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**Reductive soil disinfestation and Fe amendment improve soil microbial composition and *Fritillaria* production**

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## Abstract

The continuous obstacles of cropping cause severe economic loss, which seriously threaten agricultural sustainable development. In addition, managing excess waste, such as potato peel and mineral waste residues, is a vital burden for industry and agriculture. Therefore, we explored the feasibility of reductive soil disinfestation (RSD) with potato peel and amendment with iron mineral waste residues for the production of *Fritillaria thunbergii*, which is vulnerable to continuous obstacles. In this study, the influences of iron mineral, RSD with different organic matters, as well as the combined effects of iron mineral and RSD on *Fritillaria* rhizosphere soil physicochemical properties, microbial communities, and *Fritillaria* production were investigated. The results revealed that the RSD treatments with potato peel significantly reduced the soil salinity, and increased the soil pH, microbial activity, organic matter, and the contents of K and Ca. RSD with potato peel also significantly thrived of the beneficial microbes (*Bacillus*, *Azotobacter*, *Microvirga*, and *Chaetomium*), and down-regulated potential plant pathogens. RSD with potato peel significantly promoted *F. thunbergii* yield and quality. Moreover, the combined effects of RSD and iron mineral amendment further enhanced soil health, improved microbial community composition, increased the yield and peimisine content of *F. thunbergii* by 24.2% and 49.3%, respectively. Overall, our results demonstrated that RSD with potato peel and amendment with iron mineral waste residues can efficiently improve soil fertility, modify the microbial community, and benefit for both the sustainable production of *F. thunbergii* and the management of waste.

**Keywords:** Reductive soil disinfestation, potato peel waste, iron mineral, *Fritillaria thunbergii*, continuous cropping obstacle, microbial community

## Key points

- RSD increases soil pH, organic matter, microbial activity and mineral content
- RSD with potato peel enriches beneficial microbes and decreases plant pathogens
- PP+Fe treatment increases *Fritillaria* yield by 24.2% and peimisine content by 49.3%

## Introduction

*Fritillaria* spp., a large genus of the *Liliaceae* family with over 130 species worldwide, is a rich source of novel and bioactive steroidal alkaloids and has been used as expectorants and antitussives in traditional Chinese medicine for more than 2000 years (Wu et al. 2022). *Fritillaria thunbergii* Miq. is one of the most important medicinal varieties of *Fritillaria*, primarily cultivated in Zhejiang province, China (Liu et al. 2022). Pharmacological studies have demonstrated that several steroidal alkaloids isolated from *F. thunbergii* display a wide range of antitussive, expectorant, anticancer, and anti-inflammatory bioactivities (Nile et al. 2021). Therefore, considering its multiple medical and economic benefits, *Fritillaria* is highly valued by farmers and planted continuously. However, *Fritillaria* planted in the same field continuously for more than two years may result in short plants, little bulbs, and poor yield (Liao et al. 2011). Moreover, the continuous crop obstacle of *Fritillaria* is becoming increasingly grave with the increase in intensive agriculture and limited cultivated land. This is manifested by the aggravation of pests and diseases, the reduction in quality and yield, and ultimately, total crop failure (Liu et al. 2020). These continuous obstacles can cause a 10%-30% economic loss every year and hugely undermine the sustainable development of the *Fritillaria*-related pharmaceutical industry (Liu et al. 2022). Several factors may affect the efficacy of continuous cropping of medicinal plants, such as soil degradation (e.g., acidification and salinization), increased allelopathic autotoxicity of root exudates, and the accumulation of fungal pathogens (Li et al. 2019). Therefore, it is urgent and essential to develop effective management practices to ameliorate soil degradation and maintain the productivity of *Fritillaria* under the existing cultivation system.

Reductive soil disinfestation (RSD) has been confirmed to be a reliable and effective pre-planting approach in the prevention of various plant pathogens (Huang et al. 2015). Here, readily decomposable organic wastes (e.g., agricultural residues, molasses, etc.) are incorporated into soils, watered, and then covered with a plastic film for approximately three weeks. RSD can readily create highly reductive soil conditions that promote the

anaerobic decomposition of added organic matter (Huang et al. 2019). Potato peel is an ideal choice of organic matter for RSD since it is cheap and easily degradable. The effectiveness of RSD on the productivity of continuous cropping of *Fritillaria* nor the feasibility of potato peels used in RSD has been investigated so far. Furthermore, considering potatoes rank as the fourth most important agricultural crop based on mass and are essential to the global diet, due to their wide application, almost 50 million tons of potato peel waste would be produced worldwide, leading to a major problem for the potato industry (Yang et al. 2022). Therefore, RSD with potato peel waste could be a mutually beneficial solution to alleviate the continuous cropping obstacle of *Fritillaria* and in handling excessive potato peel waste.

