specific differences in primary and secondary metabolite production and validate quantitative contribution from vegetative growth. Front Plant Sci. 2018;9:1336. http://doi.org/10.3389/fpls.2018.01336.
52. Lopez-Angulo J, de M, la C, Chacon-Labella J, Illuminati A, Matesanz S, et al. The role of root community attributes in predicting soil fungal and bacterial community patterns. New Phytol. 2020. http://doi.org/10.1111/nph.16754.
53. Bulgarelli D, Garrido-Oter R, Munch PC, Weiman A, Droge J, Pan Y, et al. Structure and function of the bacterial root microbiota in wild and domesticated barley. Cell Host Microbe. 2015;17:392-403. http://doi.org/10.1016/j.chom.2015.01.011.
54. Perez-Jaramillo JE, de Hollander M, Ramirez CA, Mendes R, Raaijmakers JM, Carrion VJ. Deciphering rhizosphere microbiome assembly of wild and modern common bean (*Phaseolus vulgaris*) in native and agricultural soils from Colombia. Microbiome. 2019;7:114. http://doi.org/10.1186/s40168-019-0727-1.
55. Wagner MR, Lundberg DS, del Rio TG, Tringe SG, Dangl JL, Mitchell-Olds T. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun. 2016;7. http://doi.org/10.1038/ncomms12151.
56. Barriere C, Veiga-da-Cunha M, Pons N, Guedon E, van Hijum S, Kok J, et al. Fructose utilization in *Lactococcus lactis* as a model for low-GC gram-positive bacteria: Its regulator, signal, and DNA-binding site. J Bacteriol. 2005;187:3752-61. http://doi.org/10.1128/jb.187.11.3752-3761.2005.

57. Mau RL, Liu CM, Aziz M, Schwartz E, Dijkstra P, Marks JC, et al. Linking soil bacterial biodiversity and soil carbon stability. ISME J. 2015;9:1477-80. http://doi.org/10.1038/ismej.2014.205.
58. Richardson AE, Simpson RJ. Soil microorganisms mediating phosphorus availability. Plant Physiol. 2011;156:989-96. http://doi.org/10.1104/pp.111.175448.
59. Gutierrez-Manero FJ, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo FR, Talon M. The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. Physiol Plantarum. 2001;111:206-11. http://doi.org/10.1034/j.1399-3054.2001.1110211.x.
60. Vogel C, Innerebner G, Zingg J, Guder J, Vorholt JA. Forward genetic in planta screen for identification of plant-protective traits of *Sphingomonas* sp strain Fr1 against *Pseudomonas syringae* DC3000. Appl Environ Microb. 2012;78:5529-35. http://doi.org/10.1128/aem.00639-12.
61. Shi W, Li M, Wei G, Tian R, Li C, Wang B, et al. The occurrence of potato common scab correlates with the community composition and function of the geocaulosphere soil microbiome. Microbiome. 2019;7:14. http://doi.org/10.1186/s40168-019-0629-2.
62. McCormick S. Rhizobial strain-dependent restriction of nitrogen fixation in a legume-*Rhizobium* symbiosis. Plant J. 2018;93:3-4. http://doi.org/10.1111/tpj.13791.
63. Zhang L, Feng G, Declerck S. Signal beyond nutrient, fructose, exuded by an arbuscular mycorrhizal fungus triggers phytate mineralization by a phosphate solubilizing bacterium. ISME J. 2018;12:2339-51. http://doi.org/10.1038/s41396-018-0171-4.
64. Weidner S, Koller R, Latz E, Kowalchuk G, Bonkowski M, Scheu S, et al. Bacterial diversity amplifies nutrient-based plant-soil feedbacks. Funct Ecol. 2015;29:1341-9. http://doi.org/10.1111/1365-2435.12445.
65. Richardson AE, Barea J-M, McNeill AM, Prigent-Combaret C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil. 2009;321:305-39. http://doi.org/10.1007/s11104-009-9895-2.

