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Distinct roles for soil fungal and bacterial communities associated with the suppression of vanilla *Fusarium* wilt disease

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ABSTRACT

Characterizing microbial communities associated with disease-suppressive soil is an important first step toward understanding the potential of microbiota to protect crops against plant pathogens. In the present study, we compared microbial communities in suppressive- and conducive-soils associated with *Fusarium* wilt disease in a vanilla long-term continuous cropping system. Suppressive soil was associated with higher fungal diversity and lower bacterial diversity. The fungal phyla *Zygomycota* and *Basidiomycota*, and the bacterial phyla *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria* and *Firmicutes* were strongly enriched in the suppressive soil. Notably, suppressive soil was dominated by the fungal genus *Mortierella*, accounting for 37% of the total fungal sequences. The hyper-dominance of *Mortierella* spp. in suppressive soil suggests that this taxon may serve as an indicator and enhancer of *Fusarium* wilt disease suppression in vanilla. In addition, Molecular Ecological Network analysis revealed that fungal communities were more connected and showed more co-occurrence relationships in the suppressive versus conducive soils. Our results indicate that fungal communities may be important in the development of soil suppressiveness against vanilla *Fusarium* wilt disease.

1. Introduction

Some soils, referred to as disease-suppressive, can naturally suppress plant pathogens, suggesting that they harbor specific characteristics that keep pathogens in check (Cha et al., 2016; Cook and Rovira, 1976). Microorganisms, including bacteria and fungi, are the main drivers of soil suppressiveness (Chaparro et al., 2012; Garbeva et al., 2004). Understanding which microbial communities are associated with disease-suppression can provide the foundation for soil community manipulation and new opportunities to explore novel strategies to promote plant health in a sustainable way (Stone et al., 2004).

Vanilla (*Vanilla planifolia*), a high-value cash crop, is widely cropped in tropical and subtropical regions (Minoo et al., 2008). However, this crop is seriously threatened by *Fusarium* wilt disease, caused by *Fusarium oxysporum* f. sp. *vanillae* (Pinaria et al., 2010). Vanilla is typically grown as a monoculture, resulting in the rapid accumulation of *Fusarium* pathogen densities in soils (Xiong et al., 2015b). *Fusarium* pathogen accumulation can be controlled by crop rotation (Xiong et al., 2016), but such measures are generally impractical due to monetary and labor costs.

In previous field surveys, we discovered that some soils on Hainan Island China, retain a low *Fusarium* wilt disease incidence even after decades of vanilla monoculture. In these soils, the pathogen is present but remains at a low level and does not cause damage to the crop. We hypothesized that distinct soil microbial communities in these soils may explain differences in *Fusarium* wilt disease incidence between the geographically proximate fields, which otherwise share the same climatic conditions, agronomic management and fertilization regimes (Xiong et al., 2015b). Soil microbial communities play an essential role in the suppression of *Fusarium* wilt disease in several other plants, such as strawberry and banana (Cha et al., 2016; Shen et al., 2015). However, bacterial and fungal communities are rarely investigated together in disease suppressive soils (Cha et al., 2016; Mendes et al., 2011; Penton et al.,

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2014) and fungal communities are often overlooked.

In the present work, we aimed for an integrative study of soil microbial communities and analyzed the relative importance of bacterial and fungal communities for the suppression of *Fusarium* wilt. We tested whether suppressive and conducive soils differed in bacterial and fungal abundance, diversity and taxonomic composition. Further, we used association networks to examine the frequency of interactions within microbial communities associated with the suppressive-versus conducive-soils.

2. Materials and methods

2.1. Site description and sampling

We selected two model orchards continuously planted with vanilla for at least 20 years in Hainan province, China. Both two orchards have similar edaphic properties (loam soil), agronomic management history and fertilization regimes. The mean annual temperature and precipitation in this area are 24.5 °C and 2200 mm. However, the two orchards differ strongly in *Fusarium* wilt disease incidence. The first study site (later: suppressive soil), a vanilla orchard located in the town of Gaolong (18°736′N-18°738′N, 110°191′E-110°193′E), has been continuously cropped with vanilla since 1989, yet harbors a low *Fusarium* wilt disease incidence of less than 10% during the last ten years. The second orchard (later: conducive soil) is situated 4.6 km from the first field, in the town of Xinglong (18°698′N-18°700′N, 110°170′E-110°171′E). This orchard has been cropped continuously with vanilla for over 20 years and has a high disease incidence (over 65% over the last three years). Based on the accounts of the local farmers, *Fusarium* wilt was not detected at this site prior to vanilla cropping.

For each site, 9 random subplots (about 60 m^2) were chosen and 10 random cores (0–20 cm in depth) from each subplot were collected using a 2.5 cm diameter (at least 2 m between the cores) in April 2014. The 10 random cores from each subplot were mixed to form one composite sample, resulting in 9 samples per site. The 18 soil samples were placed into separate sterile plastic bags and transported to the laboratory on ice. Each soil sample was sieved through a 2-mm sieve and thoroughly homogenized. One portion of each sample was air-dried for chemical analysis according to our previous methods (Xiong et al., 2015b), and the other portion was stored at −80 °C for subsequent DNA extraction.

2.2. Assessing the disease suppressive ability of soils in pots

For the pot experiments, soils were collected with a shovel in the direct vicinity of the cores used for DNA extraction. Soils were thoroughly mixed for each site. In order to assess whether disease suppression can be attributed to microbial communities rather than differences in physicochemical properties, we performed soil suppressiveness assay based on Mendes et al. (2011) with some modifications. Briefly, we set up four treatments as follows: 1) suppressive soil (S), 2) conducive soil (C), 3) conducive soil amended with 50% (w/w) of suppressive soil (SC), and 4) conducive soil amended with 50% (w/w) of heat-treated (90 °C for 2 h) suppressive soil ($S_{90}C$). For each treatment, the soil was thoroughly mixed and poured into the sterilized pots (15 kg soil per pot). Each treatment contained three replicates, and each replicate consisted of five pots. Three vanilla seedling were planted in each pot (Xiong et al., 2015b). Pots were incubated in a greenhouse (located at the Spice and Beverage Research Institute, Wanning City, Hainan Province, China) at 30 °C and with 72% relative humidity with a randomization of all pots. Vanilla seedlings were monitored daily for the appearance and severity of vanilla *Fusarium* wilt disease. Disease symptoms typically manifested themselves approximately three weeks after planting, and disease incidence was calculated as the percentage of infected plants among the total number of plants.

