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RESEARCH ARTICLE

Changes of microbiome in response to sugars in a wilt pathogen-infested soil

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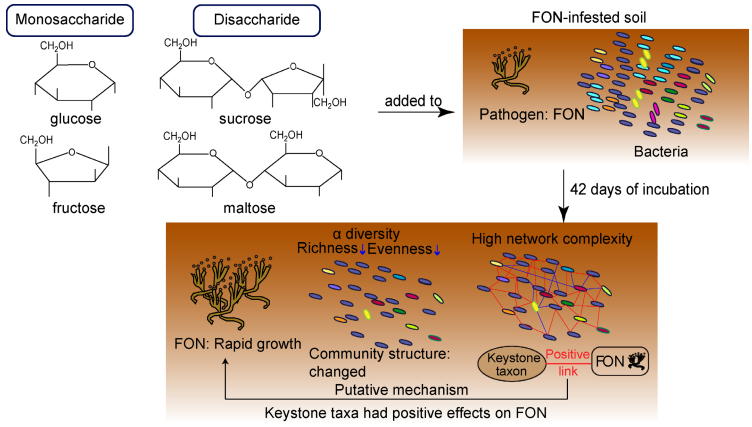
HIGHLIGHTS

- Sugar addition caused vigorous proliferation of wilt pathogen.
- Sugar addition modified bacterial community structure and decreased the diversity.
- Sugar addition caused more complex and connected networks.
- Keystone taxa formed positive links with wilt pathogen in sugar-spiked networks.

GRAPHICAL ABSTRACT



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ABSTRACT

Sugars are frequently and abundantly found in root exudates, but influence of specific sugars on the fate of soil-borne pathogens, microbiome structure, and particularly microbial interactions are not well understood. A 42-day of microcosm incubation was conducted with two soils: a natural watermelon *Fusarium* wilt pathogen (i.e., *Fusarium oxysporum* f. sp. *niveum* (FON))-infested soil (Low-FON soil) and the soil further receiving the wilt pathogen inocula (High-FON soil). Both soils were supplemented with four simple sugars before incubation. The results show that, in both soils, FON was enriched by all sugars although co-living with tremendously diverse microbes; and bacterial richness, evenness, and diversity were decreased and bacterial community structure was changed by all sugars. Bacterial richness and evenness were negatively correlated with FON quantity in both Low-FON and High-FON soils, indicating that FON may tend to live in soil with low alpha-diversity. In both Low-FON and High-FON soils, the sugar-spiked networks had more links, higher density, larger modules, and shorter harmonic geodesic distance, suggesting greater potentials for microbial interaction and niche-sharing. The positive links between some of the keystone taxa and FON indicates that these keystone taxa may have promoted FON. This may be one of reasons why FON could proliferate vigorously after sugar supplementation.

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1 Introduction

Fusarium wilt (also known as Panama disease), caused by the fungus *Fusarium oxysporum* f. sp. *niveum* (FON), is the most destructive soilborne disease of watermelons worldwide (Everts et al., 2014). Once has been introduced, the deleterious fungus, FON, could survive in soil for long periods in the absence of a host primarily because it can produce resistant spore structures (i.e., thick-walled chlamydospores) (Martyn, 2014; Everts and Himmelstein, 2015). Although it has been more than 120 years since E.F. Smith first described the disease, it continues to cause significant economic losses worldwide (Martyn, 2014). *Fusarium* wilt of watermelon occurs on every continent worldwide except Antarctica (Martyn, 2014). In China, since the arable land is limited, watermelon is usually produced on the same land for successive years or in short rotations, and this practice usually results in plant pathogen enrichment and thus more serious plant disease.

The main function of the "hidden" part of the plant, its root system, has traditionally been considered as anchorage and uptake of water and nutrients (Badri and Vivanco, 2009). However, roots can release an enormous range of compounds into the rhizosphere that are known collectively as root exudates, and this ability has been considered as one of the most remarkable metabolic features of root system. By using tracer techniques for labeling root derived C, it is estimated that up to half of photosynthesis-derived C is transferred into the soil as root exudates, forming a diverse chemical milieu (Kuzyakov and Domanski, 2000). A wealthy of literature has reported the versatile roles of root exudate such as acting as carbon and energy resources to feed the rhizosphere microorganisms, serving as signal molecules for plant and symbionts interactions, helping plants to obtain nutrients especially in a nutritionally deficient environment, and etc. There is increasing evidence that root exudates play pivotal roles in pathogen and plant disease development directly or indirectly.

Root exudates could directly influence pathogen or plant disease development though such as allelochemicals within the released compounds (Wu et al., 2010; Liu et al., 2015). The allelochemicals, ferulic acid and sinapic acid, which are two phenolic acids found in plant root exudates, could inhibit the growth and conidial germination of *F. oxysporum* f. sp. *niveum* (Wu et al., 2009; Wu et al., 2010). By contrast, exudates of tomato root have been shown to stimulate the microconidia germination of tomato pathogens *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* and the level of stimulation was affected by plant age, although the signals stimulating *F. oxysporum* germination are still unknown (Steinkellner et al., 2005). However, once treated with chitosan, an elicitor of plant defenses, root exudates from tomato plants could inhibit circa twofold growth kinetics of the tomato root parasitic fungus *F. oxysporum* f. sp. *radicis-lycopersici* (Suarez-Fernandez et al., 2020). A fraction of pea root exudate, pisatin, was reported to be negatively correlated with the extent of *F. oxysporum* f. sp. *pisi* spore germination (Bani et al., 2018). A recent study found that rose plants could reduce root secretion of valine in response to microbial colonization, and thereby reduce the colonization of pathogenic *Agrobacterium* colonization and disease severity (Chen et al., 2021). Root exudates of potato onion were found to play a critical role in alleviating clubroot of Chinese cabbage in a Chinese cabbage-potato onion-Chinese cabbage crop rotation system (Chen et al., 2018). Root exudates from grafted-root watermelon were also found to make a contribution in inhibiting *F. oxysporum* f. sp. *niveum* (Ling et al., 2013).

Released compounds could regulate plant disease development by indirectly modifying the assembly of microbiomes (Li et al., 2014b; Gu et al., 2016). Using the plant, lisianthus (*Eustoma grandiflorum*), combined with *Fusarium* wilt disease as a model system, authors have found that the pathological condition of the plant, lisianthus, was associated with fungal community succession triggered by root exudates in the plant–soil system (Huang et al., 2020). Another study found that (Gu et al., 2016) the changes of the rhizosphere community via the shifting the root exudation profile were partially responsible for the inhibition of the bacterial pathogen *Ralstonia solanacearum*. Similarly, other authors also reported that changing root exudation chemistry to assemble health-promoting microbiomes was one of critical mechanisms by which the plant adapts to biotic stresses (Rolfe et al., 2019). On the other hand, Li et al. (2014b) showed that root exudate of peanut incited the accumulation of peanut-pathogenic fungi at the expense of decreasing the level of potentially beneficial microbes, such as plant growth promoting rhizobacteria and mycorrhizal fungi, and it is the resultant modification in microbial community structure that thereby negatively affected the growth of the peanut plants rather than because of the direct autotoxicity of plant root exudate. In the last few decades, much efforts have been conducted to investigate the effect of allelochemicals, especially phenolic acids within root exudates, on pathogen growth/proliferation (Wu et al., 2010; Ling et al., 2013; Yang et al., 2014; Yang et al., 2015), soil sickness (Li et al., 2014b), and the resultant modulation in microbes (Li et al., 2014b).

Exclude root compounds include low-molecular weight compounds like sugars, amino acids, organic acids, and secondary metabolites and high-molecular weight compounds like mucilage and proteins (Badri and Vivanco, 2009). Among these compounds, sugars are released in large quantities (Kraffczyk et al., 1984; Farrar et al., 2003), comprising up to 65% of the total soluble low-molecular weight root exudates in some plant species (Kraffczyk et al., 1984). What's more, they were even found to be able to inhibit bacterial wilt of tomato caused by *Ralstonia solanacearum* because they stimulated microbial activity and decreased the survival of the pathogen in a limited number of studies (Posas et al., 2007). Sugars are also primary drivers of soil microbial diversity and activity. Although sugars are quantitatively dominant and ecologically significant, it is not yet well understood how specific sugars influence the bacterial

community structure and especially the co-occurrence network, and what's the relationship between the network keystone species and the fate of the wilt pathogen (FON).

In this study, we constructed soil microcosms with a natural watermelon *Fusarium* wilt pathogen-infested soil (which was used as Low-FON soil) and the soil further inoculated with FON (which was used as high level of FON-infested soil). Both soils were supplemented with four simple sugars: two monosaccharides (glucose and fructose) and two disaccharides (sucrose and maltose), which are representative of common sugars found in root exudates. Real-time PCR was applied to examine the quantity of FON and bacteria over the 42 d of incubation. Illumina sequencing was used to investigate the bacterial community changes. Molecular ecological network analysis was applied to understand the bacterial occurrence pattern and interactions and to illustrate the relationship between the network keystone taxa and the fate of the wilt pathogen (FON). We aimed to address three questions: (i) Can the FON be the winner in term of growth in soil environment where tremendously diverse microorganisms co-live, by preferentially utilizing given sugars? (ii) Are sugar-added bacterial community significantly different from that without addition of sugars in terms of structure, diversity, and occurrence pattern? (iii) Are the keystone taxa in the microbial network associated with the fate of the wilt pathogen (FON)?

2 Materials and methods

2.1 Soil sampling

Soil was sampled from the upper 15 cm of a greenhouse field in Huai'an, Jiangsu Province, China (119°5′ E, 33°30′ N), where *Fusarium* wilt was a consistent problem. The region is an important watermelon-producing area in China. The soil has a sandy loam texture. Soil initial physicochemical characteristics were presented as follows: pH (H_2O) 7.88, sand (2–0.02 mm) 64.0%, silt (0.02–0.002 mm) 22.7%, clay (< 0.002 mm) 13.3%, organic matter 22.2 g kg^{-1}, soil water holding capacity 58.5%, total N 1.55 g kg^{-1}, available P 40.0 mg kg^{-1}, and available K 48.0 mg kg^{-1}.

2.2 FON conidia preparation

The wilt pathogen FON (coded NJAUS-1) was provided by the Laboratory of Plant-Microbe Interactions, Nanjing Agricultural University, China. FON was cultured in a liquid mung bean medium (Bai et al., 2002) at 28°C for 4 days to generate conidia. Then, the liquid culture was filtered through a four-layer of steriled cheesecloth to remove mycelial fragments. To collect conidia, the filtrate was centrifuged at 10 000 r min^{-1} for 10 min in an Eppendorf Centrifuge 5810R (Eppendorf, Hamburg, Germany) and the supernatant was discarded. The precipitated conidia were washed three times with sterile distilled water and suspended in sterile distilled water. The conidia concentration of the suspension was determined using a hemacytometer with a microscope.

2.3 Microcosm experiment

Two soils (1200 g for each soil based on dry weight) were prepared. One soil was inoculated with FON to reach a concentration of $\times$ 10^5 conidia g^{-1} soil. The other soil was added with sterile distilled water instead of FON conidia suspension. The FON-inoculated soil is thereinafter designated as High-FON soil, and the soil without inoculation is designated as Low-FON soil. The High-FON and Low-FON soils were divided into five subsoils at an equal weight, respectively. Glucose, fructose, sucrose, and maltose were dissolved in sterile water to reach a concentration of 100 mg C mL^{-1}. These four stock solutions were added into four subsoils from the FON-inoculated treatment, respectively, to reach a concentration of 8 mg C g^{-1} dry soil. The amount of C used in our study is comparable to that used in many other studies since several mg C per gram soil was usually used when studying the involvement of root exudates in the development of soil microbial community structure (Kozdrój and van Elsas, 2000; Schutter and Dick, 2001). The subsoils receiving glucose, fructose, sucrose, and maltose from the High-FON soil were referred to as FON + Gluc, FON + Fruc, FON + Suc, and FON + Mal soil, respectively. The remaining subsoil was added with sterile water instead of sugar. This subsoil was used as control and referred to as FON. In the same manners as that used in the High-FON soil, four subsoils from the Low-FON soil were added with glucose, fructose, sucrose, and maltose stock solutions, respectively and these soils are referred to as Gluc, Fruc, Suc, and Mal soils, respectively. The remaining subsoil from the Low-FON soil was treated with sterile water rather than sugars, and this subsoil was used as control (CK).