Figures

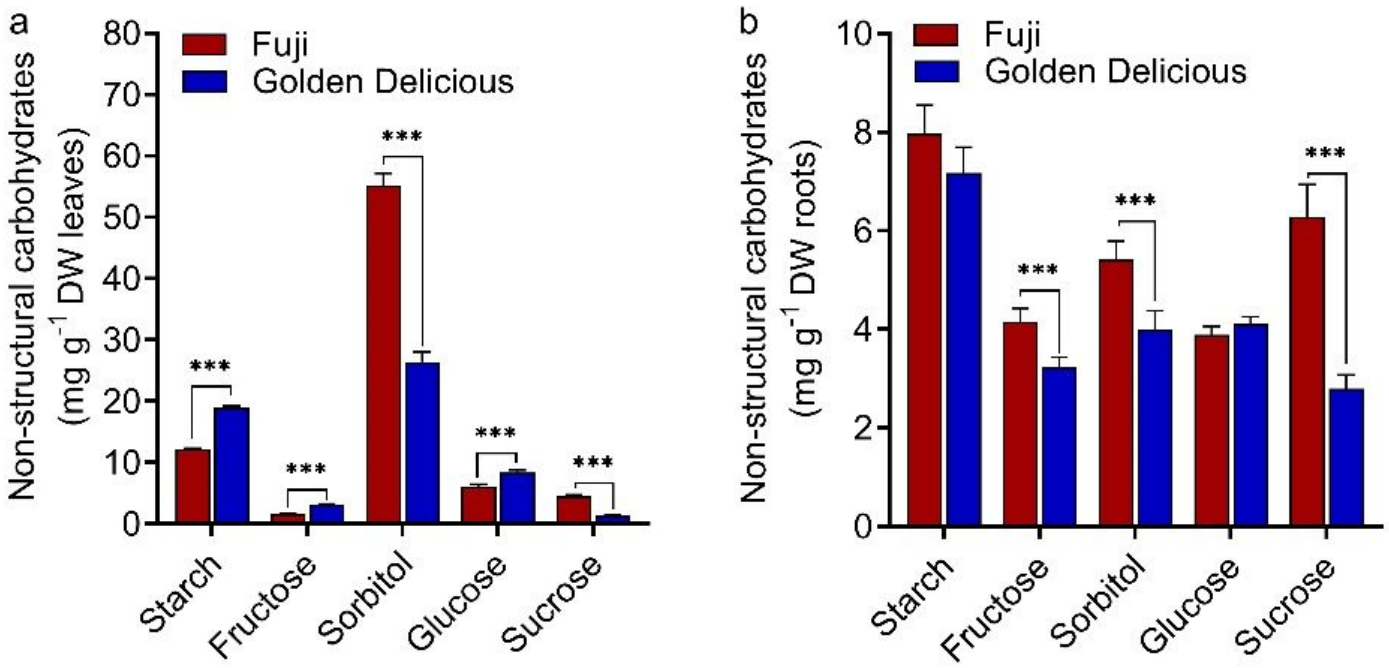

Figure 1

Quantification of non-structural carbohydrates in leaves (a) and root (b) of 'Fuji' and 'Golden Delicious' grafted apples. Values are means ± SE (n=28). Significance levels are as follows * P < 0.05, ** P < 0.01, *** P < 0.001.