Sugarcane bagasse may be applied and compared to determine the feasibility of potato peel waste, which is considered to be an effective RSD substrate and could significantly increase the functional activity and diversity of soil (Huang et al. 2019). Iron mineral is a strong reducing agent, and has been widely used in the remediation of soil pollution (Miretzky and Cirelli 2010). Therefore, the addition of iron mineral is expected to strengthen the reduction state of soil. Moreover, mineral waste residues pose a serious environmental burden, while recent studies have revealed the use of organo-mineral waste from industries in reclaiming and improving soil quality (Antonkiewicz et al. 2018). However, there is limited information on the effectiveness of RSD combined with iron mineral for soil improvement.

RSD notably has favorable effects not only on preventing soil degradation but also on improving soil microbial activity and restructuring soil microbiome (Zhao et al. 2018). Rhizosphere microorganisms have been known to play crucial roles in preserving soil health, enhancing plant nutrition, and fostering plant growth by providing a variety of beneficial functions, including decomposition of organic substances, nutrient cycling and retention, stress resistance, and protection against pathogens (Sterkenburg et al. 2015; Xiong et al. 2017). A field experiment was, therefore, conducted using different materials (sugarcane bagasse, potato peel, and iron mineral) with RSD in an *F. thunbergii* monoculture cropping field. It was hypothesized that RSD with potato peel waste could

significantly alleviate the continuous cropping obstacle of *F. thunbergii*. The purpose of this study was to (1) investigate the effects of RSD with potato peel waste and iron mineral on soil physicochemical properties, microbial activity, microbial community composition, and the yield and quality of *F. thunbergii*, and (2) explore the underlying mechanisms of RSD with potato peel on alleviating the continuous cropping obstacle of *F. thunbergii*.

## Materials and methods

### Field design and soil sampling

The field experiment was conducted in September 2021 in Pan'an County, Zhejiang Province, China (28°53' N, 120°19' E). The area is characterized by a typical subtropical monsoon climate with an annual average temperature of 16.6 °C and precipitation of 1630.7 mm. The soil demonstrated cropping obstacles considering *Fritillaria* had been constantly cultivated in the area for 30 years.

This study comprised six varied treatments: (1) CK, untreated soil; (2) CK+Fe, soil with 0.4 t ha<sup>-1</sup> iron mineral (obtained from an iron tailing located in Guangdong Province, China); (3) SB, the soil amended with 1 t ha<sup>-1</sup> sugarcane bagasse (extracted from *Saccharum officinarum* L.); (4) SB+Fe, the soil amended with 1 t ha<sup>-1</sup> sugarcane bagasse and 0.4 t ha<sup>-1</sup> iron mineral; (5) PP, soil amended with 1 t ha<sup>-1</sup> potato peel (extracted from *Solanum tuberosum* L.); (6) PP+Fe, soil amended with 1 t ha<sup>-1</sup> potato peel and 0.4 t ha<sup>-1</sup> iron mineral. The sugarcane bagasse and potato peel were dried, smashed (< 2 mm), and uniformly added to the topsoil before irrigating to saturation. A white plastic film (80 µm thick, Jiangsu Yupeng Plastic Co., Ltd., Shuyang, Jiangsu, China) was applied as soil mulch for the RSD treatments, with the film's edge buried 35 cm into the soil surface. The soil temperature was kept at 35–40 °C during the 4-week anaerobic treatment period. The plastic films were then removed after the anaerobic treatment.

The iron mineral was added to the seedling holes before planting the *Fritillaria*. The planting density of *Fritillaria* was 50 plants per square meter. The total organic carbon (C) content of sugarcane bagasse and potato peel were 330 and 358 g kg<sup>-1</sup>, respectively.

Each treatment included three replicates in a randomized block design (as presented in Supplemental Fig. S1) of a 5 m<sup>2</sup> (5 m length × 1 m width) area. Standard procedures followed by local farmers were considered for the implementation of management practices, including weeding, fertilization, disease, and pest control.

