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RESEARCH ARTICLE

Bioinformatics Analysis of the Rhizosphere Microbiota of Dangshan Su Pear in Different Soil Types

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Abstract: ***Background*:** The rhizosphere microbiota are of vital importance for plant growth and health in terrestrial ecosystems. There have been extensive studies aiming to identify the microbial communities as well as their relationship with host plants in different soil types.

***Objective*:** In the present study, we have employed the high-throughput sequencing technology to investigate the composition and structure of rhizosphere microbiota prosperous at the root of Dangshan Su pear growing in sandy soil and clay soil.

***Method*:** A high-throughput amplicon sequencing survey of the bacterial 16S rRNA genes and fungal ITS regions from rhizosphere microbiota was firstly performed. Subsequently, several common bacterial and fungal communities were found to be essential to Dangshan Su pear by using a series of bioinformatics and statistics tools. Finally, the soil-preferred microbiota were identified through variance analysis and further characterized in the genus level.

***Result*:** Dangshan Su pears host rich and diverse microbial communities in thin layer of soil adhering to their roots. The composition of dominant microbial phyla is similar across different soil types, but the quantity of each microbial community varies significantly. Specially, the relative abundance of Firmicutes increases from 9.69% to 61.66% as the soil ecosystem changes from clay to sandy, which can be not only conducive to the degradation of complex plant materials, but also responsible for the disinfestation of pathogens.

***Conclusion*:** Our results have a symbolic significance for the potential efforts of rhizosphere microbiota on the soil bioavailability and plant health. Through selecting soil types and altering microbial structures, the improvement of fruit quality of Dangshan Su pear is expected to be achieved.

Keywords: *Pyrus bretschneideri*, dangshan Su pear, soil microbiota, high-throughput sequencing, 16s rRNA, ITS.

1. INTRODUCTION

The rhizosphere iswell known small area of soils and directly sustained by living roots of plant [1]. Meanwhile, a large number of microbes also form complex and dynamic associations with host plants in the rhizosphere [2]. Recently, wide-ranging research has led to an augmented understanding of the plant-soil-microbe interactions [3, 4]. The rhizosphere acts as an important place for the interchange of substances, energy and signaling among plants and microbes. It is well established that the composition and structure of rhizosphere microbiota are mainly supported through several environmental factors, including soil types, geographic location and ambient conditions [5, 6]. Besides, the microbial communities are consistently influenced by the activities of growing plants, such as root secrete metabolites and sloughed-off cells [7, 8]. On the other hand, rhizosphere microbiota could also play critical roles in plant growth and development through mineralizing soil organic matter [9], activating plant defense mechanisms [7], and even producing antibiotics against phytopathogens *via* direct or indirect mechanisms [10, 11]. The plant-soil-microbe feedback loop ensures mutual advantage for both host plants and rhizosphere microbiota during the long-term evolution. Therefore, knowledge about the positive feedback loop will improve the understanding of soil biomass and the exploitation of a rational approach for agricultural initiatives.

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Pyrus bretschneideri 'Dangshan Su' is a white pear variety and originated in Dangshan County that has been awarded as the "Town of Pear" in China [12]. Due to its nutritional and therapeutic values, Dangshan Su pear is in high demand both at home and abroad, which has reversely promoted the cultivation of pear trees as well as the development of agribusiness industry. At present, the Dangshan Su pear orchards cover up to an area of 410,000 hectares, accounting for 48% of total cultivated area of 847,000 hectares in Dangshan County. As the old course of the Yellow River winds through the central and northern parts of Dangshan, this county possesses large alluvial plain areas that are rich in sandy soils and especially suitable for planting white pears. Accumulating studies have shown that Dangshan Su pears planted in sandy soil tend to have a lower content of stone cells and a higher quality of ripen fruits than those in clay soil [12]. Apart from the characterization of soil physical properties, however, no studies related to the plant-soil-microbe feedback loop have been conducted up to now. Therefore, it is important to better understand the structure of soil microbiota of Dangshan Su pear trees.

In the present study, we have employed the high-throughput sequencing survey of the bacterial 16S rRNA genes and fungal ITS regions to investigate the communities of microbiota prosperous at the pear root-soil interface. Combined with the bioinformatics analysis methods, we aim to (1) profile the core bacterial and fungal rhizosphere taxa contributing to the growth of Dangshan Su pear and (2) determine whether soil type could influence the structure of rhizosphere microbiota and provide specific benefits to host trees.

2. MATERIALS AND METHODS

2.1. Experimental Site and Collected Samples

The experimental site for sample collection in the present study was located at Dangshan, Anhui province, People's Republic of China (N34°47', E116°38'), which is a main cultivation region for Chinese white pears (*Pyrus bretschneideri* 'Dangshan Su') and famous as the "Town of Pear" in China. The annual average of temperature and precipitation is approximately 14 °C and 773.6 millimeters, respectively. The pear cultivar, agronomic management and fertilization regime are similar among different orchards in this study. The location of the field nursery is not privately-owned or protected in any way.

In October 2017, ripen pears were collected from 6 separate fruit orchards, including sandy soil (SS_A, SS_B and SS_C) and clay soil (CS_A, CS_B and CS_C) samples in Dangshan County. In each pear orchard, three 20-year-old healthy and robust pear trees were randomly selected. Then, 10 mid-size fruits were picked, refrigerated and transferred to the lab for physiological experiments. Meanwhile, four rhizosphere soil samples around the same tree were collected by a sterile spade close to the trunk at depths of 40 to 60 cm, separately. A total of 12 rhizosphere soil samples were collected per pear orchard. Then, the twelve samples from the same orchard were mixed to one sample, stored in a sterile bag on ice and brought to the lab for further processing.

After removing the loosely adhering soil by hand-shaking, the sampled root with rhizosphere soil particles attached was placed in flasks containing 100 mL of sterile 0.9 % NaCl solution. The flask was shaken for 30 min to detach the soil particles and then centrifuged at 8000 rpm for 10 min. The supernatant was discarded and the remaining soil fraction was used for DNA extraction.