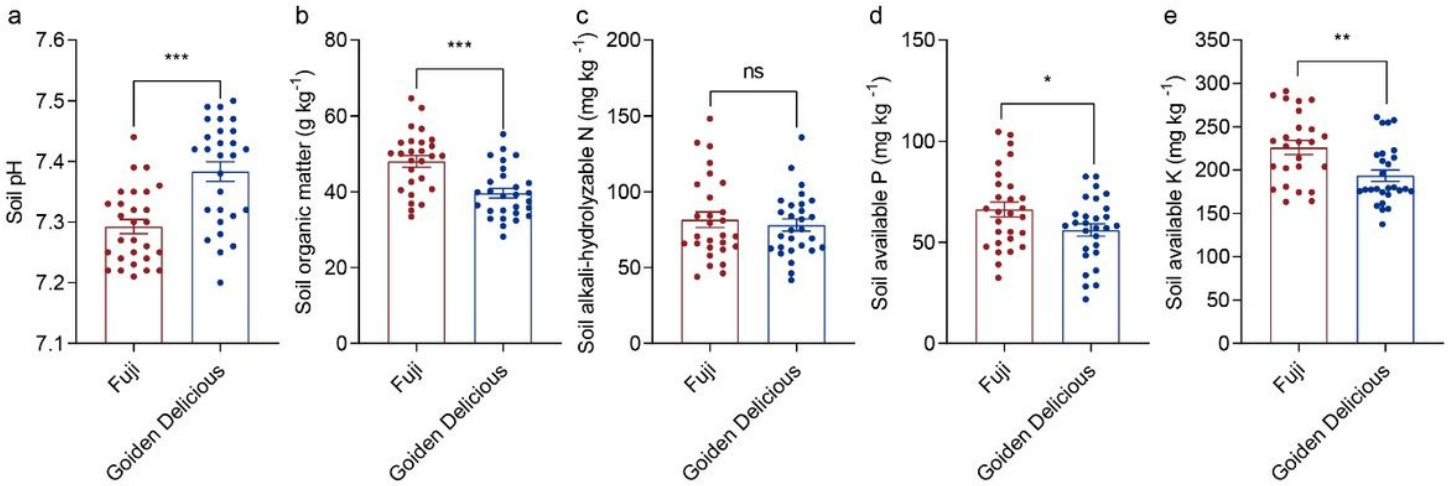

Figure 2

Rhizosphere soil chemical characteristics of different scion-rootstock combinations - pH (a), soil organic matter (b), alkali-hydrolyzable N (c), available P (d) and available K (e). Values are means ± SE (n=28). Significance levels are as follows * P < 0.05, **P < 0.01, *** P < 0.001.

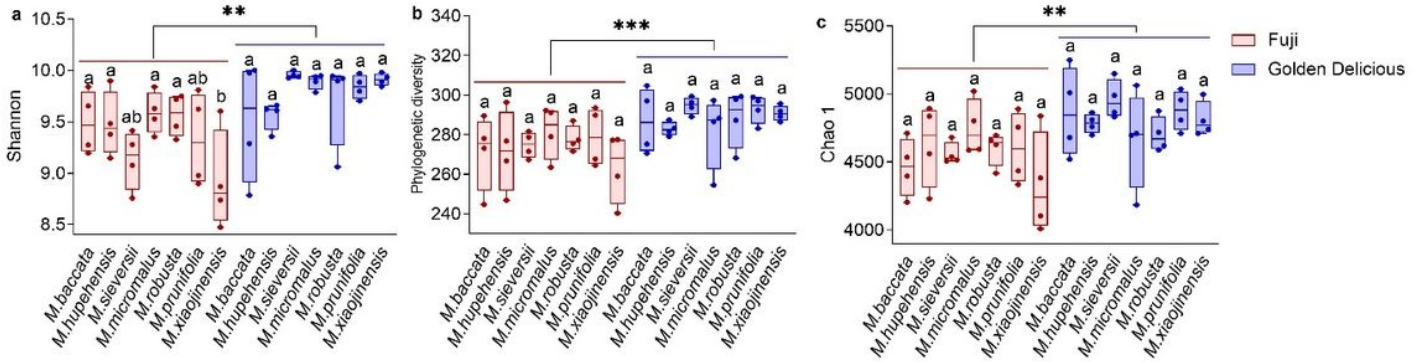

Figure 3

Box plot of the rhizobacterial bacterial alpha-diversity in in rhizosphere soil of grafted combinations. (a) Shannon index, (b) phylogenetic diversity, and (c)Chao1 were calculated among scion cultivars and rootstock genotypes. Different letters indicate significant differences among rootstocks grafted with the same apple scion cultivar. Asterisks denote significant differences between scion cultivars. Significance levels are as follows * P < 0.05, ** P < 0.01, *** P < 0.001.