Rhizosphere soil samples were obtained in April 2022. To obtain the rhizosphere soil samples, five *Fritillaria* plants were dug up from each plot. The tightly bound soil on the root surface was brushed down as rhizosphere soil after the loose adhesive soil on the root was removed by shaking (Philippot et al. 2013). The obtained soils were sieved (2 mm mesh), homogenized, and separated into two parts; one part was stored at 4 °C for measuring the microbial activity and physicochemical properties, and the other was stored at –80 °C for DNA extraction.

#### **Assay of soil characteristics, microbial activity, and enzyme activity**

Standard methods were used to measure the soil pH, electrical conductivity (EC), NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, total carbon (TC), total nitrogen (TN), and soil organic matter (SOM) (Nelson and Sommers 1982; Zhao et al. 2018). Total soil microbial activity was estimated with the fluorescein diacetate hydrolysis method, and the result was expressed as production of fluorescein in µg per g dry soil (Schnürer and Rosswall 1982). The concentrations of K, Ca, Mg, Mn, Fe, As, Cr, Cd, Pb, and V in soil were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 8300; PerkinElmer, Waltham, MA, USA) and mass spectrometry (ICP-MS, Agilent 7500, Santa Clara, CA, USA), respectively. Soil urease activities (SUE) were measured using the soil enzyme kit from Solarbio Science & Technology Co. (Beijing, China).

#### **DNA extraction, high-throughput sequencing, and bioinformatic analysis**

The soil DNA of each sample was extracted, quality-controlled, and quantified as previously described (Zhao et al. 2018). The V4–V5 region of the bacterial 16S rRNA gene was amplified using the primer pair 515F/907R (Turner et al. 1999). The primer set

ITS1F/ITS2 (Walters et al. 2016) was used to amplify the fungal ITS region. Standard PCR reaction and thermal profile followed the method by Zhao et al. (2018). The Illumina Miseq 2500 platform (Majorbio, Shanghai, China) was used to purify, pool, and sequence the PCR products. The raw sequences were eliminated if they contained ambiguous nucleotides with a low ( $Q < 20$ ) quality score and if they were short in length ( $< 100$  bp). The DADA2 pipeline in R 4.0 (<http://cran.r-project.org/>) and the package dada2 (v1.12.1) were used to generate the tables of amplicon sequence variations (ASVs). The taxonomic classification of fungi was identified using the UNITE (v8.0) databases (Nilsson et al. 2019).

#### **Assay of *Fritillaria* yield, disease index and quality**

*Fritillaria* bulbs were collected from  $5 \text{ m}^2$  of each treatment and their fresh weight was measured and used to determine the theoretical yield using the following formula:

Theoretical yield ( $\text{t ha}^{-1}$ ) = bulb yield from  $5 \text{ m}^2 \times 2000$ .

The *Fritillaria* bulbs affected by insect infestation were visually assessed, and the numbers of infected bulbs and total plants were counted at harvest. The infestation rate was calculated as Infestation rate = Number of infected bulbs/Total number of bulbs

A total of ten individual *Fritillaria* bulbs were randomly selected for laboratory analysis for each treatment. The fresh biomass of a single *Fritillaria* bulb was determined using an electronic balance (ACS-ZE14, Senssun, Zhongshan, China). According to the Chinese Pharmacopoeia (2020 edition), the primary active ingredients of *Fritillaria* bulbs are peimine and peiminine. The content of peimisine (including peimine and peiminine) was represented as the quality of *Fritillaria*, and determined using liquid chromatography (Liu et al. 2010). To determine the concentrations of K, Mg, Ca, Zn, and Fe in *Fritillaria* bulbs, 0.2 g of dried *Fritillaria* bulbs were powdered and digested with  $\text{HNO}_3$  and  $\text{HClO}_4$  (5:1, v/v) in polytetrafluoroethylene tubes and measured using ICP-OES.

## Statistical analysis

The data were analyzed using Excel 2021 and SPSS 25 software (SPSS Inc., Chicago, IL, USA). Measurement data were demonstrated as mean  $\pm$  standard deviation. Significant differences between groups were tested using analysis of variance (ANOVA) or Wilcoxon with false discovery rate (FDR) adjusted  $p$ -value. The statistical significance was defined as  $P < 0.05$  and the bar charts were generated using Origin 2022 (OriginLab, Northampton, MA, USA). The Shannon diversity of microbial communities was calculated via QIIME (<http://qiime.org/>). The “vegan” package in R was utilized to perform principal coordinate analysis (PCoA) at the ASV level to evaluate the variations in the composition of the fungal community (Oksanen et al. 2012). The fungal plant pathogens were inferred using the program FUNGuild (Nguyen et al. 2016). The Mantel tests based on Spearman’s correlation were furthermore performed with the “vegan” package to reveal the relationships between the rhizosphere microbial communities in relation to the background soil environmental properties and the quality of *Fritillaria*.