2.2. Measurement of Stone Cell Content

The content of stone cells in pear fruits was measured according to a previously described method [13]. Approximately 5 g pulp per fruit was collected and stored at -20 °C for 24 h. Subsequently, the frozen pulp was homogenized at 20,000 rpm for 3 min and then incubated in water to decant the upper suspension. For better performance, these procedures were repeated several times. Finally, the collected stone cells were oven-dried at 60 °C and then weighed with three replications. The stone cell content was calculated as follows:

$$\text{Stone cell content (\%)} = \frac{\text{DW}}{\text{FW}} \times 100$$

where DW was defined as the weight of stone cells and FW was the total weight of pulp per pear fruit.

2.3. Library Preparation and Amplicon Sequencing

Total genomic DNAs were extracted from each soil sample with the E.N.Z.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, United States) by following the manufacturer's instructions. The quality and quantity of DNA were evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Cleveland, OH, United States). Then, the extracted DNAs were eluted in 50 μL of Elution buffer and used as templates for PCR amplification. For bacterial-diversity analysis, the V3-V4 variable regions of the 16S rRNA genes were amplified using the slightly modified versions of primers 338F (5'-ACTCCTACGGGA GGCAGCAG-3') and 806R (5'-GGACTACHVGGGT WTCTAAT-3'). For fungal-diversity analysis, the ITS2 variable regions of small-subunit rRNA genes were also amplified using the primers fITS7 (5'-GTGARTCAT CGAATCTTTG-3') and ITS4 (5'-TCCTCCGCTTATTGA TATGC-3') with slight modification. The PCR conditions consisted of an initial denaturation at 98 °C for 30 seconds; 35 cycles of denaturation at 98 °C for 10 seconds, annealing at 52 °C for 30 seconds, and extension at 72 °C for 45 seconds; and then final extension at 72 °C for 10 minutes. The expected size of the 16S and ITS2 amplified fragments generated by each pair of primers was 469 and 353 bp, respectively. Additionally, these 5' ends of the primers were tagged with specific barcodes in each sample.

After amplicon reaction, the PCR products were confirmed with 2% agarose gel electrophoresis and further purified by AMPure XP beads (Beckman Coulter Genomics, Danvers, MA, United States). The obtained amplicon was quantified using the Qubit dsDNA Assay Kit (Invitrogen, Waltham, MA, United States). Equal amounts of the purified amplicon were pooled for the construction of high-throughput sequencing libraries. Finally, all the high-throughput amplicon sequencing assay was performed by

using the Illumina MiSeq 300PE platform and one library was sequenced with the standard Illumina sequencing primers (Illumina, San Diego, CA, United States).

2.4. Data Statistics and Analysis Tools

After getting the raw data, paired-end reads were firstly assigned to different samples based on their unique barcodes. Reads without either barcode sequences were discarded. The remaining reads were merged using the FLASH tool with default parameters [14]. Then, clean reads were obtained with successive usages of the CLC genomics workbench (version 8; https://www.qiagenbioinformatics.com/), including trimming the barcodes and primer sequences, removing shorter reads (a minimum size of 300 and 200 bp for 16S and ITS2 amplified fragments, respectively), filtering low quality reads with a quality value (Q) less than 20, and cleaning up contaminated reads through alignments (*E*-value ≤ *e*-05 and identity ≥ 80%) against the Dangshan Su pear genome (http://peargenome.njau.edu.cn/). Chimeric sequences were identified and filtered to obtain the high-quality clean reads by using the VSEARCH software (version 2.3.4) [15]. Finally, the UCLUST algorithm provided by the software package QIIME 1.9.1 [16] was used to cluster these high-quality clean reads through alignment with the Green genes Database (version 13.8) [17] for 16S rRNA sequences and the UNITE dynamic database [18] for ITS sequences. Sequences with ≥ 97% similarity were typically assigned to a special Operational Taxonomic Unit (OTU) and the most abundant sequences were selected as a representative for each OTU [19]. By aligning against the RDP database [20], all these representative sequences were annotated with taxonomic ranks including phylum, class, order, family, genus and species information.

Furthermore, the abundant information of OTUs across different soil samples was normalized to an even sequencing depth to eliminate sample heterogeneity. Then, the rarefied OTU data was used to calculate alpha and beta diversity indices. For alpha diversity, the richness of each sample was evaluated by the Chao1 index and the diversity within each sample was estimated using the Shannon metrics. For beta diversity, PCoA diagrams were created using the custom R scripts (scatterplot3d package) and employed to compare bacterial and fungal community structures among samples. Additionally, histograms and heatmap diagrams were also plotted by using the R scripts with the packages of ggplot2 and RcolorBrewer, respectively.

3. RESULTS

3.1. Comparison of Stone Cell Content

To assess the quality of pear fruits, their stone cell content was individually measured. The stone cell content of different pear fruits collected from different soil samples ranges from 1.34‰ to 1.72‰ on average. The SS_A sample had the lowest content of stone cells, while the CS_B involved the highest (Fig. **1**). The content distribution of stone cells exhibited an order of samples CS_B > CS_C > CS_A > SS_B > SS_C > SS_A. Globally, the stone cell content of pear fruits was significantly lower in sandy soil samples than in clay soil samples (Student's *t* test, $P < 0.05$).

3.2. High-throughput Amplicon Sequencing

By using the high-throughput sequencing assay, up to 47.24 and 164.16 Mb raw data were generated from the bacterial 16S rRNA and fungal ITS amplicon (Supplementary Table **S1** and **S2**). After deleting chimeric sequences and filtering low-quality reads, a total of 40.05 and 81.31 Mb clean data were obtained, respectively. Among them, there are 80,422 bacterial 16S rRNA and 296,276 fungal ITS high-quality reads in total (Table **1**). With a threshold of 97% sequence similarity, these high-quality reads were assigned to 5,263 bacterial and 837 fungal

