Bio-Fertilizer Amendment Alleviates The Replanting Disease By Reshaping Leaf And Root Microbiome

Hongmiao Wu (wuhongmiao2010@163.com)
Anhui Agricultural University
Zhen Zhang
Anhui Agricultural University
Juanying Wang
Fujian Agriculture and Forestry University
Xianjin Qin
Fujian Agriculture and Forestry University
Jun Chen
Fujian Agriculture and Forestry University
Linkun Wu
Fujian Agriculture and Forestry University
Sheng Lin
Fujian Agriculture and Forestry University
Christopher Rensing
Fujian Agriculture and Forestry University
Wenxiong Lin
Fujian Agriculture and Forestry University

Research Article

Keywords: Soil sickness, Phyllosphere, Crop microbiome, Allelopathy, Sustainable agriculture

Posted Date: July 6th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-582632/v1

Abstract

A growing problem in intensive agricultural systems is replanting disease. Application of bio-fertilizer containing beneficial microbes contributes to disease suppression and is a promising strategy to control replanting disease. However, the effect of both replanting disease and bio-fertilizer amendment on the assembly of crop microbiota in leaves and roots and their relationships to crop yield and quality remains elusive. In these experiments, roots and leaves of *Radix pseudostellariae* were collected from different consecutive monoculture and bio-fertilizer amended fields and characterized the associated microbiota by bacterial 16S rRNA gene sequencing and qRT-PCR. Consecutive monoculture altered the bacterial community structure and composition and significantly increased the abundance of pathogenic *Ralstonia* and *Fusarium oxysporum* in leaves and roots. Furthermore, bio-fertilizer application alleviated replanting disease by decreasing the pathogen load, increasing the beneficial genera *Pseudomonas*, *Streptomyces*, *Paenibacillus,* and *Bradyrhizobium*, and enhancing positive connections of the bacterial community across the two compartments. Bio-fertilizer had a positive and indirect effect as indicated by a structural equation models on both yield and quality by shaping the leaf microbiota rather than the root microbiota. Our findings highlight the role of leaf and root microbiota on replanting disease, showing that bio-fertilizer contributes to alleviating replanting disease by improving the plant-microbe interactions.

Introduction

80% of the 1.5 billion hectares of global agricultural cultivation can be attributed to monoculture of crops cultivated on a large scale [1]. This has resulted in replanting disease arising as a problematic challenge [2]. Replanting disease is considered to be a type of biological pollution that causes an increase in soil-borne pathogens at the expense of plant-beneficial microbes in the rhizosphere soil [3, 4]. Introduction of bio-fertilizer containing beneficial microbes is the principal strategy for the alleviation of replanting disease [5, 6]. Previous studies have indicated the bio-fertilizer treatment increased the populations of beneficial *Lysobacter* and indigenous *Pseudomonas* in the rhizosphere soil to enhance plant pathogens suppression [2, 6–8]. The research conducted to better understand the mechanisms underlying disease suppression has mainly focused on the rhizosphere soil microbiota, including its composition and diversity and its impact on the growth processes of crops [2, 6–8]. The soil microbiota has been proved to be transported from the rhizosphere soil to the plant root and phyllosphere through xylem vessels and aerosols [9, 10]. To date, less research has been focused on analyzing and characterizing the microbiota of the leaves and roots, especially their responses to agricultural replanting disease and bio-fertilizer remediation.

Recent studies have indicated that leaf and root microbial communities promote plant growth in above-ground tissues by enhancing the disease resistance to pathogens, increasing the adaptation to abiotic stresses, and improving nutrient acquisition and plant metabolic functions [11–14]. Long-term application of organic and inorganic fertilization significantly altered the phyllosphere microbial community composition and diversity [15, 16]. Understanding the effect of agricultural management practices on the distribution of leaf and root microbiome may clarify the potentiality of crop microbiota

for agricultural sustainability and productivity. However, the community-level effect of phyllosphere microbiota on plant health under different agricultural conditions remain elusive [11–14]. The emerging progress on leaf and root microbiome will open a new avenue to regulate plant microbiota community assembly and functions. With the growing interests to utilize the potential of plant microbiome for sustainable agriculture production, an insight understanding of the effect of soil amendment on the plant microbiome is highly desirable.

The focus of this study was *Radix pseudostellariae*, a highly-prized Chinese medicinal plant mainly produced in Fujian, Guizhou, and Anhui Provinces of China. This species often suffers from serious replanting disease [2, 3, 17]. Our aims were: (1) to assess the effect of consecutive monoculture on the bacterial community structure and composition in leaves and roots; and (2) to evaluate the underlying mechanism of the bio-fertilizer application in remediating replanting disease through the contribution of microbial taxa in different compartments and via biological predictors of crop yield and quality.

Materials And Methods

The field experiments and plant sampling

Zherong City, Fujian Province, China (119°55′E, 27°17′N) was the site of the field experiment. We utilized the *R. pseudostellariae* variety Zheshen 2. The experimental field was divided into four treatments, with each treatment being conducted in five replicate plots. The four treatments were designed as follows: *R. pseudostellariae* planted in never-planted soil, planted in one-year monoculture soil, planted in two-year monoculture soil, and planted in two-year monoculture soil with bio-fertilizer treatment (Table S1).

The bio-fertilizer used in this study was a mixture of *Bacillus spp.* and *Pseudomonas spp.* that were previously isolated from the soil of healthy *R. pseudostellariae*. These beneficial bacteria exhibited high antagonistic activity towards pathogens and a strong plant growth promoting capacity [18–20]. Based on the solid fermentation method, the bio-fertilizer was prepared by incubating a mixture of these beneficial microbes in an organic mixture of soybean meal and fish meal (2:1, w/w) [5, 21]. The bio-fertilizer (500kg/666.7m^2) was applied in the two-year monoculture *R. pseudostellariae* plots on November 20, 2019. Planting took place on December 28, 2019 finishing all of the *R. pseudostellariae* plots and sampled on May 14, 2020. Local planting practices were used in *R. pseudostellariae* planting and field management. In brief, the contents of fertilizers applied during the cultivation period were as follows: urea, 668–1002 kg/hm^2; fused calcium-magnesium phosphate (fertilizer), 1167–2333 kg/hm^2; potassium sulphate, 360 kg/hm^2.

We collected the leaves and roots of *R. pseudostellariae* planted in first cropping year soil (FY), second cropping year soil (SY), third cropping year soil (TY), and third cropping year soil with bio-fertilizer treatment (BIO) on May 14, 2020 (Table S1). Each treatment consisted of five replicates. Leaves and roots of *R. pseudostellariae* were cleaned using sterile water to aid removing loosely attached soil particles and then further cleaned in sterile phosphate buffered saline for three times using continuous

shaking at a speed of 180 rpm/min. After completing the washing procedure, leaves and roots were further dried on sterile filter paper and subsequently stored at − 80°C. The *R. pseudostellariae* yield in 2020 was obtained for each plot as the *R. pseudostellariae* productivity, and the contents of heterophyllin B, total polysaccharide, and total saponin of roots were determined as the *R. pseudostellariae* quality.