2.3. DNA extraction, PCR amplification and illumina sequencing

For each composite soil sample, total DNA was extracted from 0.5 g soil using the MoBioPowerSoil™ DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA concentration and purity were measured using a NanoDrop ND-2000 (NanoDrop Technologies, Wilmington, DE) spectrophotometry. The primer set: ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns, 1993) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al., 1990) was selected to target the fungal ITS1 region. 520F (5′-AYTGGGYD-TAAAGNG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) (Claesson et al., 2009) was used to amplify the V4 hypervariable regions of the bacterial 16S rRNA gene. Primer pairs were modified for sequencing by adding the forward Illumina Nextera adapter, a two basepair "linker" sequence, and a unique 7-bp barcode sequence at the 5′ end of the forward primer, and the appropriate reverse Illumina Nextera adapter and linker sequence at the 5′ end of the reverse primer. PCR was performed following previously published amplication conditions (Xiong et al., 2015b). Briefly, 27 and 25 cycles were performed to amplify fungal and bacterial templates, respectively. Then, the PCR products were then purified using a PCR Purification Kit (Axygen Bio, USA) and pooled in equimolar concentrations of 10 ng μl^{-1} before sequencing. Finally, paired-end sequencing of fungal and bacterial amplicons were carried out on the Illumina MiSeq sequencer at Personal Biotechnology Co., Ltd (Shanghai, China).

2.4. Quantification of the Fusarium oxysporum, bacterial and fungal abundances

We quantified *Fusarium oxysporum*, bacterial and fungal abundances using quantitative polymerase chain reaction (qPCR) according to the established protocols (Xiong et al., 2016, 2015a). Briefly, we set up a 20-μl reaction mixture containing 10 μl of the *Premix Ex Taq*^{TM} (2 ×) (Takara-Bio, Japan), 0.4 μl of each primer (10 μM), 0.4 μl of ROX Reference Dye II (50 ×), 2 μl of template DNA and 6.8 μl of ddH_2O. The following primer pairs used: AFP308R (5′-CGAATTAACGCGAGTCCCAAC-3′) and ITS1F (5′-CTTGGTCATTTA-GAGGAAGTAA-3′) (Lievens et al., 2005) for *Fusarium oxysporum*, ITS1F and ITS2 for fungi, and 520F and 802R for bacteria. The PCR thermal conditions were as follows: 30 s at 95 °C for initial denaturation, followed by 40 cycles of 5 s at 95 °C, and 34 s at 60 °C. Standard curves were obtained according to our previous protocols (Xiong et al., 2016, 2015a). The specificity of the amplification products was confirmed by melting curve analysis and visual inspection after agarose gel electrophoresis.

2.5. Bioinformatics analyses

After removing the adaptors and primer sequences, the raw sequences were assembled for each sample according to the unique barcode using QIIME (Caporaso et al., 2010). Split sequences for each sample were merged using FLASH V1.2.7 (Magoč and Salzberg, 2011). The sequences retained for each sample were processed following the established UPARSE pipeline (Edgar, 2013). Briefly, low-quality sequences with a quality score lower than 0.5 or a length shorter than 200 bp were discarded. After discarding singletons, the remaining reads were assigned to OTUs with a threshold of 97% identity level, followed by removal of chimeras using the UCHIME method (Edgar et al., 2011). Finally, the fungal representative OTUs were classified using the UNITE database

Table 1
Summary of soil characteristics at sites cultivated with vanilla that were disease-suppressive and -conducive to *Fusarium* wilt.

Vanilla sites	pH	EC (μs/cm)	OM (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Available Fe (mg/kg)
Gaolong (Suppressive soil)	6.32 ± 0.21	116.62 ± 14.79	24.90 ± 2.05*	83.26 ± 4.99*	120.81 ± 15.50*	146.90 ± 50.15	64.88 ± 1.09*
Xinglong (Conducive soil)	6.96 ± 0.10*	175.94 ± 8.14*	20.20 ± 1.89	69.62 ± 7.06	92.34 ± 8.27	221.44 ± 44.69*	54.34 ± 2.64

Values are means ± standard deviation, disease-suppressive and -conducive soils (n = 9).
* represents significance ($P < 0.05$) between disease-suppressive and -conducive soils according to Student's t-test.

(version 7.0) (Kõljalg et al., 2013) and the bacterial representative sequences were matched against the RDP database (version 9) (Wang et al., 2007) using the naïve Bayesian classifier implemented in Mothur with a 80% confidence threshold (Schloss et al., 2009).

We estimated fungal and bacterial diversity using the Chao1 richness and phylogenetic diversity (PD) indices (Faith, 1992). PD was calculated based upon neighbor-joining phylogenetic trees generated with the Mothur pipeline (Schloss et al., 2009). To explore variation in fungal and bacterial community structures across the soil samples analyzed, weighted UniFrac distance was also performed in Mothur. PCoA (Principal Coordinate Analysis) was performed on distance matrices, and coordinates were used to draw 2D graphical outputs. Analysis of molecular variance (AMOVA) was performed to evaluate the significant differences in community structures between disease-suppressive and -conducive soils. For Mantel tests, Bray-Curtis and Euclidean distance were used to construct dissimilarity matrices of communities and soil characteristics respectively via the vegan package of R (version 3.2.2). The Mantel tests were used to calculate the correlation between the fungal or bacterial community and soil characteristics.

The linear discriminant analysis (LDA) effect size (LEfSe) method was used to evaluate bacterial and fungal taxa significantly associated with disease-suppressive and -conducive soils (Segata et al., 2011). The alpha value employed for the factorial Kruskal–Wallis test was 0.05, and the threshold employed on the logarithmic LDA score for discriminative feature was 2.0.