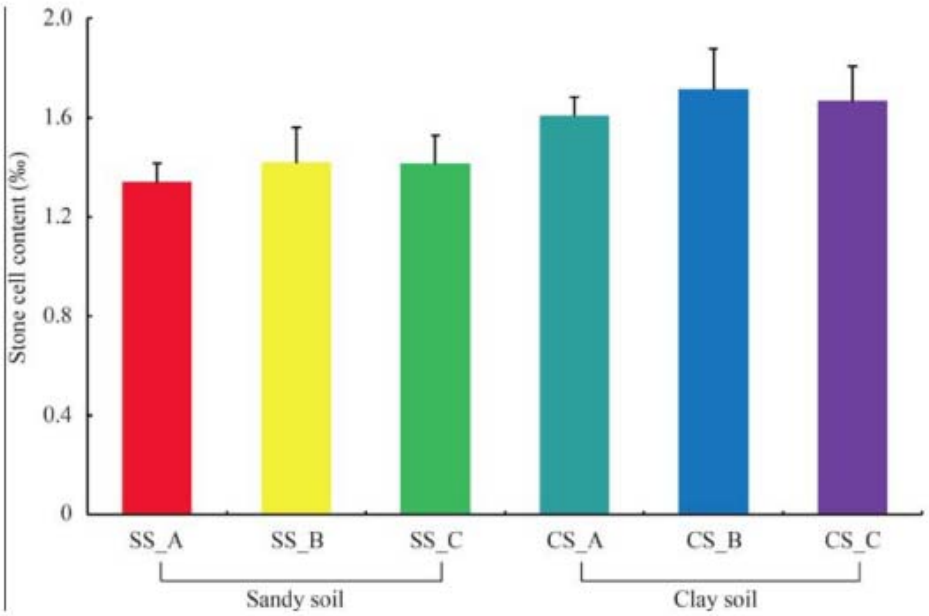

Fig. (1). Content of stone cells in riped pears collected from different orchards, including sandy soil (SS_A, SS_B and SS_C) and clay soil (CS_A, CS_B and CS_C) samples. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Statistics of sequence quality in each soil sample.

Sample	SS_A	SS_B	SS_C	CS_A	CS_B	CS_C
Bacteria						
Raw tags*	16543	18378	13655	16453	15861	18786
Clean tags	16276	18122	13436	16025	15498	18417
Valid tags	13818	14991	11225	12982	12634	14772
singletons	416	215	297	961	827	904
doubletons	161	145	134	604	560	572
Fungi						
Raw tags	33487	43572	56624	47378	84296	80969
Clean tags	32724	43452	47604	46537	83592	46082
Valid tags	32356	42975	46941	46099	82699	45206
singletons	10	11	13	18	10	14
doubletons	10	11	9	9	5	10

* The occurrence of each detect OTU was counted as tags.

Table 2. Microbiota composition and diversity in each soil sample.

Sample	Bacteria			Fungi		
	No. of OTUs	Chao1	Shannon	No. of OTUs	Chao1	Shannon
SS_A	1,014	1457.60	7.69	156	157.11	4.71
SS_B	798	953.09	7.23	104	103.62	1.37
SS_C	816	1141.60	7.50	214	209.60	4.41
CS_A	2,776	3472.51	10.23	328	321.92	6.01
CS_B	2,467	3074.04	9.84	182	175.00	5.37
CS_C	2,699	3282.01	9.80	331	345.20	5.64

OTUs, respectively (Supplementary Table **S3** and **S4**). The number of these identified OTUs could reflect the microbiota community in the rhizosphere of all sampled pear trees. Length distribution analysis of these OTU sequences showed that the predominant sizes were 422 nt in bacteria and 248 nt in fungi. Meanwhile, the length distribution in bacterial 16S rRNA OTUs is more concentrated than that in fungal ITS OTUs. Specially, the raw sequencing data in this study have been deposited at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) database under accession number PRJNA511373 (http://www.ncbi.nlm.nih.gov/bioproject/PRJNA511373).

3.3. Microbiota Diversity and Classification

The analysis of OTU data revealed microbiota composition and diversity in each soil sample (Table **2**). For bacteria, the number of OTUs ranged from 798 to 2,776, with SS_B sample exhibiting the lowest number of OTUs and CS_A sample having the highest. For fungi, the number of different OTUs ranged from 104 to 331, with SS_B and CS_C samples presenting the lowest and highest number of OTUs, respectively. Based on the Chao1 metrics, clay soil samples showed higher richness of both bacteria and fungi than sandy soil samples globally (Fig. **2A**). In addition, the Shannon index also graphically presented higher alpha diversity of both bacterial and fungal OTUs that occurred in clay soil than sandy soil samples (Fig. **2B**).

Using the default settings in QIIME [16], all the OTUs were classified with a given taxonomic rank spreading from the phylum to the genus level. Although the overall microbial phyla involved in different samples were similar, the proportion of each phylum was significantly fluctuated (Fig. **3**). At the phylum level, the bacterial OTUs in sandy soil samples were mainly assigned to Firmicutes (61.66%), Bacteroidetes (29.18%), Proteobacteria (5.66%), Candidatus Saccharibacteria (0.64%), and Acidobacteria (0.58%) (Fig. **3A**). In comparison, the top number of bacterial OTUs in clay soil samples was attributed to Proteobacteria (28.65%),