For each treatment, 6 g soil (based on dry weight) was weighted and put into 120 mL serum bottles. Soil moisture was then adjusted to 60% of the SWHC (soil water holding capacity) by adding sterile water. The bottles ware capped with butyl stoppers. A total of 36 soil microcosms were prepared for each of CK and FON treatments, and 33 soil microcosms were prepared for each of the sugar-spiked treatments in both Low-FON and High-FON soils. Triplicate soil microcosms from the CK or FON treatment were taken and stored at 4°C before use, and these samples were used as zero time (T0) samples. The remaining soil microcosms (33 microcosms for each treatment) were incubated at 28°C. On days 1, 2, 3, 4, 5, 6, 7, 9, 12, 21, and 42, triplicate soil samples from each treatment were collected for DNA extraction. The headspace of bottles was flushed with synthetic air (20% O_2 and 80% N_2) for 45s at 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, 14 d, 16 d, 18 d, 21 d, 23 d, 26 d, 29 d, 30 d, 33 d, 37 d, and 42 d.

2.4 Soil DNA extraction and real-time quantitative PCR

Soil DNA was extracted from 0.5 g soil using the FastDNA^{TM}

Spin Kit for Soil (MP Biomedicals LLC, Ohio, USA) according to the manufacture's pipeline. The biomass of FON was assessed by quantifying the ITS gene using the primer set Fon-1 (5′- CGATTAGCGAAGACATTCACAAGACT-3′) and Fon-2 (5′- ACGGTCAAGAAGATGCAGGGTAAAGGT-3′) with real-time quantitative PCR (qPCR), producing a 174 bp DNA fragment (Lin et al., 2010). The qPCR standard was made via a 10-fold serial dilution of plasmid harboring the aimed gene. The qPCR was carried out in a 20 μL reaction mixture consisting of 10 μL 2 × SYBR® *Premix Ex Taq*™ (TaKaRa Biotech, Dalian, China), 0.4 μL each primer (10 μmol L^{-1}), 0.4 μL ROX Reference Dye II (50 ×), 1 μL template DNA, and 7.8 μL ddH_2O. The qPCR was performed in a 7500 Real Time PCR System (Applied Biosystems Life Technologies, Foster City, USA) with an initial denaturing temperature of 94°C for 30 s, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C 30 s. Blanks were always run with water as template instead of DNA. For each DNA sample, three technical replications were included when qPCR for soil samples was conducted. Similar to that adopted to quantify the ITS gene of FON, the bacterial 16S rRNA gene was quantified with the qPCR technique to assess the bacterial biomass by replacing the primer set and changing the thermocycling profile. The primer set targeting the V4–V5 region of the bacterial 16S rRNA gene was 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The thermal profile was as follows: 94°C for 30 s, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C 30 s.

2.5 Illumina sequencing

For the DNA samples from T0, 1, 3, 5, 9, and 42 d, the bacterial 16S rRNA gene was amplified using the primer set 515F (5′-GTGCCAGCMGCCGCGG-30) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). To resolve different samples, each primer was fused with an 8-bp barcode. A 20 μL reaction mixture contained 4 μL of 5 × *TransStart*® *FastPfu* PCR buffer (Mg^{2+} plus), 0.25 mM of each deoxynucleoside triphosphate, 0.2 μM of each primer, 0.4 μL of *TransStart*® *FastPfu* DNA Polymerase (TransGen Biotech, Beijing, China), and 10 ng of template DNA. The PCR was performed in an ABI 9700 GeneAmp® PCR system (Applied Biosystems Inc., Foster City, USA) with an initial denature at 95°C for 3 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C 45 s. Negative controls using water as template rather than soil DNA were always run. Each DNA sample was amplified with three technical replicates. The PCR products were extracted from a 2.0% agarose gel and purified with a AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, USA). The concentration of the PCR amplicons was determined with QuantiFluor™-ST (Promega, Madison, USA). Amplicons of all samples were pooled in equimolar concentrations, and then paired-end sequencing (2 × 300 bp) was carried out on the Illumina MiSeq platform at the Shanghai Majorbio Biopharm Technology Company (Shanghai, China).

2.6 Sequence analysis

The Quantitative Insights Into Microbial Ecology (QIIME) pipeline (http://qiime.org/) was used to analyze the Illumina sequence of 16S rRNA genes. Raw sequences were assigned to each sample based on the barcode. Illumina sequences were trimmed with the trimmomatic tool (http://www.usadellab.org/cms/index.php?page = trimmomatic) (Bolger et al., 2014) by processing the steps as follows: (i) Remove trailing low quality bases (below quality 20), (ii) scan the read with a 50-base wide sliding window, cutting when the average quality per base drops below 20, and (iii) drop sequence reads below the 50 bases long. Paired sequences were merged with the FLASH software (http://ccb.jhu.edu/software/FLASH). Sequences were clustered to operational taxonomic units (OTUs) at a 97% similarity level using UPARSE (Edgar, 2013). The taxonomic classification of OTUs was obtained based on the Silva database. Total sequence number of each sample was summarized based on the OTU table. The original sequence data were deposited at the European Nucleotide Archive with accession number PRJEB29254.

Sequences of each sample were subsampled to the same sequence depth (10 290 sequences per sample) to bring the Illumina sequence data onto a common scale. The relative abundance of each phylotype at different taxonomic levels was summarized. Principal coordinate analysis (PCoA) and hierarchical clustering were performed based on the Bray-Curtis distance. Three complementary non-parametric analyses for multivariate data (Zhou et al., 2012), i.e., the analysis of similarities (ANOSIM) (Clarke, 1993), non-parametric multivariate analysis of variance using distance matrices (adonis) (Anderson, 2001), and a multi-response permutation procedure (MRPP) (Mielke and Berry, 2001; McCune and Grace, 2002), were conducted to test community structure differences between treatments. The Bray-Curtis distance was used in the ANOSIM, adonis, and MRPP analyses, and the Monte Carlo permutation was used to test the statistical significance. All three procedures (ANOSIM, adonis, and MRPP) were performed with the Vegan package in R software.

2.7 Molecular ecological network analysis

We constructed the molecular ecological networks (pMENs) (Deng et al., 2012) to understand the interactions among community members. Based on the subsampled OTU data, the co-occurrence network (i.e., pMEN) was constructed according to the molecular ecological network analyses pipeline (MENAP) (http://129.15.40.240/mena/) with the default parameters. The advantage of this approach is that the threshold to construct the network is automatically determined, and this approach shows remarkable capacity in tolerating noise, thus resulting in reliable, robust networks (Deng et al., 2012). At least eight replicate data sets are required for pMENs construction, and thus, we merged data

from CK samples of all time points into CK data set, data from FON samples of all time points into FON data set, data from Gluc, Fruc, Suc, and Mal treatments at each time point into Sugar-treatment data set, and data from FON + Gluc, FON + Fruc, FON + Suc, and FON + Mal treatments at each time point into FON + Sugar data set. These samples were merged as (i) In the Low-FON soil, the CK samples of all time points clustered closely in the PCoA data space and were distributed distantly from the Sugar-spiking treatment (Fig. S1), suggesting similar community structure in the CK soil samples of all time points but different community structure between CK and sugar-spiking treatment; and (ii) Similarly, in the High-FON soil, the FON samples from all time points clustered closely in the PCoA space and were distributed separately from the sugar-added treatment, and the FON + Gluc, FON + Fruc, FON + Suc, and FON + Mal samples of each time point clustered closely in the PCoA space (Fig. S1). For each data set, only OTUs detected in more than half of replicate samples were used for network construction. Network graphs were visualized using Cytoscape 3.2.1 software (Shannon et al., 2003).

Modules were detected with the greedy modularity optimization method (Deng et al., 2012). Nodes (i.e., OTUs) within a module are linked more tightly together than they are to nodes in other modules (Olesen et al., 2007). The connectivity of each node was determined by its within-module connectivity (*Zi*), which describes how well a node is connected to other nodes in the same module, and among-module connectivity (*Pi*), which reflects what degree a node connects to different modules (Guimerà and Amaral, 2005). The topological roles of individual nodes can be assigned based on *Zi* and *Pi*. In this study, the topological roles of individual nodes are divided into four categories: (i) Peripheral nodes ($Zi \leqslant 2.5$, $Pi \leqslant 0.62$), which have only a few links inside its own module and rarely any to other modules, (ii) Connectors ($Zi \leqslant 2.5$, $Pi > 0.62$), which are highly connected to several modules and "glues" modules together, (iii) Module hubs ($Zi > 2.5$, $Pi \leqslant 0.62$), which are highly connected to many nodes in their own modules and thus important to coherence of their own modules, and (iv) Network hubs ($Zi > 2.5$, $Pi > 0.62$), which act as both module hubs and connectors (Olesen et al., 2007; Deng et al., 2012). The latter three are termed generalist while peripherals are specialists.

3 Results

3.1 Changes in the abundance of FON and total bacteria

A Real-time PCR was carried out to quantify the FON population to assess the effects of different sugars on the growth of FON (Fig. 1). In both the Low-FON (i.e., FON uninoculated soil) and the High-FON (i.e., FON inoculated soil) soils, the abundance of FON was higher in sugar-added treatments than no sugar addition treatment, and this observation indicated that the tested four sugars promoted the growth of the wilt pathogen, FON. However, no one sugar caused the largest increase in FON abundance at all time points. Furthermore, the promotion effect induced by sugars was strong at the beginning and became weak over time. Specifically, in the Low-FON soil, after 1 d of incubation, the quantity of the ITS gene of FON strongly increased to 7.08×10^4, 6.08×10^4, 3.99×10^4, 3.83×10^4 copies g^{-1} dry soil under Gluc, Fruc, Suc, Mal treatments, respectively, resulting in 1.54–3.69 times larger than that in the original soil (1.51×10^4 copies g^{-1} dry soil) and the 1d-CK soil (1.51×10^4 copies g^{-1} dry soil) (Fig. 1A). The extent of the increase became weak gradually from 2 d to 42 d. Until 42 d, the abundance in the Gluc, Fruc, Suc, Mal treatments was only 0.06–1.02 times higher than that in the CK. Similar observation was found in the High-FON soil: the quantity of the ITS gene of FON in Sugar-added soil reached 9.94–21.7 times larger than that in sugar-unadded soil at 1 d but lowed to only 0.26–9.35 times higher at 42 d.

In addition to FON, all of the bacteria may have a better growth by utilizing given sugars as carbon/energy sources. A strong increase of the bacteria quantity was found after the addition of sugars at the beginning (1 d) in both the Low-FON and High-FON soils (Fig. S2), which was similar to the observation in FON. The promotion effect induced by sugars was relatively stable until 21 d and became weak at 42 d (Fig. S2).

3.2 Bacterial diversity

Illumina sequencing technique was applied to understand the effect of sugars on the bacterial diversity. The T0, 1, 3, 5, 9, and 42 d samples were subjected to Illumina sequencing. After quality filtering, 3 671 668 high quality sequences were recovered across all 156 samples (i.e., average = 23 536 sequences per sample) (Table S1). Observed OTU number, Heip's evenness index, and Shannon index were used to assess bacterial richness, evenness, and diversity, respectively, all three calculated with 10 290 rarefied sequences per sample. In both Low-FON and High-FON soils, the bacterial richness, evenness, and diversity were significantly ($P < 0.05$) lower under all of the sugar treatments throughout the 42 days of incubation (12.8%–64.5% lower for observed OTU number, 46.2%–95.1% lower for Heip's evenness index, 11.4%–54.3% lower for Shannon index), although the extent of the decrease resulting from the addition of sugars became weak at 42 d compared with that from 1 to 9 d (Fig. 2).

The correlation between the bacterial diversity and the FON quantity (assessed by the ITS gene copy number of FON with qPCR technique) was analyzed further. Both in Low-FON and High-FON soils, the bacterial richness and evenness were negatively correlated with the FON quantity (Low-FON soil: $r = -0.581$ for richness, $r = -0.442$ for evenness; High-FO soil: $r = -0.636$ for richness, $r = -0.498$ for evenness; $P < 0.05$ in all cases) (Fig. S3), indicating that FON tended to live in soil with low bacterial richness and evenness, whether the wilt pathogen was at a low level or at a high level in the original soil.