2.6. Network analyses

We used the phylogenetic Molecular Ecological Network (pMEN) method to construct interaction networks for disease-suppressive and -conducive soils (Zhou et al., 2010, 2011; Deng et al., 2012). In this study, the 300 most abundant fungal and bacterial OTUs from disease-suppressive and -conducive soil samples were used for the network constructions. The Random Matrix Theory (RMT) was used to automatically identify the appropriate similarity threshold (St) prior to network construction. All analyses were performed using the Molecular Ecological Network Analyses Pipeline (http://ieg2.ou.edu/MENA/main.cgi), and network graphs were visualized using Cytoscape 2.8.2 software.

2.7. Statistical analyses

Soil physicochemical characteristics, *Fusarium oxysporum* abundance, fungal and bacterial total abundances, alpha diversity indices, and the taxa (phyla and genus) in disease-suppressive and -conducive soils were compared using Student's t-test. Analysis of disease incidence from the pot experiment was performed using Tukey's HSD multiple range test. Spearman's rank correlation coefficient was used to evaluate the relationships between alpha diversity indices and soil characteristics. All analyses were performed in SPSS v20.0 (SPSS Inc., USA).

2.8. Sequence accession numbers

Sequences are available in the NCBI Sequence Read Archive (SRA) database under the accession number SRP056349.

3. Results

3.1. Soil characteristics and total Fusarium oxysporum abundance in the two vanilla planting sites

Soil characteristics for the two vanilla fields are summarized in Table 1. Compared with conducive soil (Xinglong site), suppressive soil (Gaolong site) had a significantly ($P < 0.05$; Student's t-test) higher OM, available N, P and Fe contents. In contrast, conducive soil showed a significantly ($P < 0.05$; Student's t-test) higher soil pH, EC, and the content of available K. *F. oxysporum* was present in both soils, with the suppressive soil harbouring a lower *F. oxysporum* density (1.5×10^5 copies g^{-1} soil) as compared to the conducive soil (2.8×10^5 copies g^{-1} soil) (Fig. S1).

3.2. Disease-suppressiveness of the two vanilla planting sites

In accordance with what would be expected from field observations, disease incidence was significantly lower for pots with suppressive soil, with only 11% disease incidence compared to 70% disease incidence in the conducive soil ($P < 0.001$; Tukey's HSD test) (Fig. 1). In order to assess whether soil microbial communities or physicochemical properties determined disease suppression, we transferred suppressive soil to the conducive one. Transferring 50% of the suppressive soil into the conducive soil significantly reduced disease incidence from 70% to 27% ($P < 0.05$; Tukey's HSD test). Transferring the same proportion suppressive soil after heat

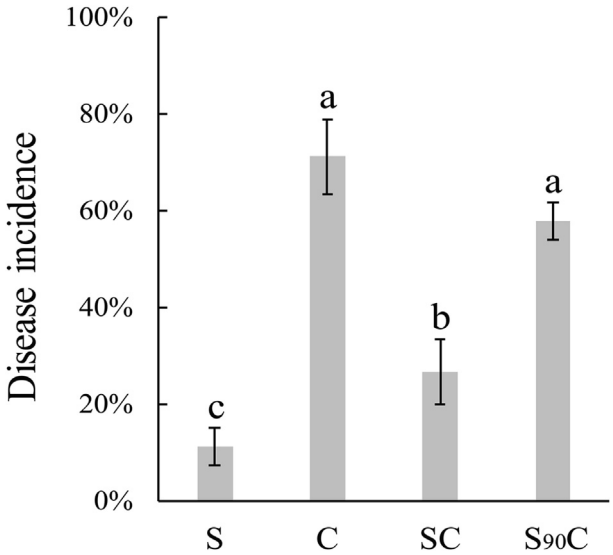

Fig. 1. Disease incidence of seedling vanilla in suppressive soil (S), conducive soil (C), conducive soil amended with 50% (w/w) of suppressive soil (SC) and conducive soil amended with 50% (w/w) of heat-treated suppressive soil ($S_{90}C$). Different letters above the bars indicate statistically significant differences according to Tukey's HSD test (n = 3, $F_{3,11} = 68.60$, $P < 0.001$, one-way ANOVA).

Table 2
Abundance, richness and phylogenetic diversity indices of fungi and bacteria from disease-suppressive and -conducive soils.

	Soil traits	Abundance*	Richness (Chao1)	Phylogenetic diversity
Fungi	Suppressive soil	9.04 ± 0.08 a	1483.30 ± 106.98 a	274.54 ± 19.44 a
	Conducive soil	8.69 ± 0.12 b	1085.81 ± 72.09 b	200.56 ± 16.05 b
Bacteria	Suppressive soil	10.51 ± 0.15 B	5917.91 ± 188.29 B	222.81 ± 23.67 B
	Conducive soil	10.92 ± 0.10 A	6816.49 ± 78.56 A	302.19 ± 12.06 A

Values are means ± standard deviation, disease-suppressive and -conducive soils (n = 9).
Means followed by a different letter for a given factor are significantly different ($P < 0.05$; Student's t-test).
* Fungal or bacterial copy numbers were $\log_{10}$-transformed in abundance.

treatment, however, did not have a significant impact on disease incidence ($P = 0.09$; Tukey's HSD test). Together, these results indicate that soil microbial communities, rather than chemical properties, are responsible for the differences in *Fusarium* wilt disease suppression between the two soils.

3.3. Microbial community abundances, diversity and structure

Fungal and bacterial abundances, richness (Chao1) and phylogenetic diversity are summarized in Table 2. We observed significantly higher fungal abundance, richness and phylogenetic diversity in suppressive soil samples ($P < 0.05$; Student's t-test). In contrast, conducive soil samples contained higher bacterial abundance and diversity.