The measurement of *R. pseudostellariae* quality

Content of heterophyllin B: The extraction of heterophyllin B followed a previously described method [22]. In addition, HPLC-MS (model: Thermo LTQ XL) was used to detect the contents of heterophyllin B in different samples. The parameters of HPLC-MS were as follows. Ion Source: APCI; Scan Mode: negative ion mode; Vaporizer Temp: 400°C; Sheath Gas Flow Rate: 50arb; Aux Gas Flow Rate: 20 arb; Sweep Gas Flow Rate: 0 arb; Capillary Temp: 350°C; Capillary Voltage: -3 V. The mobile phase consisted of water (A) and acetonitrile (B). The analysis was carried out with an elution gradient as follows: 0–2 min, 5–30% B; 2–12 min, 30–40% B; 12–15 min, 40–45% B; 15–16 min, 45–50% B; 16–20 min, 50–60% B; 20–22 min, 60–70% B; 22–22.1 min, 70–5% B; 22.1–26 min, 5% B.

Content of total polysaccharides: The total polysaccharide content was determined by water extraction and alcohol analysis [23]. In brief, a glucose standard solution was prepared, and the standard curve was drawn by the phenol-sulfuric acid method. The total polysaccharide was extracted by the above described experimental method; the absorbance was determined by phenol-sulfuric acid color reaction, and the total polysaccharide content was calculated according to the regression equation.

Content of total saponin: The extraction of saponin was performed using distilled water ultrasonic extraction [24]. In brief, we used 70% ethanol as the extractant. The extract was filtered after overnight and then volatilized until completely dry. Thereafter, distilled water was added to the dried material, and the solution was extracted with saturated n-butanol. The resultant extracts were pooled and evaporated until completely dry at 4°C. Ginsenoside Rb1 was used as the standard solution. The resultant extracts and standard solution were analyzed using a UV spectrophotometer at an absorbance of 560 nm. The total saponin content was calculated according to the regression equation.

Genomic DNA extraction, PCR and sequencing

For genomic DNA preparation, frozen leaf and root samples were twice homogenized in a homogenizer, and the genomic DNA was extracted using the FastDNA SPIN Kit (MP Biomedicals) based on the manufacturer's instructions. DNA concentrations were determined using Nanodrop (Thermo Scientific). To characterize the bacterial community of leaves and root, we amplified variable regions 5–7 (V5–V7) using the specific primers 799F and 1193R [11]. The library was then sequenced on an Illumina Miseq PE250 platform. The high-quality sequences were analyzed using the standard operating procedure in QIIME2 (https://qiime2.org/). The bacterial species annotations were determined via the Silva database [25].

Quantitative PCR analysis of *Fusarium oxysporum*

The DNA from leaves and roots was extracted using the FastDNA SPIN Kit (MP Biomedicals). Five replicates of each treatment were extracted. The abundance of pathogenic *Fusarium oxysporum* (ITS1F/AFP308R) [26] was determined by using a CFX96 Real-Time system (Bio-RDA, US). The PCR amplification conditions were as described here: 3 min for denaturation at 95°C, 35 cycles of 50 s at 95°C, 45 s for annealing at 60.4°C, 60 s for elongation at 72°C, and 10 min for a final extension at 72°C. Quantitative PCR was performed as previously described [18]. Five independent assays were performed for each treatment.

Statistical analyses

The R software package was used to calculate all of the statistical analyses unless otherwise indicated. Alpha diversity indices (Observed_species, Shannon, and Faith_PD) were calculated at the OTU level by using the QIIME2 platform based on cumulative sum scaling normalization and sub-sampling. Principal component analysis (PCA) was used to analyze the discrepancies among samples at the level of OTUs. The differential abundances of microbial taxa were screened out by using analysis of variance (ANOVA) and the least significant difference (LSD) test (significance level of P value < 0.05). Significant differences in the relative abundance of microbial taxa among all of the treatments were identified using linear discriminant analysis (LDA). Random Forest analysis was carried out with the “randomForest” package to identify the main bacterial predictors of *R. pseudostellariae* yield and quality.

The co-occurrence networks were generated using the “igraph” package [27] in R 4.0.2. The networks focused on the correlations with absolute values of Spearman’s coefficient > 0.6 and $p < 0.01$. Networks were visualized by the “Gephi” interactive platform (Ver 0.9.2, https://gephi.org). The correlations between *R. pseudostellariae* yield and quality measures and microbial abundance were then evaluated using Spearman’s rank correlation tests and visualizing by the TBtools platform [28].

Results And Discussion

Bio-fertilizer amendment shaping the *R. pseudostellariae*-associated bacterial community structure

Replanting disease is a typically negative feedback of plant-soil, and causes a significant wreck in mediating crop performance. In the current study, we found that bio-fertilizer application significantly increased the yield and quality (i.e., contents of heterophyllin B, total polysaccharide, and saponin) of *R. pseudostellariae* compared to second- and third-year monocultures (Fig. 1). Then, we collected the microbiota that were tightly attached to the surface and endogeny of leaves and roots. High-throughput sequencing showed that the roots contained a significantly higher alpha diversity of bacteria than the leaves (Figure S1 and S2A). PCA revealed significant differences in community composition between leaf and root microbiota (Figure S2B). The bio-fertilizer treatment significantly decreased the bacterial alpha diversity in both leaves and roots compared to the third-year monoculture treatment (Fig. 2). The results were in line with a previous study suggesting that the application of fertilizer would lead to lower bacterial diversity in leaves [29]. Meanwhile, the PCA analysis showed that the bacterial community composition was generally separated when comparing bio-fertilizer and different years of consecutive monoculture

treatments in each of two compartments (Fig. 2C). This implied that the application of bio-fertilizer altered the bacterial community structure in leaves and roots under agricultural field conditions.

Continuous monoculture and bio-fertilizer altered the *R. pseudostellariae*-associated bacterial community composition

Continuous monoculture significantly increased the dominant abundance of Proteobacteria and Firmicutes and decreased the Actinobacterial phyla in leaves. Meanwhile, bio-fertilizer treatment significantly enhanced the abundance of Proteobacteria, Firmicutes, and the ratio of Firmicutes/Proteobacteria and decreased Actinobacteria in leaves compared to the consecutive monoculture treatments. In addition, the bio-fertilizer treatment significantly lowered the abundance of Proteobacteria and Bacteroidetes, and the bio-fertilizer treatment increased the abundance of Acidobacteria in roots under the third-year monoculture treatment (Fig. 3). Although the key community members of microbiotas were overlap in the leaves and roots, the overall bacterial community structure and composition were different. In line with previous observations, the abundance of Firmicutes was markedly reduced and Proteobacteria were enriched; this contributed to shaping the bacterial community in leaves and subsequently causing leaf disease [13]. This result indicated that the bio-fertilizer might influence the leaf and root microbial community, possibly through plant microbe–microbe interactions.