## Results

### Soil physicochemical properties

The soil pH of all RSD treatments (SB, SB+Fe, PP, and PP+Fe) was significantly ( $P < 0.05$ ) higher than that of the treatments without RSD (CK and CK+Fe), as presented in Fig.1a. Soil salinity (EC) in all the RSD-related treatments decreased significantly ( $P < 0.05$ ) compared to that in the untreated control (CK) (Fig. 1b). In addition, soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were not significantly different among treatments, though soil  $\text{NO}_3^-$  was observed to be slightly decreased in the PP+Fe treatment (Fig. 1c and Supplemental Table S1). Compared to the CK treatment, the SOM was significantly increased ( $P < 0.05$ ) in all RSD-related treatments (Fig. 1d). Compared to the non-RSD (CK and CK+Fe) treatments, the soil microbial activity (the content of fluorescein) was observed to be significantly ( $P < 0.05$ ) increased in the SB, SB+Fe, PP, and PP+Fe treatments, where the

PP+Fe treatment demonstrated the highest impact (Fig. 1e). The concentration of K was significantly higher ( $P < 0.05$ ) in CK+Fe, SB+Fe, PP, and PP+Fe treatments than that in the CK treatment (Fig. 1f). The contents of TC and C/N in the SB+Fe treatment were observed to be the highest, while there was no significant difference observed among the other treatments (Supplemental Table S1). The treatments of PP and PP+Fe demonstrated significantly ( $P < 0.05$ ) higher urease activity than the other treatments, while there was no significant difference among the other treatments (Supplemental Table S1). The PP+Fe treatment also demonstrated the highest concentration of Ca and significantly ( $P < 0.05$ ) higher than that in CK, while the concentrations of Cd and Pb in SB-related treatments were found to be significantly higher than that in CK (Supplemental Table S2). Besides, there was no significant difference in the contents of TN, Mg, Mn, Fe, As, Cr, and V among all the treatments (Supplemental Table S1 and S2).