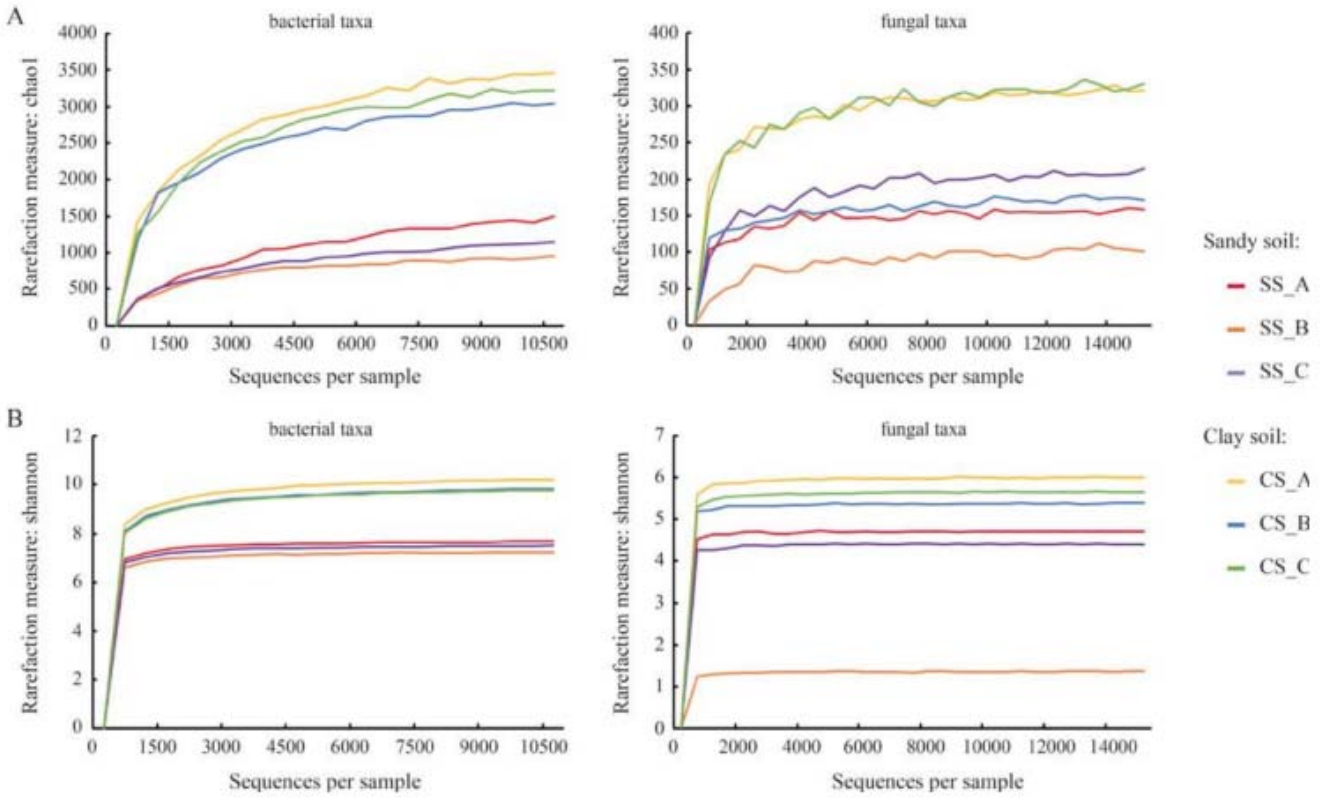

Fig. (2). Alpha diversity analysis of the bacterial and fungal taxa among different soil samples. Rarefaction curves of (A) Chao1 metrics and (B) Shannon index. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

followed by Acidobacteria (25.63%), Actinobacteria (10.28%), Firmicutes (9.69%), and Gemmatimonadetes (7.43%). On the other hand, the dominant phyla of fungi detected across all samples were shown in Fig. (**3B**). In sandy soil samples, the relative abundance of OTUs was most assigned to Ascomycota (88.09%), followed by Basidiomycota (4.04%), Zygomycota (1.97%), Glomeromycota (0.69%), and Fungi_OTU (0.57%). In clay soil samples, the most dominant phylum was also Ascomycota (74.47%). The next most dominant phyla were Zygomycota (10.34%), Basidiomycota (7.52%), Glomeromycota (1.36%), and Chytridiomycota (0.95%).

3.4. Comparison of Microbiota Composition

Microbiota structure among sandy soil and clay soil samples exhibited intuitively distinct characteristics according to the above analysis of alpha diversity. Using the Mann-Whitney U test ($P < 0.001$), the comparison between sandy soil and clay soil samples further declared a statistically significant difference in both bacterial and fungal composition (Fig. **4**). At the genus level, we performed weighted Unifrac principal coordinate analysis (PCoA) to study the relationship among these soil samples. Based on the relative values of the principal components (PCs), we have chosen the first three PCs (PC1, PC2 and PC3) to represent the microbial community in three dimensions (Fig. **5**). For bacteria, the genera in sandy soil samples were grouped on the 3-dimensional plot, indicating that their composition and structure were extremely similar. Graphically, clay soil samples were separated from sandy soil samples, suggesting a clear distinction between these two soil types. We further tested these three PCs of bacterial genera and found a statistically significant difference between sandy soils and clay soils (Mann-Whitney U test, $P < 0.05$). However, the three clay soil samples exhibited relatively scatter plots, seemingly showing a great variation in the data (Fig. **5A**). For fungi, the genera in clay soil also exhibited more dispersed distribution on the 3-dimensional plot (Fig. **5C**). Additionally, there is no statistically significant difference for fungal genera between these two soil types (Mann-Whitney U test, $P > 0.05$).

To distinguish unique and overlapped OTUs among sandy soil and clay soil samples, we have generated Venn diagrams with the abundant microbial community after removing singletons and doubletons from the original data (Supplementary Table **S5** and **S6**). As shown in Fig. (**6A**), a majority of OTUs were solely detected either in sandy soil or clay soil samples, whereas only 499 bacterial and 188 fungal OTUs were shared by these two soil types. Except for the unique OTUs, differentially abundant OTUs were also identified among overlapped OTUs and then mapped to the phylum level.

For bacteria, the differentially abundant OTUs were distributed in 20 phyla (Fig. **6B**). Among them, only NC10 and Spirochaetes were explicitly present in sandy soil, a range of species, including Actinobacteria, Armatimonadetes, Candidatus Saccharibacteria, Chloroflexi, Deinococcus-Thermus, Gemmatimonadetes, GN04,