As shown in Fig. 2, the weighted-UniFrac PCoA showed that both fungal and bacterial communities from suppressive soil were clearly separated from the conducive soil (AMOVA: Fs = 39.40, $P < 0.001$ for fungi; Fs = 30.39, $P < 0.001$ for bacteria). In addition, the weighted-UniFrac distance within suppressive soil was significantly ($P < 0.05$; Student's t-test) higher than in the conducive soil for the bacterial community system, whereas it was not significant ($P = 0.69$; Student's t-test) for the fungal community system (Fig. S2).

3.4. Taxonomic composition

A total of 474,250 ITS1 sequences and 736,751 V4 16S rRNA sequences were analyzed across the 18 soil samples, and sequences were grouped into 4071 fungal and 10,191 bacterial OTUs, respectively. Fungal OTUs were predominantly associated with the phyla *Ascomycota*, *Basidiomycota* and *Zygomycota*, and these three phyla accounted for 88.23% of the total fungal sequences (Fig. 3). *Zygomycota* and *Basidiomycota* were more abundant in suppressive soil than that in conducive soil, whereas the *Ascomycota* showed the opposite trend. Bacterial sequences were classified into a total of 21 different phyla, with the most dominant phyla being *Acidobacteria* (25.56%), *Proteobacteria* (22.99%), *Verrucomicrobia* (6.44%), *Bacteroidetes* (4.17%), *Actinobacteria* (1.74%) and *Firmicutes* (1.26%) (Fig. 3). Moreover, *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria* and *Firmicutes* were relatively more abundant in suppressive soil, whereas *Proteobacteria* and *Bacteroidetes* were more prevalent in conducive soil.

We used LEfSe to further analyze the association of the top 40 abundant fungal and bacterial genera with the disease-suppressive and -conducive soils. The fungal genera, *Mortierella*, *Ceratobasidium*, *Gliocladiopsis*, *Cylindrocladium*, *Staphylotrichum* and *Gymnopus* were more abundant in the suppressive soil (Fig. 4). *Mortierella* was hyper-dominant in the suppressive soil, accounting for 37.38% of the total fungal sequences in suppressive soil (only 1.82% in conducive soil), whereas *Fusarium* was the most dominant genus comprising 17.20% of total fungal genera in conducive soil (only 0.50% in suppressive soil) (Table S1). With respect to bacteria, 19 genera out of the top 40 bacterial genera were more abundant in conducive soil samples (Fig. 4), such as *Ohtaekwangia*,

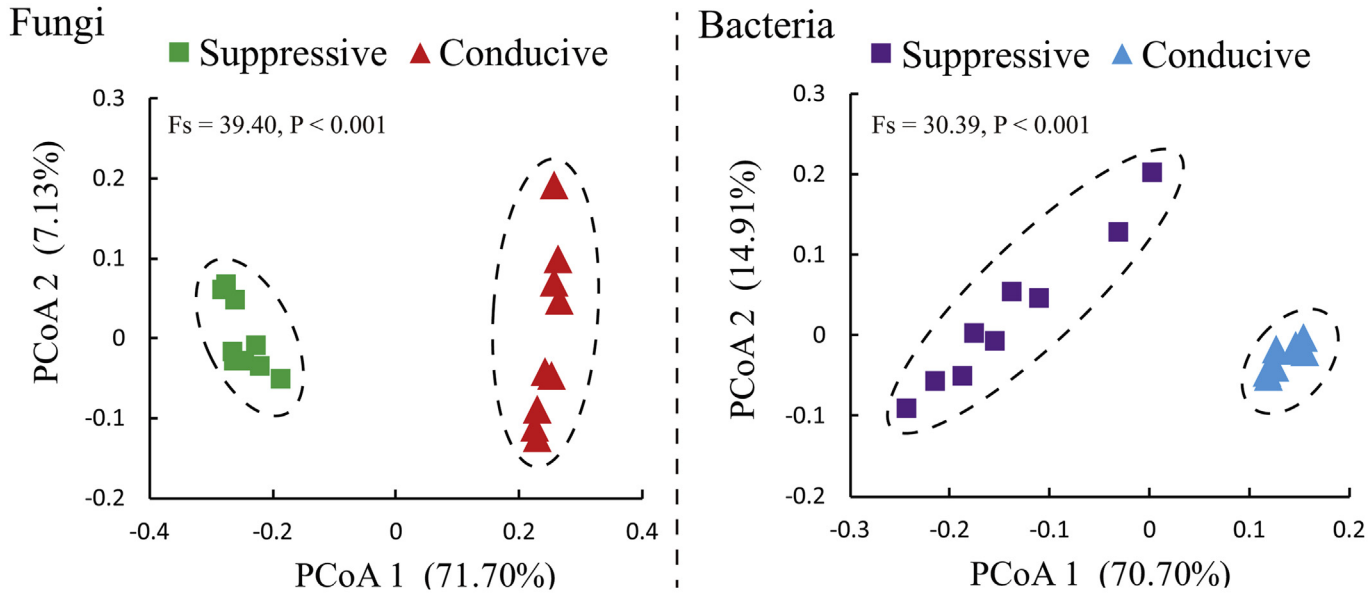

Fig. 2. UniFrac-weighted principle coordinate analysis of fungal and bacterial community structures in disease-suppressive and -conducive soils.