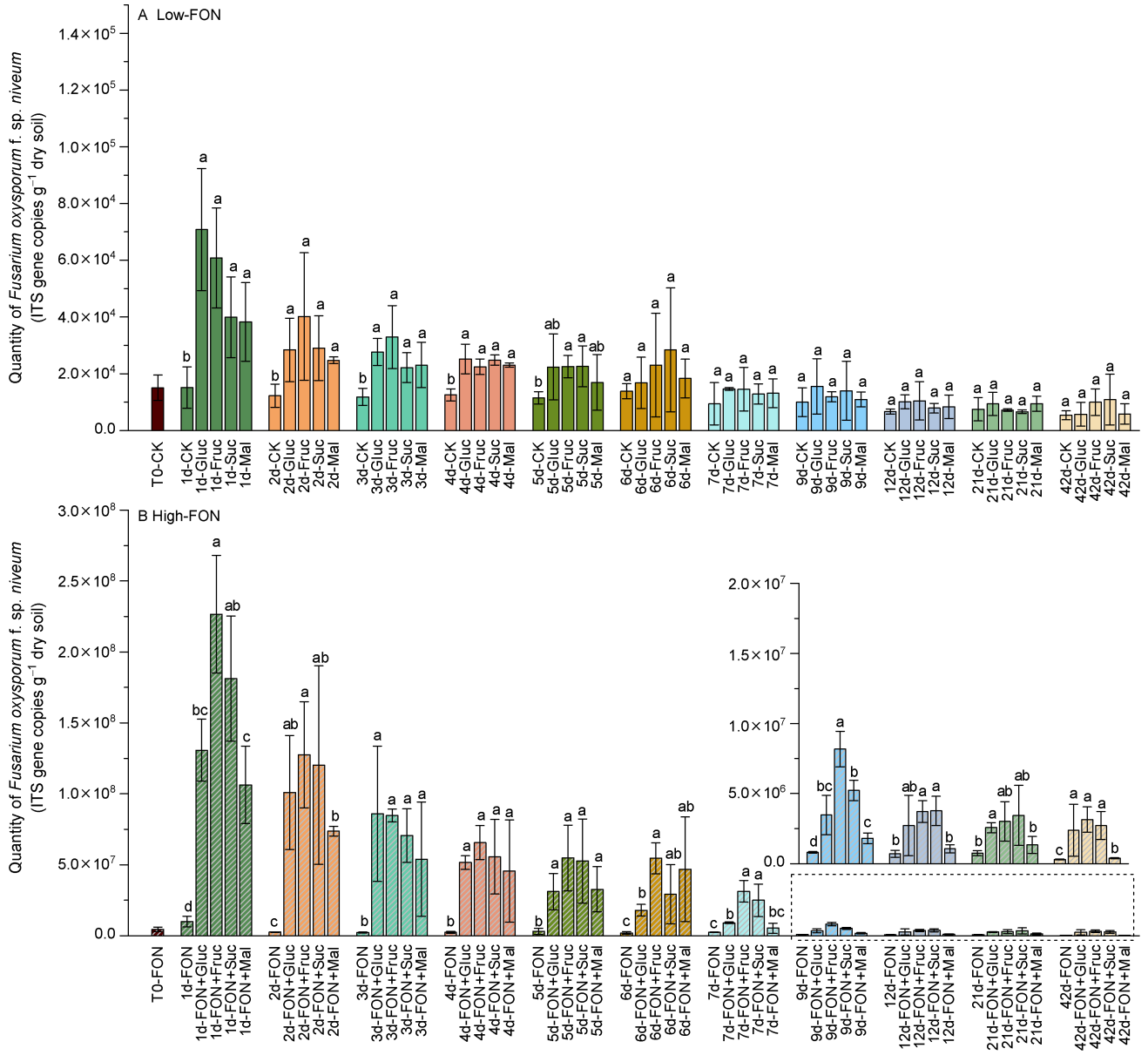


Fig. 1 *Fusarium oxysporum* f. sp. *niveum* quantity dynamics over a 42 d period in Low-FON (A) and High-FON (B) soils determined by quantifying the ITS gene of *F. oxysporum* f. sp. *niveum* with real-time PCR technique. The soil without or with *F. oxysporum* f. sp. *niveum* was referred to as Low-FON or High-FON soil, respectively. The designations CK, Gluc, Fruc, Suc, and Mal indicate that the soils were added with H_2O, glucose, fructose, sucrose solutions, respectively. The designation FON denotes the soils with FON inoculation. The designations FON + Gluc, FON + Fruc, FON + Suc, FON + Mal denote that the soil was not only inoculated with FON but also supplied with glucose, fructose, sucrose, and maltose solution, repsectivley. T0 denotes zero time and 1 d, 2 d, 3 d, …42 d denote samples collected after 1 day, 2 days, 3 days, …42 days of incubation. Different letters indicate significant differences by Duncan's multiple range test ($P < 0.05$).

3.3 Bacterial community structure

The changes in bacterial community structure were depicted in the principal coordinate analysis (PCoA) plot and clustering tree. At the time point of 1, 3, 5, 9, and 42 d, samples that were subjected to sugar addition and samples that received no sugars always distributed distantly in PCoA space (Fig. 3) or clustered into different group in the dendrogram tree (Fig. S4) in both Low- and High-FON soils. Significant differences ($P < 0.05$) in bacterial community structure between the sugar-spiking treatment and no-sugar treatment at all time points were detected by three non-parametric multivariate statistical tests (ANOSIM, adonis, and MRPP). All of the observations indicated that treatment with sugars changed the bacterial community structure. The significant impact of sugars on the bacterial community structure lasted throughout the whole 42 d of incubation (Table S2), although the impact was weaker at 42 d compared to that at previous time points (Fig. 3).

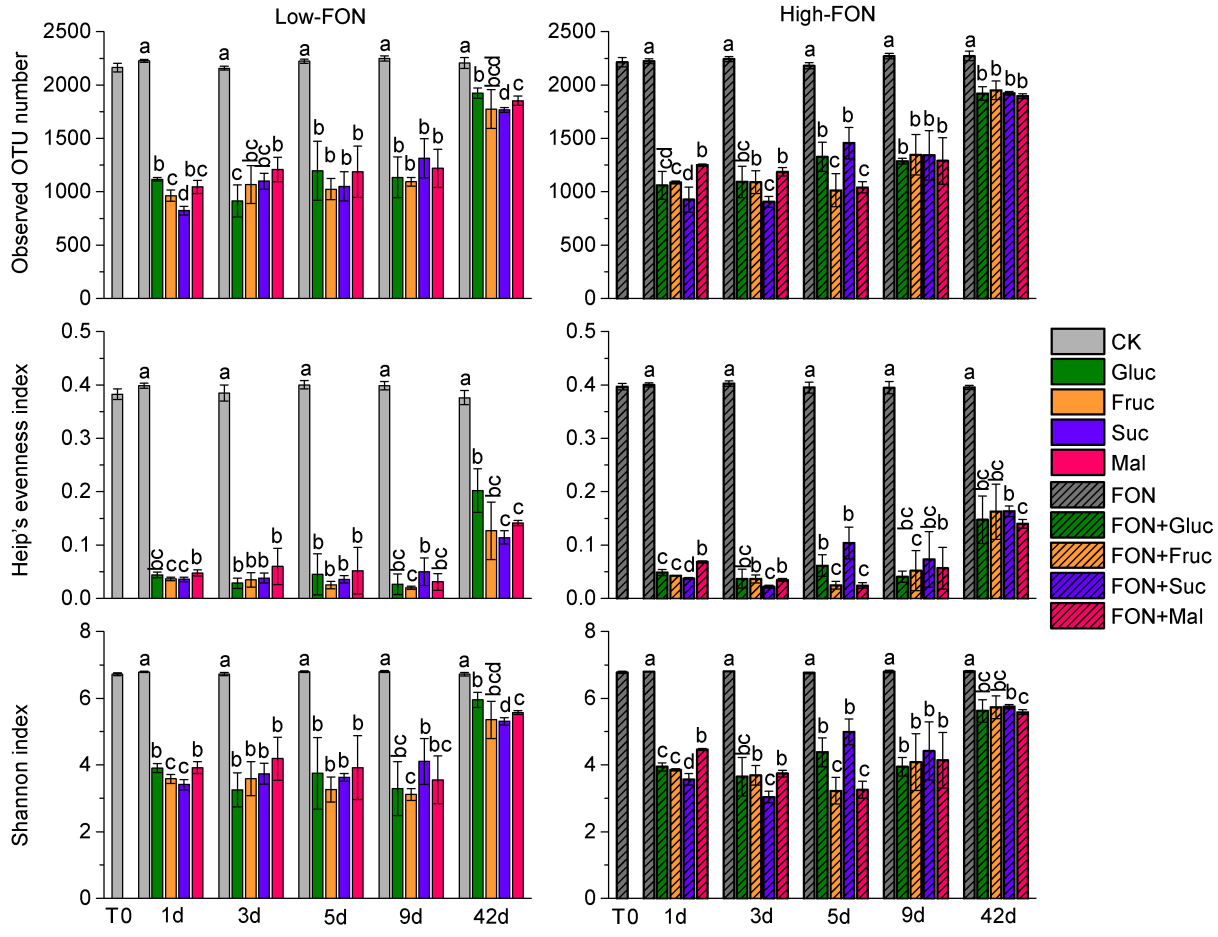


Fig. 2 Observed OTU number, Heip's evenness index, and Shannon index in Low-FON (i.e., FON-uninoculated) and High-FON (i.e., FON-inoculated) soils. The OTUs, Heip's evenness index, and Shannon index were obtained by using 10 290 subsampled sequences from each sample. The designations Gluc, Fruc, Suc, and Mal denote the soil with the supplementation with glucose, fructose, sucrose, and maltose solutions, respectively, while CK denotes the soil with the supplementation with water. FON denotes the soil with FON inoculation. FON + Gluc, FON + Fruc, FON + Suc, and FON + Mal represent the soils that were not only inoculated with FON but also supplied with glucose, sucrose, fructose, and maltose solutions, respectively. T0 denotes zero time, and 1 d, 3 d, 5 d, 9 d, and 42 d indicate that the samples were collected after 1 day, 3 days, 5 days, 9 days, and 42 days of incubation. Different letters denote significant differences by Duncan's multiple range test ($P < 0.05$). The error bars represent the standard deviation of the means.

However, soils receiving Gluc, Fruc, Suc, and Mal distributed closely in the PCoA plot (Fig. 3) or clustered in the dendrogram tree (Fig. S4) in both the Low-FON soil and in the High-FON soil and three statistical tests (ANOSIM, adonis, and MRPP) further detected no significant differences ($P > 0.05$) between the four sugar-treatments in both the Low-FON and High-FON soils (Table S2). These observations indicated similar bacterial community structures in the Gluc-, Fruc-, Suc-, and Mal-added soils no matter the original soil contained a low or a high level of wilt pathogen, FON.

3.4 Bacterial phylotypes at the high taxonomic level

To understand which phylotypes were affected by sugars, the relative abundance of main bacterial populations (those phylotypes with relative abundance >0.5% in at least one treatment were included) was summarized at the phylum/class level (Fig. 4). As shown in Fig. 4, before sugar addition, ten phylotypes including Alphaproteobacteria (9.36%–11.1%), Betaproteobacteria (8.57%–10.3%), Deltaproteobacteria (4.40%–5.53%), Gammaproteobacteria (9.21%–11.2%), Firmicutes (3.38%–5.81%), Acidobacteria (15.7%–17.2%), Actinobacteria (8.85%–12.6%), Bacteroidetes (3.85%–6.28%), Chloroflexi (7.78%–8.81%), Planctomycetes (5.38%–7.52%) were the shared and abundant populations in both Low-FON and High-FON soils, comprising 84.4%–86.7% of all sequences. Evident changes were observed in terms of their relative abundance after sugar addition. Of them, Firmicutes was found to be the most shifted phylotype in terms of relative abundance at 1 d while Gammaproteobacteria was the most shifted phylotype from 3 d to 42 d. Specifically, at 1 d, the relative abundance of the Firmicutes