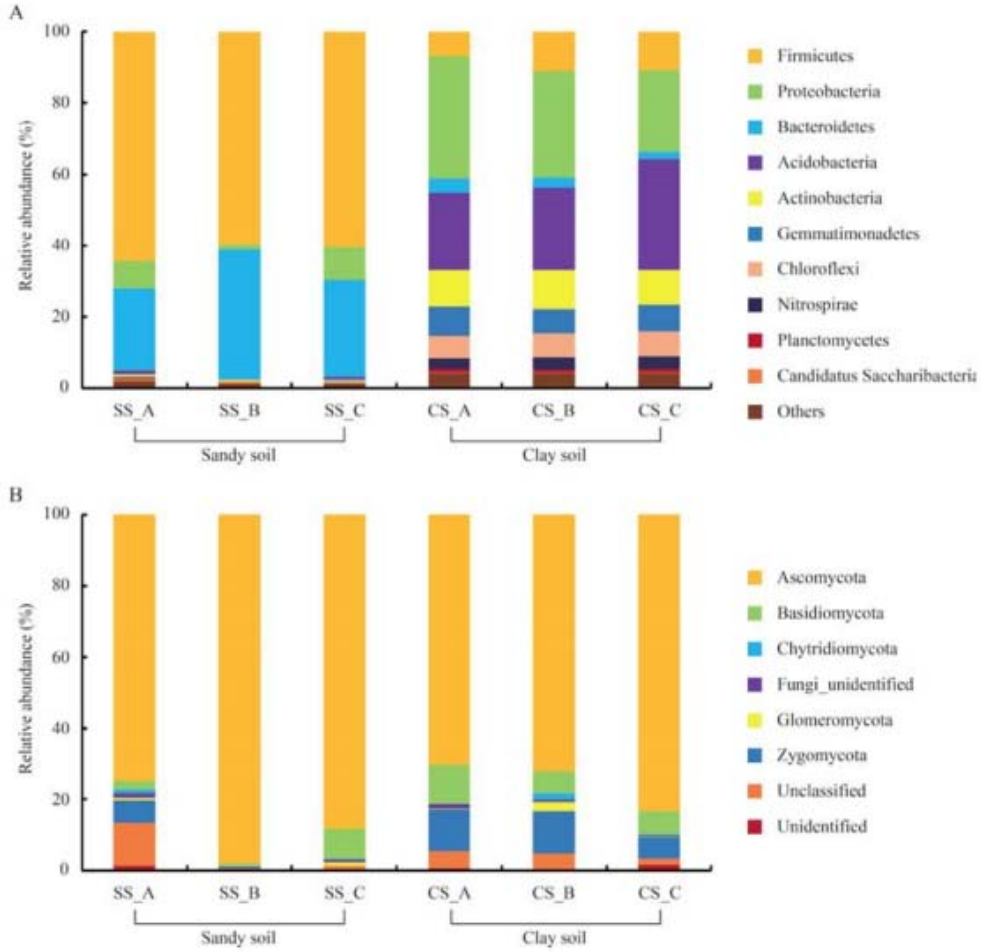

Fig. (3). Relative abundance of the dominant (A) bacterial and (B) fungal taxa in different soil samples at the phylum level. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

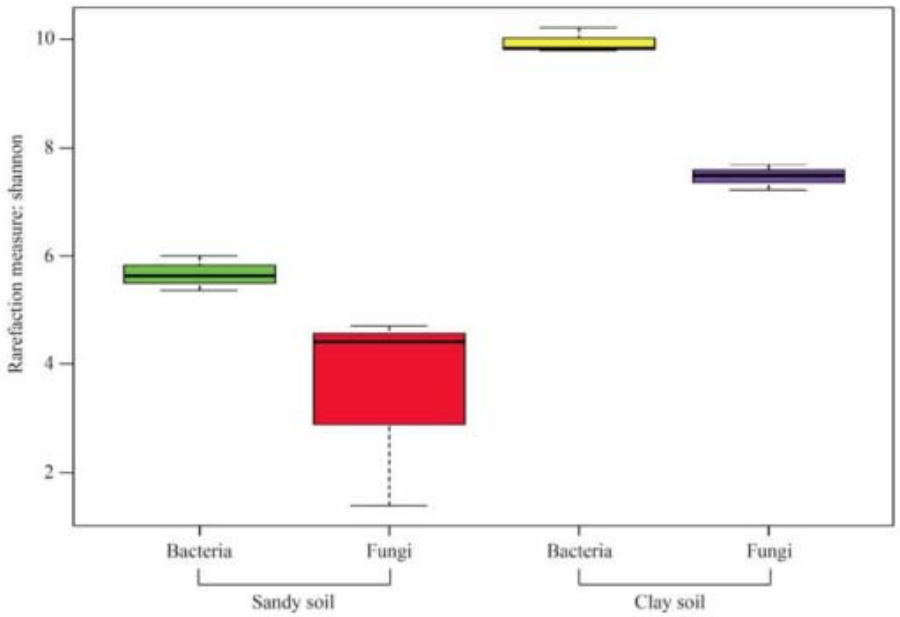

Fig. (4). Beta diversity analysis of the bacterial and fungal taxa between sandy soil and clay soil samples by using the data of Shannon index. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

