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Soil bacterial community triggered by organic matter inputs supports a high-yielding pear production

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Abstract. The roles of microorganisms in enhancing crop production have been demonstrated for a range of cropping systems. Most studies to date, however, have been confined to a limited number of locations, making it difficult to identify general soil biotic and abiotic characteristics underpinning the yield-promotion across various locations. This knowledge gap limits our capacity to harness soil microbiome to improve crop production. Here we used high-throughput amplicon sequencing to investigate the common features of bacterial community composition, ecological networks and physicochemical properties in six yield-invigorating and adjacent yield-debilitating orchards. We found that yield-

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invigorating soils exhibited higher contents of organic matter than yield-debilitating soils and harboured unique bacterial communities. Greater alpha diversity and higher relative abundances of Planctomycetes and Chloroflexi were observed in yield-debilitating soils. Co-occurrence network analysis revealed that yield-invigorating soils displayed a greater number of meta-modules and a higher proportion of negative links to positive links. Chloroflexi was recognized as a keystone taxon in manipulating the interaction of bacterial communities in yield-invigorating soils. Structural equation modelling showed that soil organic matter, beta diversity of bacterial community, and network connector (Chloroflexi) were key factors supporting high-yield pear production. Altogether, we provide evidence that yield-invigorating soils across a range of locations appear to share common features, including accumulation of soil organic matter, higher microbial diversity, enrichment of key taxa like Chloroflexi, and maintaining a competitive network. These findings have implications for science-based guidance for sustainable food production.

Keywords: Soil organic matter, Microbial diversity, Random forest prediction, Co-occurrence network, Keystone taxa

1 Introduction

Soils are essential to human wellbeing due to their great contributions to the production of food, fiber, feed, and medicine (Raaijmakers and Mazzola, 2016). Soil organisms play critical roles in maintaining these ecosystem services, such as driving nutrient cycling, maintaining soil fertility, improving plant productivity and suppressing plant diseases (Bender et al., 2016; Barrios, 2007). Microorganisms participate in nearly all soil biological processes, and the microbial abundance, community composition and activity primarily determine the sustainable productivity of agricultural lands (Philippot et al., 2013). Given that bacteria are the most diverse and abundant group of microorganisms in soil, bacterial communities and their functions can be pivotal indicators for crop production in agroecosystems (van der Heijden et al., 2008).

In general, an increase in microbial diversity is linked to a high-yielding crop production mainly through improving the host resilience to physical or chemical disturbances, modifying plant competition, and facilitating plant access to nutrients (Chaer et al., 2009, Kennedy and Smith, 1995). Since individual organisms do not live in isolation but rather form a complex system of inter-species interactions in soil, interactions among community members were found to be related to crop production in the monoculture

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system (Lu et al., 2013). Enrichments of key functional microbes in soil were deemed to serve specific soil system functions, such as suppressing soil-borne pathogens and maintaining sustainable crop production (Banerjee et al., 2018). However, the relative contributions of microbial diversity, interactions among community members, or enrichment of key taxa to crop production remain largely unknown. Therefore, it is highly desirable to identify pivotal indicators of bacterial community composition in response to high-yielding crop production.

Changes in composition of soil bacterial communities across space are often strongly correlated with soil pH (Fierer and Jackson, 2006). Soil pH has been recognized as a key driver in determining the assembly of bacterial community in arable soils by field or microcosm experiments (Rousk et al., 2010). However, recent studies have demonstrated that compositions of soil bacterial communities were driven by a myriad of soil abiotic traits, such as organic matter contents, nutrient contents and forms (Tian et al., 2018; Wang et al., 2018). For example, soil bacterial community composition, which determines the ability of soil to suppress soil-borne pathogens, was found to be strongly correlated with soil organic matter (Shen et al., 2018). An imbalanced ratio of soil nutrients, *i.e.*, ratio of nitrogen to phosphorus or potassium could be a driving force altering the bacterial community composition in long-term fertilized soils (Eo and Park, 2016). Therefore, key soil chemical properties identified in controlling the distribution and abundance of bacterial community is largely depending on soil sampling scale or treatments. As a consequence, a better understanding of the relationship between soil edaphic properties and bacterial community composition is critical to develop targeted manipulation options to increase soil service provisions.

Pear (*Pyrus*) is the third most important temperate fruit species second only to grape and apple, belonging to the subfamily *Pomoideae* in the family *Rosaceae*. As a popular fruit in the world market, pear has been cultivated globally, and China is the biggest pear producer (FAOSTAT, 2019). 'Sucui No. 1' pear, an early-maturing cultivated variety bred by the Jiangsu Academy of Agricultural Sciences, China, has displayed distinct advantages over other cultivates in Eastern and Central China, because this variety is easy to produce, adaptable to the environment, and has good quality and high economic benefits (Lin et al., 2013). With the increasing demand in China, sustainable production of high-quality pear is becoming increasingly important. Manipulation of soil microbiomes has shown to be an effective way to increase soil productivity (Chaparro et al., 2012). Considering that large-scale surveys could exhibit the diversity of soil microbial communities exceeds what is found in host-associated communities (Zorz

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et al., 2019), it is necessary to explore the general microbial characteristics of multiple yield-invigorating soils and identify key environmental drivers in assembling bacterial community.

In this study, six yield-invigorating and adjacent yield-debilitating pear orchards, which were identified through field surveys, were selected. We hypothesized that yield-invigorating pear orchard soils harbor unique bacterial communities which are manipulated by key soil abiotic factors. To address this, soil bacterial communities and edaphic properties of six yield-invigorating and adjacent yield-debilitating pear orchards were compared to (1) decipher the differences of taxonomic diversity, and composition of the bacterial community, and (2) determine the contributions of environmental variables to the changes in the structure of bacterial communities.