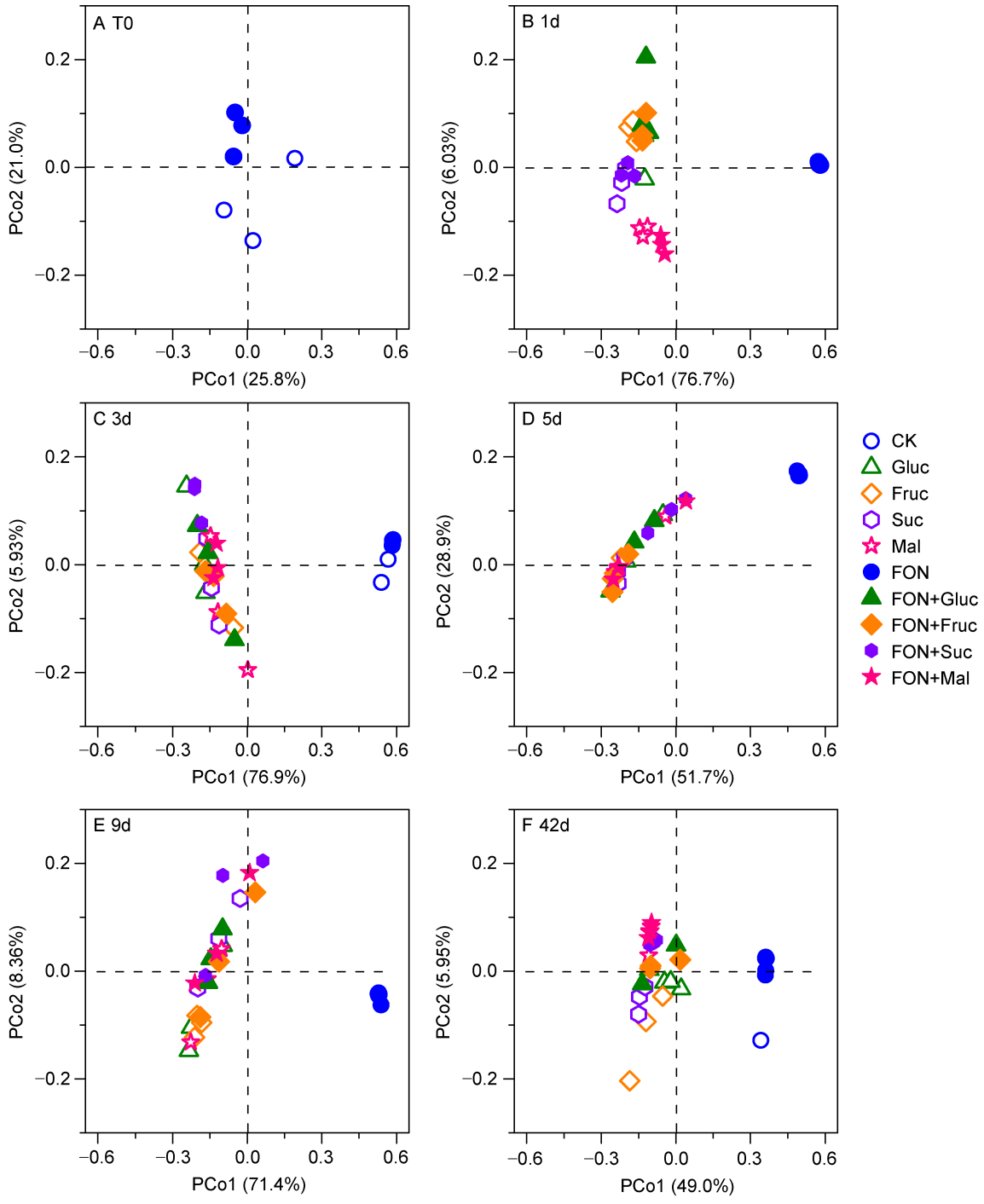


Fig. 3 Principal coordinates ananlysis (PCoA) of bacterial communities from the samples collected at zero time (A) and after 1 day (B), 3 days (C), 5 days (D), 9 days (E), and 42 days (F) of incubation. The percentages in parentheses for each axis represent the proportion of total variation explained. The PCoA plot was obtained based on the Bray-Curtis distances at a depth of 10 290 subsampled sequence reads per sample. All other designations are the same as those in Fig. 2.

increased significantly from 5.81% at sugar-unadded treatment to 68.2%–76.2% at sugar-added treatment (P = 2.92E-11 to 5.68E-11) in the Low-FON soil and increased significantly from 4.99% to 57.5%–74.4% (P = 6.70E-13 to 1.08E-11) in the High-FO soil, which resulted in 10.7–12.1 times increase in Low-FON soil and 10.5–13.9 times increase in High-FON soil. Firmicutes hence became the most dominant phylotype at 1 d after sugar addition. From 3 d to 42 d, the relative abundance of Gammaproteobacteria increased significantly from 10.0%–11.2% at sugar-unadded treatment to 30.4%–73.9% (P = 6.13E-8 to 1.08E-4) in Low-FON soil and from 9.21%–10.7% to 33.6%–72.7%

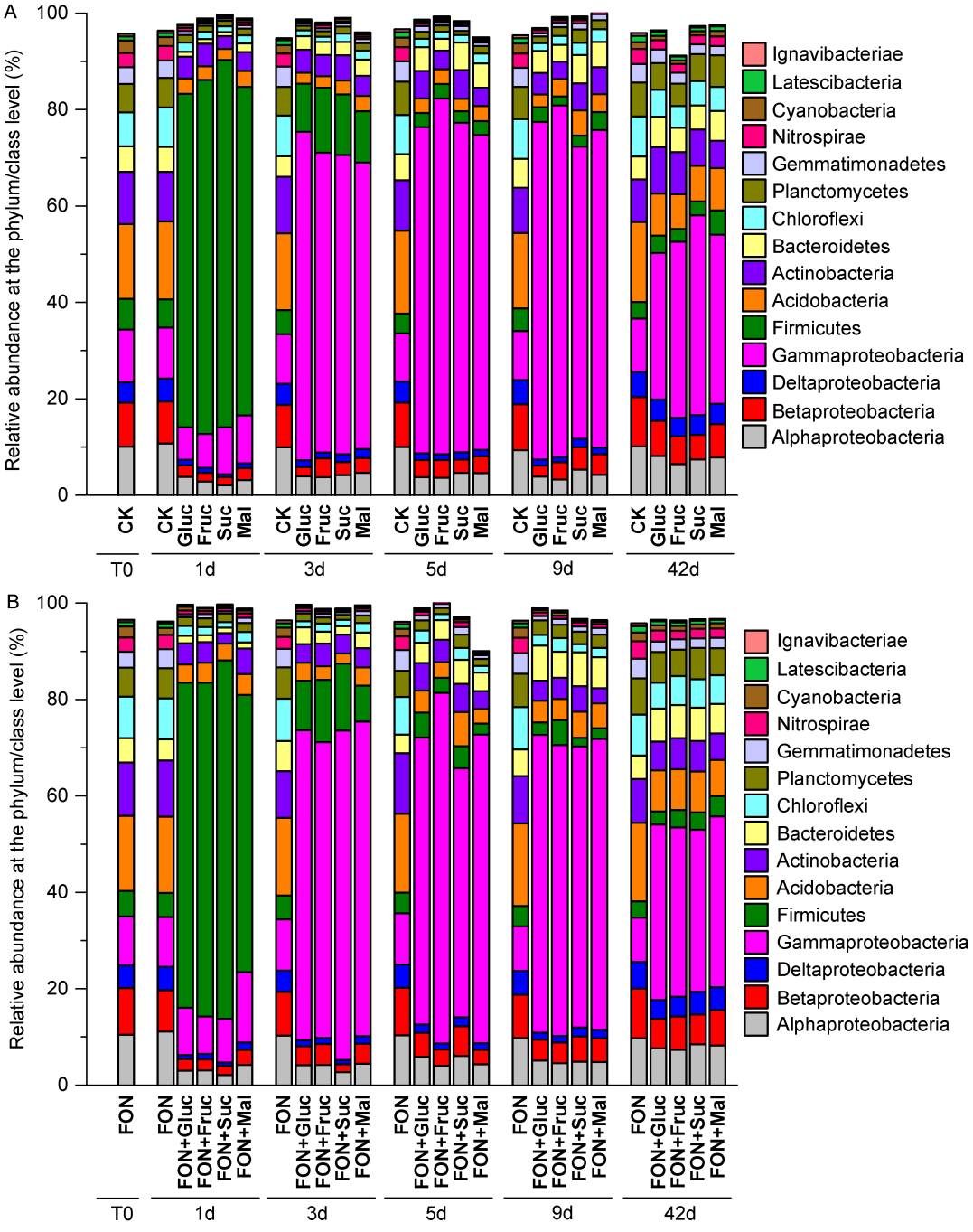


Fig. 4 Relative abundance of bacterial phylotypes at the phylum or class (only for Proteobacteria) level in Low-FON (A) and High-FON (B) soils. The phylotypes which had relative abundance >0.5% in at least one treatment are presented. All other designations are the same as those in Fig. 2.

(P = 2.11E-9 to 4.71E-04) in High-FON soil, which was 1.72–6.37 and 2.65–5.82 times increase in Low-FON and High-FON soils, respectively. Gammaproteobacteria hence became the most dominant phylotype in sugar-added treatment in both Low-FON and High-FON soils from 3 d to 42 d. The significant increase of Firmicutes at 1 d and Gammaproteobacteria at 3–42 d in relative abundance led to a much uneven bacterial community, consistent with the above result which showed a significant decrease in Heip's evenness index.

The absolute abundance of these 15 main phyla/classes were further assessed (Fig. S5) by using the following method: bacterial abundance obtained by qPCR (Fig. S2) × relative abundance of each phylum/class (Fig. 4), to understand which phylotype was promoted, suppressed, or unaffected by sugar treatments. As shown in Fig. S5, whether in Low-FON and High-FON soils, the abundance of Firmicutes and Gammaproteobacteria in all the sugar-addition soils was significantly higher ($P < 0.05$) than that in no sugar-addition

soil throughout 42 d of incubation, and this observation indicated that the growth of these two phylotypes were promoted by sugar treatments. Nevertheless, for Firmicutes and Gammaproteobacteria, the extent of the increase by sugars in abundance differed greatly at different time points. At 1 d, the abundance of Firmicutes in sugar-added soil was 34.5–54.1 times higher than in sugar-unadded soil, while Gammaproteobacteria revealed only a 0.90–4.06 times increase in Low-FON and High-FON soils. However, from 3 d to 42 d, Gammaproteobacteria had a much higher abundance increase (8.14–37.8 times higher than control) in sugar-added soils than Firmicutes (only 0.66–12.9 times higher). As for the other 13 main phylotypes, after 42 d of incubation, almost all of them (except Ignavibacteria) showed a better growth under sugar treatment based on the observation that a significant higher abundance was observed in sugar-spiking soil compared with sugar-unadded soil in almost all cases (except for a non-significant effect by Fruc in Low-FON soil and by Mal in High-FON soil at 42 d) in both Low-FON and High-FON soils.

3.5 Bacterial phylotypes at the low taxonomic level

To further understand which bacterial population was affected by sugars at a finer level, the absolute abundance of the main genera (those genera with relative abundance >1.5% in at least one treatment were included) was assessed using the following calculation method: the absolute quantity of 16S rRNA gene determined by qPCR technique × the relative abundance of each genus. As shown in Fig. S6, in both Low-FON and High-FON soils, the absolute abundance of following 7 populations, *Bacillus* (affiliated with Firmicutes), *Hydrogenophaga* (affiliated with Burkholderiales within Betaproteobacteria), and 5 Gammaproteobacteria-affilated genera, *Azotobacter*, an unclassified genus within the family Pseudomonadaceae, *Pseudomonas*, *Lysobacter*, and *Pseudoxanthomonas* at sugar-spiking treatments was significantly higher than that at no-sugar treatments throughout 42 d of incubation, and this observation indicated that the growth of these 7 genera were promoted by sugars over 42 d of incubation. *Bacillus* was promoted the most at 1 d while *Azotobacter* was promoted the most from 3 to 42 d. For the other 14 genera, the effect of sugars on their absolute abundance was variable at different points (increase, decreased, or unaffected).

For the above 7 promoted genera and FON, the extent of the increase in absolute abundance was analyzed to assess which population was more strongly promoted by the sugar-added treatment by calculating the fold increase with the following method: (Abundance in sugar-spiked treatment – Abundance in no-sugar treatment) ÷ Abundance in no-sugar treatment. As shown in Fig. 5, after only 1 d of incubation, the extent of the increase in abundance of the following two genera, *Bacillus* and *Azotobacter*, exceeded that of FON under all sugar treatments in both Low-FON and High-FON soils, which indicated a more rapid biomass increase by all the sugars at 1 d. Additionally, two Pseudomonadaceae-affiliated genera, *Pseudomonas* and an unclassified genus, also showed a more rapid abundance increase by all the sugars in the Low-FON soil at 1 d. From 3 d to 42 d, the extent of the increase in abundance of almost all genera (6 genera: *Hydrogenophaga*, *Azotobacter*, an unclassified genus within Pseudomonadaceae, *Pseudomonas*, *Lysobacter*, and *Pseudoxanthomonas*) exceeded that of FON in the Low-FON soil, suggesting a more rapid proliferation of these 6 populations than the wilt pathogen (i.e., FON) when the FON was at a low level in the original soil. Furthermore, in the High-FON soil, *Hydrogenophaga*, *Azotobacter*, and an unclassified genus within Pseudomonadaceae also suggested a more rapid abundance increase under all sugar treatments than the FON from 3 d to 42 d. *Pseudomonas*, *Lysobacter*, and *Pseudoxanthomonas* additionally revealed a rapid abundance increase under all the sugar treatments than FON at 9 d and *Pseudomonas* and *Pseudoxanthomonas* suggested a more biomass increase by all sugars at 42 d in the High-FON soil.