Ralstonia, *Pseudomonas*, and *Paenibacillus* were significantly more abundant in leaves than in roots (Figure S3 and S4). Continuous monoculture significantly increased the abundance of pathogenic *Ralstonia* and *Fusarium oxysporum* in the two compartments. However, the bio-fertilizer treatment significantly decreased the abundance of *Ralstonia* and *F. oxysporum* and increased *Bradyrhizobium* in the leaves and roots under the third-year monoculture treatment. Previous studies have reported that *Ralstonia* [30] and *F. oxysporum* [17] were among the most important plant pathogens. *Pseudomonas* [3], *Paenibacillus* [31], *Bradyrhizobium* [32], and *Streptomyces* [33] have been recognized as microbial antagonists and biological control agents in agricultural production. Furthermore, bio-fertilizer has been shown to have a positive effect on the abundances of *Pseudomonas* and *Streptomyces* in roots and on *Paenibacillus* in leaves compared to consecutive monoculture treatments, suggesting that newly available habitat niches resulting from a relative decrease of pathogens can be filled by functionally different bacteria. The potential mechanisms and soil ecological processes that are responsible for these findings might be the bio-fertilizer improving the rhizosphere micro-environment by increasing the abundance of indigenous beneficial microbes [7]. This could then alter the bacterial communities in roots and leaves. The soil microbiome was previously shown to be the major source of the leaf bacterial microbiota [10, 34–36]. Previous studies suggested that the microbes were able to be transported from the rhizosphere soil to the plant phyllosphere through xylem vessels and aerosols [9, 10]. Therefore, we assumed that the root microbiome might also serve as an important reservoir of beneficial microbes for leaves under bio-fertilizer treatment. Interestingly, the abundances of beneficial *Pseudomonas* and *Paenibacillus* were significantly increased in the leaves under continuous monoculture treatment. This might be due to the leaves harboring certain suppressive bacteria that can restrict pathogens and increase resistance [14].

Relationships between microbial communities and the yield and quality of *R. pseudostellariae*

Linear discriminant analysis (LDA) indicated that continuous monoculture tended to decrease the indicator taxa in both compartments (Figure S5 and S6). Random forest regression modelling showed that the genera belonging to Proteobacteria, Actinobacteria, and Firmicutes were incorporated as the main biological predictors for yield and quality in the two compartments (Fig. 1E and 4). The genera *Pseudomonas*, *Ralstonia*, *Acidovorax*, *Conexibacter*, and *Streptomyces* were the dominant predictors of yield and quality. These beneficial and deleterious biomarkers that have been shown to have a significant impact on microbial communities when comparing consecutive monoculture and bio-fertilizer treatments and should be taken into consideration for new strategies to improve plant health and agricultural productivity through suppressing the activity of pathogens and promoting beneficial microbes. Our results showed that the majority of the co-occurrence network connections were linked to phyla of Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Acidobacteria (Fig. 5). In addition, the modularity values of networks decreased as the number of consecutive monoculture years increased (Table S2). Previous studies have suggested modules as niches [37, 38], and the lower modularity values may therefore be linked to stronger niche overlap and interspecific competition of leaf and root microbiota under consecutive monoculture regimes. Moreover, the positive correlations of microbiota under bio-fertilizer treatment were highest among all of the treatments (Table S2), suggesting that bio-fertilizer treatment enhanced the ecological commensalism or mutualism of microorganisms.

Structural equation models (SEMs) indicated that consecutive monoculture treatment had a negative and indirect effect on the yield and quality by influencing leaf and root microbiota (Fig. 6A). The bacterial diversity and richness of roots had a significant negative effect on the bacterial diversity and richness of leaves under consecutive monoculture regimes, while the opposite pattern was observed under bio-fertilizer application. This may be due to replanting disease decreasing cell density in leaves and roots and causing malformed organs of *R. pseudostellariae* [24]. Furthermore, the addition of bio-fertilizer had a more direct influence on leaf microbiota than on root microbiota, indicating that leaf microbiota was more sensitive to bio-fertilizer than microbiota of the roots. This might be due to the root endophytes being more influenced by host genetic control [39] or phyllosphere microbes being more vulnerable to anthropogenic disturbance than soil microbes [34]. Spearman correlation analysis indicated that yield and quality were negatively correlated with the abundance of potentially pathogenic *Ralstonia* in the two compartments and positively correlated with beneficial *Pseudomonas*, *Streptomyces*, and *Bradyrhizobium* in roots (Fig. 6B). Moreover, the abundance of *Ralstonia* was significantly negatively correlated to beneficial bacteria. The beneficial microbiota also negatively affected the abundance of pathogenic *F. oxysporum* (Fig. 7). This indicated that plant-microbe–microbe interactions in turn not only impacted microbial abundance, but it also impacted plant disease by antagonizing plant pathogens.

Conclusions

Understanding the responses of leaf and root microbiomes under abiotic and biotic stimuli has recently gained increased attention [14, 16, 34]. We provide novel evidence that consecutive monoculture and bio-

fertilizer treatments significantly influenced the microbial community structure and composition of *R. pseudostellariae* across the two compartments. We thus highlight the finding that application of bio-fertilizer was able to promote the abundance of specific beneficial microbes and provide protection against the pathogens in leaves and roots, thereby improving the interaction of plant-microbes–microbes and additionally alleviating serious replanting disease. Our results support the conclusion that the management strategy led to changes in crop-associated microbiomes that may reduce crop disease and promote crop productivity.

Declarations

Acknowledgements

We thank the National Science Foundation of China and the Project Funded by China Postdoctoral Science Foundation for providing the funds used in this work.

Funding

This work was supported by National Science Foundation of China (U1205021, 82003884, 81573530), and the Project Funded by China Postdoctoral Science Foundation (No. 2019M650150).

Author Contributions

WX Lin and HM Wu conceived the study; HM Wu wrote the paper. HM Wu, JY Wang, XQ Qin and J Chen performed experiments; HM Wu and Z Zhang performed the statistical analyses; LK Wu and S Lin were involved in soil sampling. C Rensing and WX Lin have revised the manuscript. All authors discussed the results and commented on the manuscript.

Ethics Approval: Not applicable.

Consent for Publication: All authors agreed with the publication of this manuscript.

Competing interests: The authors declare no competing interests.

Data availability

All data generated or analyzed were included in this manuscript and the supporting information.

References

1. Altieri MA, Nicholls CI (2020) Agroecology and the reconstruction of a post-COVID-19 agriculture. The Journal of Peasant Studies 47:881–898. doi:10.1080/03066150.2020.1782891
2. Wu H, Wu H, Qin X, Lin M, Zhao Y, Rensing C, Lin W (2021) Replanting disease alters the faunal community composition and diversity in the rhizosphere soil of Radix pseudostellariae. Agr Ecosyst Environ 310:107304. doi:https://doi.org/10.1016/j.agee.2021.107304