2 Methods

2.1 Study sites and experimental design

From July - August 2019, a field production survey of orchards cultivated with 'Suci No. 1' pear was performed after pear fruits harvest to compare the differences of soil nutrients and microbiota between yield-invigorating (YI) with yield-debilitating (YD) orchards. The locations, planting density, cropping years, soil type and total yield were recorded. To minimize the effects of microclimate at each site, only pair-located pear orchards with invigorating and debilitating yield and at similar growth stage were selected for this research. In total, six pair-located yield-invigorating and -debilitating pear orchards distributed in four cities of Jiangsu province, China, were selected in the main pear production areas (Fig. 1A, Table S1).

Paired yield-invigorating and yield-debilitating orchards from Fengxian (FX), Suining (SN) and Tongshan (TS) were maintained in the Xuzhou city under the warm temperate sub-humid monsoon climate. This site has a mean annual temperature (MAT) of 14.5 °C and mean annual precipitation (MAP) of 847 mm. Orchards from Taixing (TX) were located in the Taizhou city under the humid southern subtropical climate with a MAT of 15.3 °C and MAP of 1055 mm. Orchards from Gaochun (GC) were located in the Nanjing city under the humid subtropical monsoon climate with a MAT of 15.4 °C and MAP of 1106 mm. Orchards from Zhangjiagang (ZJ) were located in the Suzhou city under the humid subtropical monsoon climate with a MAT of 15.7 °C and MAP of 1094 mm. For paired yield-invigorating and-debilitating orchards, the irrigation and pesticide management practices were similar according to farm records. However, yield-invigorating orchard was usually amended with more organic fertilizer under integrated nutrients management whereas the co-located yield-debilitating orchard received more

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chemical fertilizer under intensive management. The yield per tree was obtained by dividing the total yield per hectare by plant density. Detailed information about each orchard is shown in Table S1.

2.2 Soil sample collection and chemical properties determination

Along with the field survey, soil sampling campaigns were performed from July - August 2019 after pear fruits harvest. For each yield-invigorating or -debilitating orchard, four subplots with three pear trees in each subplot were randomly selected for soil sampling. Subsequently three soil cores (0-20 cm) under the trunk base for each tree were collected using a 25 mm soil auger. In total, nine soil cores for each subplot were pooled as a composite sample and finally four composite soil samples for each orchard were collected and promptly transported on ice to the laboratory. After sifting through a 2 mm sieve and thoroughly mixing, one portion of each soil sample was air-dried for chemical property analyses while the remainder was stored at -70 °C for DNA extraction.

Soil chemical properties including soil pH, content of organic matter (OM), total nitrogen (TN), available phosphorus (AP), available potassium (AK), alkali-hydrolyzale nitrogen (N), exchangeable calcium (Ca), effective magnesium (Mg), effective iron (Fe), effective manganese (Mn), effective copper (Cu) and effective zinc (Zn), were measured according to methods described by Shen et al. (2018) and Huang et al. (2019). Briefly, soil pH was determined using a glass electrode meter in a suspension with a 1:5 soil/water ratio (w/v). Soil OM was determined using the potassium dichromate external heating method. TN was determined using a dry combustion method on an Element Analyzer (Vario EL, Germany). AP and AK were determined using the molybdenum blue method after soil was extracted with sodium bicarbonate and flame photometry after soil was extracted with ammonium acetate, respectively. Soil alkaline hydrolysable nitrogen (N) was measured by the alkaline hydrolysable diffusion method. Contents of soil Ca, Mg, Fe, Mn, Cu and Zn were determined by the atomic absorption spectroscopy method using ICE 3300 AAS Atomic Absorption Spectrometer (ThermoScientific, USA) after acid hydrolysis.

2.3 Soil DNA extraction and bacterial abundance quantification

Genomic DNA from 0.25 g soil for each sample was extracted by using the DNeasy® PowerSoil® Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions. The abundances of soil bacteria were determined with Eub338F/Eub518R primer using a 7500 Real Time PCR System (Applied Biosystems, USA). Standard curves were generated by using 10-fold serial dilutions of a plasmid containing a full-length copy of the 16S rRNA gene from *Escherichia coli*. Quantitative PCR analysis

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was performed in 96-well plates with a 20-µl mixture for each reaction using SYBR®Premix Ex Taq™ (TaKaRa, Japan). Thermal cycling was conducted according to a standard procedure with three replicates, and the results were expressed as log copy numbers g^{-1} dry soil.

2.4 Sequencing library construction and sequencing

The gene-specific primers 515F/806R with 12 bp barcode were used to amplify the V4 region of bacterial 16S rRNA gene on the BioRad S1000 (Bio-Rad Laboratory, CA) roughly according to the protocols described by Caporaso et al. (2011). All constructed libraries were sequenced using the Illumina NovaSeq 6000 at the Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China).

2.5 Sequence processing

Quality filtering of the paired-end raw reads was performed to obtain the high-quality clean reads according to the Trimmomatic (V0.33) quality control process. Sequences were assigned to each sample based on their unique barcode, after which the barcodes and primers were removed. Paired-end clean reads were merged using FLASH (V1.2.11). Raw tags were processed to generate the final ASV (Amplicon Sequence Variant) table file at 97% pairwise identity according to the QIIME2 pipeline (Bolyen et al., 2019). The nonbacterial and mitochondrial ASVs and extremely low frequency ASVs (relative abundance < 0.01%) were removed. A representative sequence for each ASV was selected and classified using the RDP classifier (Wang et al., 2007) against the RDP Bacterial 16S database.

