Suppression of Banana *Fusarium* Wilt Disease With Soil Microbial Mechanisms Via Pineapple Rotation and Residue Amendment

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Abstract

Aims The large outbreak of banana *Fusarium* wilt has become a bottleneck limiting the industry's development, and crop rotation is a cost-effective and essential measure to overcome the obstacles of banana crop monoculture. The present work was carried out to explore the mechanisms of how changes in physicochemical properties and the reestablishment of soil microorganisms in high-incidence soils are affected by crop rotation and plant residue.

Methods In this study, pineapple-banana crop rotation and pineapple residue amendment were used to alleviate banana *Fusarium* wilt, and their effects on bacterial and fungal communities were studied using the MiSeq Illumina sequencing platform.

Results Both pineapple-banana rotation and residue addition significantly reduced disease incidence. Moreover, pineapple rotation and residue amendment altered the bacterial and fungal community composition. The taxonomic and phylogenetic alpha diversity of bacteria and fungi significantly increased against disease suppression and nutrition competition. The relative abundances of the *Burkholderia*, *Pseudomonas*, *Elaphocordyceps, Penicillium,* and *Talaromyces genera* were higher, and the number of *Fusarium* was significantly lower in rotational soil than in banana monoculture soil. Finally, linear models (LM) was used to show that the *Burkholderia and Talaromyces* in crop rotation, and *Aspergillus* in residue amendment have significant negative relationship to disease incidence, which plays a key role in *Fusarium* reduction.

Conclusions To consider the economic benefits and protect the vitality of the soil, this study suggested that pineapple-banana rotation and pineapple residue amendment both could be considered for the sustainable management of banana wilt.

Introduction

Currently, soil-borne diseases are a serious threat to soil health and crop productivity (Dita et al. 2010; Mendes et al. 2013) and have become one of the major problems for the sustainable development of intensive agriculture (Butler 2013). Banana (*Musa* spp.) is among the crops more severely affected by succession disorders caused by multiple abiotic (poor soil fertility, drought, high temperature and salinity) and biotic (fungi, bacteria, viruses and pests) factors (Mendes et al. 2014; Huang et al. 2012; Hwang and Ko 2004), particularly strains of the fungal pathogen *Fusarium oxysporum* f. sp. cubense (Foc4), this fungus has an extremely serious impact on the banana industry worldwide (Zhang et al. 2014; Wang et al. 2013; Sun et al. 2018; Ploetz and Churchill 2011).

For a long time, physical (e.g., exposure), chemical (e.g., soil fumigation) and biological (e.g., antagonistic microorganisms) methods have reduced the pathogens in the soil to some extent and have made important contributions to the effective prevention and control of soil-borne diseases (Lakshmanan et al. 2008; Xue et al. 2019; Li et al. 2019; Su et al. 2017). However, currently, in high-incidence banana soil, the soil microbiota is severely imbalanced, and the short-term reduction in the pathogenic population does not fundamentally improve the imbalance of the soil microbiota (Ploetz 2015). Therefore, in high-incidence fields, to gradually restore a healthy soil microbiota, crop rotation may be an important measure for the gradual modification of soil ecology. Studies show that soil pathogens can be reduced by using specific agricultural practices, for example, the incorporation of plant residues in the soil and crop rotation (Larkin 2015; Bonanomi et al. 2018).

Crop rotation is an important measure for gradually changing the soil ecology (Li et al. 2021). Different crop rotation systems have different effects on the suppression of different diseases, such as obstructing the inbreak of pathogens, chemosensitive substances, and antagonistic microorganisms, improving soil fertility through root exudates and residues, and increasing soil microbial biomass and activity (Christen and Sieling 1995; Wang et al. 2010; Larkin and Halloran 2014; Wright et al. 2014). These strategies mostly rely on promoting the emergence of protective microbiomes or directly impacting the population of the pathogen, both of which account for effective disease control (Cunfer et al. 2006; Wang et al. 2016; Tao et al. 2020).

The soil microbiome plays an important role in promoting plant growth and development, nutrient uptake, disease resistance and adaptation to environmental stresses. Their abundance, composition and activity largely determine the sustainability of agriculture. Recent studies have shown that modulation of the soil microbiome can reduce the number of pathogens in the soil and enhance rhizosphere immunity, thereby reducing or suppressing disease occurrence. For example, Wei et al. (2019) showed that phages can "target" tomato cyanobacteria to disrupt their ability to survive while structuring the rhizosphere soil flora to restore community diversity and increasing the abundance of beneficial microbes. In addition, the abundance and activity of *Pseudomonas*, *Sphingomonas*, *Penicillium* and *Trichoderma* spp. were found to be significantly higher than the *Fusarium* number in the soil (Hong et al. 2020). The soil microbiome can enhance rhizosphere immunity, improve soil fertility and crop yield, maintain the persistence of plants, animals and humans, and protect soil health (Bender et al. 2016). Additionally, there are many protozoans that protect crop health by feeding on or producing beneficial antibacterial

substances to defend against the invasion of other *Fusarium* pathogens (Guo et al. 2021). More interestingly, the interactions between soil microbial communities are also closely related to the ability of *Fusarium* to invade plants and affect their health. Studies have shown that immune rhizosphere microbial communities are more diverse and have a more complex microbial network (Ge et al. 2021). It was shown that rhizosphere competitive intercropping communities could produce more bacterial inhibitory substances or fully occupy limited ecological niches, thus effectively suppressing pathogen invasion and reducing widespread outbreaks of soil-borne diseases.

Furthermore, studies have shown that plant root exudates and residues in soil can have a long-lasting effect on pathogen control as a result of crop rotation and relieve soil pressure by improving soil properties (Hu et al. 2018; Yuan et al. 2018; Zhou et al. 2020). As such, it is clear that crop rotation and plant residue manipulation in the field are important strategies for soil-borne disease management (Mawar and Lodha 2015). However, these strategies have received relatively little attention, as efforts have mostly been given to investigate how such approaches relate to overall aspects of soil quality, nutrient cycle, and crop performance (Wang et al. 2018; Martinez-Feria et al. 2018; Chen et al. 2018). Therefore, understanding how these approaches impact the soil microbiome composition and its ecological function remains elusive (Afshan et al. 2015). This opens up potential opportunities to explore beneficial outcomes of agricultural management associated with soil-borne disease control (De et al. 2020).

In our previous work, banana-pineapple rotation was picked out for its high-efficiency in banana *Fusarium* wilt disease prevention and control (Wang et al. 2015). In normal farming operations, pineapple residue is returned to the field after rotation. Therefore, the effect of crop rotation consists of two parts: simple crop rotation and residue return. We hypothesize that both crop rotation and residue amendment have inhibitory effects on banana *Fusarium* wilt, while, how they work was still unknown. In this study, two pot experiments, crop rotation and residue amendment, were designed to study the improvement of soil physicochemical properties and their effect on soil microbiota. Further, we hypothesise that pineapple-banana crop rotation and pineapple residue addition provides greater suppression of *Fusarium* wilt due to improved soil physicochemical properties that stimulate the growth of indigenous beneficial microorganisms, resulting in a significant reduction in disease incidence in bananas compared to that under monoculture. The aims of this study were (1) to evaluate the effect of the pineapple-banana crop rotation system on wilt disease in banana seedlings during the seedling period, (2) to estimate the changes in microbial community structure from the bulk soil to the rhizosphere soil zone, (3) to analyse the diversity of soil microorganisms, and (4) to study the relationship between the soil microbial community composition and environmental factors.

Materials And Methods

Crop residues, root exudates and soil sampling

Preparation of banana and pineapple residues: Banana and pineapple residues were collected from a field located at Paigou village in Zhongyuan town, Qionghai City, Hainan Province, China (110°30’E, 19°5’N). Briefly, the whole plants were put into plastic packaging bags, kept on ice, and transported to the laboratory (< 6 h). These crop residues were carefully washed five times with sterile deionized water in the laboratory and divided into root, stem leaf and fruit plant parts. CK: no residue, B_L: banana stem leaf residue, B_R: banana root residue, P_L: pineapple stem leaf residue, P_R: pineapple root residue, and P_F: pineapple fruit residue. These crop residues were chopped into tiny pieces and ground down to powder. Each residue type was sieved through a 4-mm mesh, and the total nutrient content was measured. Fresh plant samples were dried, ground and extracted at a ratio of 1:5 plant residue: deionised water for 48 hours and aseptically filtered to obtain the extract master batch.