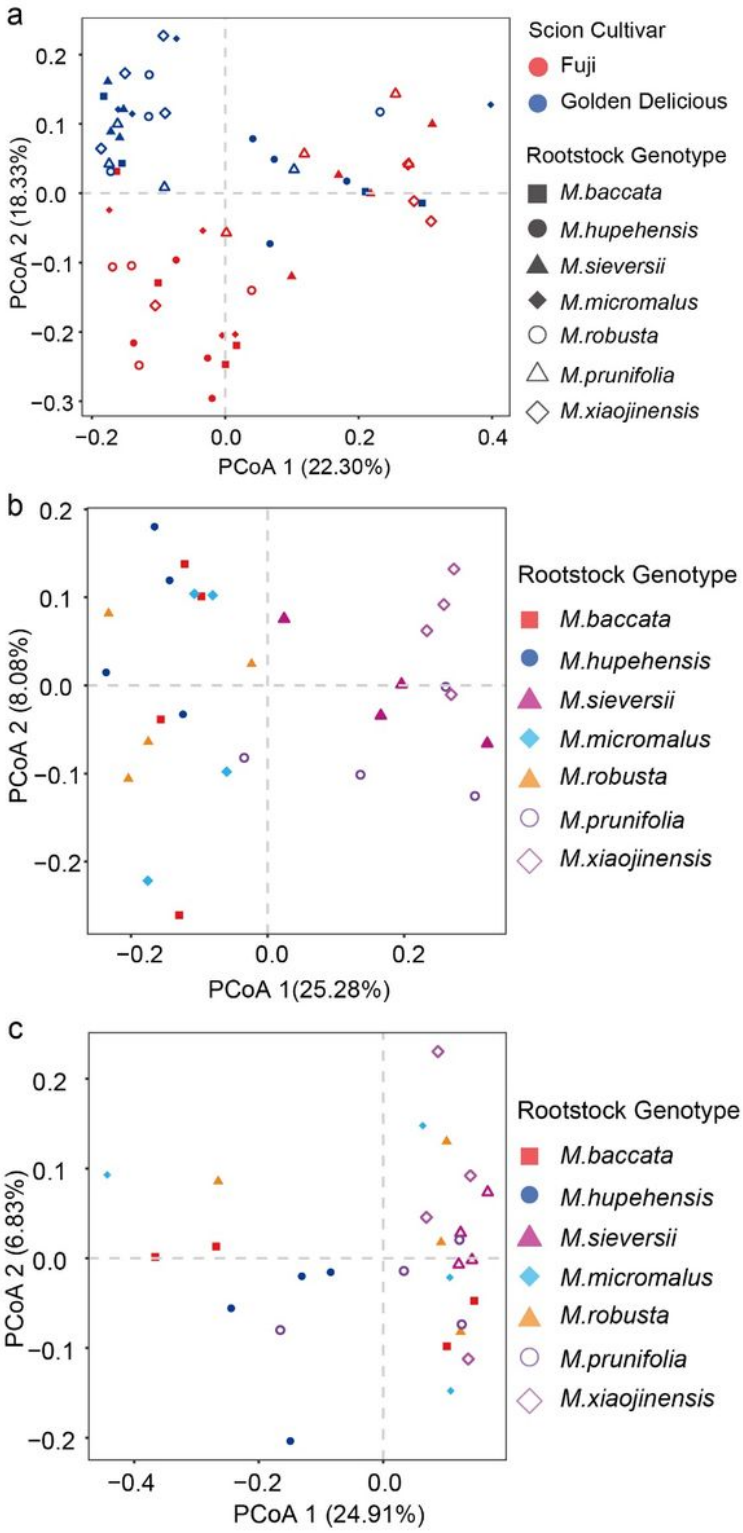

Figure 4

Rhizosphere bacterial community structure of scion-rootstock combinations. Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity. (a) Rhizosphere bacterial community of ‘Fuji’ (red) and ‘Golden delicious’ (blue) grafted on the common rootstocks. (b) PCoA among seven rootstocks only grafted with ‘Fuji’. Rootstock genotype explained 37.62% of the total variability (PERMANOVA, $P < 0.05$).