2.6 Statistical analyses

Statistical analyses were performed using the software SPSS 20.0 and R. Non-normal data were square-root or log transformed when necessary. The significance of soil properties or microbial taxa in yield-invigorating or-debilitating orchards was determined based on paired Wilcoxon rank sum test, and adjusted *P* values (< 0.05) were obtained by the FDR method. Mantel tests were used to identify the correlations between microbial community composition and pear yield, and soil chemical properties using the 'vegan' package in R. The linear regression analyses relating yield to selected microbial taxa or soil chemical properties were conducted using the 'basicTrendline' package in R.

Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance was performed in MOTHUR (V1.38.1) (Schloss et al., 2009) and visualized by the 'ggplot2' package in R to explore the differences in microbial community composition. Permutational multivariate analysis of variance (PERMANOVA) was performed to evaluate the significant differences of microbial community composition according to sample locations and orchard yield using the 'vegan' package in R. Microbial

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alpha diversity indexes (Chao, Shannon) were calculated based on randomly resampled ASV abundance matrices at the same depth (23,800 sequences) in MOTHUR. A Venn diagram was generated based on the final ASVs to compare microbial community composition between yield-invigorating and -debilitating orchard soils. The affiliations of unique and shared ASVs in yield-invigorating and -debilitating soils were compared to evaluate the differences of the bacterial community composition and plotted using the 'pheatmap' package in R. Fold changes (log2 transformed) of shared ASVs across yield-invigorating and -debilitating soils were calculated. The ASVs with fold change ratios > 2 and unique ASVs in yield-invigorating soils were recognized as potential responders to yield promotion. In addition, to better understand the bacterial community composition, relative abundances at the genus level were compared by STAMP software v2.1.3 (Parks et al., 2014).

Potential ecological interactions among bacteria were determined by modeling the microbial community using Molecular Ecological Network Analysis (http://ieg2.ou.edu/MENA) based on pear yield. After removal of ASVs whose abundances were lower than 0.01%, the ASVs table appeared in at least half soil samples were merged for phylogenetic molecular ecological network (pMEN) construction (Deng et al., 2012). The microbial network was constructed using random matrix theory-based at 0.94 similarity threshold and visualized using Cytoscape 2.8.3 software (V3.5.1, http://cytoscape.org/). Module clustering and composition in yield-invigorating and -debilitating networks were compared and plotted in R using the 'pheatmap' and 'ggplot2' packages. Redundancy analysis (RDA) was performed in the R 'vegan' package to examine the relationship among frequencies of ASVs, samples and soil variables, which were selected using 'stepAIC' in R. Variance partitioning analysis (VPA) was used to determine the contributions of soil properties, sample location, and yield, as well as interactions among the variation in a microbial community with hellinger-transformed data. The predictors of selected soil properties for explaining the pear yield were identified by random forest regression analysis (Boulesteix, et al., 2012). The significance of each predictor in the response variables was assessed with the 'rfPermute' package with 1000 permutations based on 1000 trees. Structural equation modelling was applied to evaluate relative contributions of soil chemical properties and bacterial community to pear yield (Schermelleh-Engel et al., 2003). The conceptual SEM fitness was examined on the basis of a non-significant chi-square test ($P > 0.05$) and the goodness-of-fit index (GFI). Model was fitted using the 'lavaan' package in R (Rosseel, 2012).

3 Results

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3.1 Overview of sequencing data

In total, 1,622,858 16S rRNA sequences were retained after quality control and a total of 9,394 ASVs were obtained for the 16S rRNA gene sequences based on 97% similarity. Among the total 16S rRNA gene sequences, 159 ASVs with 74,372 sequences were classified as Archaea while 9,235 ASVs with 1,548,486 sequences were identified as Bacteria. Among Bacteria, Acidobacteria, Proteobacteria, Chloroflexi, Planctomycetes and Actinobacteria were the most abundant phyla (Fig. S1).

3.2 Bacterial abundances and community compositions

Yield-invigorating orchards together displayed significantly higher abundances of total bacteria than that in co-located yield-debilitating orchards based on real time PCR result (Fig. 2B). Meanwhile, bacterial community compositions at the ASV level were significantly correlated to pear yield (r = 0.460, p = 0.001) (Fig. 1C).

Fig. 1 here

PCoA based on Bray-Curtis distance matrices clearly revealed treatment-based differences in bacterial community compositions (Fig. 2A). Six distinct groups representing samples from different locations (FX, GC, SN, TS, TX and ZJ) were obviously separated and confirmed by PERMANOVA test (F = 14.9, P = 0.001). At each location, soil bacterial community composition in yield-invigorating orchards was significantly separated from that in co-located yield-debilitating orchards, which was also confirmed by PERMANOVA test (F = 3.6, P = 0.001). Although only the Shannon diversity in yield-invigorating orchards from GC and ZJ was significantly higher than that in co-located yield-debilitating orchards (Fig. S2), the mean alpha diversity indices of Chao and Shannon in all yield-invigorating orchards were significantly higher than those in all yield-debilitating orchards based on the paired Wilcoxon test (Fig. 2B).

Fig. 2 here

The Venn diagram showed that 4540 ASVs occupying over 90% of total sequences were shared between yield-invigorating and -debilitating orchards (Fig. 2C). Among these shared ASVs, the fold changes larger than 2 of ASVs in yield-invigorating compared to yield-debilitating orchards were potentially linked to yield improvement. Surprisingly, none of these ASVs potentially linked to yield improvement were shared among six separated collocated orchards (Fig. S3). A total of 2546 unique ASVs with 53,222 sequences were found in all yield-invigorating orchards and 2308 unique ASVs with 44,389 sequences were observed in all yield-invigorating orchards, among which almost 70% of total

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ASVs were shared among these unique ASVs between yield-invigorating orchards and -debilitating orchards. However, no shared unique ASVs were found among six separately located orchards. The affiliation of unique and shared ASVs at the phylum level exhibited that the Proteobacteria, Planctomycetes, Chloroflexi, Acidobacteria and Actinobacteria were the top five phyla (Fig. 2D).