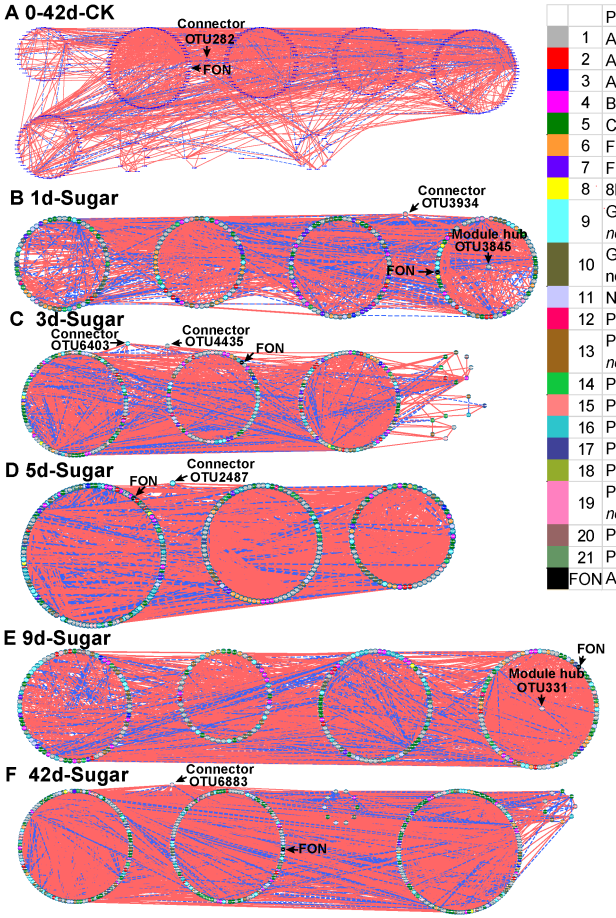
3.6 Molecular ecological network

We constructed phylogenetic molecular ecological networks (pMENs) to understand the microbial occurrence patterns and hence identify potential microbe-microbe interactions in response to sugars (Fig. 6). The assemblages in sugar-spiking soils formed more complex and more closely connected networks than that in sugar-unadded soil at all time points in both Low-FON and High-FON soils, although the network size was variable at different time (Fig. 6A–F, Fig. 6H–M). Specifically, in Low-FON and High-FON soils, sugar-treated networks contained more connections (links) between nodes than sugar-unadded networks at all time points, which increased the density of connections in sugar-spiking soils and created more intricate network pattern (Fig. 6A–F, Fig. 6H–M, Table S3). The increased complexity in the sugar-treated networks was also reflected by the increased average degree (i.e., average links per node), as well as the shorter harmonic geodesic distance (Table S3) (Shi et al., 2016). Additionally, a smaller average path distance in sugar-treated networks at all time points in Low-FON and High-FON soils indicated a closely connected network under sugar treatments. Overall, taxa tended to co-occur (positive correlations, red lines) rather than co-exclude (negative correlations, blue lines), with positive correlations 75%–87% and 69%–85% of the potential interactions at all time points in the Low-FON and High-FON soil, respectively (Fig. 6A–F, Fig. 6H–M, Table S3). A module in the network is a group of OTUs which are well connected among themselves, but are less connected with OTUs outside the group. Sugar-spiking treatment tended to form less but larger modules while no-sugar treatment tended to form more but smaller modules: 10 modules at CK versus 3–6 modules at sugar-spiking treatment in Low-FON soil; 9 modules at FON versus 3–7 modules at sugar-spiking treatment in High-FON soil (Fig. 6A–F, Fig. 6H–M).

		Abundance (10^8 copies g^{-1} dry soil)							Abundance (10^5 copies g^{-1} dry soil)	Increment fold (Sugar-added treatment - no sugar-added treatment)/no sugar-added treatment							
	Phylum	Firmicutes	Proteobacteria							Firmicutes	Proteobacteria						FON
	Class; Order; Family; Genus	Bacilli; Bacillales; Bacillaceae; *Bacillus*	Betaproteobacteria; Burkholderiales; Comamonadaceae; *Hydrogenophaga*	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Azotobacter*	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; unclassified	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*	Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Lysobacter*	Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Pseudoxanthomonas*	FON	Bacilli; Bacillales; Bacillaceae; *Bacillus*	Betaproteobacteria; Burkholderiales; Comamonadaceae; *Hydrogenophaga*	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Azotobacter*	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; unclassified	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*	Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Lysobacter*	Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Pseudoxanthomonas*	
		6	12	16	17	18	20	21		6	12	16	17	18	20	21	
1d	CK	38.7±3.1 c	0.43±0.15 d	0.25±0.06 c	0.27±0.08 d	9.44±1.62 d	20.2±1.7 d	1.62±0.15 c	0.15±0.07 b								
	Gluc	2225±130 b	2.58±0.46 b	31.3±6.2 b	6.87±1.65 c	89.1±3.9 c	26.9±4.6 bc	5.48±0.91 b	0.71±0.21 a	**56.5**	**4.96**	**123**	**24.4**	**8.44**	0.33	2.38	3.68
	Fruc	3249±110 a	1.52±0.31 c	35.9±8.1 b	8.82±0.90 b	151±13 b	36.6±5.5 ab	5.42±0.92 b	0.61±0.18 a	**83.0**	2.51	**141**	**31.6**	**15.0**	0.81	2.34	3.02
	Suc	3425±111 a	3.35±0.32 a	132±10 a	23.1±2.3 a	210±8 a	25.5±3.2 c	3.73±0.93 b	0.40±0.14 a	**87.6**	**6.76**	**523**	**84.5**	**21.3**	0.26	1.30	1.64
	Mal	2365±124 b	3.43±1.79 abc	33.3±7.9 b	12.1±2.8 b	210±65 ab	40.7±8.1 a	7.23±0.36 a	0.38±0.14 a	**60.2**	**6.94**	**131**	**43.8**	**21.2**	1.01	**3.46**	1.53
3d	CK	46.8±16.0 c	0.43±0.12 c	1.62±0.49 b	0.18±0.05 d	6.75±1.19 d	11.4±0.8 c	0.69±0.15 c	0.12±0.03 b								
	Gluc	198±11 b	24.9±7.2 b	1766±199 a	61.5±3.3 ab	202±41 b	146±22 a	59.4±5.7 a	0.28±0.05 a	**3.23**	**57.2**	**1091**	**344**	**28.9**	**11.8**	**85.1**	1.33
	Fruc	305±32 a	42.7±3.1 a	1763±299 a	54.1±3.5 c	182±9 b	100±10 b	54.0±12.2 ab	0.33±0.11 a	**5.52**	**98.7**	**1089**	**302**	**25.9**	**7.79**	**77.3**	1.78
	Suc	282±59 a	29.8±7.0 b	1894±204 a	73.7±9.5 a	299±29 a	153±30 a	56.6±7.2 ab	0.22±0.05 a	**5.02**	**68.5**	**1170**	**412**	**43.3**	**12.4**	**81.1**	0.87
	Mal	187±31 b	25.5±10.0 b	1545±255 a	61.9±0.6 b	235±59 ab	113±27 ab	45.1±7.9 b	0.23±0.08 a	**2.99**	**58.6**	**954**	**346**	**33.8**	**8.94**	**64.4**	0.94
5d	CK	23.3±1.5 d	0.69±0.00 c	0.15±0.07 c	0.10±0.00 c	7.13±0.10 c	13.1±1.0 b	0.92±0.15 b	0.11±0.02 b								
	Gluc	35.4±9.5 c	42.2±14.6 ab	1668±556 ab	39.4±9.1 b	109±20 b	150±13 a	88.4±20.5 a	0.22±0.12 ab	0.52	**59.9**	**11231**	**397**	**14.2**	**10.5**	**94.7**	0.95
	Fruc	101±5 a	50.6±16.1 a	2195±251 a	69.5±13.3 a	218±34 a	178±34 a	84.1±24.8 a	0.23±0.04 a	**3.34**	**72.0**	**14781**	**701**	**29.6**	**12.6**	**90.0**	0.97
	Suc	52.6±3.4 b	25.1±4.2 b	1700±128 b	53.9±14.0 ab	244±78 a	159±22 a	100±29 a	0.23±0.07 a	**1.26**	**35.2**	**11449**	**543**	**33.3**	**11.2**	**107**	0.97
	Mal	49.2±9.1 bc	45.1±9.8 a	1832±75 b	65.3±9.3 a	186±41 a	184±36 a	83.9±12.2 a	0.17±0.10 ab	**1.11**	**64.1**	**12337**	**659**	**25.2**	**13.1**	**89.8**	0.48
9d	CK	25.8±1.8 c	0.61±0.20 c	1.86±0.49 c	0.65±0.13 c	8.14±1.16 c	9.44±1.89 d	1.12±0.34 c	0.10±0.05 a								
	Gluc	32.3±4.2 b	35.5±7.5 b	2640±538 ab	63.5±20.4 ab	190±60 ab	104±16 b	55.9±7.3 ab	0.16±0.10 a	0.25	**57.6**	**1416**	**96.3**	**22.3**	**10.0**	**49.0**	0.56
	Fruc	65.2±18.8 a	35.9±7.9 b	2711±119 a	71.2±12.4 ab	176±31 b	84.7±1.6 c	43.8±8.7 b	0.12±0.02 a	**1.52**	**58.3**	**1454**	**108**	**20.6**	**7.97**	**38.2**	0.19
	Suc	63.1±18.9 a	52.6±4.7 a	2029±492 b	81.3±13.5 a	273±58 a	139±9 a	60.0±6.6 a	0.14±0.10 a	**1.44**	**85.8**	**1088**	**124**	**32.5**	**13.7**	**52.7**	0.41
	Mal	63.4±22.5 a	51.3±14.6 ab	2447±487 ab	59.0±8.0 b	188±47 ab	92.0±21.8 bc	44.9±5.9 b	0.11±0.03 a	**1.45**	**83.7**	**1312**	**89.5**	**22.0**	**8.75**	**39.2**	0.10
42d	CK	15.2±0.6 d	0.32±0.00 d	1.05±0.23 c	0.32±0.00 c	7.00±1.09 c	4.08±0.40 c	0.24±0.08 c	0.05±0.02 a								
	Gluc	27.1±4.3 b	5.20±0.70 c	270±73 b	13.9±1.0 b	78.6±6.7 b	13.9±2.6 b	4.03±0.69 b	0.06±0.04 a	**0.78**	**15.1**	**256**	**41.8**	**10.2**	**2.42**	**15.6**	0.06
	Fruc	21.5±0.0 c	7.32±0.98 b	601±106 a	29.2±7.7 a	165±49 a	18.6±3.4 ab	5.87±0.61 a	0.10±0.05 a	0.41	**21.6**	**570**	**89.2**	**22.5**	**3.56**	**23.2**	0.85
	Suc	36.4±3.5 a	6.89±0.40 b	744±77 a	32.8±4.9 a	142±17 a	17.2±2.7 ab	7.07±1.11 a	0.11±0.09 a	**1.39**	**20.3**	**705**	**100**	**19.3**	**3.22**	**28.1**	1.02
	Mal	44.0±8.0 a	19.5±5.4 a	678±20 a	25.5±5.5 a	94.5±20.6 b	22.6±3.5 a	6.23±1.20 a	0.06±0.04 a	**1.89**	**59.1**	**643**	**77.8**	**12.5**	**4.54**	**24.6**	0.08
1d	FON	31.2±0.9 d	0.36±0.07 b	0.42±0.10 d	0.47±0.07 c	9.62±1.09 d	17.7±0.5 d	1.04±0.27 e	100±37 d								
	FON+Gluc	2487±120 b	4.76±1.04 a	70.0±10.4 b	6.84±2.02 b	95.1±26.9 c	39.9±3.1 a	11.2±3.3 a	1,307±218 bc	**78.6**	12.1	**168**	**13.6**	8.89	1.25	9.82	12.1
	FON+Fruc	2386±37 b	4.57±0.24 a	29.4±4.6 c	6.88±1.86 b	121±20 bc	28.4±2.1 c	2.37±0.00 d	2,265±414 a	**75.4**	11.6	**69.9**	13.7	11.6	0.60	1.28	21.7
	FON+Suc	2874±104 a	5.11±0.97 a	77.6±9.2 b	16.1±2.5 a	166±37 ab	27.1±1.7 c	3.83±0.38 c	1,812±442 ab	**91.0**	13.1	**186**	**33.5**	16.3	0.53	2.69	17.1
	FON+Mal	1919±117 c	5.81±1.19 a	169±25 a	20.1±3.2 a	224±36 a	34.9±2.2 b	6.06±1.67 b	1,064±273 c	**60.4**	**15.0**	**405**	**42.1**	**22.2**	0.96	4.84	9.64
3d	FON	27.8±3.7 d	0.69±0.00 d	0.62±0.15 c	0.54±0.07 c	8.94±2.03 c	13.7±0.9 d	1.28±0.14 d	22.7±3.4 b								
	FON+Gluc	212±38 b	59.7±15.4 ab	2404±464 a	82.0±13.9 ab	314±54 a	154±18 a	77.0±6.4 a	859±477 a	6.62	**85.8**	**3864**	**151**	34.1	10.3	**59.3**	36.9
	FON+Fruc	453±66 a	87.5±18.4 a	2317±211 a	63.4±6.7 b	292±13 a	123±21 ab	63.6±20.3 ab	847±45 a	15.3	**126**	**3724**	**116**	31.7	8.03	**48.8**	36.3
	FON+Suc	135±20 c	28.5±10.6 c	2249±65 a	83.8±5.8 a	255±36 ab	67.7±15.4 c	30.2±4.4 c	706±189 a	3.86	**40.4**	**3615**	**154**	27.5	3.96	22.7	30.1
	FON+Mal	107±10 c	42.8±16.5 bc	1569±68 b	65.6±17.4 ab	205±50 b	98.4±13.5 b	36.8±7.1 bc	540±402 a	2.86	**61.2**	**2521**	**120**	22.0	6.21	**27.8**	22.8
5d	FON	25.4±2.1 d	0.71±0.07 c	0.33±0.20 d	0.22±0.05 c	8.40±1.40 d	12.2±1.3 c	0.92±0.27 c	30.7±21.4 b								
	FON+Gluc	155±14 a	57.1±2.3 a	1296±223 b	57.9±7.2 ab	234±18 a	173±13 a	52.5±1.4 b	311±127 a	5.11	**79.1**	**3895**	**260**	**26.9**	13.2	**56.1**	9.14
	FON+Fruc	90.1±24.7 b	30.1±9.5 b	1987±203 a	47.4±13.5 ab	149±8 b	124±24 b	98.4±8.3 a	549±232 a	2.55	**41.2**	**5971**	**213**	16.7	9.19	**106**	16.9
	FON+Suc	182±32 a	62.7±16.0 a	847±220 c	74.0±22.6 a	273±54 a	190±24 a	55.5±15.2 b	527±297 a	6.18	**87.0**	**2546**	**333**	**31.5**	14.6	**59.4**	16.2
	FON+Mal	39.1±7.2 c	32.2±0.9 b	1756±145 a	42.6±8.4 b	99.1±4.3 c	173±21 a	116±30 a	328±160 a	0.54	**44.2**	**5279**	**191**	**10.8**	13.2	**126**	9.69
9d	FON	22.5±4.0 c	0.37±0.00 d	0.73±0.26 c	0.24±0.05 c	7.68±0.51 b	9.41±1.69 c	0.96±0.32 c	8.13±0.48 d								
	FON+Gluc	92.2±5.6 a	47.4±5.9 c	2091±216 a	55.2±7.3 b	175±8 a	146±37 a	59.7±9.9 a	34.7±14.1 bc	3.11	**129**	**2859**	**225**	**21.7**	**14.5**	**61.3**	3.27
	FON+Fruc	73.1±15.8 ab	64.7±11.1 ab	1876±578 ab	58.9±13.2 ab	171±24 a	95.7±11.4 b	30.5±0.6 b	81.8±12.7 a	2.26	**176**	**2564**	**241**	**21.3**	**9.16**	**30.7**	9.06
	FON+Suc	52.2±6.8 b	97.0±21.5 a	1278±363 b	74.7±10.8 a	237±60 a	162±5 a	55.3±16.6 a	52.3±7.3 b	1.33	**264**	**1746**	**305**	**29.8**	**16.2**	**56.6**	5.43
	FON+Mal	58.6±18.0 b	61.4±11.3 bc	1796±554 ab	65.5±7.8 ab	164±19 a	166±36 a	74.6±10.2 a	18.1±3.8 c	1.61	**167**	**2455**	**268**	**20.4**	**16.7**	**76.7**	1.23
42d	FON	18.3±0.8 d	0.30±0.00 c	0.13±0.06 d	0.17±0.06 c	6.64±1.36 c	5.85±0.30 c	0.23±0.06 d	3.04±0.32 c								
	FON+Gluc	28.8±2.1 c	6.95±1.99 a	562±134 a	19.6±4.0 ab	108±18 a	17.1±2.8 a	7.84±0.63 a	23.8±18.5 a	0.57	**22.2**	**4230**	**117**	**15.2**	1.93	**32.7**	6.84
	FON+Fruc	45.3±6.3 a	4.14±0.47 b	412±4 b	14.0±2.4 b	73.1±6.4 b	9.78±1.64 b	3.83±0.45 c	31.4±9.1 a	1.47	**12.8**	**3100**	**83.1**	**10.0**	0.67	**15.5**	9.35
	FON+Suc	34.1±2.8 b	6.07±0.87 a	339±18 c	15.9±2.0 ab	69.2±2.7 b	10.5±2.3 b	4.38±0.52 c	27.2±9.9 a	0.86	**19.3**	**2551**	**94.5**	**9.42**	0.80	**17.8**	7.96
	FON+Mal	44.2±9.3 ab	9.77±2.94 a	556±34 a	19.2±2.8 a	72.7±6.8 b	12.5±2.5 ab	5.74±0.12 b	3.83±0.36 b	1.41	**31.7**	**4180**	**115**	**9.94**	1.14	**23.7**	0.26

