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Supplementary Materials and methods

Quantitative PCR analysis

The abundances of bacteria and fungi were quantified with gene-specific primers (Table S1) and SYBR® *Premix Ex Taq*™ II (Takara Bio Inc., Kusatsu, Japan), following the manufacturer's instructions. qPCRs amplifications were performed with a total volume of 20 µl reactions on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Thermal cycling conditions were 30 s at 95°C, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. Standard curves of bacteria and fungi were carried out as previously described [1]. Each sample was performed in three replicates, and the results were expressed as $\log_{10}$ target copy numbers g^{-1} soil.

Data processing

Resulting raw sequences were first demultiplexed to each sample and to remove the adaptor and primer sequences in QIMME (1.8.0) [2], then sequences were trimmed and filtered by using the built-in "filterAndTrim" function of DADA2 (v1.6) [3] in R (v3.4.3), with the following parameters: truncLen = c(180, 180), maxN = 0, maxEE = c (2, 2), truncQ = 2. Error rates were learned, an amplicon sequence variants (ASVs) table was generated through the de-noising, pair-merging, and chimera-removing steps of the DADA2 pipeline by using default settings. Representative sequence for bacteria were classified using the RDP naïve Bayesian classifier [4] with dada2 package, and fungal ASVs were classified using UNITE database (version 7.2) in mothur [5].

Prior to statistical tests and community metrics analysis, the ASV tables were

rarefied at depths of 10227 sequences for bacteria and 18356 sequences for fungi, per sample. Shannon diversity index were calculated using *vegan* package in R (v3.4.3). β-diversity for both bacterial and fungal communities were determined using principal coordinate analysis (PCoA) analyses based on the Bray-Curtis distance matrices with *ape*, *stats* and *vegan* package in R (v3.4.3). Permutational multivariate analyses of variance (PERMANOVA, permutations=999) [6] were performed to test the significant difference among microbial composition of samples via *vegan* package in R (v3.4.3).

In order to characterize the abundance of microbial taxa, the estimated absolute abundance (EAA) was adopted and calculated as the relative abundance of bacterial and fungal ASVs multiplied by the total bacterial and fungal count (qPCR), respectively [7]. Linear discriminant analysis (LDA) effect size (LEfSe) method, which was implemented in the online Galaxy framework (http://huttenhower.sph.harvard.edu/galaxy/), was applied to analyze the differential abundance of soil microbial taxa between the rhizosphere soil of healthy and diseased banana plants [8]. The alpha value employed for the Wilcoxon rank test was 0.05, and the threshold employed on the logarithmic LDA score for discriminative feature was 4.0. Especially, to identify and compare the EAA of pathogen, all the sequences affiliated to *Fusarium* genus were aligned with FOC Race 4 (FOC4) rDNA sequences in NCBI GenBank (accession number: LT571434.1), and the phylogenetic tree was constructed with MEGA 7.0.26 by neighbor joining method with 1000 bootstrap replications and 50% consensus.

Co-occurrence networks were constructed to elucidate correlations between pathogen and microbial taxa. ASVs with less than twenty sequences were filtered and appeared in at least five samples were retained to contrast the co-occurrence network

for each sample, respectively. The co-occurrence relationship was implemented with Sparse Correlations for Compositional data algorithm (SparCC) method in python (five iterations), together with a two-sided pseudo *P* values less than 0.05 based on bootstrapping of 500 iterations [9]. The nodes topological roles played in the network were determined and classified based on the value of within-module connectivity (*Zi*) and among-modules connectivity (*Pi*), which were calculated by *rnetcarto* packages in R (v3.4.3). In general, the nodes in the network were distributed into four categories: peripherals ($Zi \leq 2.5$ and $Pi \leq 0.62$, nodes almost connected within modules with few links), connectors ($Zi \leq 2.5$, $Pi > 0.62$, nodes highly connected to modules), module hubs ($Zi > 2.5$, $Pi \leq 0.62$, nodes highly connected within modules), and network hubs (nodes highly connected within entire network, $Zi > 2.5$ and $Pi > 0.62$) [10]. Network graphs were visualized using Cytoscape 3.5.0 software.

Statistical analyses

All statistical tests were performed in R v3.4.3 [11] and considered significant at $P < 0.05$. Non-normal data were log-transformed or square-root to meet test assumptions. Significant differences in Shannon diversity index, EAA of *Fusarium* and fSeq2 across samples were determined by the Kruskal-Wallis rank test followed by pairwise Wilcoxon rank sum tests with FDR corrected.