At the phylum level, the relative abundances of bacterial dominant phyla varied across the location and orchard yield condition (Fig. 3A). Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi and Planctomycetes were the top five abundant phyla. The mean abundance of Chloroflexi and Planctomycetes was significantly higher while Firmicutes was significantly lower in yield-invigorating orchards compared to yield-debilitating orchards based on Wilcoxon test (Fig. 3A, Fig. S4).

At a finer resolution, 967 genera were observed for all soil samples, among which 299 genera appeared in more than half of soil samples in yield-invigorating or -debilitating orchards. However, only 34 genera displayed significant differences between yield-invigorating or -debilitating orchard soils based on STAMP analysis (Fig. 3B). Interestingly, *Ornatilinea*, *Ktedonobacter*, *Longilinea*, belonging to Chloroflexi, were significantly enriched in yield-invigorating orchard soils. *Gimesia* in Planctomycetes and *Arenimonas* in Proteobacteria showed significantly higher relative abundances in yield-invigorating orchard soils than in yield-debilitating orchard soils.

Fig. 3 here

3.3 Co-occurrence patterns of bacterial community

The phylogenetic molecular ecological networks were constructed using the random matrix theory-based approach to explore the organization of bacterial communities in yield-invigorating (YI) or yield-debilitating (YD) soil samples. After filtering ASVs that occurred in less than half of soil samples, 591 ASVs for yield-invigorating samples and 485 ASVs for yield-debilitating samples were used to construct the networks. The YI network contained 302 nodes, 448 edges, and 11 larger modules (> 5 nodes), with an average connectivity (avgK) of 2.967, average path distance of 5.494 and clustering coefficient (avgCC) of 0.152, while the values in the YD network were 235, 334, 9, 2.843, 6.232 and 0.131, respectively (Fig. 4A, Table S2). The module eigengene network analysis revealed a difference in the higher-order organization between the two networks. Notably, the node composition was substantially different between the two networks as the relative abundances of dominant phyla were obviously different among different modules (Fig. 4B and C). A higher proportion of nodes in the module of YI network was unique. ASVs affiliated to Acidobacteria, Chloroflexi, Proteobacteria, Actinobacteria, and

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Planctomycetes within the unique modules (M9, M10 and M11) were observed in the YI versus YD network.

Analysis using the threshold values of *Zi* and *Pi* showed that majority of nodes from both networks were categorized as peripherals that had only a few links and almost always linked to the nodes within their own modules (Fig. 4D). Although only three nodes affiliated to Acidobacteria were categorized as module hubs in the YI network, seven nodes belonging to Acidobacteria, Actinobacteria and Proteobacteria were categorized as module hubs in the YD network. Interestingly, four nodes including *Longilinea* species from Chloroflexi in the YI network whereas only one node in the YD network was categorized as module connectors (Table S3).

Fig. 4 here

3.4 Relationships between soil chemical properties and microbial community composition

Soil chemical properties differed significantly among the locations and orchard yield types (Table S4). Together, yield-invigorating orchards exhibited a significantly higher content of soil organic matter (OM) compared to yield-debilitating orchards based on the Wilcoxon test. Soil chemical properties were significantly correlated to the bacterial community compositions (Mantel: r = 0.803, p = 0.001). Soil chemical properties, location, and orchard explained 44.9% of the observed variation, leaving 55.1% of the variation unexplained for bacterial community composition based on VPA analysis (Fig. 5A). Variation in the community composition was largely explained by soil properties (42.3%), and was also influenced by locations and orchard yield types.

After forward stepwise selection, the module including soil OM, TN, alkaline N, AP and AK, available calcium (Ca), copper (Cu) and manganese (Mn) explained the majority of the variation in bacterial community composition (Fig. 5B). As evidenced by the RDA vectors, OM was among the most important soil properties in shaping bacterial community composition. Random forest analysis showed that contents of soil Mn, OM and Ca were the top parameters for predicting the orchard yield (Fig. 5C). Furthermore, soil OM was also significantly correlated with bacterial communities as revealed by Mantel test (Fig. 5D, Table S5).

Fig. 5 here

3.5 Relationships of soil chemical and microbial indicators with orchard yield

Soil OM as potentially key soil chemical properties and bacterial alpha diversity, beta diversity and relative abundance of Chloroflexi and Planctomycetes as potentially key microbial indicators in

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determining pear yield were used to construct a model to explain yield improvement. Final structural equation modelling (path analysis) (Fig. 6) showed that the strongest driver explaining yield improvement was beta diversity of bacterial community (PCoA) ($r = 0.959$, $P < 0.001$), which was positively affected by content of soil OM ($r = 0.843$, $P< 0.001$). Alpha diversity (Chao) of bacterial community also determined yield improvement to a large extent ($r = 0.542$, $P = 0.009$). However, alpha diversity was not significantly correlated with content of soil OM.

Fig. 6 here

4 Discussion

Although pear is among the most important fruits worldwide, soil microbial communities in pear orchards have been largely under-investigated (Huang et al., 2019). The present study attempts to decipher the bacterial community linked to high-yield production of pear. Our results based on Mantel analysis suggested a directly significant correlation between bacterial community and pear yield. Microbial characteristics responding to yield promotion have repeatedly been observed on several crops depending on single experimental site (Zhong et al., 2020; Qiao et al., 2019; Shen et al., 2013). It remained unclear, however, whether these distinctions are ubiquitous at a large-scale. By comparing multiple co-located yield-invigorating and -debilitating orchards, we demonstrate that high-yielding pear production soils harbored shared bacterial communities with high diversity, significantly enriched indigenous microbes and well-organized interaction network, which was triggered by soil organic matter. Here we discussed these main results and potential mechanisms in detail.