(c) PCoA among seven rootstocks only grafted with ‘Golden Delicious’. Rootstock genotype explained 32.7% of the total variability (PERMANOVA, P > 0.05).

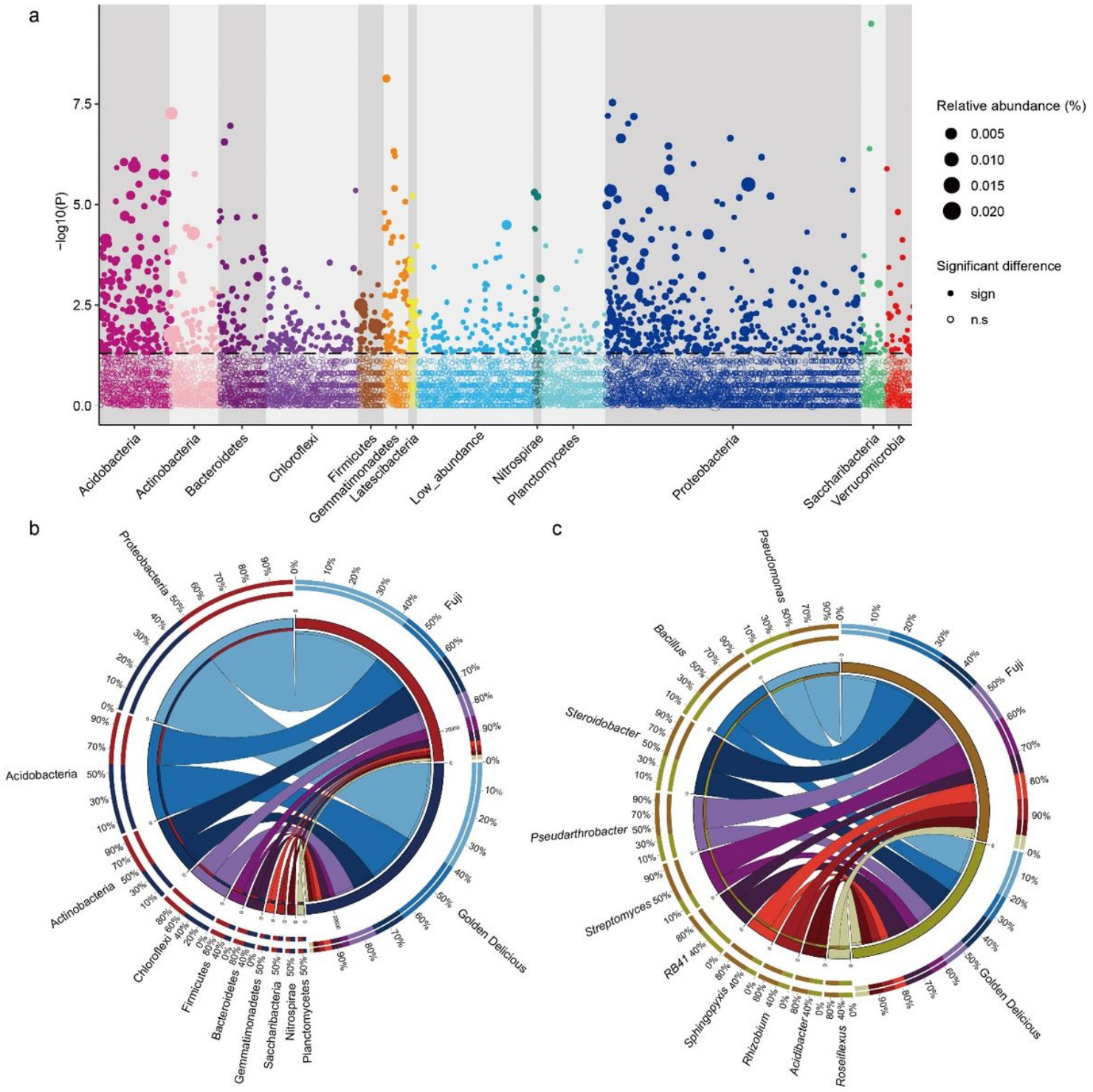

Figure 5

Apple rhizosphere bacterial composition. (a) Manhattan plots showing the OTUs with difference between ‘Fuji’ and ‘Golden Delicious’ rhizosphere soil. OTUs that are significantly different are depicted as full circles. The color of each dot represents the different taxonomic affiliation of the OTUs (phylum level), and the size corresponds to their relative abundance. Distribution of ten most abundant bacterial phyla