References

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Supplementary Figures

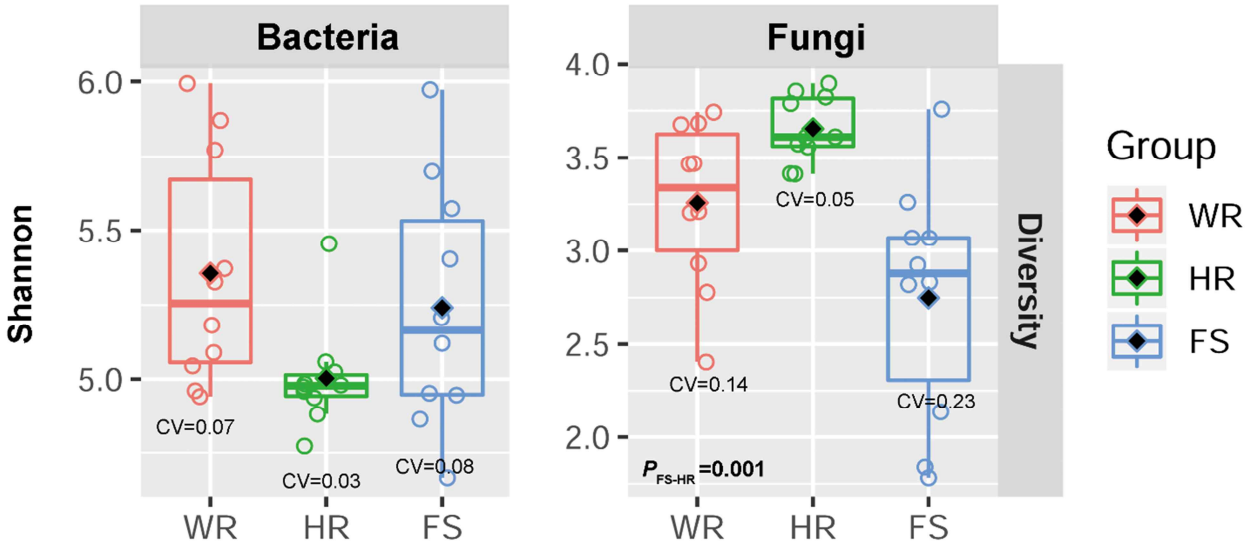

Fig. S1 Bacterial and fungal Shannon diversity index. The bottoms and tops of boxes represent the 25 and 75% quartiles, respectively. The horizontal bars and diamond within boxes represent the median and mean, respectively. WR, rhizosphere soil of diseased banana plants; HR, rhizosphere soil of healthy banana plants; FS, forest soil. Only significant *P* values between soil samples are shown. The coefficient of variability (CV, standard deviation divided by the mean) in Shannon diversity index were also calculated and are displayed.

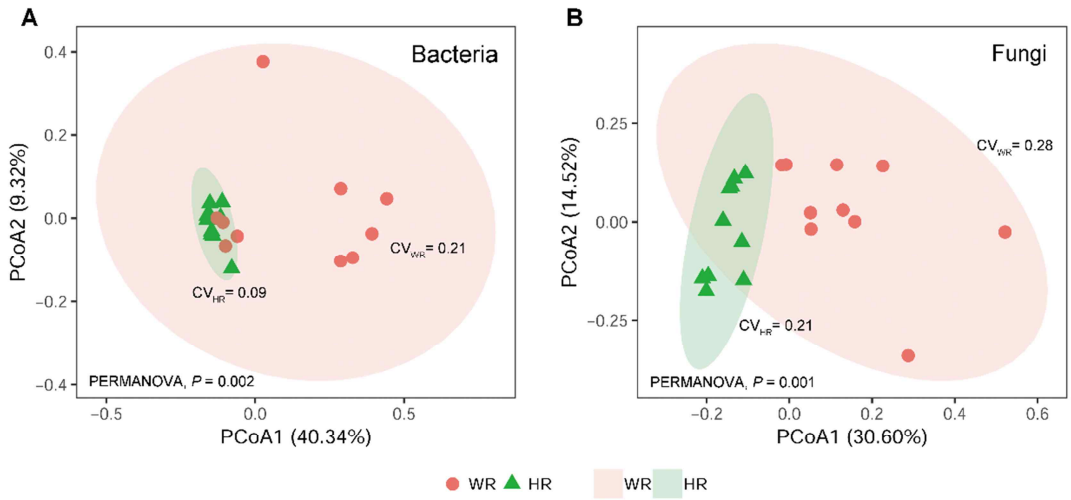

Fig. S2 Principal coordinate analysis (PCoA) of bacterial (A) and fungal (B) community structure based on Bray-Curtis distance metric between WR and HR soil samples. WR, rhizosphere soil of diseased banana plants; HR, rhizosphere soil of healthy banana plants. The coefficient of variability (CV, standard deviation divided by the mean) in community structure based on Bray-Curtis distance within group were also calculated and shown.