Microbial diversity is critical to soil ecosystems in maintaining the integrity, function and long-term sustainability (Kennedy and Smith, 1995). Higher soil biodiversity is considered to be linked to a more stable system and enhance the combination of vital microbial functions and processes (Cardinale et al., 2006; Bell et al., 2005). In line with a previous report that crop yield was correlated to the soil bacterial diversity (Zhao et al., 2014), greater diversity of bacterial community in yield-invigorating soils was observed in the present study. Our results indicate that higher microbial diversity may result in a more stable agroecosystem, contributing to sustainable pear production.

In this study, we found that Proteobacteria, Acidobacteria, Actinobacteria, Planctomycetes and Chloroflexi were the top abundant phyla. This result roughly agreed with previous studies showing that Proteobacteria, Acidobacteria and Actinobacteria are usually dominant bacterial taxa in agricultural soils (Xun et al., 2019; Dai et al., 2018), while Planctomycetes and Chloroflexi exhibit an unexpectedly high

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relative abundance in rice cropped soil (Edwards et al., 2015) and sandy loam soil (Pathan et al., 2021). The highest relative abundance of Proteobacteria was probably explained by the fact that Proteobacteria are considered as copiotrophic bacteria and always flourish in soils with large amounts of available nutrients (Fierer et al., 2007).

Moreover, a significantly higher abundance of Planctomycetes and Chloroflexi was observed in yield-invigorating orchards, indicating that Planctomycetes and Chloroflexi may be responsible for pear yield-improvement. There is no direct evidence showing that Planctomycetes could improve plant growth, however, Planctomycetes has been reported to be involved in the soil biological processes such as ammoxidation, carbohydrate and polysaccharide metabolic (Fuerst, 2017). This implies that Planctomycetes may promote plant production through improving soil biological fertility. Chloroflexi is a facultative anaerobic phylum including autotrophic, heterotrophic and mixotrophic taxa (Speirs et al., 2019). Considering that soil amended with organic fertilizer could enhance the soil water holding capacity, the yield-invigorating soils with more organic material input have a higher soil moisture content, especially after irrigation, probably leading to the enrichment of Chloroflexi in soil. Furthermore, it has been well documented that Chloroflexi could grow well in drought conditions (Ullah et al., 2019), implying that yield-invigorating soils with a higher relative abundance of Chloroflexi may exhibit excellent resistance to environmental stress to support sustainable crop production.

Network analysis is a systems-level method to explore interactions within an ecosystem that cannot be directly observed through co-occurrence analysis (Fath et al., 2007). Similar to the food web network analyses in macro ecosystems, microorganisms also form complex interactions with other species (Faust and Raes, 2012) and have been widely investigated to explore the linkage of microbial network with soil function, such as nutrient supply (Fan et al., 2021) and disease suppression (Lu et al., 2013). Overall, in line with previous findings (Hu et al., 2020), the topological properties of the constructed networks, including connectivity, average clustering coefficients, average degree distance, and modularity indicate that these networks are scale-free, modular and “small world”. Our comparative network analysis indicated that microbial co-occurrence patterns in soils were correlated to pear production. As a meta-module is usually considered as a group of modules functionally interrelated (Langfelder and Horvath, 2007), a greater number of meta-modules were identified in the network constructed from yield-invigorating soils, suggesting that a greater number of network nodes in the yield-invigorating soils were functionally interrelated than those in the yield-debilitating soils. A majority of nodes in the meta-

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modules were not shared between yield-invigorating and -debilitating networks, indicating basal shifts in network architecture during pear production with contrasting yield performance.

Furthermore, a higher proportion of negative interactions to positive interactions were identified in the network constructed from yield-invigorating network than the yield-debilitating network. Our results indicated that stronger resource competitions in yield-invigorating soils, which means that the soil co-occurrence network was more stable to maintain soil ecosystem function (Coyte et al., 2015). In this study, three module connectors and three module hubs were identified as potentially key taxa in the yield-invigorating network. Interestingly, among those key species, ASV357 affiliated to *Longilinea*, belonging to the Chloroflexi, was recognized as a key phylum in improving pear yield. Similarly, Chloroflexi was reported to be key-stone taxa in the constructed network from agricultural soils with 40-years fertilization (Fan et al., 2021). Chloroflexi play key roles in manipulating soil microbiome probably due to that Chloroflexi could participate in degrading plant compounds to create more niches via pathways for the degradation of starch, pyrogallol, cellulose, and longchain sugars, as it is positively correlated with genes for amino sugars, sugar alcohols and simple carbohydrate metabolic pathways (Hug et al., 2013).

Soil pH is generally recognized as the main driver in the assembly of bacterial community, especially in the studies related to geographic distribution of microorganisms (Fierer and Jackson, 2006). Soil pH varying across a wide range allows insights into the relationships between pH and soil bacterial communities in those researches. Therefore, we speculated that there are important factors other than pH in shaping soil bacterial communities in our study, given that soil pH only ranged within two units. In this study, a significantly higher content of soil organic matter was observed in yield-invigorating orchards, demonstrating that soil organic matter can also drive the assembly of bacterial community. Consensus is emerging that microbial materials are an important constituent of soil organic matter (Kallenbach et al., 2016), a higher content of soil organic matter usually supports a more diverse microbial community, which participate in almost all soil biological processes (Fierer, 2017).