(b) and ten most abundant bacterial genus (c) in rhizosphere soil. The length of the bars of each sample on the outer-ring represented the percentage of phyla and genus in each sample. Asterisks denote significant differences in abundance between scion cultivars. * P < 0.05, ** P < 0.01, *** P < 0.001.

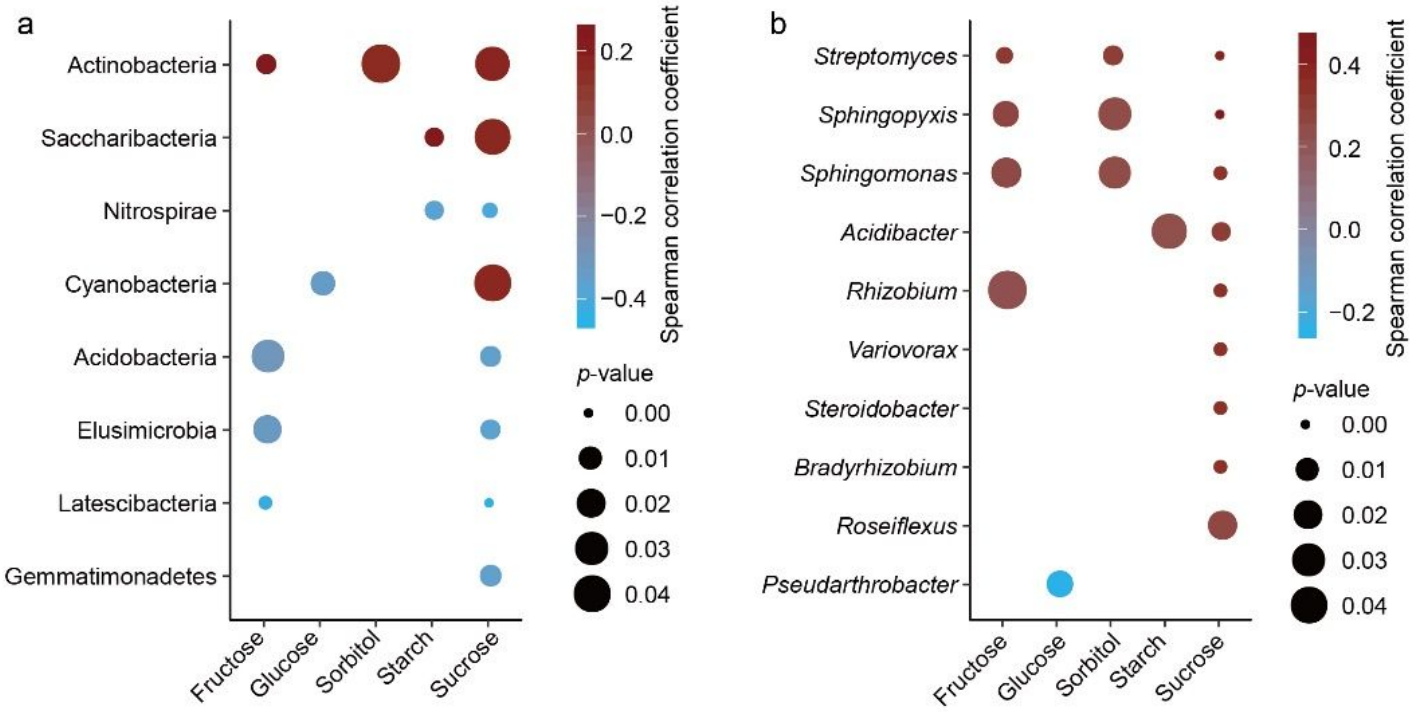

Figure 6

Heatmap of the Spearman's rank correlation coefficients between root sugars and relative abundance of rhizosphere bacteria. Bacterial phyla (relative abundance > 1%) (a) and genera (relative abundance > 0.5%) (b) interacted with root sugars. Only shown the significant correlations (P < 0.05). The color grading indicated the spearman correlation coefficients. Positive correlations were shown in red and negative in blue. The size of the points indicated P-value.