3. Wu H, Qin X, Wang J, Wu L, Chen J, Fan J, Zheng L, Tangtai H, Arafat Y, Lin W (2019) Rhizosphere responses to environmental conditions in Radix pseudostellariae under continuous monoculture regimes. Agr Ecosyst Environ 270:19–31
4. Li Y, Dai S, Wang B, Jiang Y, Ma Y, Pan L, Wu K, Huang X, Zhang J, Cai Z, Zhao J (2020) Autotoxic Ginsenoside Disrupts Soil Fungal Microbiomes by Stimulating Potentially Pathogenic Microbes. Appl Environ Microbiol 86. doi:10.1128/aem.00130-20
5. Wu L, Yang B, Li M, Chen J, Xiao Z, Wu H, Tong Q, Luo X, Lin W (2019) Modification of Rhizosphere Bacterial Community Structure and Functional Potentials to Control Pseudostellaria heterophylla Replant Disease. Plant Dis 104:25–24
6. Xiong W, Guo S, Jousset A, Zhao Q, Wu H, Li R, Kowalchuk GA, Shen Q (2017) Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol Biochem 114:238–247. doi:10.1016/j.soilbio.2017.07.016
7. Tao C, Li R, Xiong W, Shen Z, Liu S, Wang B, Ruan Y, Geisen S, Shen Q, Kowalchuk GA (2020) Bio-organic fertilizers stimulate indigenous soil Pseudomonas populations to enhance plant disease suppression. Microbiome 8. doi:10.1186/s40168-020-00892-z
8. Fu L, Penton CR, Ruan Y, Shen Z, Xue C, Li R, Shen Q (2017) Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. Soil Biol Biochem 104:39–48
9. Compant S, Mitter B, Gualberto Colli-Mull J, Gangl H, Sessitsch A (2011) Endophytes of Grapevine Flowers, Berries, and Seeds: Identification of Cultivable Bacteria, Comparison with Other Plant Parts, and Visualization of Niches of Colonization. Microb Ecol 62:188–197. doi:10.1007/s00248-011-9883-y
10. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and Functions of the Bacterial Microbiota of Plants. In: Merchant, SS (ed.) Annual Review of Plant Biology, Vol 64, pp. 807–838
11. Zhang J, Zhang N, Liu Y-X, Zhang X, Hu B, Qin Y, Xu H, Wang H, Guo X, Qian J, Wang W, Zhang P, Jin T, Chu C, Bai Y (2018) Root microbiota shift in rice correlates with resident time in the field and developmental stage. Science China-Life Sciences 61:613–621. doi:10.1007/s11427-018-9284-4
12. Gong T, Xin X-F (2021) Phyllosphere microbiota: Community dynamics and its interaction with plant hosts. J Integr Plant Biol 63:297–304. doi:10.1111/jipb.13060
13. Chen T, Nomura K, Wang X, Sohrabi R, Xu J, Yao L, Paasch BC, Ma L, Kremer J, Cheng Y, Zhang L, Wang N, Wang E, Xin X-F, He SY (2020) A plant genetic network for preventing dysbiosis in the phyllosphere. Nature 580:653–653+. doi:10.1038/s41586-020-2185-0
14. Chaudhry V, Runge P, Sengupta P, Doehlemann G, Parker JE, Kemen E (2021) Shaping the leaf microbiota: plant-microbe-microbe interactions. J Exp Bot 72:36–56. doi:10.1093/jxb/eraa417
15. Chen Q-L, An X-L, Zheng B-X, Ma Y-B, Su J-Q (2018) Long-term organic fertilization increased antibiotic resistome in phyllosphere of maize. Sci Total Environ 645:1230–1237. doi:10.1016/j.scitotenv.2018.07.260

16. Sun A, Jiao X-Y, Chen Q, Wu A-L, Zheng Y, Lin Y-X, He J-Z, Hu H-W (2021) Microbial communities in crop phyllosphere and root endosphere are more resistant than soil microbiota to fertilization. Soil Biol Biochem 153. doi:10.1016/j.soilbio.2020.108113
17. Wu H, Qin X, Wu H, Li F, Wu J, Zheng L, Wang J, Chen J, Zhao Y, Lin S (2020) Biochar mediates microbial communities and their metabolic characteristics under continuous monoculture. Chemosphere: 125835
18. Wu H, Wu L, Wang J, Zhu Q, Lin S, Xu J, Zheng C, Chen J, Qin X, Fang C (2016) Mixed phenolic acids mediated proliferation of pathogens Talaromyces helicus and Kosakonia sacchari in continuously monocultured Radix pseudostellariae rhizosphere soil. Front Microbiol 7:335
19. Wu H, Xu J, Wang J, Qin X, Wu L, Li Z, Lin S, Lin W, Zhu Q, Khan M, Lin W (2017) Insights into the mechanism of proliferation on the special microbes mediated by phenolic acids in the Radix pseudostellariae rhizosphere under continuous monoculture regimes. Front Plant Sci 8. doi:10.3389/fpls.2017.00659
20. Chen J, Wu L, Xiao Z, Wu Y, Wu H, Qin X, Wang J, Wei X, Khan MU, Lin S, Lin W (2017) Assessment of the Diversity of Pseudomonas spp. and Fusarium spp. in Radix pseudostellariae Rhizosphere under Monoculture by Combining DGGE and Quantitative PCR. Front Microbiol 8. doi:10.3389/fmicb.2017.01748
21. Wu L, Chen J, Wu H, Qin X, Wang J, Wu Y, Khan MU, Lin S, Xiao Z, Luo X (2016) Insights into the regulation of rhizosphere bacterial communities by application of bio-organic fertilizer in Pseudostellaria heterophylla monoculture regime. Frontiers in microbiology 7:1788
22. Wang M, Song J, Han L, Liu X, Wang L, Zou L, Fu X (2010) Content Analysis and Dynamic Research of heterophyllin B in the R. pseudostellariae. Journal of Chinese Medicinal Materials 33:1225–1227
23. Wu H, Wang X, Wang L, Xiang Y, Shi J (2012) The Antibiotic Activity of Polysaccharide from Houttuynia cordata Thunb. Chinese Wild Plant Resources 31:24–26
24. Zeng L, Lin M, Dai L, Li J, Li J, Zhang Z, Lin W (2012) Effects of Continuous Cropping on Photosynthesis and Medicinal Quality of *Pseudostellariae heterophylla*. ACTA AGRONOMICA SINICA 38:1522–1528
25. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. doi:10.1093/nar/gks1219
26. Lievens B, Brouwer M, Vanachter A, Levesque CA, Cammue BPA, Thomma B (2005) Quantitative assessment of phytopathogenic fungi in various substrates using a DNA macroarray. Environ Microbiol 7:1698–1710. doi:10.1111/j.1462-2920.2005.00816.x
27. Csardi G, Nepusz T (2006) The igraph software package for complex network research. InterJournal Complex Systems 1695:1–9
28. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020) TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Mol Plant 13:1194–1202. doi:10.1016/j.molp.2020.06.009

29. Venkatachalam S, Ranjan K, Prasanna R, Ramakrishnan B, Thapa S, Kanchan A (2016) Diversity and functional traits of culturable microbiome members, including cyanobacteria in the rice phyllosphere. Plant Biol 18:627–637. doi:10.1111/plb.12441
30. Gu S, Wei Z, Shao Z, Friman V-P, Cao K, Yang T, Kramer J, Wang X, Li M, Mei X, Xu Y, Shen Q, Kuemmerli R, Jousset A (2020) Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. Nature Microbiology 5:1002–1002+. doi:10.1038/s41564-020-0719-8
31. Olishevska S, Nickzad A, Deziel E (2019) Bacillus and Paenibacillus secreted polyketides and peptides involved in controlling human and plant pathogens. Appl Microbiol Biotechnol 103:1189–1215. doi:10.1007/s00253-018-9541-0
32. Terakado-Tonooka J, Fujihara S, Ohwaki Y (2013) Possible contribution of Bradyrhizobium on nitrogen fixation in sweet potatoes. Plant Soil 367:639–650. doi:10.1007/s11104-012-1495-x
33. Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) Streptomyces as a plant's best friend? FEMS Microbiology Ecology 92. doi: 10.1093/femsec/fiw119
34. Xiang Q, Chen Q-L, Zhu D, Yang X-R, Qiao M, Hu H-W, Zhu Y-G (2020) Microbial functional traits in phyllosphere are more sensitive to anthropogenic disturbance than in soil. Environ Pollut 265. doi:10.1016/j.envpol.2020.114954
35. Dini-Andreote F (2020) Endophytes: The Second Layer of Plant Defense. Trends Plant Sci 25:319–322. doi:10.1016/j.tplants.2020.01.007
36. Tkacz A, Bestion E, Bo Z, Hortala M, Poole PS (2020) Influence of Plant Fraction, Soil, and Plant Species on Microbiota: a Multikingdom Comparison. Mbio 11. doi:10.1128/mBio.02785-19
37. Wu L, Yang Y, Chen S, Zhao M, Zhu Z, Yang S, Qu Y, Ma Q, He Z, Zhou J (2016) Long-term successional dynamics of microbial association networks in anaerobic digestion processes. Water Res 104:1–10
38. Eiler A, Heinrich F, Bertilsson S (2012) Coherent dynamics and association networks among lake bacterioplankton taxa. ISME J 6:330
39. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK (2020) Plant-microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607–621. doi:10.1038/s41579-020-0412-1