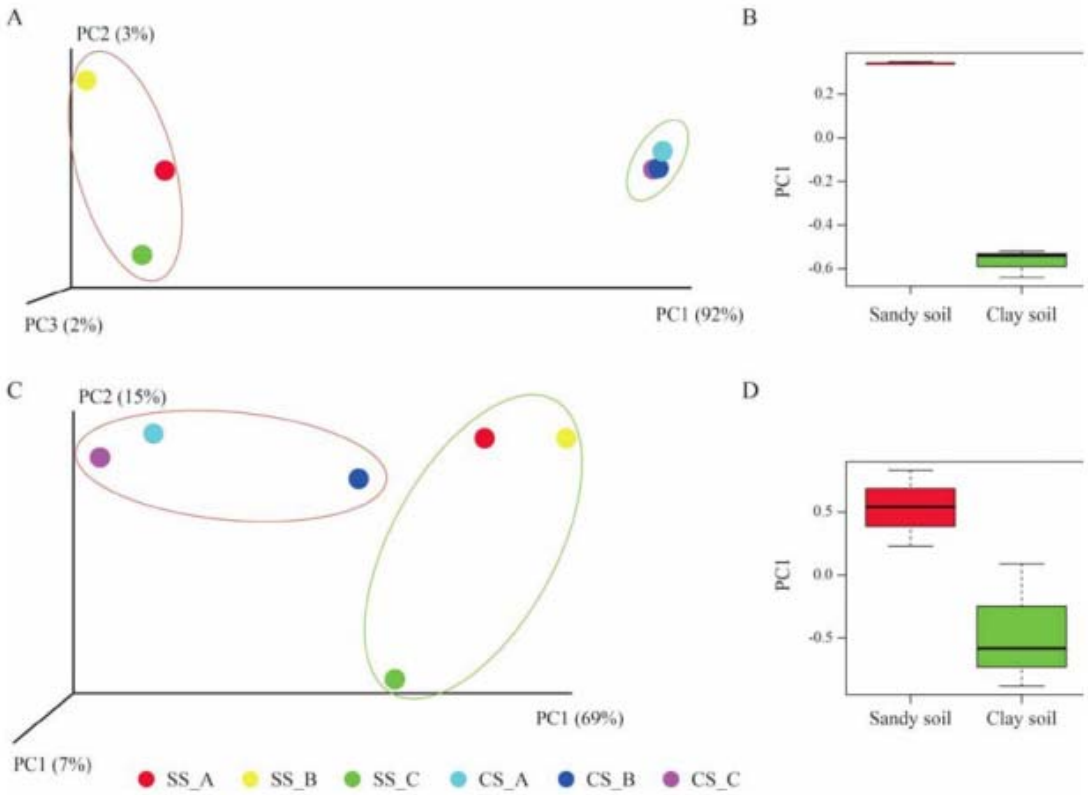

Fig. (5). Weighted Unifrac PCoA analysis of the OTU counts in all soil samples was represented by the first three principal components (PCs). (A) Bacterial 16S rRNA amplicon. (B) The distribution of PC1 for bacterial 16S rRNA amplicon. (C) Fungal ITS amplicon. (D) The distribution of PC1 for fungal ITS amplicon. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Latescibacteria, Nitrospirae, OD1, Planctomycetes, SBR1093, Verrucomicrobia and WS3, were specifically expressed in clay soil. At the genus level, the NC10 and Spirochaetes were represented by the sole class wb1-A12 and Spirochaetia, respectively.

For fungi, the differentially abundant OTUs detected were from 7 phyla (Fig. **6C**). Almost all these phyla except the unidentified phylum were enriched in sandy clay. The dominant phylum Ascomycota was largely identified as a member of the genera Ascomycota_OTU, Faurelina, Fusarium, Herpotrichiellaceae_OTU, Nectria, Nectriaceae_OTU, and Stachybotrys. Additionally, the phylum Zygomycota was unique to clay soil and represented by the sole genus Mortierella. Whereas, three differentially abundant OTUs (OTU22, OTU32 and OTU394) were only detected in sandy soil but with unidentified annotation up to now.

4. DISCUSSION

In the present study, we have characterized the rhizosphere microbiota of Dangshan Su pears grown in different soil types by using the high-throughput sequencing and bioinformatics analysis. The results indicated that Dangshan Su pears host rich and diverse microbial communities in the thin layer of soil adhering to their roots. Both in sandy soil and clay soil samples, the abundant bacterial phyla include Firmicutes, Proteobacteria, Bacteroidetes, Acidobacteria, Actinobacteria, Gemmatimonadetes, Chloroflexi, Nitrospirae, Planctomycetes and Candidatus Saccharibacteria, consistent with previous studies on the soils from Tibetan Plateau [21], northeast China [22], America [23] and Arctic region [24]. On the other hand, the common fungal phyla were Ascomycota, Basidiomycota, and Zygomycota, which were also frequently detected in soils [25, 26]. Since a majority of microbes belonging to these phyla could not be easily isolated, the application of high-throughput sequencing to soil microbial communities would extend our knowledge of what is happening underground *in situ*.

As mentioned above, the composition of dominant microbial phyla was similar across different soil types, but the quantity of each microbial community varied significantly. Between sandy soil and clay soil, microbial diversity was observed due to their individual chemical and physical characteristics, and then continued to shift by the interaction with host plants. As shown in Fig. (**3**), the results displayed a strong correlation of the rhizosphere microbiota to soil characteristics, supporting previous studies that environmental factors play vital roles in shaping microbial diversity [27, 28]. The leading phyla of bacteria and fungi in sandy soil were Firmicutes (61.66%) and Ascomycota (88.09%). Towards the other side, the dominant bacterial community in clay soil was Proteobacteria (28.65%),