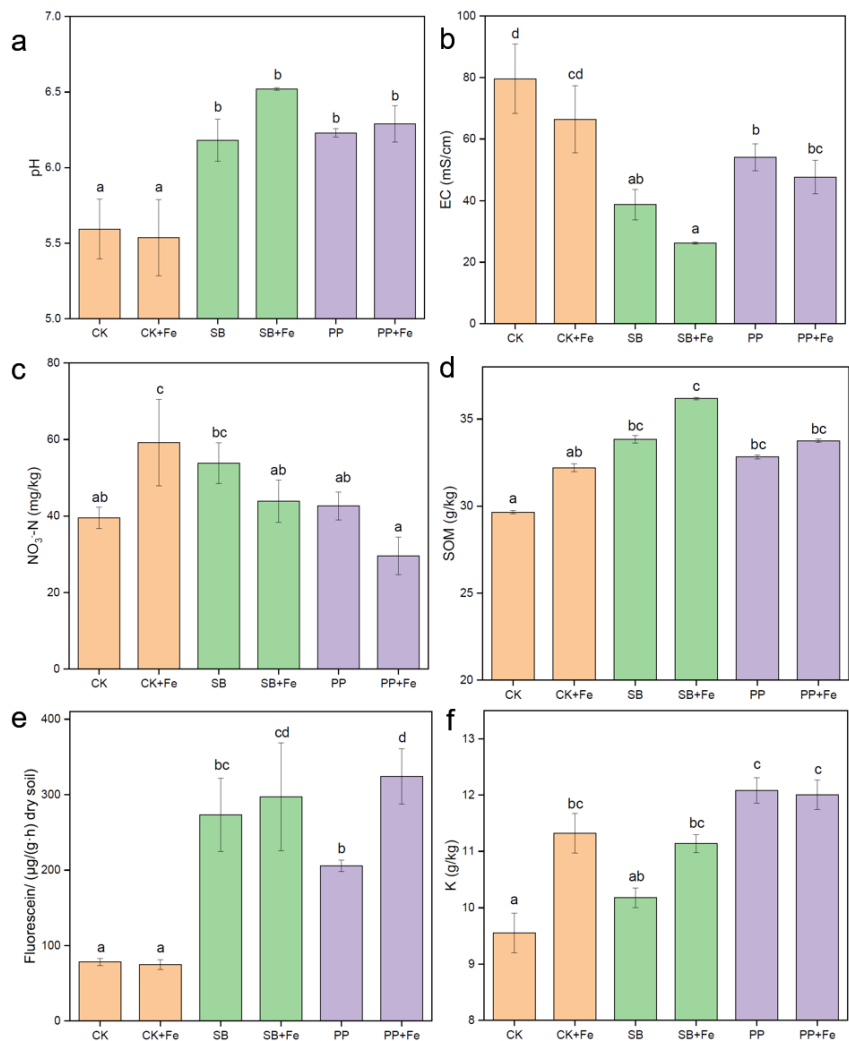


**Fig.1** Effects of different soil treatments on selected soil properties, including pH (a), electrical conductivity (EC) (b), nitrate nitrogen ( $\text{NO}_3^-$ -N) (c), organic matter (OM) (d), soil microbial activity (the content of fluorescein) (e), and the content of potassium (K) (f). The data shown are the means  $\pm$  standard deviation (SD) of three replicates. The lowercase letters above the columns indicate significant differences between treatments ( $P < 0.05$ ), with the same letter indicating no significant difference ( $P > 0.05$ ). The treatments are denoted as follows: CK for untreated soil, CK+Fe for soil with added iron mineral, SB for soil with added sugarcane bagasse, SB+Fe for soil with added sugarcane bagasse and iron mineral, PP for soil with added potato peel, and PP+Fe for soil with added potato peel and iron mineral. The soils in treatments of SB, SB+Fe, PP, and PP+Fe were flooded and mulched with plastic film

#### ***Fritillaria* yield, infestation rate, fresh biomass and quality**

The highest *Fritillaria* yield was obtained under PP+Fe treatment, with a 24.2% increase compared to CK, while there were no significant differences among the other treatments (Fig. 2a). Additionally, the infestation rate in the PP+Fe treatment was the lowest (0), while that in CK treatment had the highest rate among all the treatments (0.8) (Fig. 2b). The average fresh biomass of a single *Fritillaria* bulb did not show significant differences among all the treatments (Fig. 2c). The PP+Fe treatment had the highest peimisine content, increasing by 49.3% compared to CK (Fig. 2d). In the PP+Fe treatment, the content of peimine and peiminine increased by 52.7% ( $P < 0.05$ ; Fig. 2e) and 41.6% ( $P < 0.05$ ; Fig. 2f), respectively, compared to CK. The concentrations of K, Mg, Ca, Zn, and Fe in *Fritillaria* bulbs of different treatment groups are shown in Supplemental Table S3. Overall, the PP-treated bulbs exhibited significantly higher Mg and Zn concentrations than CK and SB-related treatments, particularly in the PP+Fe treatment, which also exhibited relatively higher Ca and Fe concentrations than other treatments (Supplemental Table S3). Furthermore, the concentration of K in *Fritillaria* bulbs was the highest in CK, while the concentrations of K and Zn in SB+Fe treatment were the lowest among all the treatments (Table S3).