Preparation of banana and pineapple root exudates: The plants were removed from the soil, rinsed five times with tap water and five times with deionised water, and incubated in a plastic cup containing 300 mL of deionised water for 24 h. The solution containing the root exudate was combined and then slowly filtered (filter membrane pore size: 0.45 μm, size: Φ25 mm), and the filtrate was freeze-dried to dryness. All roots of pineapple plants involved in root exudate collection were then dried and weighed separately, the freeze-dried material was dissolved separately in an appropriate amount of deionized water, and the volume was fixed to one gram of dry weight root exudate per mL of root exudate master batch (i.e., 1 g of dry root exudate per mL) and stored at -20 °C.

Plates were poured according to different volume ratios (1:10, 1:100, 1:1000) of residue extracts to water agar. Pathogen cakes were inoculated into the centre of the prepared plates with a 0.8-cm-diameter hole punch to obtain uniform growth of Fusarium acuminatum cakes of uniform media thickness. Effect of mycelia growth (%) = (diameter of treated colonies - diameter of control colonies) / (diameter of control colonies - 0.8 cm) × 100.

Soil with high banana *Fusarium* wilt disease incidence (> 65%) was collected from the field trial site (Lingao Xinxing Farm, Hainan Province, China (109°77’E, 19°77’N), where bananas were continuously cropped for 11 years. The field trial site has a tropical monsoon climate with an average annual temperature of 23.8 °C, an average precipitation of 1,786 mm, and an annual average of 2,059 hours of

sunshine. The soil is a dry red soil with a pH of 5.31, an organic matter content of 3.64 $g \cdot kg^{-1}$, an available phosphorus content of 15.64 $mg \cdot kg^{-1}$, an available potassium content of 411.67 $mg \cdot kg^{-1}$, and an available nitrogen content of 91.23 $mg \cdot kg^{-1}$. The excavated field soil was collected within the drip line next to the banana plant approximately 20 cm deep, thoroughly mixed and immediately transferred to a greenhouse (average temperature of 36 °C and 37% humidity) at the College of Agriculture, Hainan University, Haikou, Hainan Province, China (110°34'E , 20°06'N).

Pot rotation experimental design

A pot experiment was conducted from May 2018 to February 2019. It was completely randomized and included three treatments (i.e., Treatments I-III): fallow treatment (Treatment I), banana monoculture (Treatment II), and pineapple rotation (Treatment III). Each polypropylene pot (35 × 25 × 25 cm, length × width × height) had 10 kg of soil per pot, with six replicates of 10 pots per replicate. Before transplanting, commercial bio-organic fertilizer (a pure plant-derived bio-organic fertilizer containing 1.3% nitrogen (N), 1.1% phosphorus (P_2O_5), 1.0% potassium (K_2O), organic matter ≥ 40%, and moisture ≤ 30%) was applied at a rate of 2% soil as a base fertilizer in the pots. After 5 days of transplanting, evenly grown pineapple and banana seedlings were transplanted into the pots. After 6 months of cultivation, planted banana seedlings exhibited mild *Fusarium* wilt symptoms, such as yellow leaves and scabs at the base of the stem. Subsequently, the next crop of banana seedlings was continued by pulling out pineapple and banana seedlings and planting the banana seedlings. When the maximum incidence of treatment banana (B) was greater than 80% (4 months), the incidence of banana seedlings was counted for all treatments.

At this time, the whole banana plant was removed, and the bulk and rhizosphere soil were collected with sterile tweezers or brushes. The bulk soils of fallow, banana and pineapple were defined as CBf, BBm and PBr, respectively, while the rhizosphere soils of fallow, banana and pineapple were defined as CRf, BRm and PRr, respectively (Fig. 1A).

Pineapple residue amendment pot experimental design

For the experiment (September 2018 to March 2019) on the effects of soil amendment with crop residue on disease severity, field experiment available crop residue, banana and pineapple (Qionghai, Hainan) were chopped and finely ground using an Oster blender and were then passed through a 2-mm mesh sieve. Crop residue was then thoroughly mixed with the pasteurized soil at a rate of 3.0% (v/v). There were three treatments (i.e., Treatments VI-IV) designed for soil collected from the field with the same potting specifications as above. Treatment VI was the control: no residue added, Treatment V was banana residue added, Treatment VI was pineapple residue added. The experiment ended when the incidence of banana seedlings treated with added banana residues was over 60% (at six months). At this time, the whole banana plant was removed, and the bulk and rhizosphere soil were collected with sterile tweezers or brushes. The bulk soils of fallow, banana and pineapple were defined as CBn, BBb and PBp, respectively, while the rhizosphere soils of fallow, banana and pineapple were defined as CRn, BRb and PRp, respectively (Fig. 1B).

Disease incidence determination

Banana plants with typical wilt symptoms, such as yellow leaves, wilted leaves, and root rot, were counted as diseased plants, and the incidence rate was expressed as the percentage of the number of diseased banana plants to the total number of planted banana plants. In other words, the incidence rate (%) = banana plants with incidence/total number of banana plants × 100%.

Soil sampling collection and DNA extraction

Bulk soil samples were collected by removing banana plants from soil cores to a depth of 10 cm using a soil auger. The soil was mixed, homogenized and sieved through a 2-mm nylon sieve to remove plant debris and was then divided equally into three subsamples. One subsample was air-dried for physicochemical analysis, and the other two were stored at 4 °C and -80 °C for microbiological analysis and DNA extraction, respectively. At this time, the whole plant was removed, and the bulk soil was removed by careful shaking. Soil still adhering to the roots was collected with sterile tweezers and defined as rhizosphere soil. Total soil DNA was extracted using a PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol. Bulk soil and rhizosphere soil samples of banana and pineapple collected from pot planting and residue addition tests were accurately weighed to 0.7000 g and 0.4000 g, respectively. Soil DNA microorganisms were extracted using a kit (MoBio products, USA: PowerSoil® DNA Isolation Kit, 12888) (Wang et al. 2016). The extraction results were verified by nucleic acid assay and stored at -20 °C in a refrigerator. A volume of 20 μL of DNA samples from each treatment was selected and sent to "Nanjing aomike Bio" for Illumina MiSeq sequencing.

Quantitative PCR of *Fusarium oxysporum* in bulk and rhizosphere soil

Quantitative real-time PCR amplification (qPCR) was performed to determine the number of *F.oxysporum* using total bulk and rhizosphere soil DNA as a template. The primers used were FocSc-1 and FocSc-2 (Table S1). The system for real-time fluorescence absolute quantification of pathogens at 50 μL was 2 μL of DNA template sample, 25 μL of premix reagent, 1 μL of primer 1 (FocSc-1) (10 pmol/L), 1 μL of primer 2 (FocSc-2) (10 pmol/L), and 21 μL of ddH_2O. All soil DNA samples and the standard curve were analysed using a 7500 Real-Time PCR System (Applied Biosystems™, Foster City, USA). The melting curve and amplification efficiency were confirmed as standards for amplification. Six replicates of each sample were estimated, and the results were converted to log10 copy numbers (copy numbers g^{-1} soil) (Xiong et al. 2017a).

Soil chemical analysis and culturable microorganism determination

Soil pH was assayed using a soil-water ratio of 1:5 (w/v) suspensions. The organic matter (OM) content was measured using the $K_2Cr_2O_7$-H_2SO_4 oxidation method. Available potassium (AK) was extracted with ammonium acetate and analysed by flame photometry. Available phosphorus (AP) was determined by molybdenum antimony colorimetry, and the available nitrogen (AN) content was determined following Liu et al. (2016).

The total numbers of culturable *Fusarium*, bacteria, fungi and actinomycetes were determined following Shen et al. (2015a). The counts were as follows: the number of colonies forming units (CFU) formed on the plate was converted to the number of colonies formed per gram of dry soil and are expressed as CFU/g (dry soil) (Shen et al. 2015b).

Illumina MiSeq sequencing and data processing

The V4-V5 region of bacterial 16S rDNA and the fungal ITS region were amplified using the individual barcoded primers 520F/802R and ITS5F/ITS2R, respectively (Table S1) (Shen et al., 2018b). The PCR amplification procedures, including a 25-μL reaction volume containing 5 μL of 5 × reaction buffer, 5 μL of 5 × GC buffer, 1 μL of the 10 μM primer set, 2 μL of template DNA, 8.75 μL of ddH_2O, and 5 μL of 100 mM dNTPs for 16S or 2 μL of 100 mM dNTPs and 0.25 μL of DNA polymerase for ITS, were conducted according to Shen et al. (2015a). The resulting PCR products were generated using the following PCR conditions: a temperature programme of 2 min at 98 °C, followed by 28-30 cycles of 15 s at 98 °C, 55 °C or 50 °C for 30 s, and 30 s at 72 °C. The purified amplicons were pooled in equimolar amounts and analysed with the Illumina MiSeq Aomike at Personal Biotechnology Co., Ltd. (Nanjing, China).