Structural equation modelling approach has been widely used to decipher keystone indicators associated with soil function and crop production in agroecosystems (Jiang et al., 2020; Chen et al., 2019). In the present study, we observed that soil organic matter, beta diversity of bacterial community, and network connector were key indicators in supporting high-yield pear production based on the structural equation modelling results. Therefore, we proposed that yield-invigorating soils harbour unique bacterial

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communities that could improve soil biological fertility, which could be driven by soil organic matter and manipulated by keystone species (Chloroflexi) through altering the bacterial interactions.

5 Conclusions

In conclusion, by comparing six paired-located orchards, our results demonstrated that yield-invigorating soils showed a higher content of organic matter and harboured unique bacterial community with greater diversity than yield-debilitating soils. We further highlight that Chloroflexi was significantly enriched and served as a keystone taxon in manipulating the interaction of bacterial community in yield-invigorating soils. These findings help elucidate the role of soil microbiome in maintaining crop production and factors controlling the assembly of soil microbiome. Such knowledge is a first step toward harnessing soil microbiome in support of sustainable agroecosystems.

Data availability. Raw amplicon sequencing data for each sample used in this study was deposited at the National Center for Biotechnology Information (NCBI) in the FASTQ format and is available under the accession number PRJNA749397. Other data that support the findings of this study are available on request from the corresponding author (Xiaomei Ye).

Authors' contributions

L. Wang: performed all experiments; L. Wang, X. Ye, and Z. Shen: designed the study, and wrote the majority of the manuscript; L. Wang and Z. Shen and C. Tao: analyzed the data; H. Hu, J. Du, Y. Xi, J. Lin, and D. Chen: participated in the design of the study, provided comments and edited the manuscript. The authors read and approved the final manuscript.

Competing interests. The authors declare that they have no conflict of interest.

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Supplementary data

Supplementary figures and tables to this article can be found in the supplemental material.

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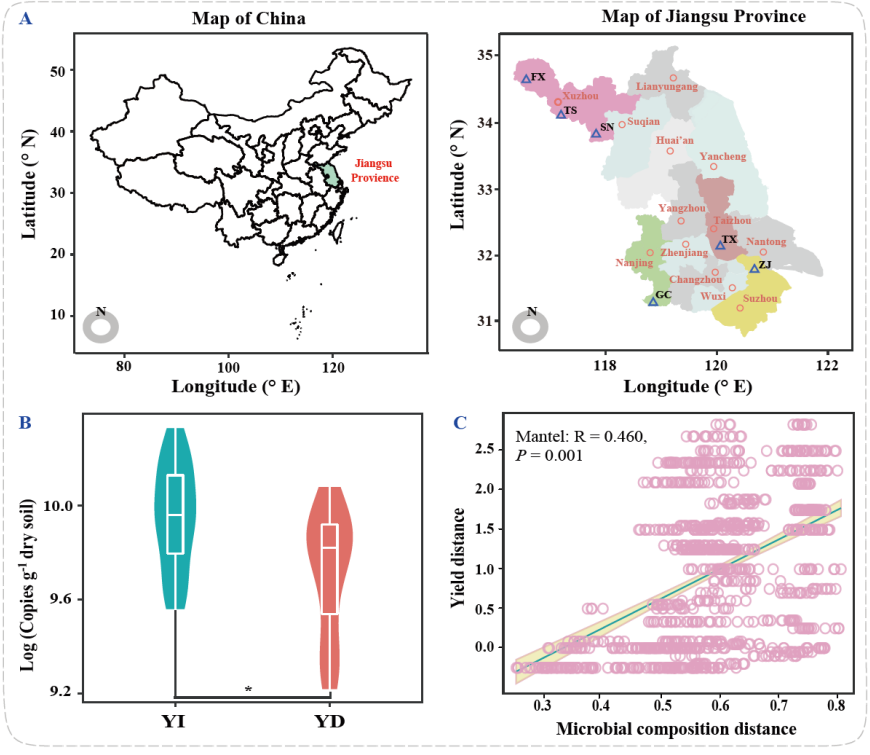
Figure legends

Fig. 1 Distribution of field sites, quantitation of the abundance of bacteria population, and linkage of microbial composition to pear yield. (A) Map showing the sites of six pair-located orchards sampled in this study. (B) Violin plot showing the abundance of total bacteria for all selected orchards. * indicates a significant difference between yield-invigorating (YI) and yield-debilitating (YD) orchards based on Wilcoxon tests ($p < 0.05$). (C) Correlation plot showing the relationship of microbial composition and yield based on braycurtis distances calculated by Mantel test.