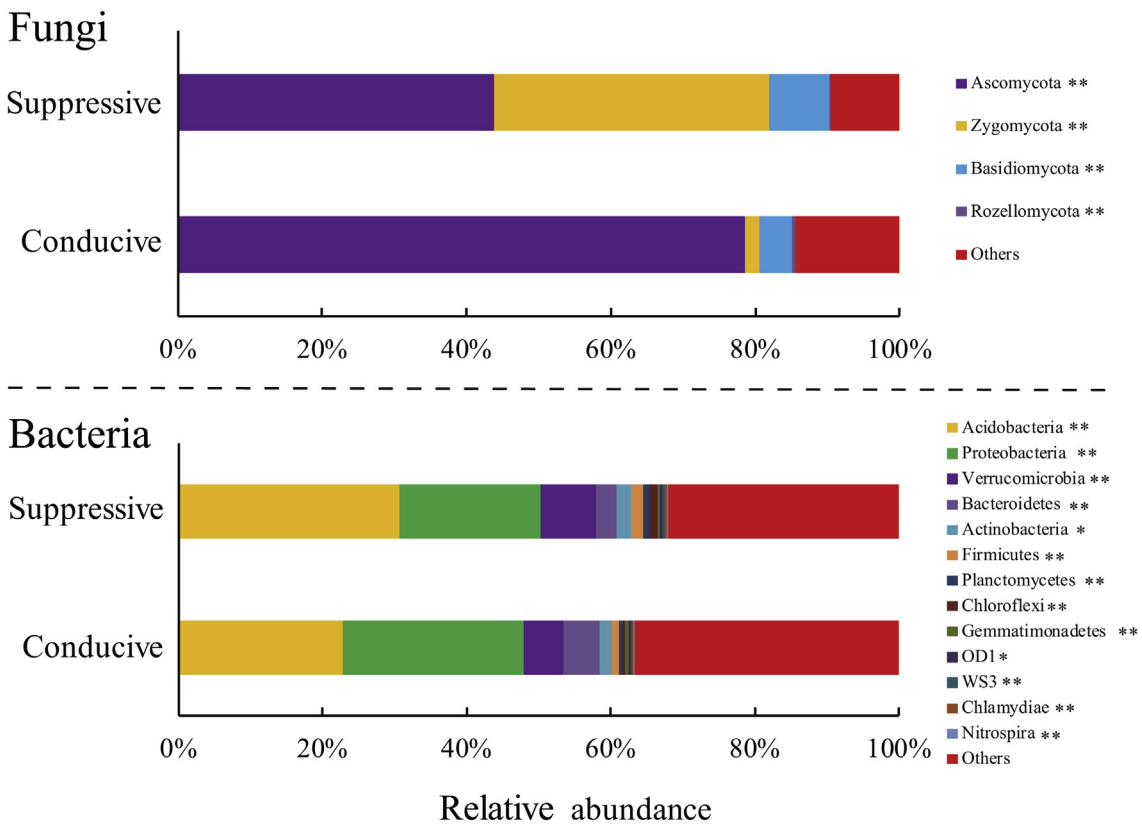

Fig. 3. The average relative abundances of fungal and bacterial phyla from disease-suppressive and -conducive soils. Others includes phyla below 0.1% of relative abundance and the unclassified phyla.*and ** represent significance ($P < 0.05$ and $P < 0.01$) between disease-suppressive and -conducive soils according to Student's *t*-test (n = 9).

Ignavibacterium, *Solirubrobacter* and some groups of *Acidobacteria* (*Gp4*, *Gp6*, *Gp17* and *Gp22*), whereas the *Acidobacteria* groups *Gp2*, *Gp1*, *Gp3*, *Gp13* and *Ktedonobacter* were more abundant in the disease-suppressive soil.

3.5. Fungal and bacterial community networks

In the fungal phylogenetic Molecular Ecological Networks (pMENs), disease-suppressive and -conducive soils resulted in networks with similar sizes (225 and 230 nodes, respectively; Fig. 5). For suppressive and conducive networks, the average connectivity (connectivity or degree of distribution is the number of links of a node to other nodes) was 6.85 and 5.48, respectively (Table 3); the average path length (path length is the shortest path between two nodes) was 3.28 and 2.42, respectively. The suppressive soil (0.34) had a higher value of average clustering coefficient (clustering coefficient describes how close among the neighbors of a node) than the conducive soil (0.27). Moreover, suppressive and conducive soils harbored 24 and 37 modules with modularity values 0.56 and 0.50, respectively. Modularity measures the degree to which the network is organized into clearly delimited modules, networks with high modularity have denser connections between the nodes within modules but sparser connections between nodes in different modules. Suppressive soil (771 links) had higher link numbers than conducive soil (630 links). Strikingly, the positive link/negative link ratio (P/N) in suppressive soil (P/N = 15.76) was much higher than that in conducive soil (P/N ratio = 1.68), demonstrating that the suppressive soil contained more positive co-occurrence relationships than the conducive soil. In line with the community overview data, the 3 most abundant

Table 3
Major topological properties of the empirical phylogenetic Molecular Ecological Networks (pMENs) of fungal and bacterial communities in disease-suppressive and -conducive soils and their associated random pMENs.

	Empirical networks									Random networks[d]		
	Soil traits	No. of original OTUs[a]	Similarity threshold (S_t)	Network size (n)[b]	R^2 of Power law	Avg connect (avgK)	Avg path length (GD)[c]	Avg clustering coefficient (avgCC)	Modularity (No. of modules)	Avg path distance (GD)	Avg cluster coefficient (avgCC)	Modularity (M)
Fungi	Suppressive	300	0.86	225	0.85	6.85	3.28	0.34	0.56 (24)	2.91 ± 0.11	0.11 ± 0.01	0.31 ± 0.01
	Conducive	300	0.89	230	0.73	5.48	2.42	0.27	0.50 (37)	3.00 ± 0.12	0.08 ± 0.01	0.36 ± 0.01
Bacteria	Suppressive	300	0.90	186	0.87	4.25	3.46	0.24	0.62 (21)	3.21 ± 0.16	0.04 ± 0.01	0.45 ± 0.01
	Conducive	300	0.86	225	0.85	3.56	4.34	0.24	0.71 (21)	3.89 ± 0.15	0.02 ± 0.01	0.53 ± 0.01

[a] The number of OTUs that were originally used for network construction using the random matrix theory (RMT)-based approach.
[b] The number of OTUs (i.e., nodes) in the network.
[c] GD, geodesic distance.
[d] The random networks were generated by rewiring all of the links of a pMEN with the identical numbers of nodes and links to the corresponding empirical pMEN.