Raw sequences based on unique barcodes were assigned to soil samples following the QIIME software package (version 1.9.1) tutorials after elimination of the adaptors and primer sequences (Caporaso et al. 2010), and pairs of reads were merged using the FLASH software tool (version 1.2.7). The paired sequences were processed by the UPARSE pipeline to produce an operational taxonomic unit (OTU) table with USEARCH 11 and Perl scripts (Edgar et al. 2011). Briefly, sequences with a quality score < 0.5 or length < 200 bp were eliminated. The retained sequences were assembled to identify OTUs at 97% similarity, and chimaeras were removed based on the UCHIME method. Then, high-quality OTUs were classified with the RDP Bacterial 16S database and the UNITE Fungal ITS database of the RDP classifier procedure for bacteria and fungi, respectively (Wang et al. 2007; Kõljalg et al. 2013).

The raw sequence data have been deposited in the NCBI Sequence Read Archive (SRA) database under accession numbers PRJNA745388 and PRJNA746047.

Statistical analysis

Microsoft Excel 2010 was used to process and graph the obtained data. Data were analysed with SPSS 20.0 software, and one-way analysis of variance (ANOVA) was used to compare the data. The significance of differences between treatments was tested using the new complex polar difference method (Duncan's test). R (3.6.0) language (vegan, mvpart, ggplot2 and graphics packages) was used to perform principal coordinate analysis (PCoA) and Pearson and Spearman correlation analyses. Permutational multivariate analysis of variance (PERMANOVA) and variance partitioning analysis (VPA) were based on the Bray-Curtis distance using the *adonis* and *varpart* functions within the R package *vegan*, respectively (Oksanen et al. 2012).

Results

Effects of pineapple rotation and residue amendment on banana *Fusarium* wilt disease incidence and relative abundance of *Fusarium*

The effects of different concentrations of pineapple and banana residues (0.1%, 1.0% and 10.0%) on spore germination were evaluated (Fig. 2A). The infusion of all parts of banana had a promoting effect on spore germination of the pathogen, while the infusion of all parts of pineapple had an inhibiting effect on spore germination, with the fruit infusion of pineapple having a highly significant effect on spore

germination. The effect of pineapple and banana root exudates on spore germination, compared to banana root exudates, pineapple root exudates were significantly less able to promote spore germination of the pathogen. The fungus significantly increased the number of pathogenic spores compared to the control (Fig. 2B). We observed an overall significantly reduced *Fusarium* wilt disease incidence in both the pineapple rotation and residue amendment systems (Duncan's *t*-test, $p < 0.001$) (Pr and Pp), which was significantly lower than those in the fallow and monoculture treatments ($p < 0.001$) (Fig. 2C). Moreover, the abundance of rhizosphere Fusarium (rotation treatment) ($p = 0.001$), as indicated by the residue treatment bulk Fusarium ($p = 0.118$), and the relative abundance of rhizosphere Fusarium ($p < 0.001$) were both positively correlated with banana Fusarium wilt DI (Fig. 2D), demonstrating disease suppression ability after rotation and residue addition with pineapple in the banana orchard. In the rhizosphere soil, the relative abundance of *Fusarium* was significantly lower in the rotation than in the monoculture treatments ($p < 0.05$, Fig. 2C). The qPCR results showed that significantly fewer *Fusarium oxysporum* were detected in the rotation rhizosphere (Duncan's t-test, $p < 0.05$, Fig. S1).

Effects of pineapple rotation and residue amendment on soil microbial community structure

The PCoA plots (Fig. 3A, B, C and D) showed significant differences in the bacterial and fungal community composition in the rotation and residue amendment systems (PERMANOVA, $p < 0.001$), in the rotation system, there was a significant difference in bacteria (p (bulk) < 0.01, p (rhizosphere) < 0.01), moreover, the different rhizosphere soil microbial communities changed significantly from the corresponding initial soil microbial communities, and the microbial communities of different plants grown under the same soil type also differed significantly ($p < 0.001$) (Fig. S2). The sequencing results were analysed and detailed, and the microbial community richness and diversity sequencing results are shown in the Supplementary Material (Table S3). PERMANOVA and VPA analyses revealed that soil rhizosphere microbial communities were significantly influenced by soil type ($p < 0.001$) and soil pH ($p < 0.001$). The relative importance of soil type and its soil pH on soil and rhizosphere microbial communities was greater than that of plant species (Fig. 3 E, F, G and H).

Effect of pineapple rotation and residue amendment on taxonomic composition

Volcano plot analysis of the sequence results revealed rotation and residue amendment bacterial and fungal community compositions with specific respective sets of OTUs (Fig. 4A and B). With red indicating bulk soil and black indicating rhizosphere soil, we found both that the pineapple rotation residue amendment treatment had a higher OTU number in the bulk soil, and selecting an OTU with a relative abundance greater than 0.1% for Venn analysis revealed that rotation and residue treatments had more bacteria, while fungi in the bulk soil had significantly higher OTU numbers than those in the rhizosphere soil (Fig. 4C and D).

At the phylum level, the pineapple rotations and residue amendment, which had the same composition, consisting mainly of the bacterial phyla *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, *Actinobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Cyanobacteria/Chloroplast*, and *Thaumarchaeota* and the fungal phyla *Ascomycota*, and *Basidiomycetes*, had the most abundant phyla in all samples (Fig. 5A and B). The pathogens were significantly negatively correlated with the bacterial phyla *Thaumarchaeota* (-0.482**), *Nitrospirae* (-0.401*), *Firmicutes* (-0.515**), and *Ascomycota* (-0.608**) (Table S5), which were generally consistent with previous findings on disease-suppressing soils (Shen et al. 2018a).

Analysis of the variability of the top 30 genera of bacteria and fungi showed that the variability of genera significantly increased in the soil. In the rotation, we found that the *Burkholderia*, *Bacillus*, *Rhizobium*, *Sphingosinicella*, *Pseudomonas and Talaromyces* genera were significantly increased in rhizosphere soils and in bulk soil, along with *Gemmatimonas*, *Gp6*, *Nitrososphaera*, *Gp7*, *Penicillium and Mortierella* (Fig. 5C). Among them, in the pineapple rotation, there were significant negative correlations with the number of *Fusarium,* such as *Burkholderia* spp. (Pearson: -0.558**, Spearman: -0.443**), *Pseudomonas* spp. (Pearson: NS, Spearman: -0.363*), and *Talaromyces* spp. (Pearson: -0.687**, Spearman: -0.530**) (Tables S6 and S8).

In the residue amendment, we discovered that the *Pseudomonas*, *Sphingobium*, *Azohydromonas*, *Bacillus*, *Georgfuchsia*, *Rhizobium*, *Pseudoduganella*, *Klebsiella*, *Sphingomonas*, *Elaphocordyceps* and *Penicillium* genera were significantly increased in rhizosphere soils, in the bulk soil, the *Gemmatimonas*, *Gp6*, *Nitrososphaera*, *Opitutus*, *Gp3*, *GpXIII*, *Aspergillus* and *Chaetomium* genera were significantly increased (Fig. 5D). Among them, in the residue treatment, there were significant negative correlations with the number of *Fusarium*, such as *Pseudomonas* spp. (Pearson: -0.524**, Spearman: -0.440**), *Aspergillus* spp. (Pearson: -0.378*, Spearman: -0.354*), and *Penicillium* spp. (Pearson: -0.453**, Spearman: -0.540**) (Tables S7 and S8).

Bulk and rhizosphere network construction through effects on specific microbial taxa

In this study, we constructed co-occurrence networks using random matrix theory (RMT) to determine the differences in bacterial and fungal assemblages (OTU relative abundance > 0.1%) in bulk and rhizosphere soils of the different treatments. All values of the calculated modularity index were larger than 0.4 (Table S9), suggesting typical module structures (Chen et al., 2020). Overall, pineapple rotation and

residue amendment showed marked effects on the soil microbial network: the average path distance (GD), the average clustering coefficient (avgCC) and the modularity of the empirical networks were higher than those of the corresponding, identically sized random networks (Table S9). Here, we found that residue assemblages (in Fig. 6 C and D) formed more connected and more complex networks with fewer nodes but more connections (edges) between nodes compared with the bulk soil. There were many keystone taxa in the microbial communities whose removal could cause a dramatic shift in microbiome structure and function. Keystone taxa in network analysis can be computationally identified as hubs with a high within-module degree Zi (Zi ≥ 0.5 indicates that the nodes are “well connected” to other nodes in the module). The PBr and PRr treatments had some keystone taxa, such as *Burkholderia* and *Pseudomonas*, and no hub was found in the bulk or rhizosphere soil (Cf, Cn, Bm and Bb) (Fig. S3).