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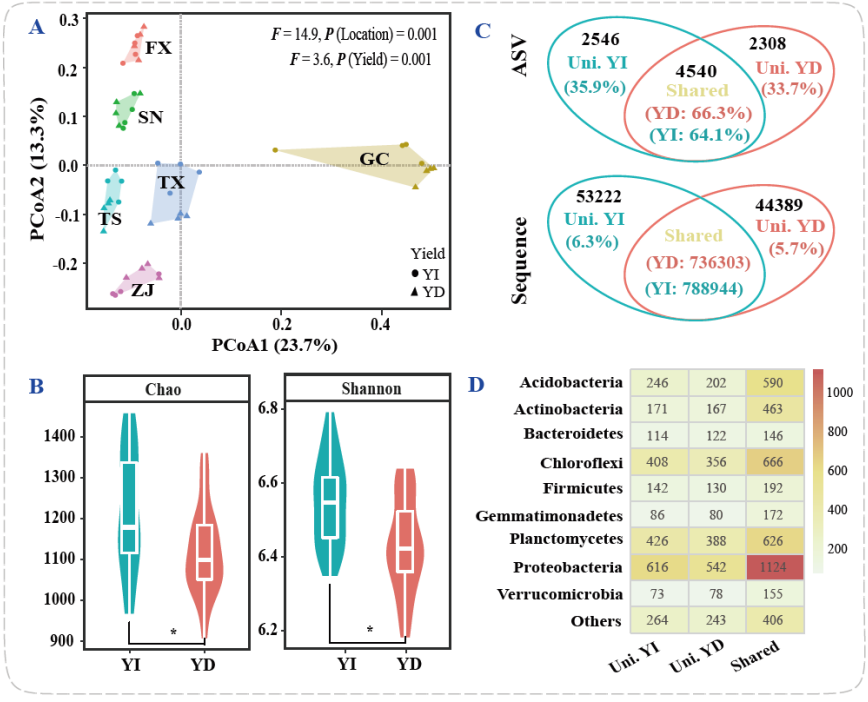
576 **Fig. 2 Overview of bacterial composition and alpha diversity.** (A) Principal Coordinates Analysis
577 (PCoA) plot displaying the bacterial community composition calculated based on braycurtis distances.
578 (B) Violin plot showing the alpha diversity indices (Chao and Shannon) for all selected orchards. *
579 indicates a significant difference between yield-invigorating (YI) and yield-debilitating (YD) orchards
580 based on Wilcoxon tests ($p < 0.05$). (C) Venn plot depicting the unique and shared bacterial ASVs
581 between yield-invigorating (YI) and yield-debilitating (YD) orchards at ASV and sequence insights.
582 Uni. YI and Uni. YD represent unique ASVs or sequences in the YI or YD soils while Shared represent
583 shared ASVs or sequences between the YI and YD soils. (D) Heatmap displaying the composition of
584 unique and shared ASVs at phylum level in YI and YD soils. Numbers in the cell represent the number
585 of ASVs affiliated to that phylum.



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588 **Fig. 3 Key taxonomic groups in distinguishing yield-invigorating (YI) and yield-debilitating (YD)**
589 **orchards.** (A) Stacked bar chart (left panel) showing dominant phyla affiliation in YI and YD soils for
590 six pair-located sites while horizontal histogram (right panel) depicting relative changes of dominant
591 phyla in YI soils compared to those in YD soils. (B) Genus-level taxonomic analysis of bacterial
592 sequences obtained from yield-invigorating (YI) and yield-debilitating (YD) orchards using the STAMP
593 software. Cyan bars represent the YI soils and pink bars represent the YD soils.

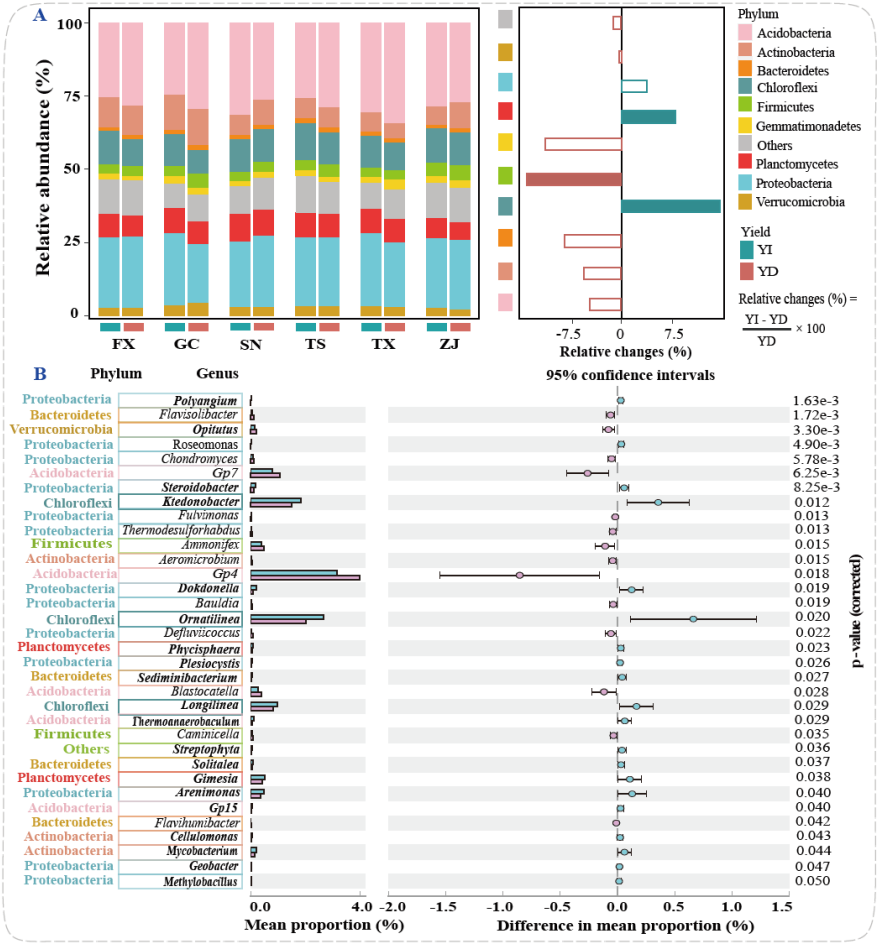

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Fig. 4 Co-occurrence networks of bacterial community and identified keystone taxa in distinguishing yield-invigorating (YI) and yield-debilitating (YD) orchards. (A) An overview of microbial phylogenetic molecular ecological networks constructed from YI and YD soils. Line with blue color indicates positive correlations whereas lines with red color signifies negative correlations in each network. Modules containing larger than five nodes in the networks are labeled with corresponding letter followed by a number. Circular node colors indicate different bacterial phyla. (B) Bubble graph showing the relative abundance of nodes in each module within each network at the phylum level. (C) Venn plot depicting the unique and shared bacterial ASVs between two networks construed from YI and YD soils. Left panel is plotted based on the original nodes used in building network while right panel is plotted based on the nodes from modules. Uni. YI and Uni. YD represent unique ASVs in the YI or YD networks while Shared represent shared ASVs between the YI and YD networks. (D) Zi-Pi plot showing the distribution of nodes based on their topological roles. The threshold values of Zi and Pi for categorizing OTUs were 2.5 and 0.62 respectively. Node colors indicate different bacterial phyla and node size represent the relative abundance in each network.