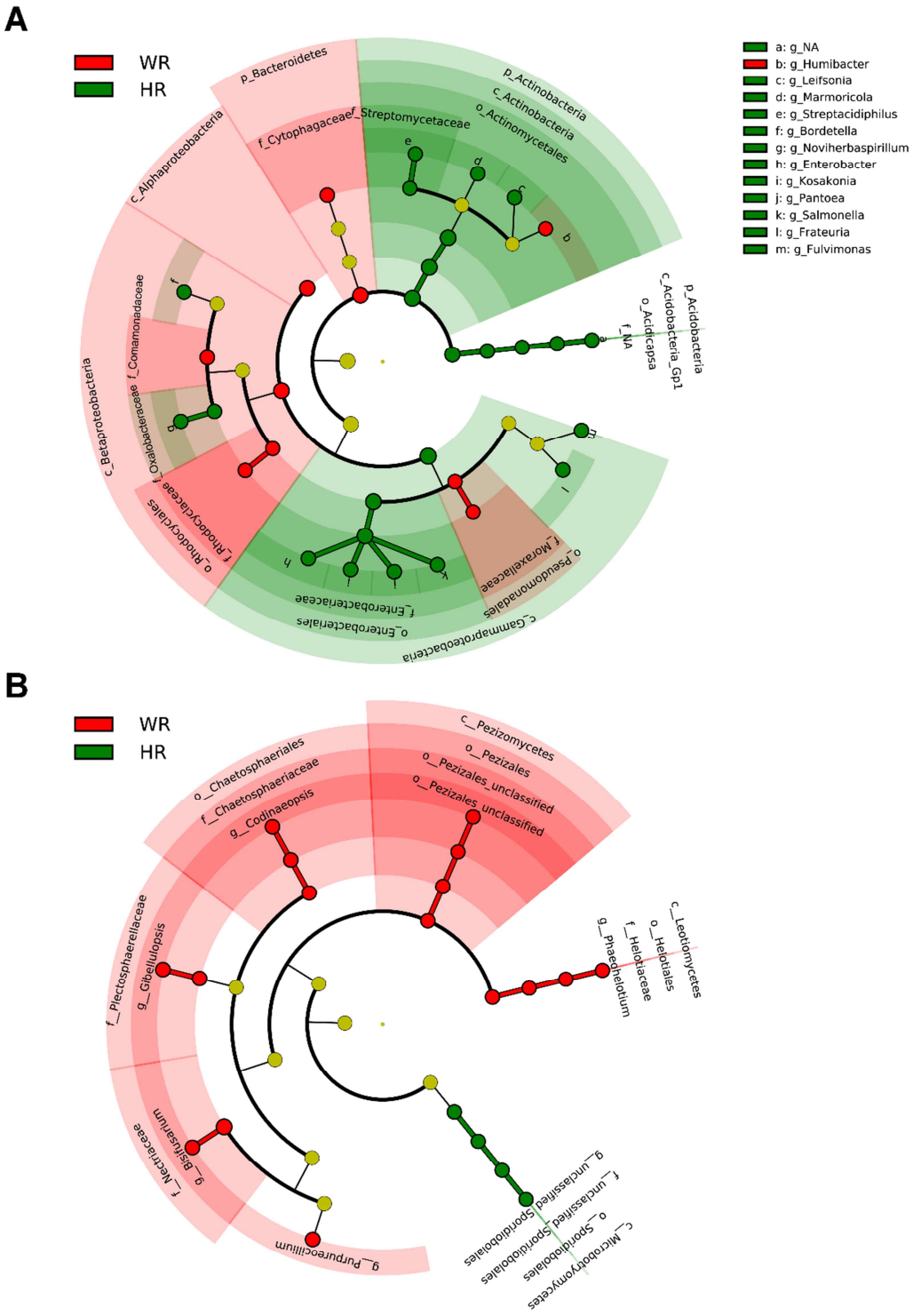

Fig. S3 Taxonomic representation of statistically and biologically consistent differences in bacteria (A) and fungi (B) between rhizosphere soil of healthy (HR) and diseased (WR) banana plants. Differences are represented in the color of the most abundant class (red indicating WR, green indicating HR, yellow non-significant).