Figures

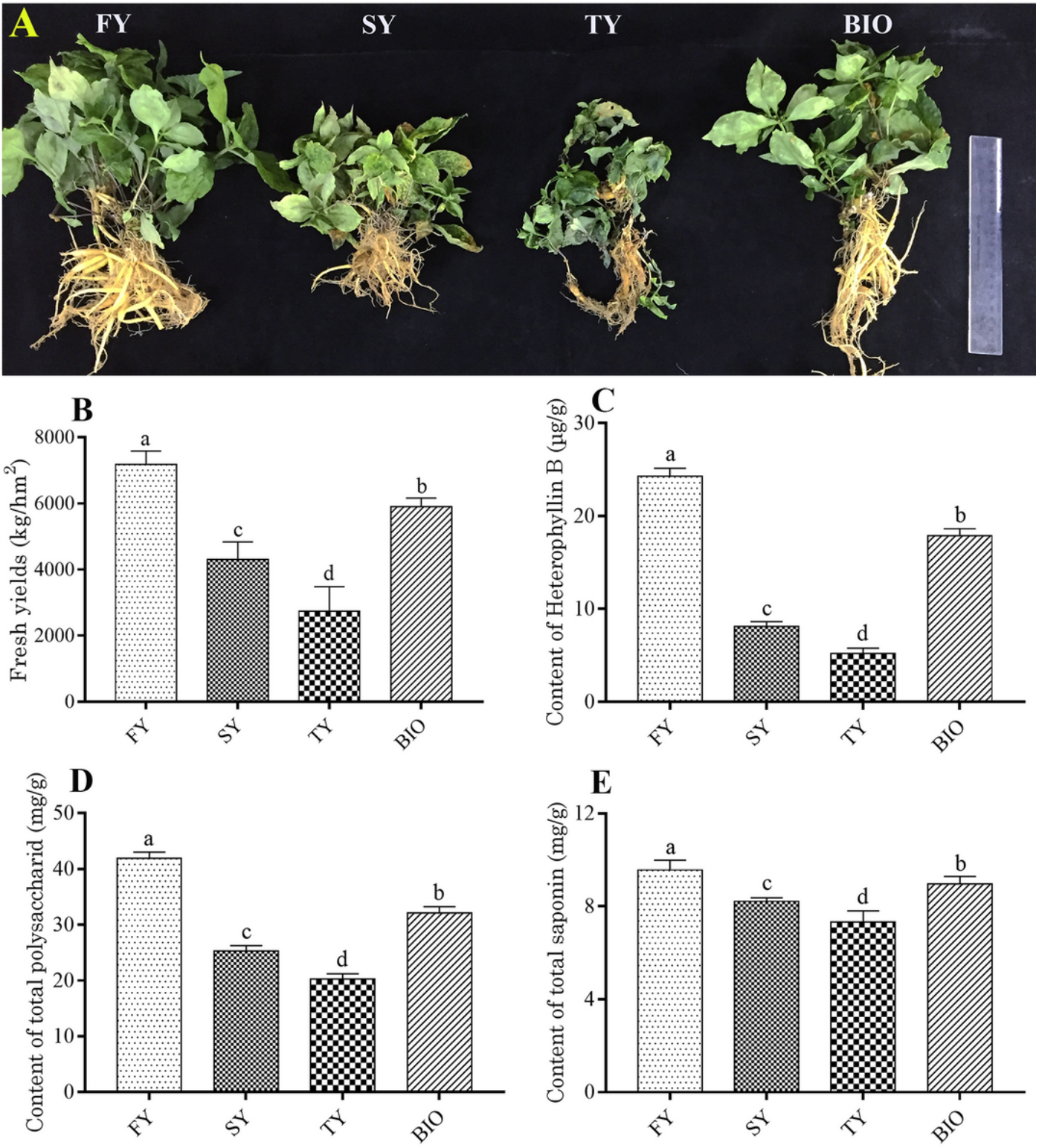

Figure 1

Photographs, fresh yields and the contents of heterophyllin B, total polysaccharide, total saponin of R. pseudostellariae under different treatments. FY: First cropping year of R. pseudostellariae; SY: Second cropping year of R. pseudostellariae; TY: Third cropping year of R. pseudostellariae; BIO: Third cropping year of R. pseudostellariae with bio-fertilizer treatment.