Fig. 5 Abundance of the significantly enriched genera and fold changes after sugar addition. The significantly enriched genera refer to those bacterial population (shown in Fig. S6) which had a significantly ($P < 0.05$) higher abundance in sugar-spiking treatment than in no-sugar treatment throughout 42 d of incubation. The data shown are the mean value of three replicates±the standard deviation. The abundance of the genus was calculated as the relative abundance of each genus × the total bacterial abundance determined by qPCR technique (shown in Fig. S2). The values in blue bold font indicate a higher fold increase for the genera than FON. Different letters indicate significant differences by Ducan's multiple range test ($P < 0.05$). All the other designations are the same as those in Fig. 2.

To assess topological roles of taxa in the networks and to explore the interactions between the keystone species and targeted pathogen (FON), the nodes (OTUs) were classified into 4 categories according to the within-module connectivity (*Zi*) and among-module connectivity (*Pi*): peripheral, connectors, module hubs, and network hubs (Deng et al., 2012). As shown in Fig. 7, the majority of OTUs (64.5%–90.8%) are under all treatments were peripherals with most of their links inside their own modules. Under no-sugar spiking condition, 25% OTUs (92 OTUs) and 20.9% OTUs (69 OTUs) are classified as connectors in Low-FON soil and High-FON soil, respectively (Fig. 7A, G). While under sugar-spiking condition,

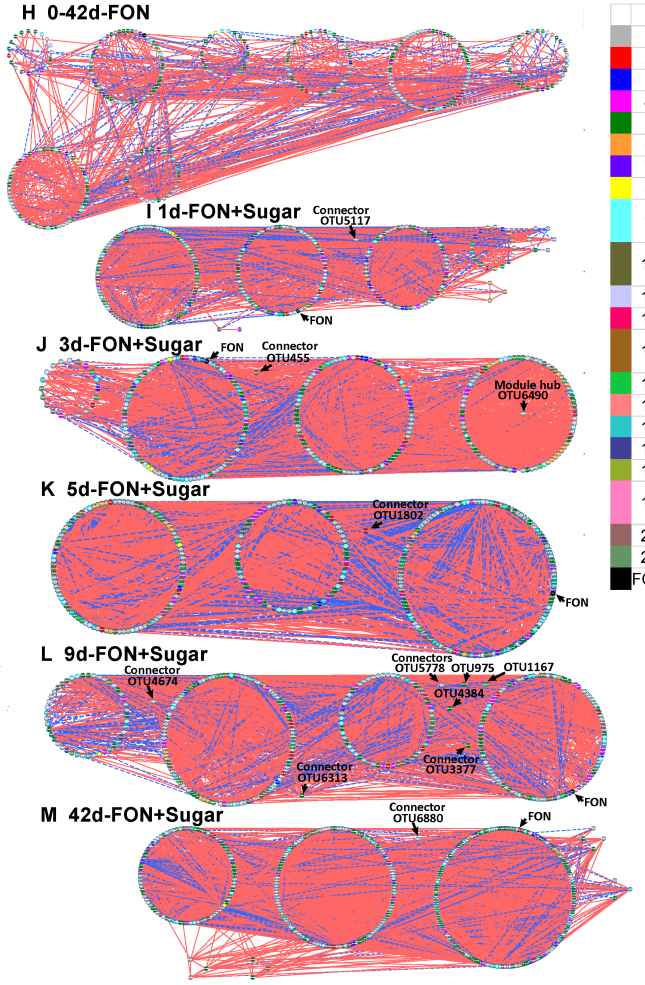


		Phylum; Class; Order; Family; Genus
	1	Acidobacteria; Acidobacteria; norank_Acidobacteria; norank_Acidobacteria; *norank_Acidobacteria*
	2	Actinobacteria; Actinobacteria; Gaiellales; Gaiellaceae; *GaiellaJJ4:J22*
	3	Actinobacteria; Actinobacteria; Propionibacteriales; Nocardioidaceae; *Marmoricola*
	4	Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; *norank_Cytophagaceae*
	5	Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae; *norank_Anaerolineaceae*
	6	Firmicutes; Bacilli; Bacillales; Bacillaceae; *Bacillus*
	7	Firmicutes; Bacilli; Bacillales; Paenibacillaceae; *Paenibacillus*
	8	8Firmicutes; Clostridia; Clostridiales; Clostridiaceae_1; *Clostridium_sensu_stricto_1*
	9	Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; *norank_Gemmatimonadaceae*
	10	Gemmatimonadetes; Gemmatimonadetes; norank_Gemmatimonadetes; norank_Gemmatimonadetes; *norank_Gemmatimonadetes*
	11	Nitrospirae; Nitrospira; norank_Nitrospira; norank_Nitrospira; *Nitrospira*
	12	Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Hydrogenophaga*
	13	Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae; *norank_Nitrosomonadaceae*
	14	Proteobacteria; Betaproteobacteria; TRA3-20; norank_TRA3-20; *norank_TRA3-20*
	15	Proteobacteria; Deltaproteobacteria; Desulfurellales; Desulfurellaceae; *H16*
	16	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Azotobacter*
	17	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; unclassified
	18	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
	19	Proteobacteria; Gammaproteobacteria; Xanthomonadales; norank_Xanthomonadales; *norank_Xanthomonadales*
	20	Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Lysobacter*
	21	Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Pseudoxanthomonas*
	FON	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; *Fusarium*

G Links between keystone taxa and FON in Low-FON soil

Treatment	Keystone taxa	*Pi*/*Zi*	Roles of keystone taxa	Target taxon	Link between keystone taxa and FON
0-42d-CK	OTU282	*Pi*>0.6	Connector	FON	negative
1d-Sugar	OTU3845	*Zi*>2.5	Module hub	FON	positive
	OTU3934	*Pi*>0.62	Connector	FON	positive
3d-Sugar	OTU4435	*Pi*>0.62	Connector	FON	positive
	OTU6403	*Pi*>0.62	Connector	FON	positive
5d-Sugar	OTU2487	*Pi*>0.62	Connector	FON	positive
9d-Sugar	OTU331	*Zi*>2.5	Module hub	FON	positive
42d-Sugar	OTU6883	*Pi*>0.62	Connector	FON	positive

Fig. 6 Network interactions of microbial communities in FON-uninoculated (A–F) and FON-inoculated (H–M) soils over time, and links between keystone taxa and FON. Those keystone OTUs (i.e., connectors, module hubs, or network hubs) which were connected with FON are indicated by the black arrow in panels A–F and H–M, and the links between these keystone taxa and FON shown in panels G and N. The red line denotes a positive connection (link), and the blue line denotes a negative connection (link). The designations CK and Sugar indicate that the soil was supplied with water and sugars (i.e., glucose, fructose, sucrose, and maltose), respectively. FON indicates the soil inoculated with FON. The samples collected after 0 day (i.e., zero time), 1 day, 3 days, 5 days, 9 days, and 42 days are referred to as 0, 1d, 3d, 5d, 9d, and 42d, respectively.