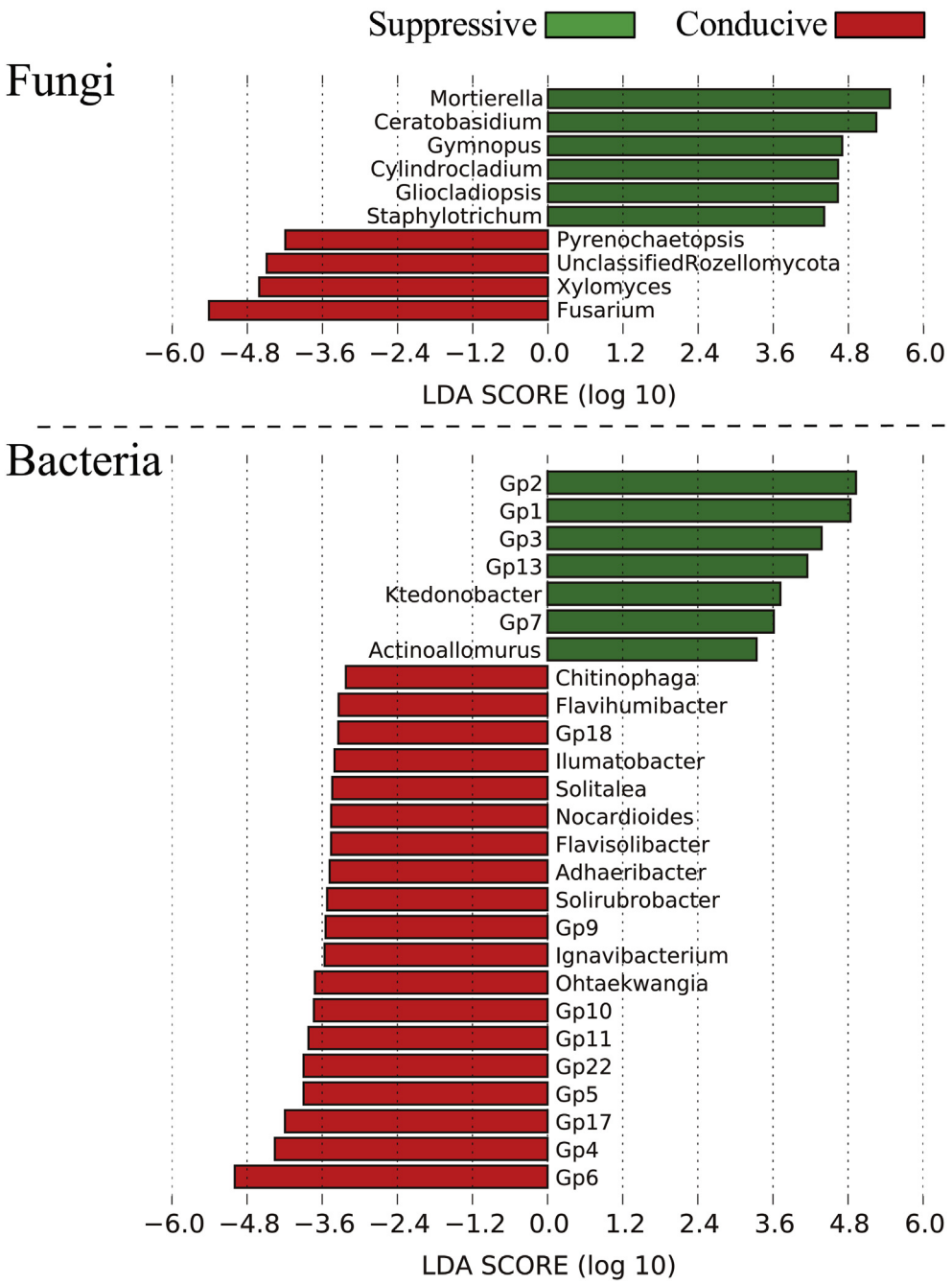

Fig. 4. Histogram of the LDA scores computed for differentially abundant fungal and bacterial genera between the disease-suppressive and -conducive soils.

nodes in suppressive soil (OTU_2, OTU_5 and OTU_81) belonged to *Mortierellales*, whereas the most abundant species in the conducive soil network (OTU_3: *Fusarium pseudensiforme*) belonged to *Fusarium* genus. It is worth noting that this OTU does not correspond to the pathogen *F. oxysporum*, suggesting that this co-generic species may also play a role in the vanilla *Fusarium* wilt disease patterns.

With respect to bacteria, networks with 186 and 225 nodes were constructed from suppressive and conducive soil samples, respectively (Fig. S3 and Table 3). The average connectivity was 4.25 and 3.56, and the average path length was 3.46 and 4.34 for suppressive and conducive networks, respectively. The suppressive and conducive soil networks had the same average clustering coefficient value (0.24), and the modularity value in suppressive networks (0.62) was lower than that in conducive networks (0.71). The total link numbers in suppressive and conducive soils were 395 and 401 (P/N = 2.31 for suppressive soil and P/N = 1.49 for conducive soil), respectively.

3.6. Effects of soil chemical properties on microbial communities

Mantel tests revealed that soil chemical properties were significantly correlated with fungal and bacterial communities ($R = 0.51$, $P < 0.01$ for fungi and $R = 0.57$, $P < 0.001$ for bacteria). In addition, Spearman's rank correlation coefficient was used to evaluate the relationships between the soil properties and alpha diversity indices (Table S2). Bacterial richness and phylogenetic