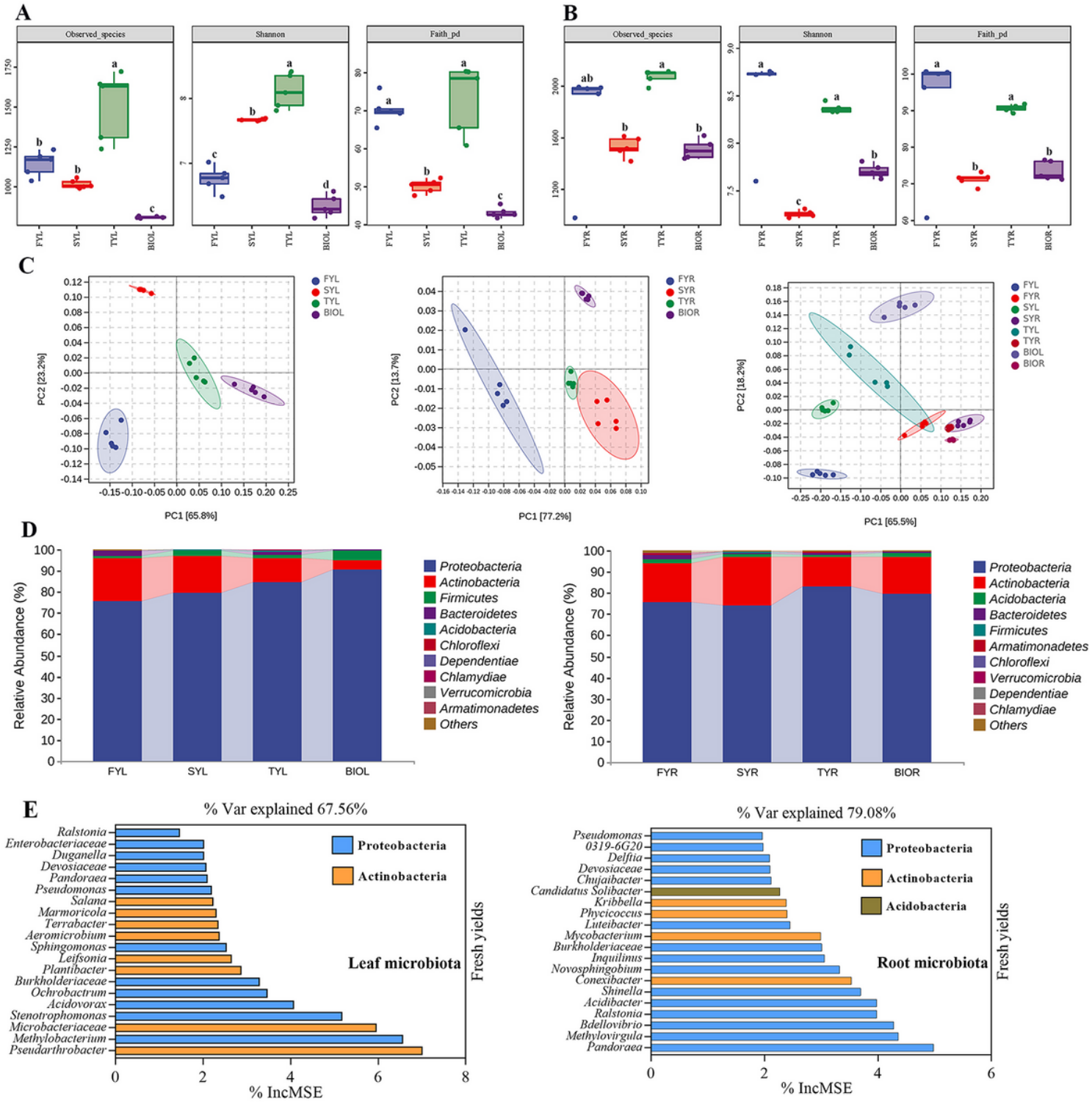

Figure 2

Relative abundance of the differentially microbial phyla among all samples. The different letters in each column indicate the significant differences (LSD-test, $p < 0.05$, $n = 5$).

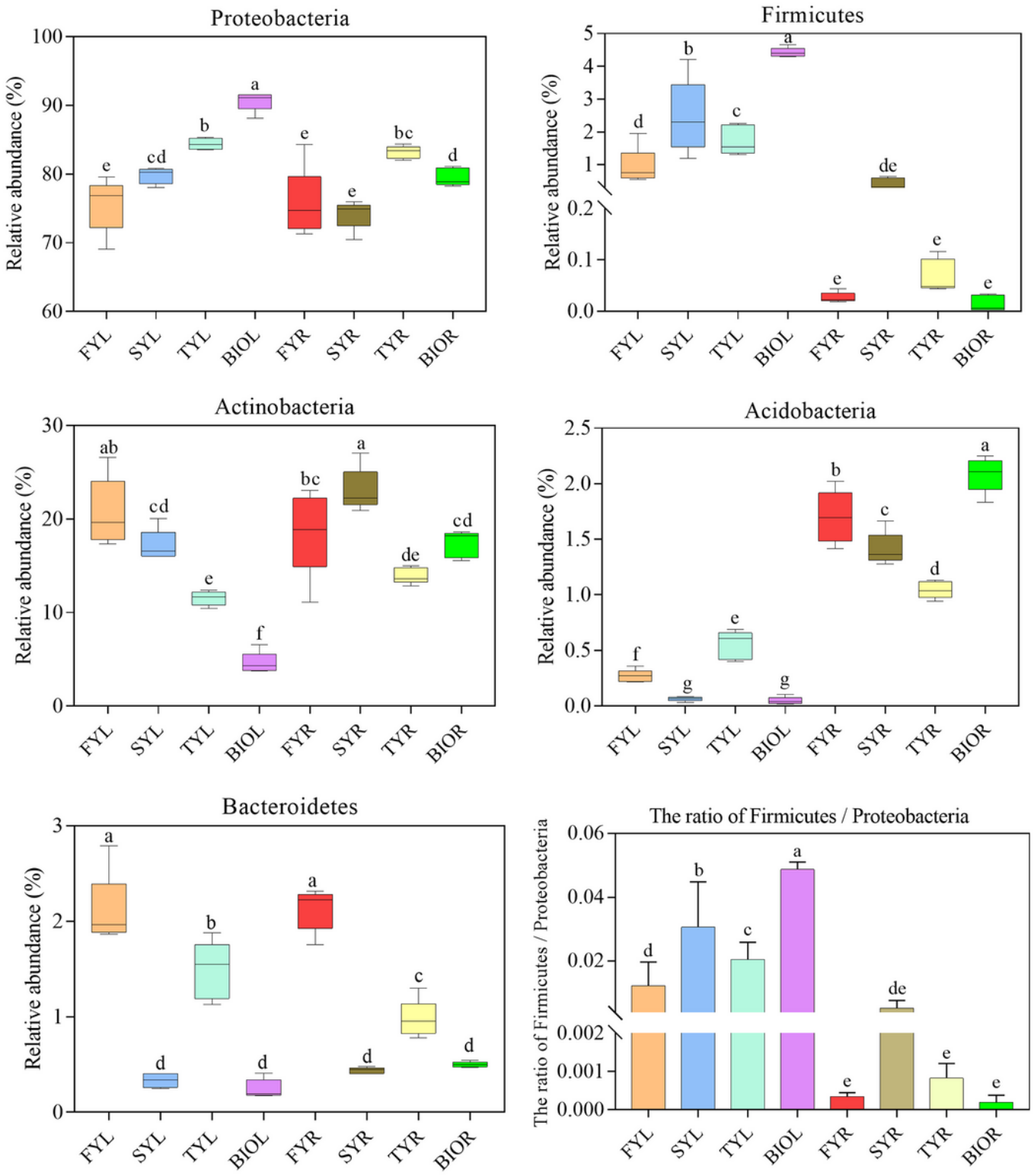

Figure 3

Random Forest regression model shows the top 20 most important taxa of bacteria at the genus level in leaves and roots samples as key drivers of heterophyllin B (A and B), total polysaccharide (C and D) and total saponin (E and F), respectively.