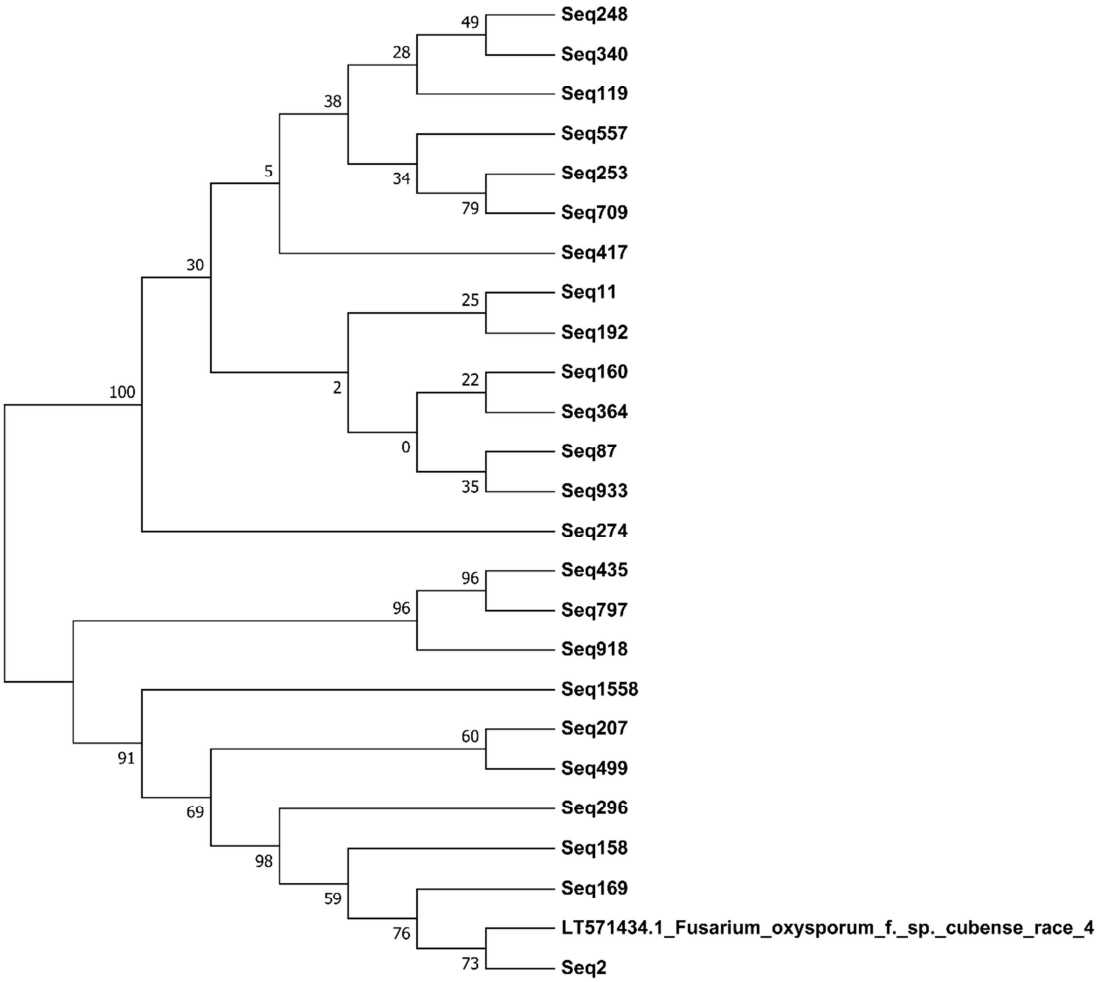

Fig. S4 Phylogenetic tree of ITS sequences from *Fusarium* ASVs and reference strain *F. oxysporum cubense* Race 4 with neighbor joining method.

Supplementary Table

Table S1 Sequences of oligonucleotide primers required for real time quantitative PCR

Target group	Primer name and sequence (5′−3′)
Bacteria	Eub338F, ACTCCTACGGGAGGCAGCAG
	Eub518R, ATTACCGCGGCTGCTGG
Fungi	ITS1f, TCCGTAGGTGAACCTGCGG
	5.8s, CGCTGCGTTCTTCATCG