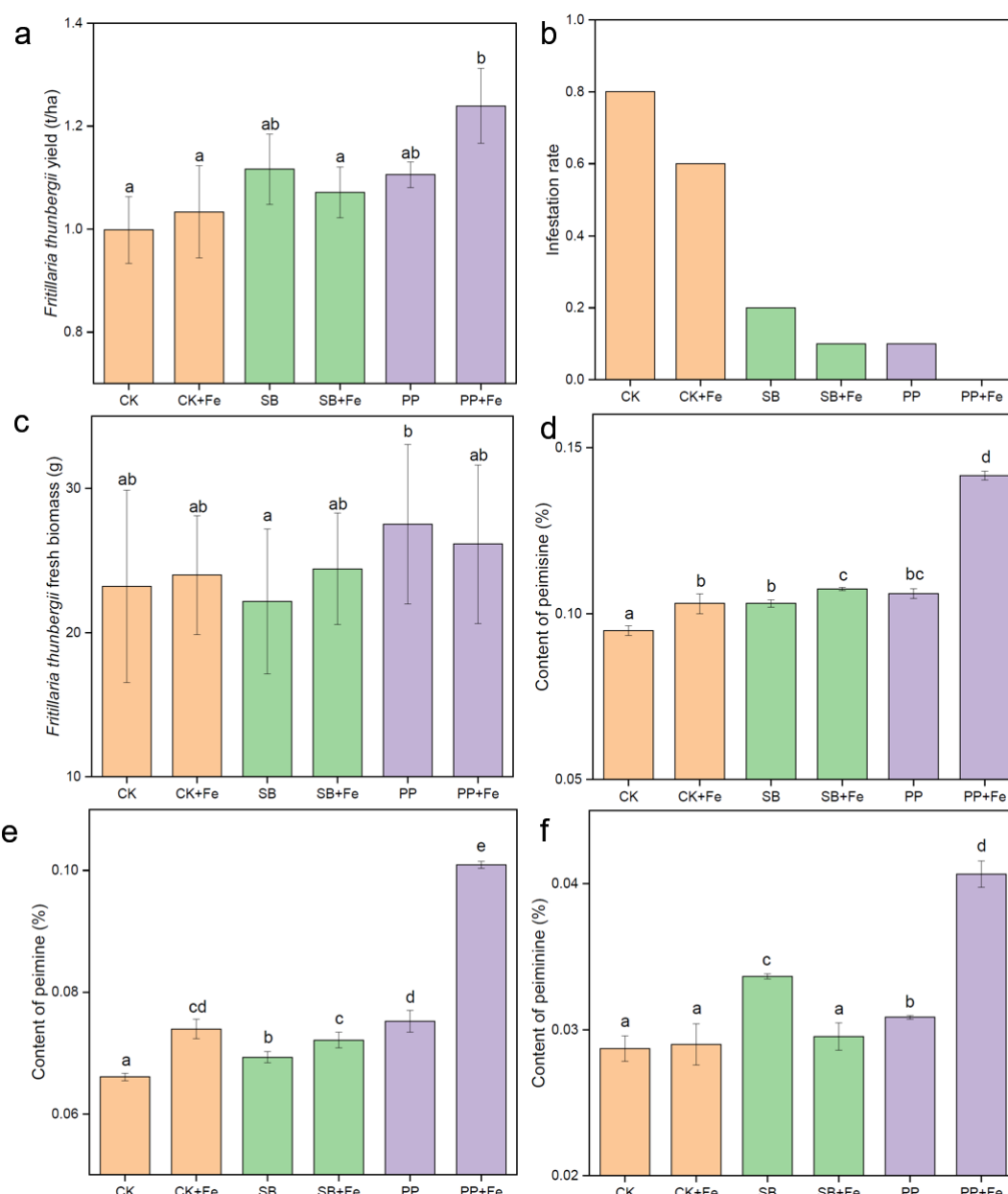


**Fig.2** Effects of different treatments on yield (a), infestation rate (b), fresh biomass (c), peimisine (d), peimine (e), peiminine (f) of *Fritillaria*. The data presented are means  $\pm$ SD (n = 3). Different lowercase letters above the columns indicate significant differences at  $P < 0.05$ . The treatments are denoted as follows: CK for untreated soil, CK+Fe for soil with added iron mineral, SB for soil with added sugarcane bagasse, SB+Fe for soil with added sugarcane bagasse and iron mineral, PP for soil with added potato peel, and PP+Fe for soil with added potato peel and iron mineral. The soils in treatments of SB, SB+Fe, PP, and PP+Fe were flooded and mulched with plastic film

## Microbial diversity

A total of 1,076,328 bacterial and 1,388,916 fungal high-quality sequencing reads were obtained from all samples, which were clustered into 11,618 bacterial amplicon sequence variants (ASVs) and 6,179 fungal ASVs, respectively. The bacterial Shannon diversity was the highest in the SB+Fe treatment, while there were no significant differences among the other treatments (Fig. 3a). The fungal Shannon diversity was significantly reduced ( $P < 0.05$ ) in the PP and PP+Fe treatments compared to the CK treatment, and the Shannon index was lower in the PP treatment than in the PP+Fe treatment (Fig. 3b). The soil bacterial and fungal community structure was significantly altered (PERMANOVA,  $P < 0.01$ ) in the RSD treatments (Fig. 3c and 3d). Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance revealed significant separation of the bacterial and fungal community at the ASV level among different treatments. The first and the second principal coordinate axes explained 25.01% and 21.57% of the total variance for bacteria (Fig. 3c), and explained 34.46% and 16.49% for fungi, respectively (Fig. 3d).