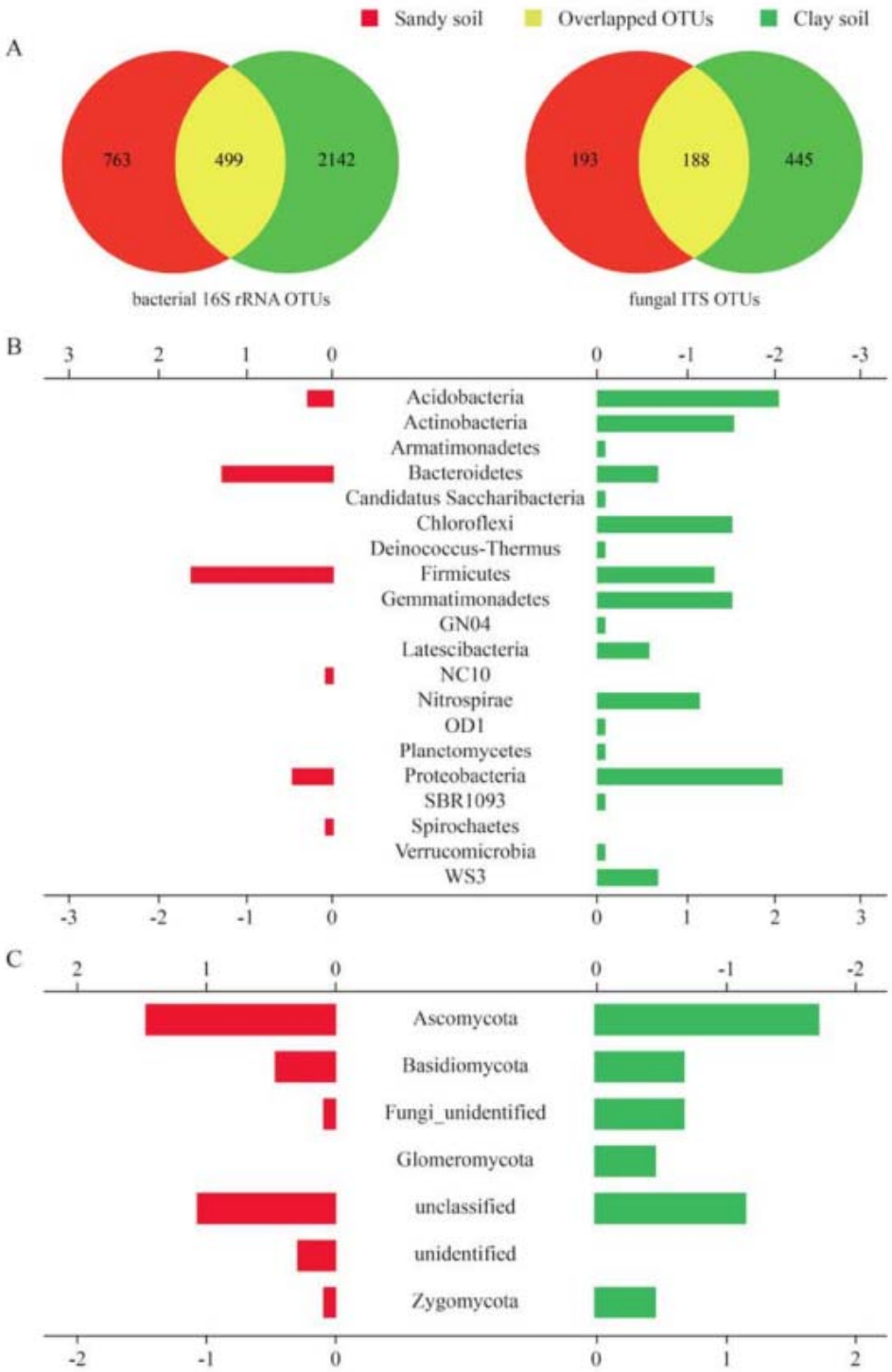

Fig. (6). Significant difference between sandy soil and clay soil samples. (A) Venn diagram showing the number of unique and overlapped bacterial and fungal OTUs. (B) Differentially abundant bacterial OTUs. (C) Differentially abundant fungal OTUs. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

whereas the relative abundance of Firmicutes decreased from 61.66% to 9.69%. Subsequently, the abundant OTUs between sandy soil and clay soil were studied with a quantitative comparison. Generally, clay soil showed higher diversity indices than sandy soil, consistent with the quantitative assays [29, 30].

As the impact of soil type on microbial community was significant, we would like to determine whether their correlation is absolute or casual. For this purpose, we have further performed the PCoA method to explore the relationship between soil type and microbial diversity. Consequently, PCoA plots (Fig. **5**) revealed that the bacterial

communities of different soils could be separated into distinct groups (Mann-Whitney U test, $P < 0.05$), while the fungal members were relatively dispersed without any distinct clusters (Mann-Whitney U test, $P > 0.05$). Hence, it is suggested that soil type does have a powerful influence on the composition of the bacterial community but a minor impact on the fungal community. The lack of significant difference for the fungal community might be due to the limited sample size used in this study, as they still possess obvious alpha and beta diversity (Fig. **2**). Nevertheless, the influence of soil type (sandy soil and clay soil) on rhizosphere microbiota is very evident. In this regard, a strong link was built between soil type and the diversity of soil microbiota, especially the bacterial community.

While soil type has shaped the composition and structure of rhizosphere microbiota, microbes could also mutually alter soil bioavailability and ultimately affect the health of host plants living in it [31]. It is well established that the optimization of microbial structure could enhance soil biodiversity and increase agricultural yield [32, 33]. In lettuce, rhizosphere microbiota were reported to affect root biomass under field conditions [34, 35]. In Arabidopsis, rhizosphere microbiota could help host plants escape abiotic stresses by altering flowering time [36, 37]. Likewise, the composition and structure of rhizosphere microbiota affected the growth of Dangshan Su pear under the same management condition. Dangshan Su pear is preferred to grow in sandy soil in Dangshan County. Under the identical cropping history and agricultural management, Dangshan Su pear grown in sandy soil tends to have a lower content of stone cells and a higher quality of riped fruits than those in clay soil [12]. Similarly, the symbiotic microbes maintained in sandy soil should be, at least partially, responsible for the high quality of Dangshan Su pear growing in it. Therefore, understanding the diversity of rhizosphere microbiota in sandy soil is a key to elucidate the relationship between soil ecosystem and Dangshan Su pear.

By comparison with microbes detected in clay soil, we have identified a range of common and unique microbes located in sandy soil. Among them, the common microbes comprised of 499 bacterial taxa and 188 fungal taxa, which formed a core set of microbial communities (Fig. **6**). The core microbiota was thought to constitute a basic mutual relationship with Dangshan Su pear trees. For instance, Acidobacteria have been reported to function in the degradation of lignocellulosic plant biomass [38, 39]. Actinobacteria played diverse roles in the decomposition of organic materials due to their different lifestyles and physiological properties [40]. Proteobacteria could influence plant health and productivity by participating in global carbon, nitrogen and sulfur cycling [41]. On the other hand, the fungal phyla Ascomycota and Basidiomycota were able to decompose crop residue and transform plant biopolymers [42].

In contrast, the unique and higher statistical microbes in sandy soil, hereafter referred to as Sandy Soil Preferred (SSP) microbiota, might provide additional benefits to the growth of Dangshan Su pear by affecting the absorption and transformation of nutrients [43]. In this study, SSP microbiota were mainly confined to bacterial taxa, with some phyla being previously reported. For instance, bacterial taxa belonging to Bacteroidetes carried genes related to denitrification, indicating their roles in *N* cycling and nutrient turnover [44]. Candidatus Saccharibacteria might serve as a validated biomarker in suppression of bacterial wilt disease [45]. Interestingly, the relative abundance of Firmicutes in this study increased from 9.69% to 61.66% as the soil ecosystem changed from clay to sandy. In fact, the presence of Firmicutes as a dominant phylum in ecological soils is quite rare up to date [46]. Here, extremely high proportion of Firmicutes may suggest that they perform essential functions to the health of Dangshan Su pear trees.