Effects of the soil properties, number of *F.oxysporum*, the bulk and rhizosphere microbial communities and key microorganism on banana *Fusarium* wilt disease incidence

To investigate the potentially relative important suppression predictors of banana *Fusarium* wilt disease incidence, we then used Linear models (LM) to identify the potential positive or negative effects of the number of *Fusarium* (including the number of *F.oxysporum* and the relative abundance of *Fusarium*), bacterial and fungal communities (including bulk and rhizosphere communities), and key microorganism *Burkholderia*, *Pseudomonas*, Talaromyces and *Pseudomonas*, *Penicillium*, *Aspergillus* (bulk and rhizosphere soil) in the crop rotation and residue amendment system on banana *Fusarium* wilt disease incidence, respectively (Table 1).

For microbial linear model in the banana-pineapple rotation system, importantly, Fungal-pcoa1 (rhizosphere) (F = 23.41, $p = 0.001$, Relative Importance = 10.89%), *Fusarium* relative abundance (rhizosphere) (F = 201.74, $p < 0.001$, Relative Importance = 12.70%), *Burkholderia* (rhizosphere) (F = 3.56, p = 0.092, Relative Importance = 13.79%), *Talaromyces* (bulk) (F = 64.34, $p < 0.001$, Relative Importance = 23.61%) and *Talaromyces* (rhizosphere) (F = 1.41, $p = 0.265$, Relative Importance = 19.09%) constrained disease incidence the most (with a relative importance more than 10%). Besides, based on linear regression analyses between disease incidence and selected microbial indicators, Fungal-pcoa1 (rhizosphere) (p =0.001), *Fusarium* relative abundance (bulk) ($p < 0.001$), *Fusarium* relative abundance (rhizosphere) ($p < 0.001$) and *Talaromyces* (bulk) ($p < 0.001$) have significant negative relationship to disease incidence (Table 1).

For microbial linear model in the pineapple residue amendment system, importantly, *Fusarium* relative abundance (rhizosphere) (F = 363.96, $p < 0.001$, Relative Importance = 25.49%) and *Aspergillus* (rhizosphere) (F = 16.15, $p = 0.003$, Relative Importance = 40.83%) constrained disease incidence the most (with a relative importance more than 10%). Besides, based on linear regression analyses between disease incidence and selected microbial indicators, *Penicillium* (bulk) (p =0.051) and *Aspergillus* (rhizosphere) ($p = 0.003$) have significant negative relationship to disease incidence (Table 1).

For physicochemical linear model, the content of soil organic matter (F = 8.94, $p = 0.011$, Relative Importance = 22.22%), the available potassium (F = 19.45, $p = 0.001$, Relative Importance = 36.45%) and the available phosphorus (F = 89.40, $p < 0.001$, Relative Importance = 16.37%), the available potassium (F = 223.59, $p < 0.001$, Relative Importance = 28.88%) and the available nitrogen (F = 7.69, $p = 0.016$, Relative Importance = 45.10%) constrained disease incidence the most (with a relative importance more than 10%) in the rotation and residue amendment system, respectively (Table S10).

Based on the above results, a conceptual model illustrating potentially path with important suppression predictors in intercropping system was constructed (Fig. 7). The conceptual mode indicated that two ways in banana plantations reduced the relative abundance of *Fusarium* by the soil characteristics and microbial community structure regulation. Among all the suppression predictors, the key physicochemical factors AP (in rotation system) and OM (in residue system) contents are significantly affected by pineapple rotation and rseidue amendment, respectively. And they were significant leading to changes of fungal community and beneficial microorganism. And the significant increases in bacterial genera (*Burkholderia*) and fungal genera (*Talaromyces* and *Aspergillus*) can directly affect the relative abundance of *Fusarium*, thereby reduce the incidence of banana (Fig. 7).

Discussion

Banana with different crop rotations can effectively reduce banana *Fusarium* wilt (Hong et al. 2020; Fan et al. 2020). In this study, the results of pot experiments revealed that pineapple-banana rotation and residue amendment systems could significantly enhance the suppression of *Fusarium* wilt disease compared with banana monocropping and residue addition. This pattern is consistent with the results reported in a previous field study (Wang et al. 2015). Notably, through the study of microbial community changes, we found that the reduction in disease incidence in crop rotation and residue amendment treatments could be attributed to a reduction in the number of *Fusarium* as well as an increase in beneficial genera and changes in soil physicochemistry. We propose two systems in which rotation and residue amendment could inhibit fungal infections. Similar results have been shown for strawberry soil disease (Fang et al. 2012), likely

due to the interruption of the host pathogen cycle in the root. Both high-throughput sequencing and qPCR showed that the *Fusarium* number was smaller in the pineapple rotation and residue amendment treatments than in the banana monoculture and residue addition and exhibited significant positive correlations with disease incidence in our study (Fig. 2 and Fig. S1). The incorporation of both pineapple residues (P_L, P_R and P_F) into the soil was found to significantly decrease the pathogen density in the soil and the incidence of Fusarium wilt disease. This finding aligns with our previous studies showing that using a pineapple-banana crop rotation system can effectively minimize the incidence of this pathogen (Wang et al. 2015). This study thus provides strong evidence that pineapple-banana rotation and residue amendment are effective approaches for suppressing disease.

In our study, pineapple rotation may have resulted in a significant decline in pathogen number via increased abundance of some beneficial microorganisms and disease suppression by antagonistic and nutritional competition with harmful microorganisms, parasitism, predation, induction of plant resistance, and interference with pathogenic signals, leading to a decrease in nutrient availability to pathogens. In addition, it seems possible that pineapple crops can stimulate beneficial bacterial microbiomes through root exudates to suppress pathogens during the crop rotation season (Hao et al. 2010).

The composition of the rhizosphere fungal community was clearly divided into different groups based on the PCoA results of the crop rotation and residue amendments, the fungal communities were clearly clustered between the pineapple-banana rotation and residue pineapple addition treatments and the banana continuous crop and banana residue addition treatments (Fig. 3). We hypothesize that soil fungi are less redundant than bacteria when exposed to environmental stresses of crop rotation and under the effective nutrients of residue addition. Additionally, VPA analysis showed that the variations in bacterial and fungal communities were best explained by the type of soil, followed by the pattern of cultivation. It has been previously reported that cultivated crops have a greater impact on microbial structure than cultivation patterns. Future research elucidating the mechanisms of plant species-root exudate-soil microbial community interactions may be an effective way to study the suppression of pathogens. Specifically, in our experiment, this suppressive status emerged as a function of changes in the soil fungal communities rather than one that was mediated by changes in soil physicochemical properties. This was also further corroborated by Linear models (LM), which traced the relationships among microbial communities, pathogen density and banana disease incidence (Table S10).

Consistent with previous findings, at the phylum level of bacteria and fungi, the major phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, *Actinobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Cyanobacteria/Chloroplast*, and *Thaumarchaeota*, and the fungal phyla were *Ascomycota* and *Basidiomycetes* (Fig. 5A and B). In this study, only the relative abundance of *Firmicutes* and *Ascomycota* increased after pineapple rotation and residue addition and showed a strong negative correlation with *F. oxysporum* abundance (Table S5). This finding suggests that *Firmicutes* and *Ascomycota* may be involved in disease suppression through the production of biocontrol agents.

The pineapple-banana rotation significantly stimulated the relative abundance of the *Burkholderia*, *Pseudomonas* and *Talaromyces* genera in the bulk and rhizosphere soils (Fig. 5C and Fig. S2). These microorganisms showed significantly negative correlations with the abundance of *F. oxysporum*, and there is a large body of work demonstrating the stable suppression of disease by these beneficial microorganisms. For example, *Burkholderia* spp. has been shown to be abundant in golden pineapple-banana rotations (Wang et al. 2015), and *Pseudomonas* spp. plays a key role in pathogen antagonism by stimulating the synthesis of a beneficial microbiome common to the native beneficial microorganisms in the soil, after the application of bioorganic fertilizers, to reduce the number of *Fusarium* in bananas (Hong et al. 2020). Thus, they are widely reported as biological control agents. These microorganisms are capable of producing antifungal metabolites and can colonize the roots of plants, making them targets for biocontrol. Additionally, studies have shown that some fungal microorganisms pre-empt the microecological environment with pathogens, such as the effect of the mass production of *Talaromyces* spp. on pathogens populations (Bahramian et al. 2016). This result is the same as that reported by Xiong et al. (2017b), who studied two treatments of vanilla wilt, where the crop rotation treatment significantly reduced the number of *Fusarium oxysporum* in the soil. In addition, both pineapple crop rotation and residue addition significantly reduced the incidence of banana seedling wilt, closely related to the decrease in the number of *Fusarium oxysporum* in the soil after pineapple cultivation.