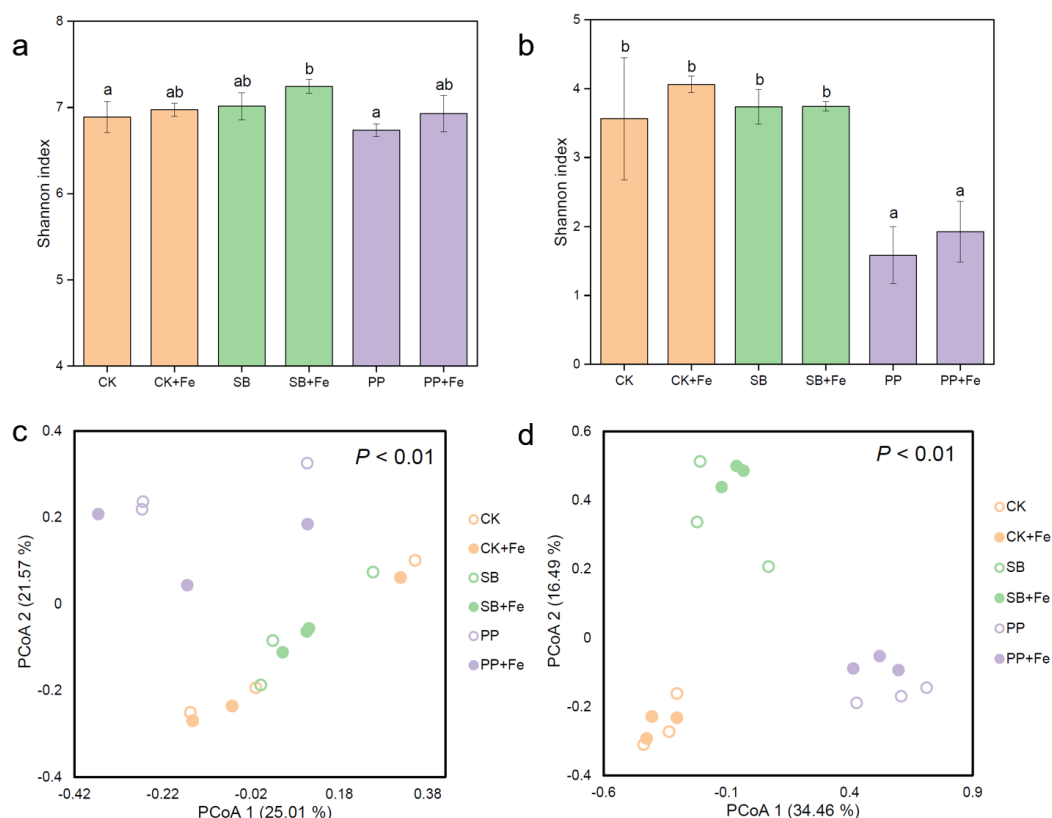


**Fig.3** Taxonomic profiles of microbial communities in different treatments. The Shannon diversity index of bacterial (a) and fungal (b) communities. Principal coordinate analysis (PCoA) plot of bacterial (c) and fungal (d) communities at ASV level based on the Bray-Curtis dissimilarity in soils subjected to different treatments. The treatments are denoted as follows: CK for untreated soil, CK+Fe for soil with added iron mineral, SB for soil with added sugarcane bagasse, SB+Fe for soil with added sugarcane bagasse and iron mineral, PP for soil with added potato peel, and PP+Fe for soil with added potato peel and iron mineral. The soils in treatments of SB, SB+Fe, PP, and PP+Fe were flooded and mulched with plastic film

### Microbial community composition

The results demonstrated that *Proteobacteria*, *Acidobacteriota*, *Actinobacteriota*, *Firmicutes*, *Chloroflexi*, *Planctomycetota*, and *Bacteroidota* were the predominant phyla across all samples and accounted for approximately 86.7–91.0% of the total bacterial community (Fig. 4a). The abundance of *Firmicutes* was notably increased in PP and