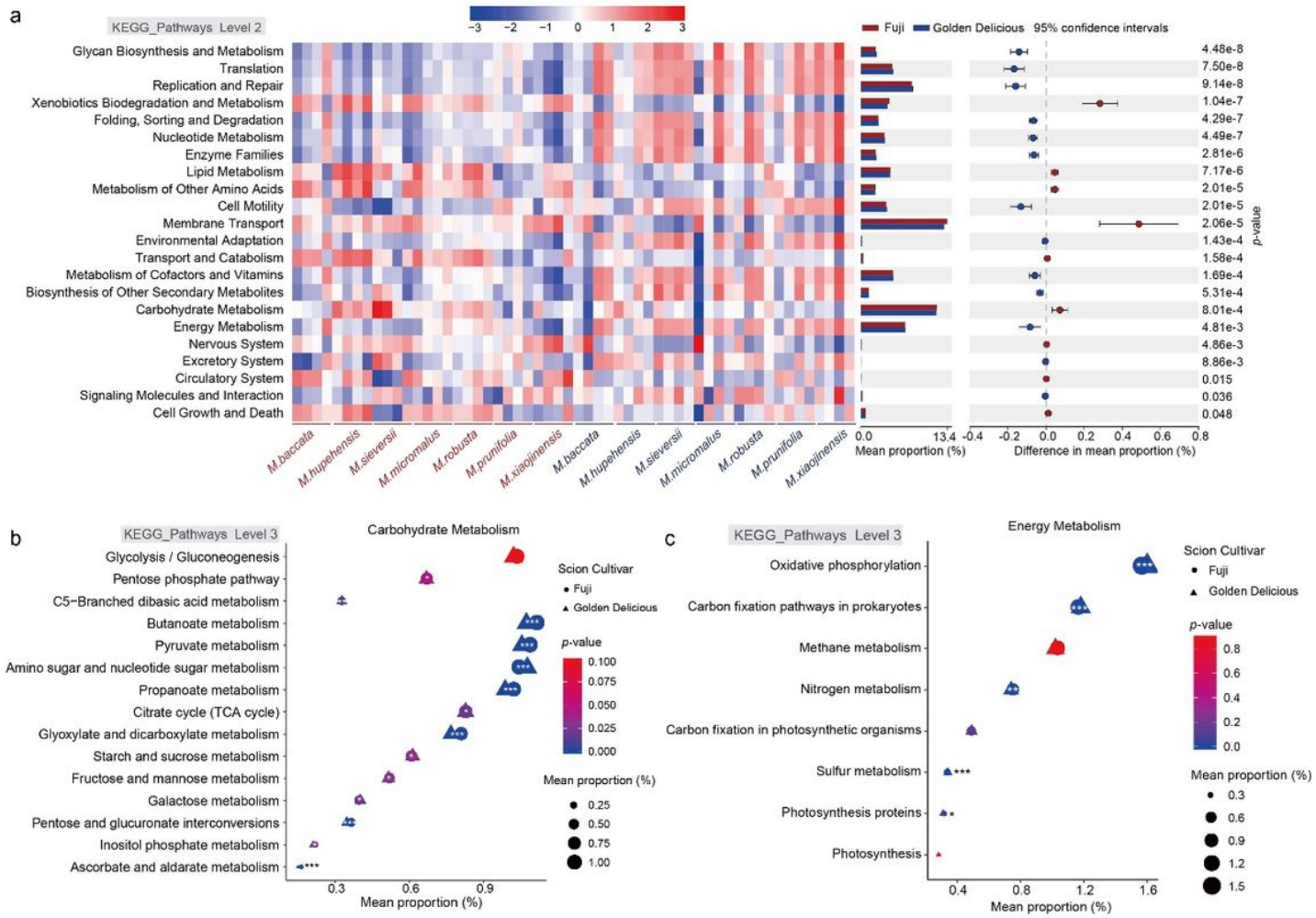

Figure 7

The predicted functional gene of bacterial metabolism related to KEGG pathways. (a) Heat map and bar plot exhibited differential abundance between ‘Fuji’ and ‘Golden Delicious’ rhizosphere soil elated to KEGG pathways at level 2. (b) Bubble chart of carbohydrate metabolism and (c) energy metabolism showed differential