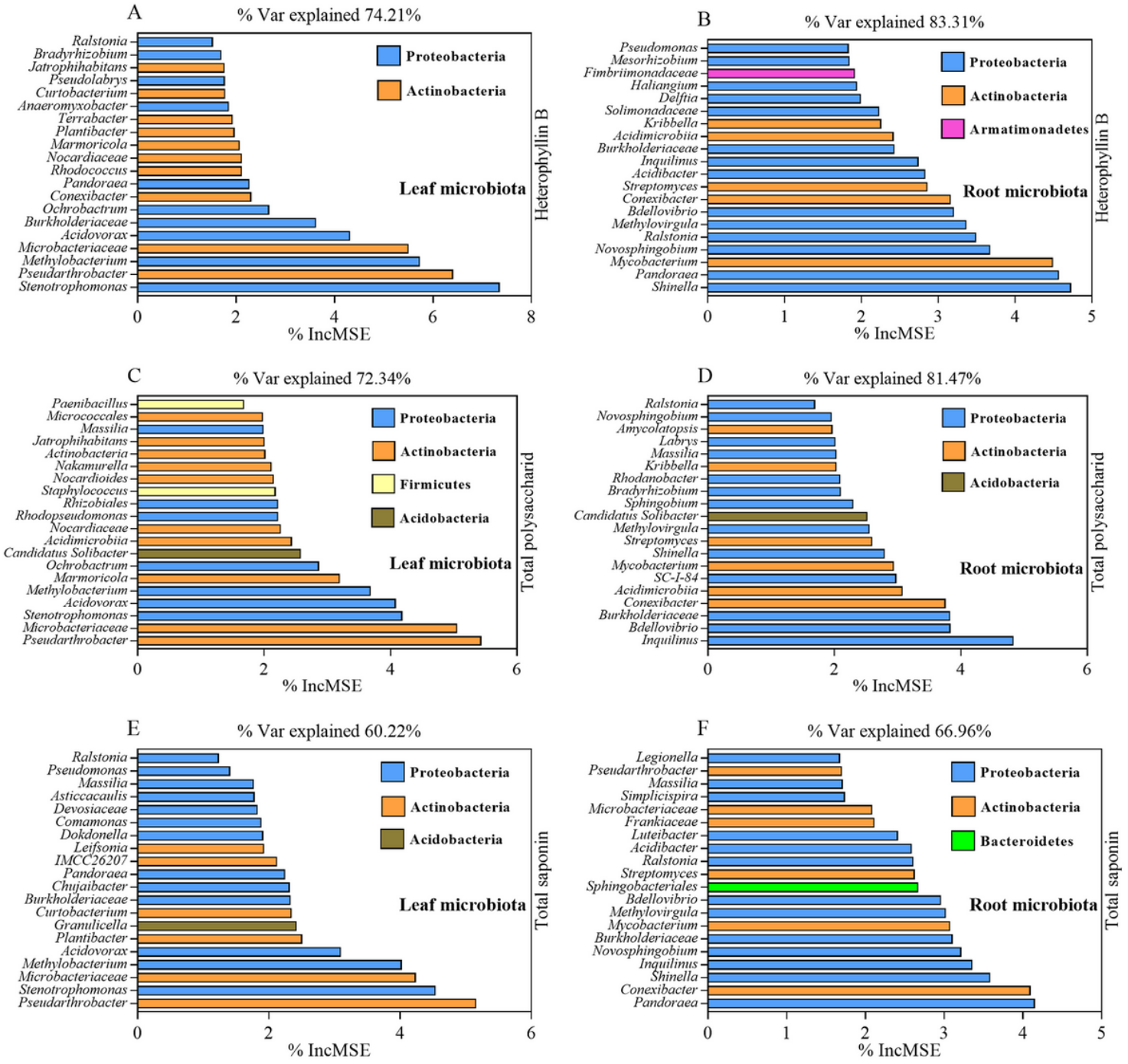

Figure 4

Alpha diversity of leaf (A) and root (B) bacteria under different treatments. Principal component analysis of leaf and root bacteria (C). Distribution of bacterial phyla across all leaf and root samples (D). Random Forest regression model showing the top 20 most important taxa of microbiota at the genus level in leaf and root samples as key drivers of fresh yields (E). FYL, SYL, and TYL represent the leaves in the first, second, and third cropping years of R. pseudostellariae, respectively; BIOL represents the leaves in the third cropping year of R. pseudostellariae with bio-fertilizer treatment. FYR, SYR, and TYR represent the roots in the first, second, and third cropping years of R. pseudostellariae, respectively; BIOR represents the roots in the third cropping year of R. pseudostellariae with bio-fertilizer treatment.

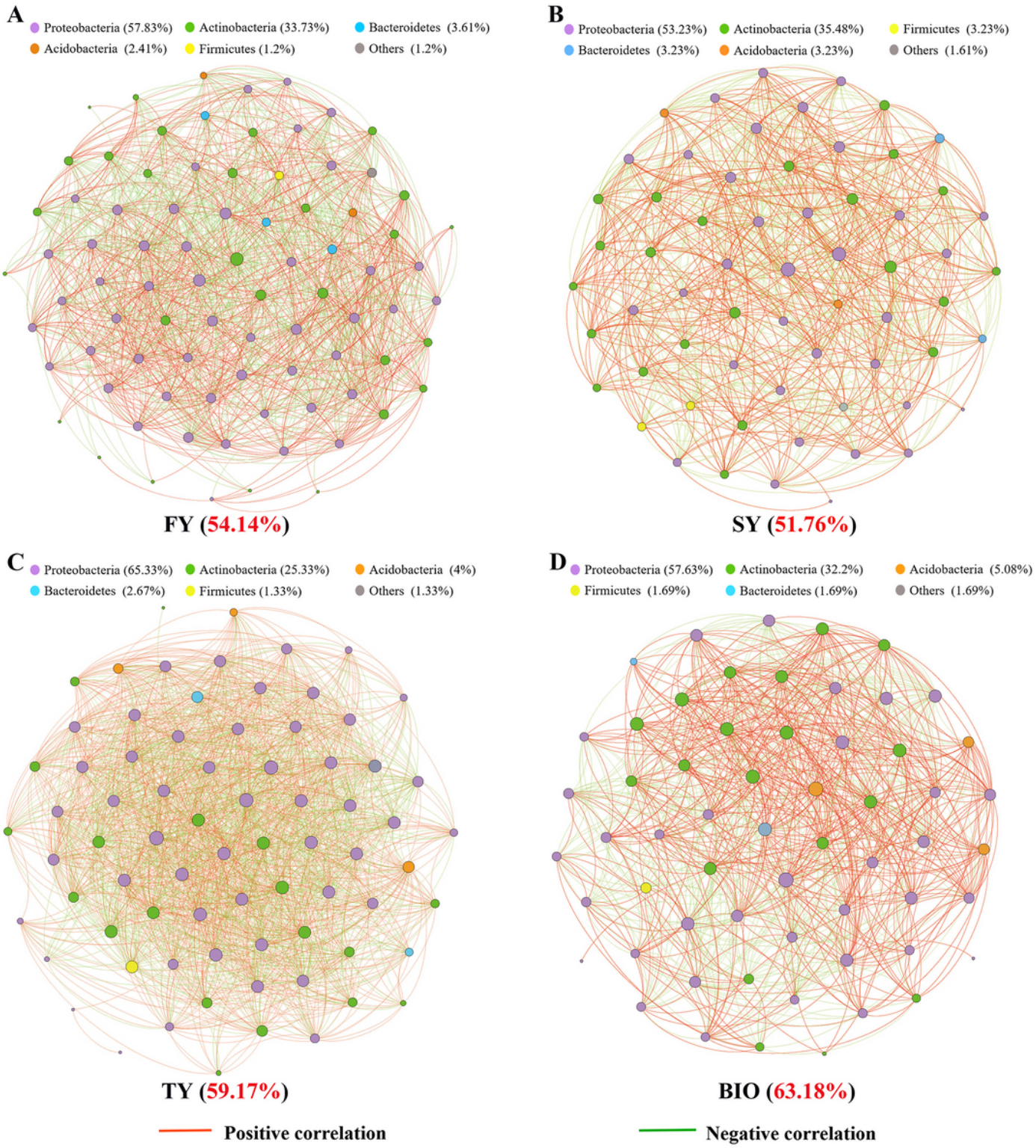

Figure 5

Network of co-occurring bacterial genera under altering treatments based on correlation analysis. FY: First cropping year of R. pseudostellariae; SY: Second cropping year of R. pseudostellariae; TY: Third cropping year of R. pseudostellariae; BIO: Third cropping year of R. pseudostellariae with bio-fertilizer treatment.