	Phylum; Class; Order; Family; Genus
1	Acidobacteria; Acidobacteria; norank_Acidobacteria; norank_Acidobacteria; *norank_Acidobacteria*
2	Actinobacteria; Actinobacteria; Gaiellales; Gaiellaceae; *GaiellaJJ4:J22*
3	Actinobacteria; Actinobacteria; Propionibacteriales; Nocardioidaceae; *Marmoricola*
4	Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae; *norank_Cytophagaceae*
5	Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae; *norank_Anaerolineaceae*
6	Firmicutes; Bacilli; Bacillales; Bacillaceae; *Bacillus*
7	Firmicutes; Bacilli; Bacillales; Paenibacillaceae; *Paenibacillus*
8	8Firmicutes; Clostridia; Clostridiales; Clostridiaceae_1; *Clostridium_sensu_stricto_1*
9	Gemmatimonadetes; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; *norank_Gemmatimonadaceae*
10	Gemmatimonadetes; Gemmatimonadetes; norank_Gemmatimonadetes; norank_Gemmatimonadetes; *norank_Gemmatimonadetes*
11	Nitrospirae; Nitrospira; norank_Nitrospira; norank_Nitrospira; *Nitrospira*
12	Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; *Hydrogenophaga*
13	Proteobacteria; Betaproteobacteria; Nitrosomonadales; Nitrosomonadaceae; *norank_Nitrosomonadaceae*
14	Proteobacteria; Betaproteobacteria; TRA3-20; norank_TRA3-20; *norank_TRA3-20*
15	Proteobacteria; Deltaproteobacteria; Desulfurellales; Desulfurellaceae; *H16*
16	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Azotobacter*
17	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; unclassified
18	Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; *Pseudomonas*
19	Proteobacteria; Gammaproteobacteria; Xanthomonadales; norank_Xanthomonadales; *norank_Xanthomonadales*
20	Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Lysobacter*
21	Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; *Pseudoxanthomonas*
FON	Ascomycota; Sordariomycetes; Hypocreales; Nectriaceae; *Fusarium*

N Links between keystone taxa and FON in High-FON soil

Treatment	Keystone taxa	*Pi*/*Zi*	Roles of keystone taxa	Target taxon	Link between keystone taxa and FON
0-42d-FON	None			FON	none
1d-FON-Sugar	OTU5117	*Pi*>0.62	Connector	FON	positive
3d-FON-Sugar	OTU6490	*Zi*>2.5	Module hub	FON	positive
	OTU455	*Pi*>0.62	Connector	FON	positive
5d-FON-Sugar	OTU1802	*Pi*>0.62	Connector	FON	positive
9d-FON-Sugar	OTU4674	*Pi*>0.62	Connector	FON	positive
	OTU5778	*Pi*>0.62	Connector	FON	positive
	OTU975	*Pi*>0.62	Connector	FON	positive
	OTU3377	*Pi*>0.62	Connector	FON	positive
	OTU4384	*Pi*>0.62	Connector	FON	positive
	OTU6313	*Pi*>0.62	Connector	FON	positive
	OTU1167	*Pi*>0.62	Connector	FON	positive
42d-FON-Sugar	OTU6880	*Pi*>0.62	Connector	FON	positive

Fig. 6 *Continued*

6.90%–33.5% OTUs were detected as connectors in Low-FON soil (Fig. 7B–F) and 14.2%–26.9% OTUs as connector in High-FON soil (Fig. 7H–L) over 42 d of incubation. These connectors originated from a variety of taxonomic groups: 20 genera within 8 phyla in Low-FON soil and 21 genera within 8 phyla in High-FON soil (Fig. S7). Members of Chloroflexi were the most prominent connectors at no-sugar treatment whether in Low-FON and High-FON soils as they accounted for the largest percentage (Low-FON: 33.7%; High-FON: 40.6%) of all connectors (Fig. S7). However, after sugar addition, Acidobacteria-affiliated OTUs, rather than Chloroflexi-affiliated OTUs, became the most prominent connectors in most cases (except under sugar treatment at 5 d), accounting for 17.0%–30.1%% and 19.5%–28.3% of total connectors in

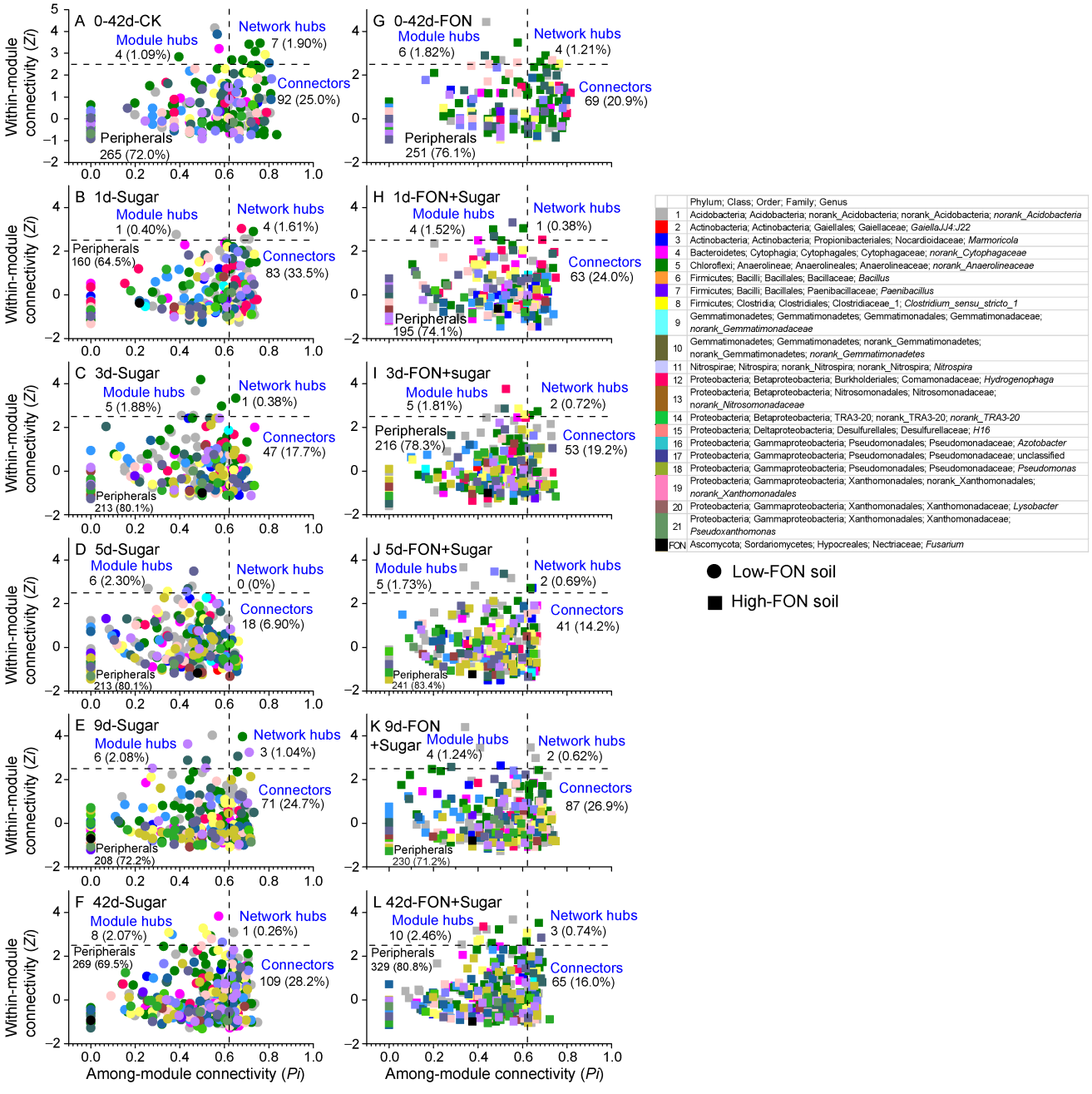


Fig. 7 Classification of nodes to identify putative keystone species within the networks. Each symbol represents an OTU. Module hubs have *Zi* >2.5, whereas connectors have *Pi* >0.62. Network hubs have *Zi* >2.5 and *Pi* >0.62. The number of peripherals, connectors, module hubs, or network hubs and their proportion in the network are shown below or next to the symbols "Peripherals", "Connectors", "module hubs", or "Network hubs". The Low-FON denotes the FON-uninoculated soil and the High-FON denotes the FON-inoculated soil. OTUs from Low-FON (dots) and High-FON (squares) soils were colored by genus membership. CK and Sugar represent the soil supplied with water and sugars (i.e., glucose, fructose, sucrose, and maltose), respectively. FON denotes the soil inoculated with FON. The samples collected after 0 day (i.e., zero time), 1 day, 3 days, 5 days, 9 days, and 42 days are referred to as 0, 1 d, 3 d, 5 d, 9 d, and 42 d, respectively.

Low-FON soil and High-FON soil, respectively (Fig. S7). OTUs of Chloroflexi also were the most prominent network hubs before sugar addition as 5 out of 7 OTUs, and 2 out of 4 OTUs, which are identified as network hubs in Low-FON and High-FON soils, respectively, belonged to Chloroflexi when sugar was not added (Fig. S8). While after sugar addition, besides Chloroflexi OTUs, OTUs affiliated to Acidobacteria, Firmicutes, Gemmatimonadetes were detected as network hubs in both Low-FON and High-FON soils and one Actinobacteria OTU (OTU4753) was additionally detected as network hub in High-FON soil (Fig. S8).

As for module hubs, when sugar was not added, 4 OTUs from Acidobacteria, Bacteroidetes, Chloroflexi, and Betaproteobacteria were identified as module hubs in Low-FON soil and 6 OTUs (2 from Acidobacteria, 2 from Chloroflexi, 1 from Gemmatimonadetes, and 1 from Nitrospirae) belonged to module hubs in High-FON soil (Fig. S9). Under sugar-spiking treatment, 1 OTU-8 OTUs from 8 phyla/classes (Acidobacteria, Chloroflexi, Gemmatimonadetes, Delataproteobacteria, Gammataproteobacteria, Bacteroidetes, Firmicutes, Nitrospirae) were identified as module hubs in Low-FON soil and 4-12 OTUs from 7 phyla (Acidobacteria, Chloroflexi, Gemmatimonadetes, Gammaproteobacteria, Firmicutes, Deltaproteobacteria, Actinobacteria) were identified as module hubs in High-FON soil (Fig. S9). Of these module hubs, Acidobacteria-affiliated OTUs were still the most prominent module hubs in Low-FON soil under sugar-spiking treatment as they were detected as module hubs in almost all times points (4 out of 5 time points, except at 42 d in Low-FON soil and at 1 d in High-FON soil) (Fig. S9).

Connectors, module hubs, and network hubs are proposed to be keystone species due to their important topological roles in network (Deng et al., 2012). Based on this criteria, at no sugar treatment, Chloroflexi-affiliated OTUs (belonging to the family *Anaerolineaceae* but norank at the genus level) would be the most prominent keystone taxa as they accounted for the largest percentage of the total connectors (Fig.S7) and of the total network hubs (Fig. S8) and were also detected as module hubs (Fig. S9) in both Low-FON and High-FON soil. While under sugar-added treatment, Acidobacteria-affiliated OTUs (norank at the genus level) became the most prominent keystone taxa as it accounted for the largest proportion of all connectors in most cases (Fig.S7) and detected as module hubs in almost all time points (Fig. S9) in Low-FON and High-FON soils. Almost all keystone species had a low relative abundance: for connectors, relative abundance ranged from 0.005% to 2.54% (except 14.9% for only one OTU) (Fig. S10); for network hubs, relative abundance spanned from 0.005% to 0.052% (Fig. S8); and for module hubs, relative abundance ranged from 0.005% to 0.015%(Fig. S9).

The FON did not act as connector, module hub, or network hub in all networks, indicating it was not the keystone species in all cases. Interestingly, when sugar was not added, the keystone species (OTU282, acting as connector) negatively linked with FON under no-sugar treatment in the Low-FON soil (Fig. 6G) and none keystone species linked with FON in High-FON soil (Fig. 6N), suggesting a potential suppressive effect of the keystone population on the growth of FON in the Low-FON soil but no influence of the keystone species on the growth of FON. However, after the addition of sugars, some keystone species (i.e., module hubs or connectors) were positively linked with FON at all time points in both Low-FON and High-FON soils (Fig. 6G, N), and this observation indicated that the keystone populations showed a potential promotion effect on FON.