According to Wang et al. (2015), golden pineapple rotation can increase the organic matter and available phosphorus content in the soil, with a decrease in the soil pH, the results of this experiment after planting pineapple were consistent with previous studies. PCoA, volcano plot and VPA analyses revealed significant differences in microbial community structure after pineapple rotation, which is consistent with the results of Wang et al. (2015), indicating that crop rotation did change the soil microbial community composition (Zhalnina et al. 2018; Wright et al. 2014; Su et al. 2015).

Plant and soil microflora have important roles in fighting soil-borne diseases (Hong et al. 2020). The species and number of beneficial microflora in soil microorganisms are crucial for suppressing pathogens (Shen et al. 2013; Tian et al. 2020). At the bulk and rhizosphere

soil bacterial and fungal genus levels, the pineapple rotation treatment significantly increased the numbers of *Burkholderia*, *Pseudomonas*, *Penicillium*, and *Talaromyces*, in the residue amendment, *Pseudomonas*, *Elaphocordyceps*, and *Aspergillus* significantly reduced the relative abundance of *Fusarium oxysporum* in the soil. Wang et al. (2015) and Steensels et al. (2019) showed that the numbers of culturable bacteria and *Burkholderia* increased significantly, and the number of *Fusarium oxysporum* decreased significantly in pineapple rotation with bioorganic fertilizer. In herb and "maize-potato" rotations, we found that the *Pseudomonas* genus was abundant and significantly suppressed *Fusarium oxysporum* populations (Xiong et al. 2017a; Li et al. 2019). Studies have shown that *Talaromyces* and *Aspergillus* can have multiple plant protection functions and are corresponding antagonists against plant diseases (Zhai et al. 2015; Naraghi et al. 2010; Moreno et al. 2005). Although *Talaromyces* and *Aspergillus* are fungal diseases in some crops, a significantly negative correlation was found between these two genera and *Fusarium oxysporum* in this experiment, which may be because the fungi *Talaromyces* and *Aspergillus* compete with *Fusarium oxysporum* for nutrients in the soil, resulting in a decrease in the number of *Fusarium oxysporum*, the exact cause of which needs to be further investigated.

The incidence of banana seedlings replanted after pineapple residue addition was highly significant and positively correlated with *Fusarium oxysporum* and culturable *Fusarium oxysporum*, except that it was significantly and negatively correlated with soil pH, *Pseudomonas*, and *Aspergillus* and significantly and negatively correlated with culturable bacteria. *Penicillium* and *Burkholderia* were abundantly present in the bulk and rhizosphere soils after pineapple residue addition, while *Talaromyces* and *Aspergillus* could pass through the pineapple rhizosphere to the soil and then survive in the next banana soil, and *Talaromyces* could colonize the next banana soil. Therefore, they are also potential key microorganisms (Baileya and Lazarovitsb 2003). In summary, these results provide some directions for our next work and further research on the mechanism of banana-pineapple crop rotation and residue amendment.

Conclusion

Our rotation pot experiment further demonstrated the effectiveness of banana-pineapple rotation in alleviating banana wilt (Wang et al. 2015). In addition, our study contributes to this body of research by showing that the addition of pineapple plant residues in the soil can trigger soil suppression of a serious soil-borne pathogen (*Fusarium* spp.). Here, we described that this state of suppression is modulated by changes in the soil bacterial and fungal communities and highlighted one bacterial taxa (*Burkholderia* spp.) and two fungal taxa (*Aspergillus* spp. and *Talaromyces* spp.) that may be directly involved in pathogen suppression. We provided further evidence for their suggested different and complementary modes of action and subsequently validated their suppressive potential in well-controlled indoor experiments (Fig. 2A, B), which offers great promise for the search for beneficial substances in root exudates and residues in the future. Taken together, our research may provide new avenues for exploring agricultural practices with a focus on beneficial outcomes that directly affect soil health and crop productivity in a viable and sustainable manner.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

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Authors' contributions

Jinming Yang: Conceptualization, Methodology, Software, Visualization, Writing - review & editing. Zongzhuan Shen: Resources, Supervision, Funding acquisition. Xiangyu Ren: Resources, Supervision. Wei Gao: Resources, Supervision. Yutong Wang: Resources, Supervision. Manyi Liu: Resources, Supervision. Shan Hong: Resources, Supervision. Mingze Sun: Resources, Supervision. Yan Zhao: Resources, Supervision. Yunze Ruan: Resources, Supervision. Beibei Wang: Investigation, Conceptualization, Methodology, Visualization, Writing - review & editing, Supervision, Funding acquisition.

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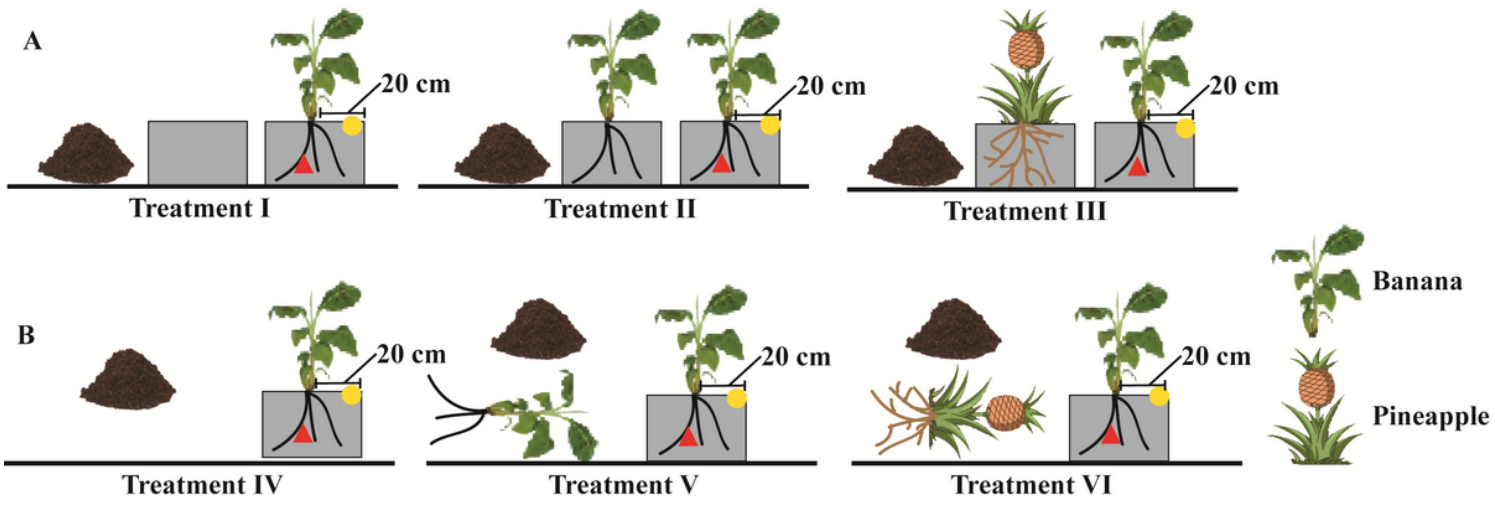
Tables

Table 1 Linear models (LM) for the relationships of microbial indicators with disease incidence and the relative importance of each indicators in the crop rotation and residue amendment system. *P* was results of ANOVAs.