In order to further inspect their potential functions, the dominant microbes included in the Firmicutes phylum were studied in detail (Fig. **7**). Clostridia, a kind of gram-positive anaerobic bacteria, were the most abundant class, accounting for 76.2% of the bacterial communities in this phylum. They are natural inhabitants of the soil and regarded as major players in the decomposition of various organic compounds [47]. It is well established that Clostridia possess an excellent source of extracellular enzymes that could hydrolyze polysaccharides such as starch, cellulose and hemicellulose. The features of several hydrolytic enzymes are remarkable as they are unique to Firmicutes even through different microbes, such as nitrogenase iron protein in *Clostridium pasteurianum* [48], superoxide dismutase in *Clostridium acetobutylicum* [49], cellulose in *Clostridium thermocellum* [50] and ADP-glucose pyrophosphorylase in *Ruminococcus albus* [51]. Generally, the Lachnospiraceae and Ruminococcaceae families tend to possess more abundant and diverse carbohydrate-active enzymes than the Clostridiaceae family, which makes them special for the degradation of complex plant materials [52]. In this study, specific enrichments of the Lachnospiraceae and Ruminococcaceae families (Fig. **7**) should be considered as a profitable indicator for complete degradation of organic materials. Furthermore, they may be also responsible for the disinfestation of pathogens through producing antifungal enzymes and decomposing mycelial cells, which is supported by negative relationship in reductive soil disinfestation [53, 54]. Thus, significant roles played by Firmicutes as well as other SSP microbiota should not be ignored in future studies.

CONCLUSION

In this study, the composition and structure of rhizosphere microbiota of Dangshan Su pear growth in different soil types were investigated through a combination of high-throughput sequencing technology and bioinformatics analysis. Consequently, a range of the common bacterial and fungal communities was found to be essential to Dangshan Su pear. Furthermore, the SSP microbiota in the core rhizosphere were also identified and mainly confined to the bacterial taxa. Notably, the significant enrichments of the Lachnospiraceae, Ruminococcaceae and Clostridiaceae families involved in the Firmicutes phylum could possess an excellent source of extracellular enzymes and antifungal enzymes, which may provide additional host functions for the growth and health of Dangshan Su pear. These findings have significant implications for sustainable soil

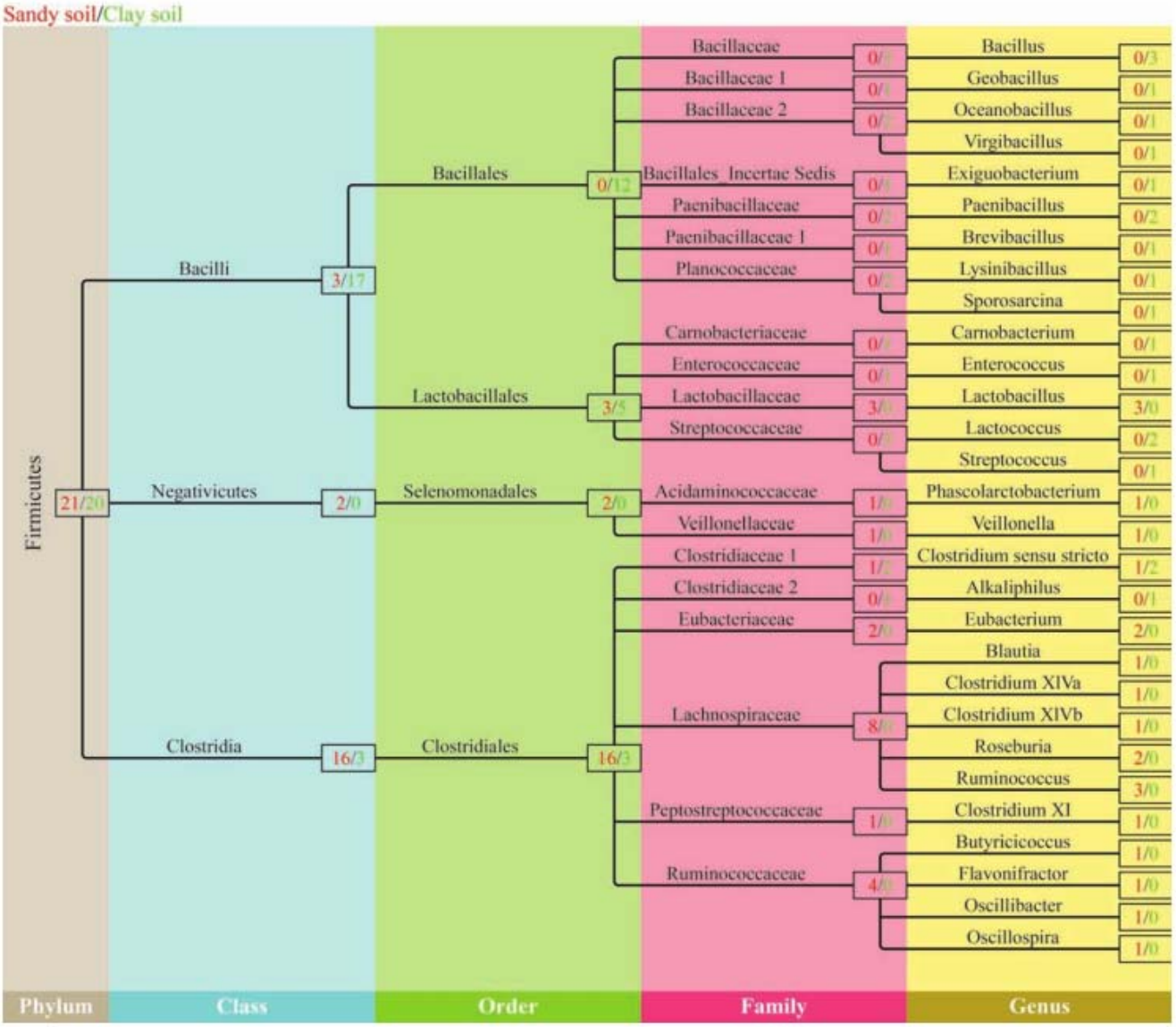

Fig. (7). Microbial taxa involved in the Firmicutes phylum were displayed at the level of Class, Order, Family and Genus, with their biological names showed on the lines. The digits in the boxes indicate the number of each taxa identified from sandy soil (red) and clay soil (green), respectively. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

management of orchards with altering microbial biomass in agriculture production.

AUTHOR CONTRIBUTIONS

Xiaojing Ma and Junyang Yue planned the project and designed the experiments. Sambhaji Thakar and Junyang Yue prepared bioinformatics analysis. Huimin Zhang, Zequan Yu and Li Meng performed the experimental work. Xiaojing Ma and Junyang Yue participated in the discussion and production of the first draft manuscript. All authors critically reviewed and edited the manuscript and approved its publication.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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