4 Discussion

Although sugar serves as a common C and energy source for all microorganisms, probably not every microbial community member could have a rapid growth and proliferation when sugars are provided because of complex microbial interactions and substrate preference (Zhalnina et al., 2018). In this study, the FON showed a rapid growth once all the sugars were provided, suggesting that this pathogen may be a fast-growing r-strategist and could preferentially metabolize all test sugars since the r-strategist have generally been characterized by high growth rate (Torsvik et al., 2002) and respond positively to low-molecular-weight substrates (Fierer et al., 2007; Goldfarb et al., 2011). Further, as copiotrophic organisms are described as thriving under conditions where resource availability, particularly C availability is high (e.g., soils with labile rhizodeposited C), while oligotrophs are relatively more abundant under resource-limited conditions (e.g., bulk soil) (Fierer et al., 2007; Eilers et al., 2010), the FON in our study may also represent important copiotroph, with increase in the quantity potentially tied to supply of labile C substrate in soil. As root exudate are usually secreted abundantly in mature plants, our study hence provided experimental bases to explain why the pathogen could be enriched after crop planting and why continuous cropping usually leads to pathogen accumulation and enhances severity of soil-borne diseases.

In this study, addition of all tested sugars decreased the bacterial richness, evenness, and diversity in both Low-FON and High-FON soils. Similarly, decrease in bacterial richness and diversity have been observed in rhizosphere community compared with the community from bulk soil (Kowalchuk et al., 2002; Chaparro et al., 2014; Shi et al., 2015). Niche differentiation has long been the focus to maintain and explain biodiversity (Leibold and McPeek, 2006). Soil is considered as one of the most diverse microbial habitat on Earth, which is commonly attributed to microsite niche heterogeneity (Shi et al., 2015). Substrate preferences of soil microbes also drive patterns in microbial community assembly (Zhalnina et al., 2018). Therefore, we speculate that presence of sugars overwhelmed and homogenized differences among soil microsites, hence reduced extent of niche heterogeneity in soil, and then led to the decrease in bacterial richness and diversity. The current study further revealed that the FON quantity was negatively related with bacterial richness. This

result is in line with other studies that has shown a negative correlation between microbial biomass and species richness (Nielsen et al., 2011; Wang et al., 2021).

Previouse studies on the rhizosphere effect on microbita have revealed a evident different bacterial community structure between the rhizosphere and bulk soil (Uroz et al., 2010; Li et al., 2014a; Shi et al., 2015). Similarly, we observed a significant difference in bacterial community structure between the sugar-added and sugar-unadded soil, and this "sugar effect" on the commuity structure occurred as early as 1 day and lasted until at 42 d. *Bacillus* was the most shifted taxon in response to sugars at 1 d while *Azotobacteria* was the most shifted phylotype from 3 d to 42 d. Therefore, at 1 d, *Bacillus* was speculated as the main contributor that lead to the overal bacterial community structure changes induced by sugar supplemenation while *Azotobacteria* contributed the most from 3 d to 42 d. Moreover, although a few sugar-supplementation studies associated with soil microbial communities have been reported (Baudoin et al., 2003; Eilers et al., 2010), these studies focused on grassland soil, forest soil (Eilers et al., 2010), or corn field soil (Baudoin et al., 2003) rather than pathogen-infested soil. In this study, no signficiant difference was found between the soils that were treated with different tested sugars (including two monosaccharides and two disaccharides) in terms of the bacterial community structure over the entire 42 d of incubation. Carbohydrates are completely metabolized to CO_2 and H_2O under aerobic conditions via four steps: EMP pathway (Embden-Meyerhof-Parnas pathway), acetyl CoA production, tricarboxylic acid cycle, and oxidative phosphorylation. Sucrose and maltose could also enter the first step after just a simple hydrolization and glucose and fructose could enter the first step directly. Therefore, the tested four sugars experienced almost the same metabolic processes, and thus the difference between sugar-specific microorganisms may be too subtle to be detectable at the community level. Moreover, the microbes commonly exhibit a broad array of functions in soil and this functional redundancy can often obscure the linkages between microbial taxa and functional traits (Allison and Martiny, 2008; Green et al., 2008; Eilers et al., 2010).

At the specific taxonomic phylotype level, members of Gammaproteobacteria were the most evident positive responder to all the tested sugars based on the observation that 5 of 7 positive responders at the genus level affiliated with Gammaproteobacteria throughout 42 d of incubation. Several studies have reported the Gammaproteobacteria to respond to low-molecular-weight C substrate (Padmanabhan et al., 2003; Cleveland et al., 2007; Eilers et al., 2010). Eilers et al. (2010) found a significantly increased relative abundance of Gammaproteobacteria after supply of glucose in a coniferous forest soil. A similar increase in Gammaproteobacteria was found by Fierer et al. (2007) after the addition of sucrose and by Bernard et al. (2007) after the supplementation of fresh organic matter (representing a readily available source of C, energy, and nutrients for soil microbes), leading these authors to draw conclusion about the copiotrophic or r-strategic features of these organisms. Such conclusion seems to be verified in our present study.

Additionally, Firmicutes-affiliated *Bacillus* and Betaproteobacteria-affiliated *Hydrogenophaga* also showed a significantly positive response to all the sugars simultaneously in both Low-FON and High-FON soils throughout 42 d of incubation, indicating a potential copiotrohic lifestyle of them. This observation was similar to a previous study (Cleveland et al., 2007) showing increased relative abundance of the opportunistic *Bacillus* sequences following labile carbon addition. More importantly, *Bacillus* and *Hydrogenophaga* showed a more rapid biomass increase than FON after addition of sugars at certain time points in Low-FON and High-FON soils. The positive response of FON, Gammaproteobacteria members, *Hydrogenophaga*, and *Bacillus* indicated a similar resource utilization spectrum. As trophic niche overlap usually leads to competition, it is hypothesized that when living in a resource-limited environment, competition may occur. However, considering the diversity of nutritional resources in soil and the metabolic styles of microbes, we cannot rule out a potential collaboration between FON and other positive responders mentioned above (i.e., 5 genera of Gammaproteobacteria, *Bacillus* of Firmicutes and *Hydrogenophaga* of Betaproteobacteria). Examining their relationships in future work will help to explore the potential of the positive responders to control FON through competitive exclusion. Furthermore, *Bacillus* was the most sensitive population as it was enriched the most after only 1 d of incubation following sugar addition. *Bacillus* spp. have been considered as important biocontrol agents for plant disease because of the presence of cLP (cyclolipopeptides) and ISR (induced systemic resistance) genes and the production of broad spectra of antifungal metabolites (Haddoudi et al., 2021). Member of *Bacillus* have also shown plant growth promoting activity for its ability to produce lipopeptides, indoleacetic acid, and siderophores, and solubilize phosphate (Haddoudi et al., 2021). Therefore, sufficient supplementary of labile C (such as sugar) to soil may be a vital way to enrich *Bacillus*, which is a crucial plant growth promoting bacteria and disease control agent.

In this study, the bacterial assemblages formed more complex networks in sugar-spiking soil than that without sugar addition, which was similar to previous researches on the rhizosphere effect on the bacterial network showing that high network complexity dominates rhizosphere assemblages (Shi et al., 2016), as well as the effect of organic amendments on fungal networks (Xue et al., 2018). Improved network connectivity and complexity are previously undescribed characteristics of bacterial assemblages in sugar-spiking soils. Network often reveal co-variation pattern, where members' abundance covary when responding to environmental factors (Shi et al., 2016). In particular, resource and food availability have been considered as critical drivers of social network structures in macrobiological studies (Henzi et al., 2009; Foster et al., 2012). It is hence speculated that the increased connectivity and complexity after the addition of

sugars may be attributed to more food and resources for all microbe. This speculation was supported by previous studies which proposed that the increased phylogenetic (Zhou et al., 2011) and functional (Zhou et al., 2010) complexity of soil bacterial network under elevated CO_2 was likely own to the increase of C input into soil. The finding that the rhizosphere bacterial networks were substantially more complex than those in surrounding soil may be explained by a significant amount of C import into the rhizosphere during plant growth (Shi et al., 2016). Furthermore, the covariation not only indicates direct or indirect interactions, but also represents niche sharing (Berry and Widder, 2014; Shi et al., 2016). Therefore, the more connected and complex network in sugar-added soils can be interpreted as the combination of both enhanced bacterial interactions and the development of shared niches. While the relatively simple networks in no-sugar addition soils may result from a minimal bacterial interactions or niche sharing compared to that in the sugar-addition soil. This speculation was supported by the study of the bulk soil where organisms were thought to live in a heterogeneous, disconnected habitat at the microscale (Torsvik et al., 2002; Fierer and Lennon, 2011; Shi et al., 2016). As well, this speculation seemed to be verified in our study by the observation that sugar-spiking soils harbored larger network modules as the OTUs within a module are considered to be likely to have a similar ecological niche (Yao et al., 2014).

Several studies have focused on the identification of keystone phylotypes (Banerjee et al., 2016; Shi et al., 2016; Tipton et al., 2018) due to the its significance in maintaining network structure. Hundreds of keystone taxa have been reported in soil, plant, and marine ecosystems and the human microbiome (Banerjee et al., 2018). Module hubs (i.e., nodes highly connected within a module), connectors (i.e., nodes linking different modules), and network hubs (i.e., acting as both module hubs and connectors) are usually considered as keystone species as these organisms play a disproportionately pivotal role in maintaining module assembly, network structure, and even ecosystem stability (Olesen et al., 2007; Shi et al., 2016). In this study, the sugar-spiking soil harbored distinct keystone taxa (connectors, module hubs, or network hubs) compared with no-sugar addition soil, which supports the context dependency theory that holds that keystone species play keystone roles only under certain conditions (Power et al., 1996). For example, as conditions, such as land use or plant growth season, change, the keystone taxa changed accordingly (Lupatini et al., 2014; Shi et al., 2016). Interestingly, the keystone taxa were found to have relatively low relative abundance in most cases in our study. Similar observation was found in soil and rhizosphere bacterial networks (Lupatini et al., 2014; Shi et al., 2016) and in skin and lung microbial networks (Tipton et al., 2018) where the rare microbes or the low abundant populations were the keystone taxa. Indeed, some authors have proposed that the keystone taxa were not related to their abundance (Power et al., 1996; Banerjee et al., 2018) and this conclusion seems to be verified in our study and studies of others.

Detecting microbial interactions, especially the interactions between keystone species and the pathogen (FON) would provide a new viewpoint for explaining why the pathogen could have a profuse proliferation after exposure to sugars. Here, in sugar-added networks, some keystone taxa formed positive connections with *Fusarium* wilt pathogen of watermelon (i.e., FON) at all time points but they exhibited negative links or no links with this pathogen in no-sugar spiking networks. This phenomenon indicates that the keystone phylotypes may have had a promotion effect on FON after addition of sugars while they may have had a suppressive effect or no effect on FON in sugar-unadded soil. This may be one of the reasons why the pathogen (FON) could have a vigorous proliferation after sugar addition. Acidobacteria-affiliated OTUs were the most prominent connectors and frequently detected module hubs under sugar-spiking condition in Low-FON and High-FO soils, although Acidobacteria is a slower growing group (Jiang et al., 2017) and was found to be substantially enriched in bulk soil (Jones et al., 2009). Similar to our observation, in some rhizosphere bacterial network studies, Acidobacteria members have also been identified as connectors or module hubs in the rhizosphere bacterial networks of wild oat (Shi et al., 2016) and blueberry (Jiang et al., 2017). Acidobacteria-affilaited OTUs have also been detected as module hubs in a grassland soil under unwarming condition (Deng et al., 2012). The observation that Chloroflexi-affiliated OTUs (belonging to the family *Anaerolineaceae* but no rank at the genus level) were the most prominent connectors and network hubs may partially result from that this group is a slower growing population (Jiang et al., 2017) and could have a better growth in resource limited soil such as bulk soil (Jones et al., 2009).

5 Conclusion

While the *Fusarium* wilt pathogen of watermelon, FON, lives in an environment where tremendously diverse community coexist and intensely interact, it could still be the beneficiary in growth and proliferation through preferentially utilization of the sugars. Meanwhile, sugar supplementation changed bacterial community structure, decreased bacterial richness, diversity, and evenness, and led to higher complex and connected community networks. Positive connections between FON and some keystone taxa in sugar-treated networks suggested a potential promotion effect of the keystone taxa on FON, which may explain why FON could have a vigorous proliferation after sugar addition. Overall, this study not only enhanced our understanding of sugars serving as drivers for the rapid growth of FON, the evident changes of bacterial community structure, and the intense microbial interactions, but also revealed the possible mechanism that enable FON to have a vigorous growth and proliferation after addition of sugars from the viewpoint of microbial interactions.

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Electronic supplementary material

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