Crop rotation	df	F	*P*	r	Relative importance	**Residue amendment**	df	F	*P*	r	Relative importance
Bacterial-pcoa1 (bulk)	1	2.35	0.159	2.06	4.19%	*F.oxysporum* (bulk)	1	154.60	0.000	2.33	6.44%
Fungal-pcoa1 (rhizosphere)	1	23.41	0.001	-2.47	10.89%	*F.oxysporum* (rhizosphere)	1	91.47	0.000	2.27	7.95%
F.oxysporum (rhizosphere)	1	278.24	0.000	2.05	7.73%	*Fusarium* relative abundance (rhizosphere)	1	363.96	0.000	2.55	25.49%
Fusarium relative abundance (bulk)	1	68.97	0.000	-1.80	6.62%	Fungal-pcoa1 (bulk)	1	1.89	0.203	-1.50	6.78%
Fusarium relative abundance (rhizosphere)	1	201.74	0.000	-3.36	12.70%	*Pseudomonas* (bulk)	1	98.76	0.000	2.70	4.85%
Burkholderia (rhizosphere)	1	3.56	0.029	-2.22	13.79%	*Penicillium* (bulk)	1	5.06	0.051	-1.48	3.48%
Talaromyces (bulk)	1	64.32	0.000	-3.52	23.61%	*Aspergillus* (bulk)	1	28.96	0.000	1.64	3.01%
Talaromyces (rhizosphere)	1	1.41	0.265	1.19	19.09%	*Aspergillus* (rhizosphere)	1	16.15	0.003	-4.02	40.83%
Residuals	9					Residuals	9				
Model summary: R^2 = 0.974, AIC = 66.00, p < 0.001						**Model summary: R2 = 0.978, AIC = 53.43, p < 0.001**					
Proportion of variance explained by model: 98.62%						Proportion of variance explained by model: 98.83%					

Figures



Treatment	I	II	III	VI	V	IV
Sampling site	Bulk Rhizosphere	Bulk Rhizosphere	Bulk Rhizosphere	Bulk Rhizosphere	Bulk Rhizosphere	Bulk Rhizosphere
Soil sampling	CBf CRf	BBm BRm	PBr PRr	CBn CRn	BBb BRb	PBp PBp

Figure 1

Diagram of crop rotation, monocropping and residue addition sampling sites in the pot experiments. CBf: fallow in the bulk soil, CRf: fallow in the rhizosphere soil, BBm: banana monoculture in the bulk soil, BRm: banana monoculture in the rhizosphere soil, PBr: pineapple-banana rotation in the bulk soil, PRr: pineapple-banana rotation in the rhizosphere soil, CBn: fallow in the bulk soil, CRn: fallow in the rhizosphere soil, BBb: adding banana residue in the bulk soil, BRb: adding banana in the rhizosphere soil, PBp: adding pineapple in the bulk soil, PBp: adding pineapple in the rhizosphere soil.

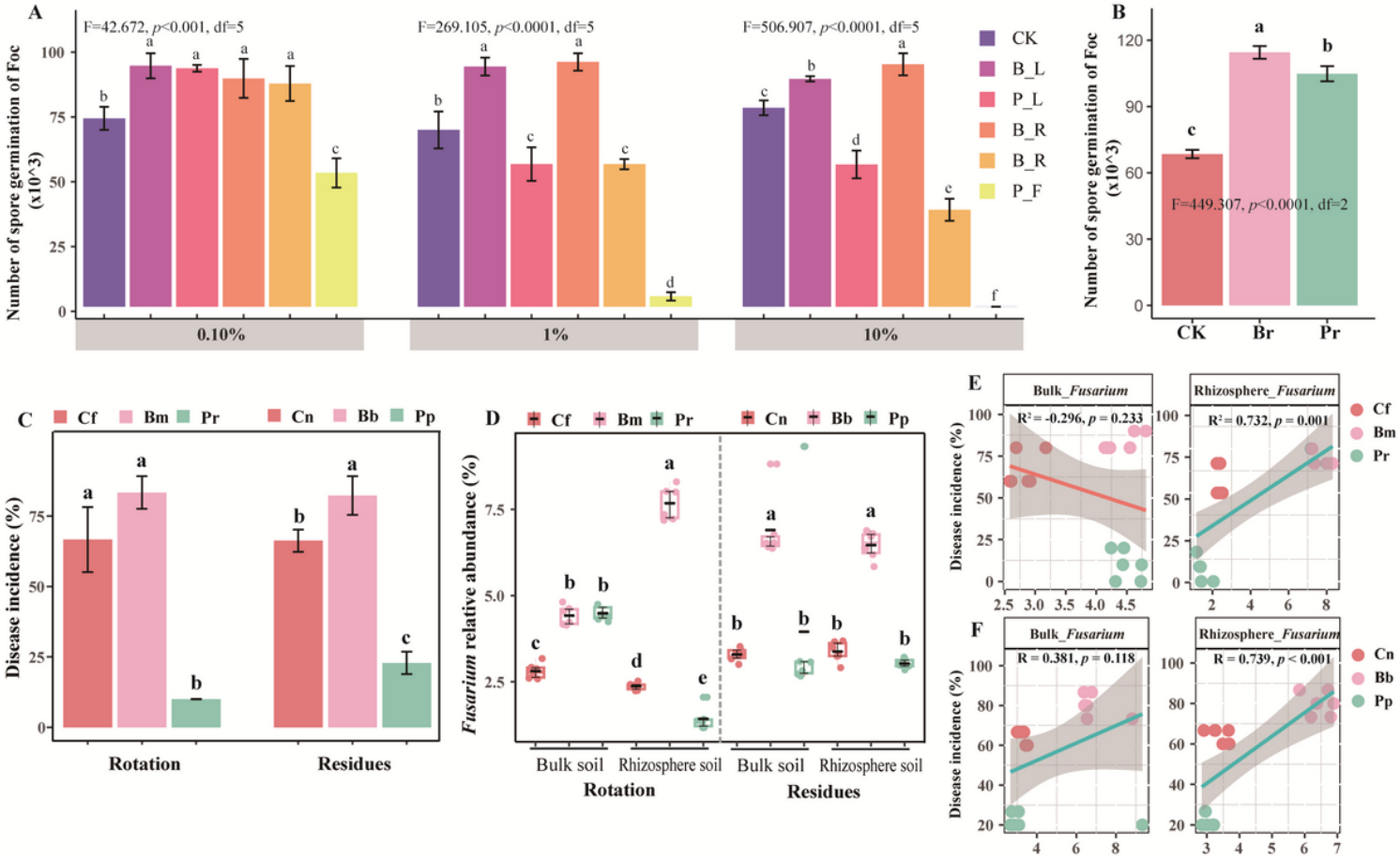


Figure 2

Effects of exudates from pineapple and banana on the spore germination of Foc (A). Effects of root exudates from pineapple and banana on the spore germination of Foc (B). Disease incidence of banana (%) and the relative abundance of Fusarium (C), and linear regression of DI and pathogen abundance (D) across all samples from six replicates. An asterisk indicates a statistically significant difference ($p < 0.05$, $p < 0.01$, $p < 0.001$) based on Duncan's t-test. The treatment abbreviations are defined in Fig. 1.

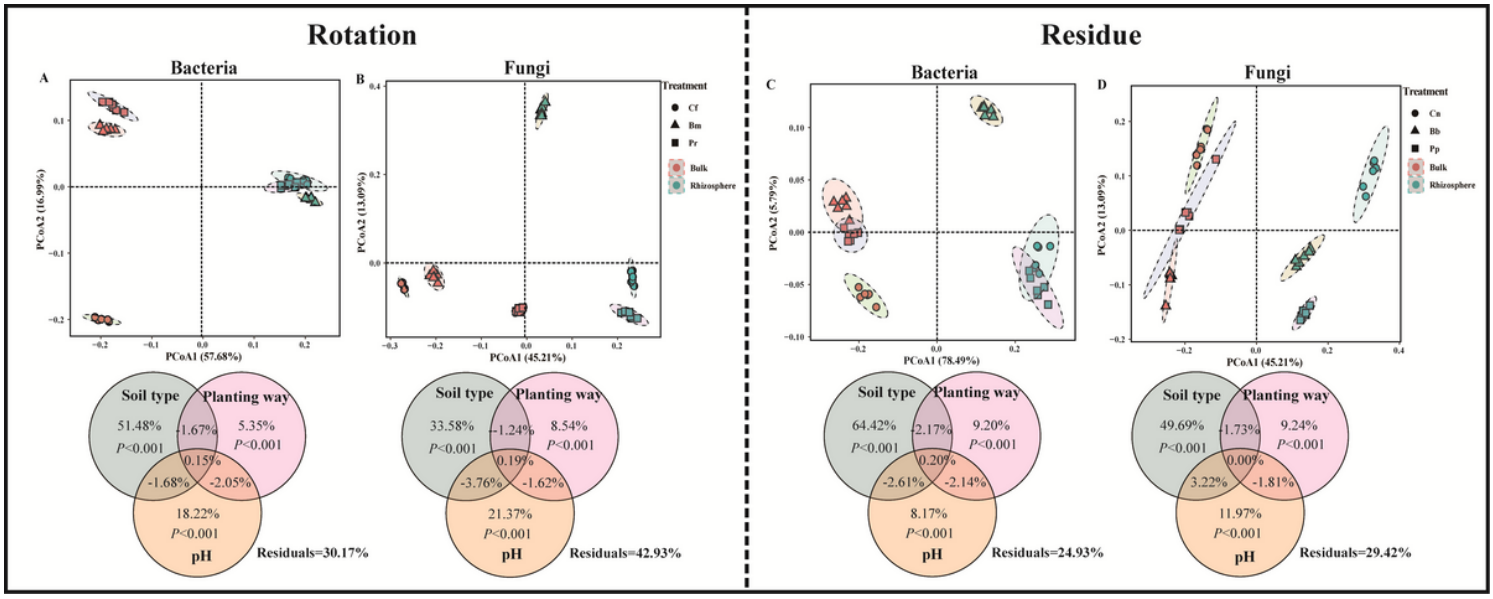


Figure 3

PCoA based on Bray–Curtis distance of rotation (bacteria (A), fungi (B)) and residue (bacteria (C), fungi (D)) of soil community structure. Contributions of soil type, planting method, and soil chemicals (pH) to the assembly of soil bacterial (E,G) and fungal (F,H) communities were calculated based on variance partitioning analyses (VPAss) in rotation and residue, respectively, and the p value was determined by PERMANOVA. The treatmentabbreviations are defined in Fig. 1.