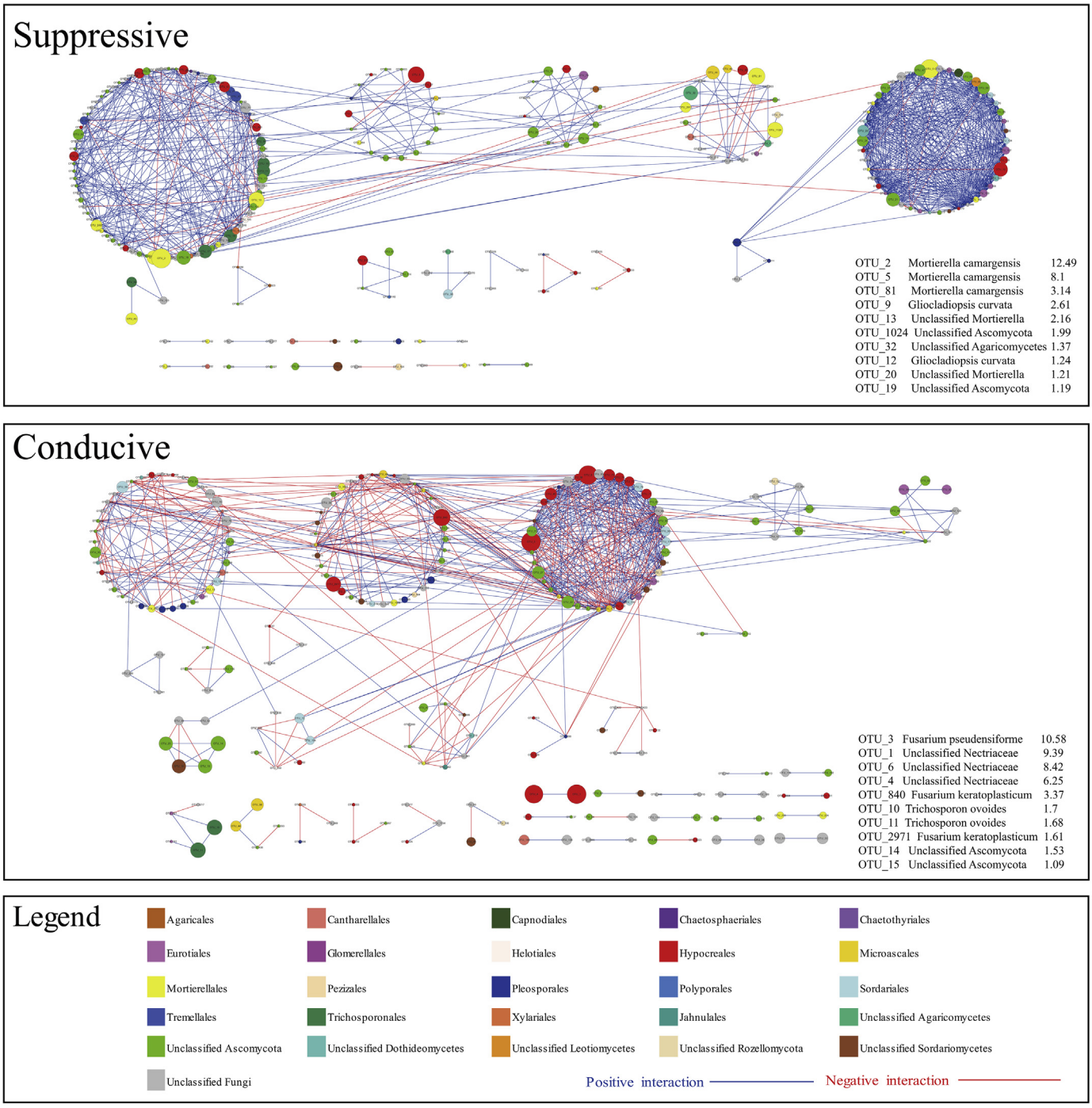

Fig. 5. Network plots of fungal communities in disease-suppressive and -conducive soils. The node size is proportional to an OTU's relative abundance. Classification of the 10 most abundant OTUs from disease-suppressive and -conducive soils were showed in the figure with the relative abundance (%) behind. Node colors indicate different phylogenetic associations. Lines connecting nodes (edges) represent positive (blue) or negative (red) co-occurrence relationships. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

diversity showed positive correlations with soil pH and EC, whereas the fungal richness and phylogenetic diversity were negatively correlated with these two soil properties. In contrast, fungal richness and phylogenetic diversity were positively correlated with available N, P and Fe contents.

4. Discussion

Fusarium wilt disease is an economically important fungal disease of many crops, including cucumber (Klein et al., 2016), watermelon (Ling et al., 2011), banana (Wang et al., 2015) and vanilla (Pinaria et al., 2010). Characterizing microbial communities in disease-suppressive soil is an important first step toward understanding the potential impacts of these communities on plant fitness (Rosenzweig et al., 2012). In the present study, we compared the soils from two similar orchards that were free from *Fusarium* wilt disease prior to vanilla cultivation. Since the initiation of vanilla cultivation, these two orchards have diverged in terms of the

incidence of *Fusarium* wilt disease and the density of *Fusarium oxysporum*, thereby yielding one disease-conducive and one disease-suppressive soil. Both soil fungal and bacterial communities varied in abundance, diversity, structure, taxonomic composition and microbial interactions in relation to vanilla *Fusarium* wilt disease suppression. Results showed the suppressive capacity of the soil was linked to the resident microbial communities, and this could be transferred from one soil to the other.

Based on the qPCR results, *Fusarium oxysporum* absolute abundance differed by a factor of less than two between the conducive and suppressive soils. This variation may explain a part of the difference in disease incidence between the two soils (Pinaria et al., 2010; Xiong et al., 2015b). This is in line with previous findings that total *Fusarium oxysporum* abundance significantly correlates with vanilla *Fusarium* wilt disease (Xiong et al., 2015b). However, the difference we detected is too small by itself to drive the sharp contrast in disease incidence between conducive (11%) and suppressive (70%) soils. We hypothesize that even though the pathogen is present in the suppressive soil, full manifestation of the disease is impeded by the actions of the resident microbial community. Indeed, mixing the two soils in equal quantity resulted in a low *Fusarium* wilt disease incidence, while mixing conducive soil with heat-treated suppressive soil resulted in disease incidence levels comparable to the conducive soil alone. Together, these findings indicate that not only the pathogen absolute abundance, but also other microbial communities, determined the significant difference in disease incidence.

The suppressive soil harbored a significantly higher fungal abundance and diversity than the conducive soil. This is in line with a previous study that highlighted the importance of fungal diversity for soil health in an intensive potato cropping system (Manici and Caputo, 2009). Conversely, our suppressive soil showed lower bacterial diversity. This result is in contrast with previous results from Rosenzweig et al. (2012), who showed that more diverse bacterial communities were found in potato common scab-suppressive soil. The lower bacterial diversity in our suppressive soil may be due to its lower pH, as we previously observed that bacterial diversity increased with higher soil pH values (Liu et al., 2014a; Shen et al., 2013).