abundance between ‘Fuji’ and ‘Golden Delicious’ rhizosphere soil elated to KEGG pathways at level 3.

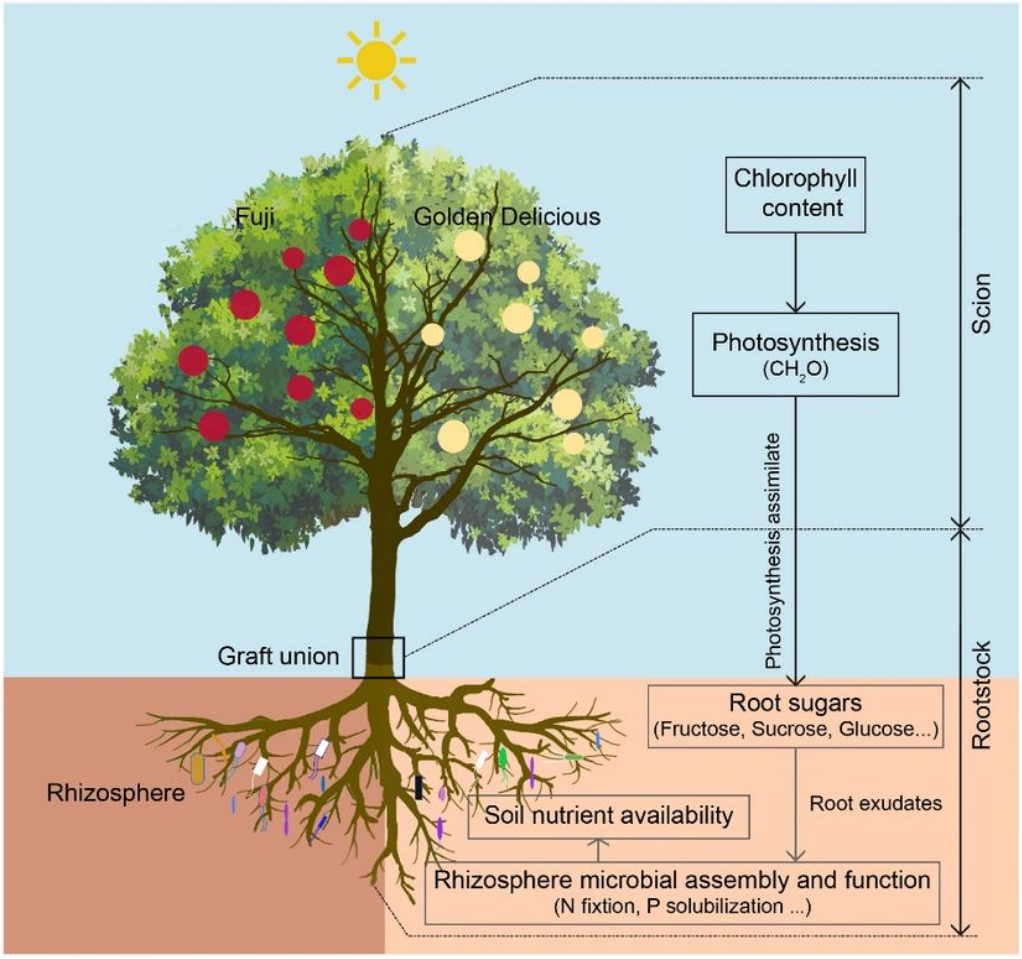

Figure 8

Schematic of regulation of scion to rhizosphere microbiota in different scion/ rootstock combinations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.docx

- Supplementaryinformation.docx
- Supplementaryinformation.docx