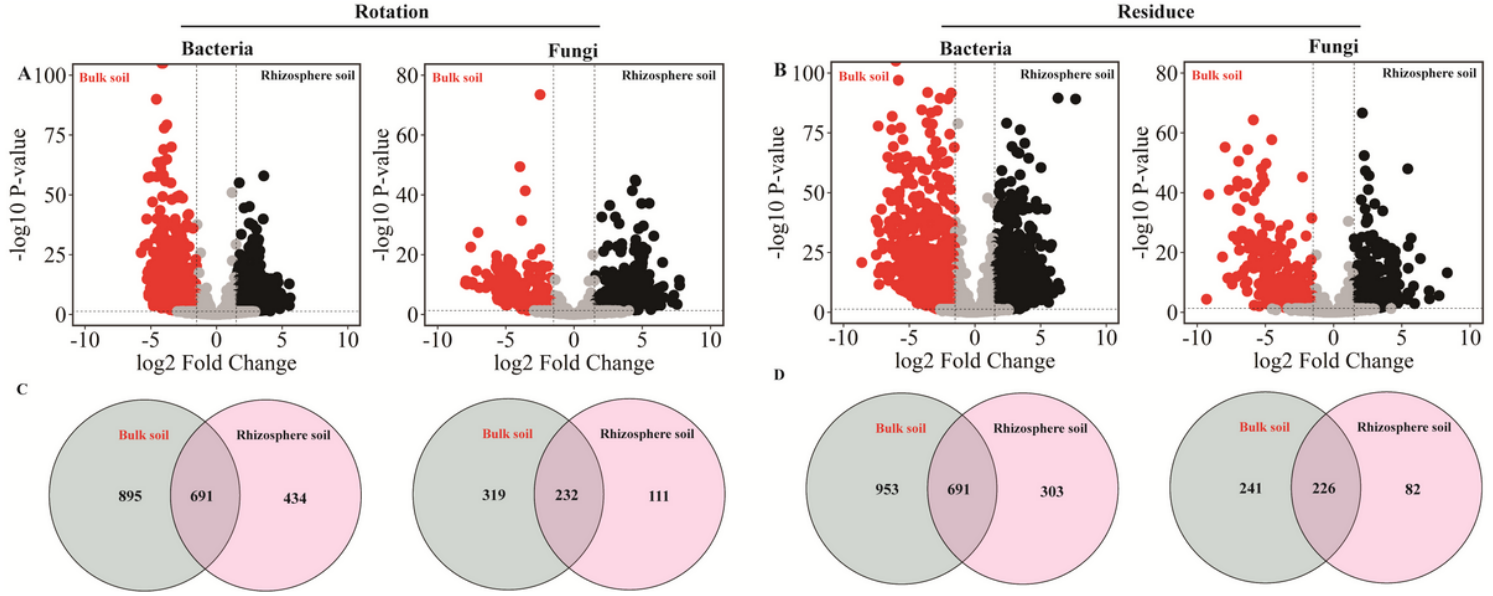


Figure 4

The effects of soil type (bulk and rhizosphere soils) in the pineapple rotation and residue identity (following banana and pineapple cultivation) on microbial compositions. The rotation bacterial (A) and fungal OTUs with relative abundances that were significantly (log2-fold change > |1.5| and FDR adjusted p value < 0.05) different between the bulk (red) and rhizosphere (black) soils are coloured in the volcano plot. Venn diagrams C and D represent the numbers of bacterial and fungal OTUs in the bulk and rhizosphere soils originating from the rice soil, respectively. Venn diagrams e and f represent the numbers of bacterial and fungal OTUs in rhizosphere soils originating from the forest soil, respectively. The treatment abbreviations are defined in Fig. 1.

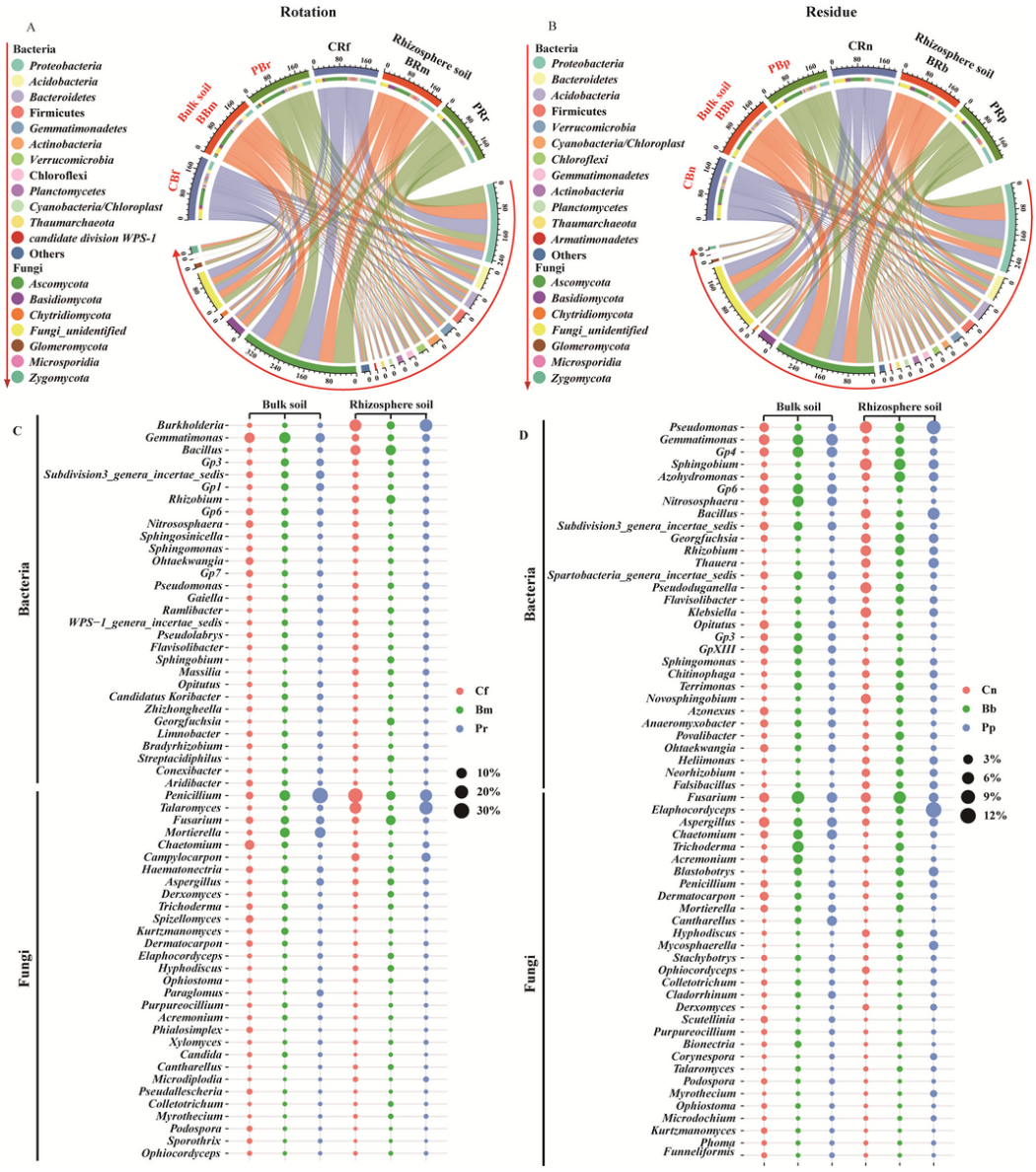


Figure 5

Relative abundances (%) of the phyla and top 30 genera containing the rotation (a, c) and residue (b, d). Circle sizes in c and d represent the relative abundances of the genera. The treatment abbreviations are defined in Fig. 1.