Microbial taxonomic composition strongly varied between disease-suppressive and -conducive soils. *Ascomycota* and *Basidiomycota* were the most abundant fungal phyla identified in the conducive soil, in agreement with our previous work investigating microflora in the vanilla continuous cropping soil (conducive soil) (Xiong et al., 2015b). This result is also in agreement with a previous study in which *Ascomycota* and *Basidiomycota* were the top two prevalent fungal phyla in a continuous cropping peanut system (Li et al., 2014). In contrast, members of the phylum *Zygomycota* strongly dominated the soil fungal community in suppressive soil. This was somewhat surprising, as this phylum is often rare in agricultural soils (Wang et al., 2015; Xu et al., 2012). We speculate that the dominant *Zygomycota* species may play a role in disease suppressiveness. For instance, they may inhibit *Fusarium* pathogen via competition for niche space and resources (Pal and Gardener, 2006). For bacteria, the phyla *Actinobacteria* and *Firmicutes* were more abundant in our suppressive soil, and these two phyla are known for species that produce high levels of secondary metabolites (Kim et al., 2011; Palaniyandi et al., 2013). Previous studies have also found higher abundances of *Actinobacteria* and *Firmicutes* in *Rhizoctonia*-suppressive soil (Mendes et al., 2011). Our results suggest that members of these phyla may play a similar role in *Fusarium* wilt disease suppression.

The linear discriminant analysis (LDA) effect size (LEfSe) method revealed some of the specific microbial groups (at the genus level) associated with vanilla *Fusarium* wilt disease-suppression. For fungi, *Fusarium* was the most dominant genus in conducive soil, comprising 17.20% of the total fungal sequences. *Mortierella* was the most abundant genus in the suppressive soil, accounting for 37.38% of the total fungal sequences. Previous studies have shown that some species of *Mortierella* can produce antibiotics, and several isolates have been investigated as potential antagonistic agents against various plant pathogens (Tagawa et al., 2010; Wills and Lambe, 1980). This taxon may serve as an important indicator of *Fusarium* wilt disease suppression in vanilla cropping systems. For bacteria, the structure of the *Acidobacteria* phylum highly discriminated between suppressive and conducive soils. *Gp4* and *Gp6* were more abundant in the conducive soil with higher pH, while other groups of *Acidobacteria* such as *Gp1*, *Gp2* and *Gp3* were more prevalent in suppressive soil with low pH. These results were in line with several previous studies demonstrating the importance of pH as a global regulator of *Acidobacterial* communities, with *Gp4* and *Gp6* being positively correlated with pH, and *Gp1*, *Gp2* and *Gp3* associated with low pH (Bartram et al., 2014; Jones et al., 2009).

In additional to microbial community traits, soil characteristics may also be important indicators of disease suppression. In this study, suppressive soil had moderately higher OM, available N, P and Fe contents compared to the conducive soil. Liu et al. (2014b) found that *Fusarium* abundance was negatively correlated with soil OM in a potato monoculture system. Research by Shen et al. (2015) also indicated that higher soil available P was associated with lower banana *Fusarium* wilt disease incidence in naturally suppressive soil. It may be that higher soil OM, available P, N and Fe stimulates plant growth and general plant health, and thereby enhancing the plant's capacity to resist disease. In our pot experiments, however, these soil chemical properties by themselves were not sufficient to induce disease suppression, as pasteurized suppressive soils could not transfer suppressive capabilities. We propose that the process of degradation from suppressive to conducive soil may induce feedback loops between soil properties such as pH and microbial communities. Slightly lower pH in the suppressive soil may have selected for specific microbiota such as *Gp1*, which often acts as a plant growth-promoting bacterium (Kielak et al., 2016). The altered microbial community may in turn contribute to a higher disease suppression in this system. Future studies using a time series in long-term experiments would be helpful for disentangling the relationships between soil characteristics, microbiota and *Fusarium* wilt disease.

Microbial molecular ecological networks revealed distinct patterns within the microbial communities of suppressive- and conducive-soils. Not surprisingly, the putatively beneficial microbe *Mortierella* spp. held a dominant position in the suppressive soil. In contrast, *Fusarium* species had a dominant position in the conducive soil. However, most *Fusarium* sequences were not affiliated with the wilt pathogenic species itself (*Fusarium oxysporum*), suggesting that high disease incidence may also be associated with an increased abundance of other co-generic species. Future studies would be required to determine the specific role of these species in promoting *Fusarium* wilt disease, either via facilitation of the pathogen or as pathogenic agents themselves. The suppressive soil showed a higher number of positive co-occurrence relationships than the conducive soil for both fungal and bacterial networks (especially in fungal network). More positive interactions may suggest more cooperation in the complex microbial community ecosystems (Zhang et al., 2014). Although high levels of cooperation may be linked to a higher community function, such interactions can also lead to a destabilization (Coyte et al., 2015). In addition, the suppressive soil had higher average connectivity than the conducive soil for both fungal and bacterial networks. A highly connected network may provide resistance to disturbance (Scheffer et al., 2012) up to a critical threshold that is still undefined at our study

site. Now that we have determined the differences between suppressive and conducive soils with respect to microbial communities and soil properties, this opens up future research perspectives for understanding how transitions develop between conducive and suppressive states.

5. Conclusions

We showed that similar soils that differ in their abilities to suppress vanilla *Fusarium* wilt disease have contrasting patterns of fungal and bacterial community structures. Although the *F. oxysporum* pathogen was present in both soils, it only leads to *Fusarium* wilt disease in the conducive soil. We propose that fungal communities may play a particularly important role in keeping *F. oxysporum* infection under control in the suppressive soil. The genus *Mortierella*, which accounted for 37% of the total fungal sequences, may be a key player in *F. oxysporum* suppression. Further studies identifying *Mortierella* spp. isolates with effective disease suppression ability and revealing the functional mechanisms involved in *Mortierella-F. oxysporum* interactions may open new avenues for the development of informed bio-control strategies.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.01.010.

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