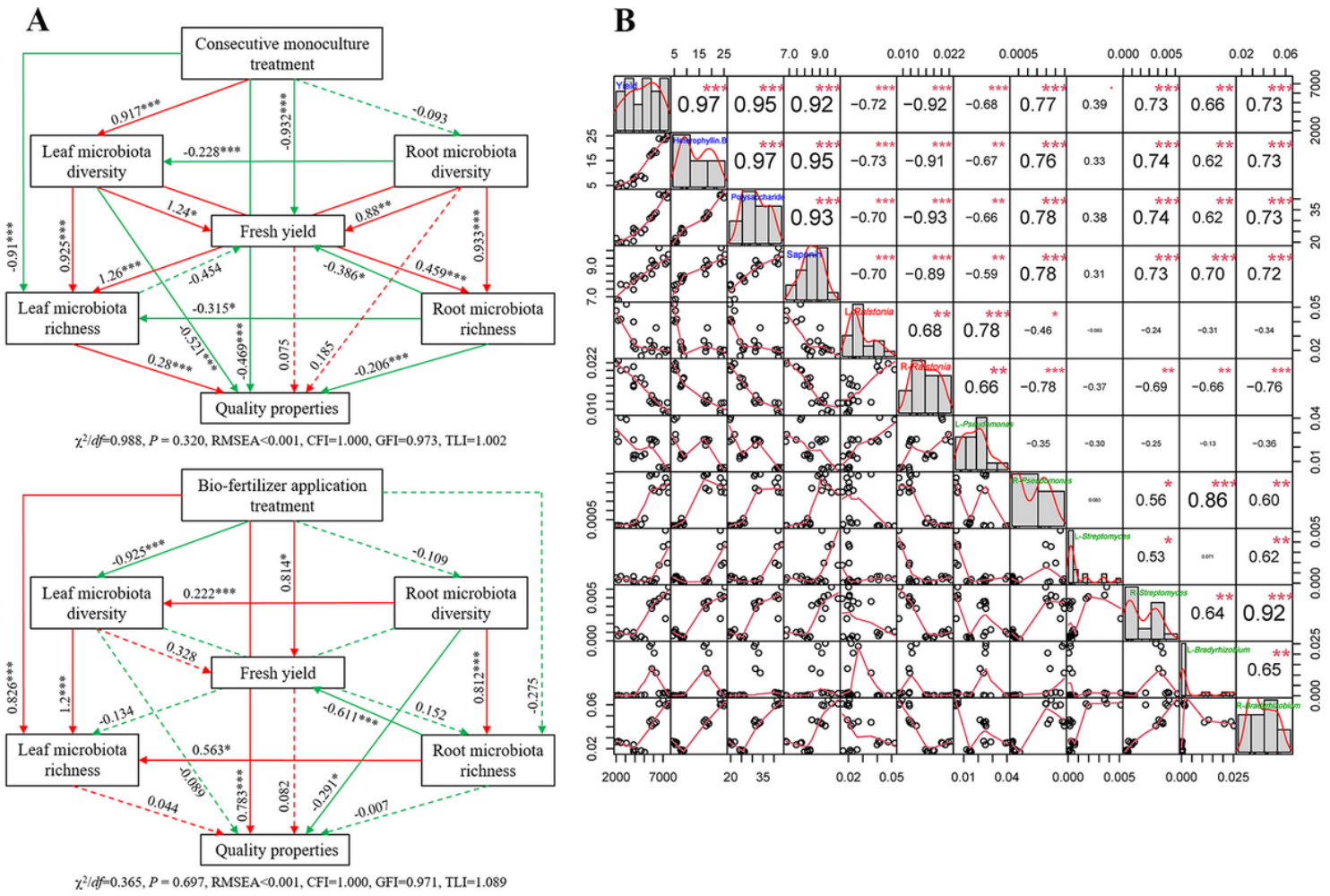

Figure 6

Correlation between the relative abundances of microbial genera and abundance of Fusarium oxysporum.

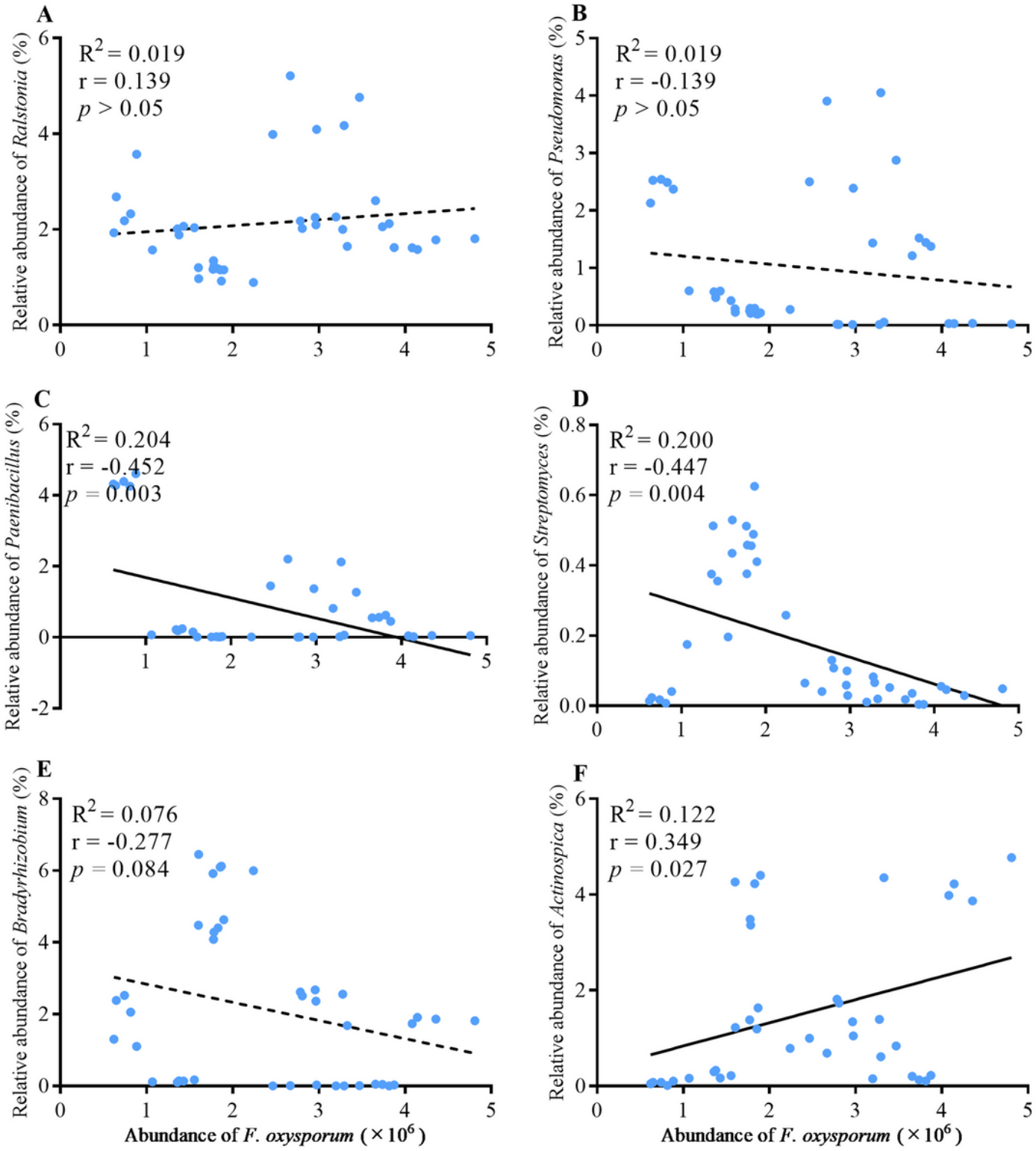

Figure 7

Structural equation models (SEM) evaluating the effects of consecutive monoculture and bio-fertilizer treatments on plant microbiota and properties, respectively (A). Correlations between plant properties and specific bacterial taxa based on Spearman correlation coefficients (B). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportinginformation.docx