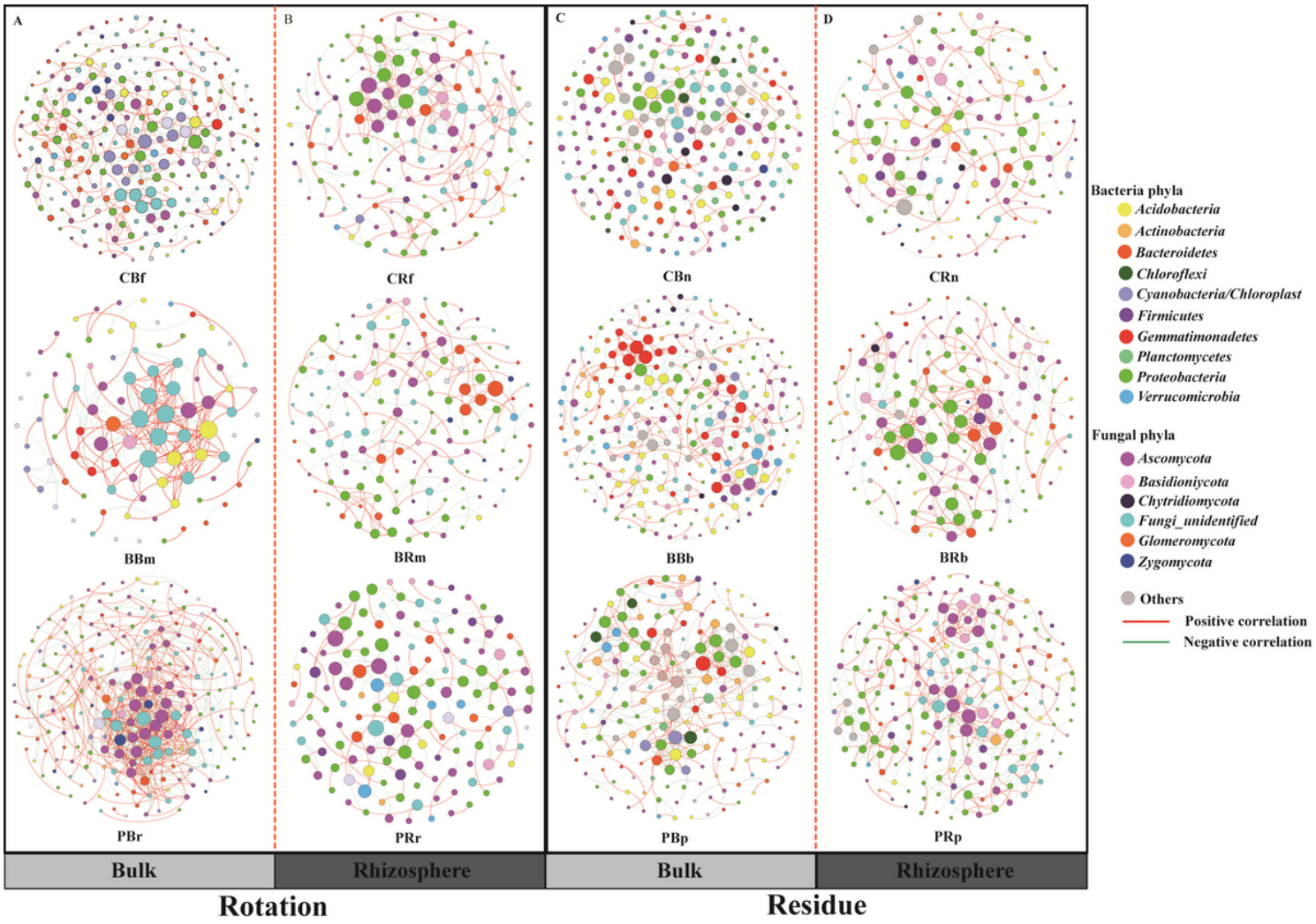


Figure 6

Plant rhizosphere and the corresponding bulk soil networks in the rotation and residue addition systems. Networks represent random matrix theory co-occurrence models derived from 6 biological replicates at each site, where nodes represent OTUs, and the edges between the nodes indicate significant correlations. A green edge indicates a negative covariation between two individual nodes, while a red edge indicates a positive covariation. The treatment abbreviations are defined in Fig. 1.

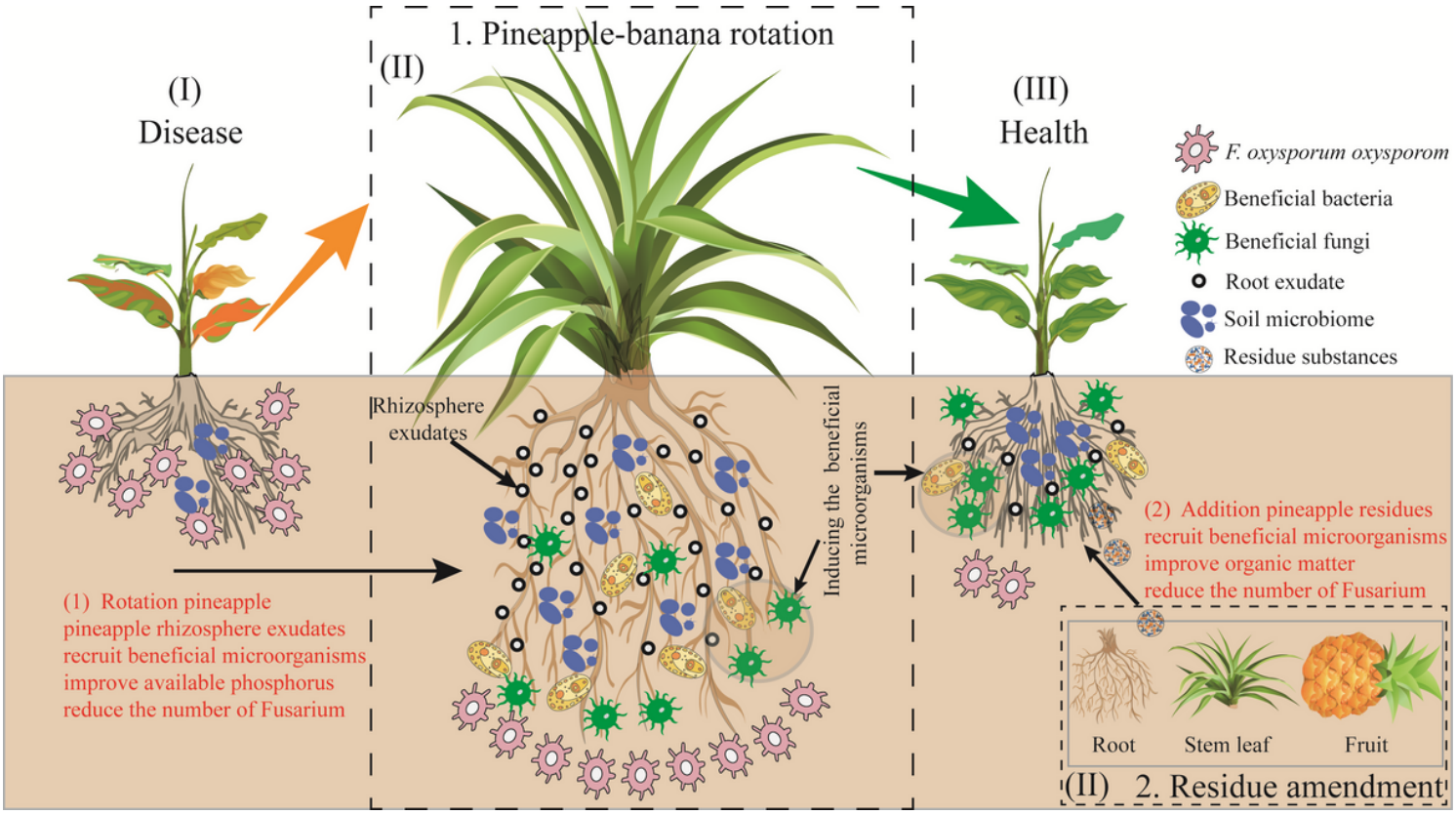


Figure 7

The overview of the mechanism by which pineapple mediates microbiota to increase available nutrients in the banana-pineapple crop rotation and residue amendment system, and reduce the incidence of banana.

Supplementary Files

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- Graphicformanuscript.docx
- SupplementaryMaterials.docx