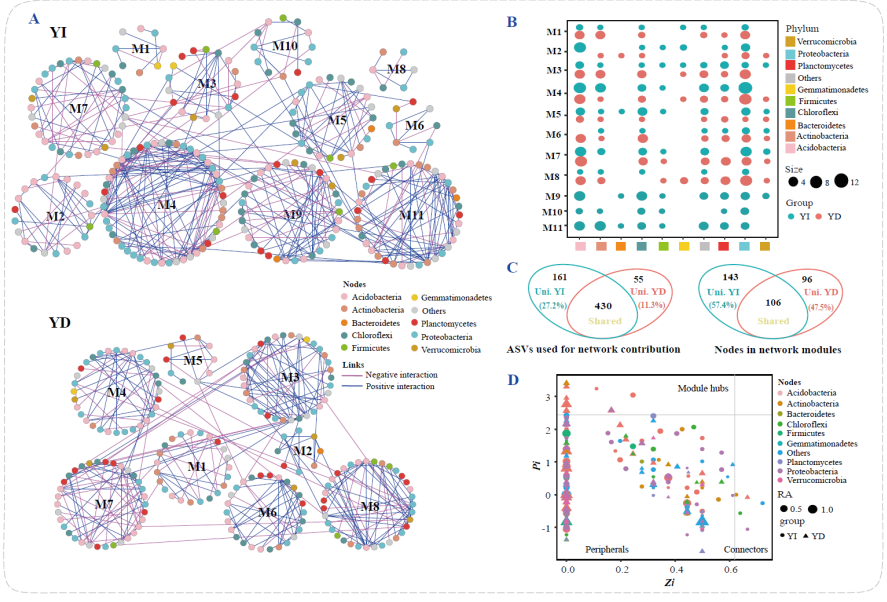

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Fig. 5 Relationships among bacterial community, soil edaphic factors and pear yield. (A) Variance partitioning analysis (VPA) map of the effects of soil edaphic properties, sample locations, pear yield and interactions of these factors on the bacterial community. (B) Redundancy analysis (RDA) plot showing the relationships among all assigned bacterial ASVs and measured soil edaphic properties for all soils after stepwise selection. (C) Random forest mean predictor importance of selected soil edaphic properties used in the as drivers in predicting the pear yield. Red bar indicates that the given predictor is significant while black bar indicates that the given predictor is non-significant. (D) Correlation plot showing the relationship of microbial composition and soil organic matter based on braycurtis distances calculated by Mantel test.

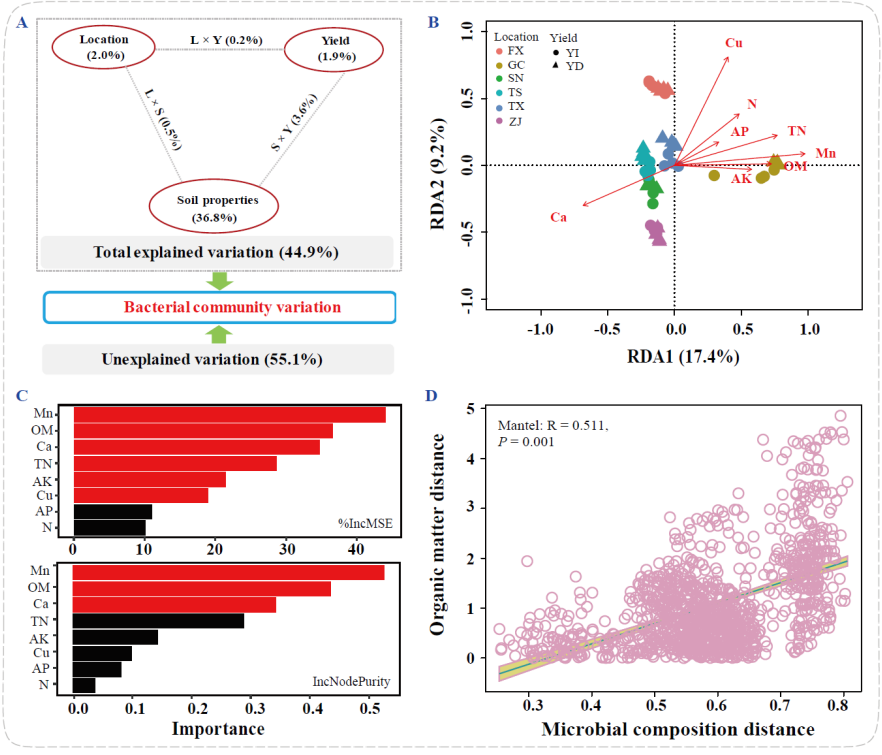

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Fig. 6 Structural equation modeling (SEM) describing the biotic and abiotic factors in affecting the crop production. Structural equation model was built incorporating soil organic matter, microbial biomass, beta diversity (PCoA), key taxa, network hubs including module hubs and network connectors, and yield. The path analysis numbers adjacent to arrows indicate the relationship's effect size and the associated bootstrap *P*-value. Cyan and red arrows indicate positive and negative relationships, respectively. Paths with non-significant coefficients are presented as gray lines.