PP+Fe treatments compared to other treatments (Fig. 4a). For fungi, *Ascomycota*,  
*Rozellomycota*, *Mortierellomycota*, and *Basidiomycota* were the predominant phyla  
 across all samples, accounting for approximately 87.0–95.7% of the total fungal  
 community (Fig. 4b). The abundance of *Ascomycota* at the phylum level was significantly  
 higher in the PP+Fe treatments compared to other treatments, representing up to 93.3%  
 of the total fungal community (Fig. 4b). Moreover, the PP+Fe treatment significantly  
 decreased the abundance of *Rozellomycota*, whereas PP treatment significantly increased  
 the abundance of *Rozellomycota* compared to the CK, while no differences were observed  
 among other treatments (Fig. 4b). At the genus level, compared to the CK, CK+Fe, SB,  
 and SB+Fe treatments, both PP and PP+Fe treatments significantly increased the relative  
 abundance of *Bacillus*, *Microvirga*, and *Azotobacter* genera while reduced the relative  
 abundance of *SC\_I\_84* and *Gaiella* genera (Fig. 4c). Additionally, the relative abundance  
 of the *Nitrospira* genus in all RSD treatments was lower than in the non-RSD (CK and  
 CK+Fe) treatments. Among the fungal genera, the relative abundance of *Chaetomium*  
 notably increased from the CK treatment (2.7%) to the PP (64.8%), and PP+Fe (69.3%)  
 treatments (Fig. 4d). In contrast, the relative abundances of *Fusarium*, *Mycochlamys*, and  
*Botryotrichum* were significantly decreased by the RSD treatments compared to the CK  
 treatment. Moreover, PP and PP+Fe treatments significantly reduced the relative  
 abundance of *Mortierella*, while SB and SB+Fe treatments notably increased the relative  
 abundance of *Pseudothielavia* compared to other treatments (Fig. 4d).

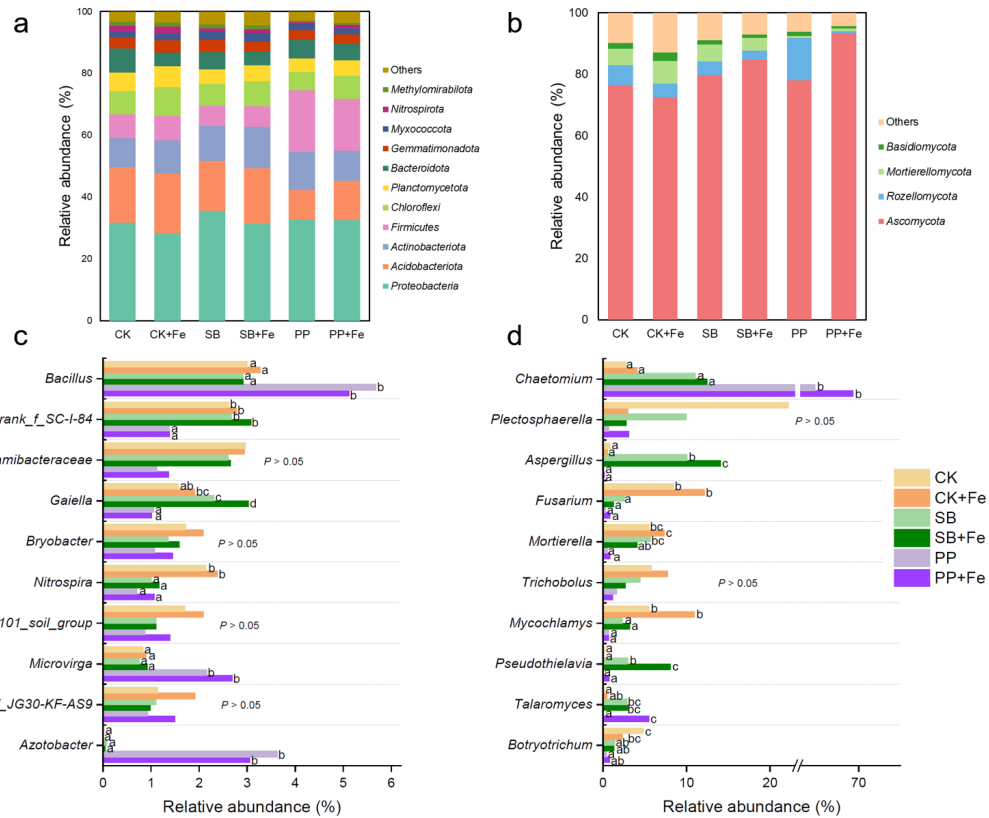


**Fig.4** Microbial community composition. Relative abundances (%) of the bacterial (a) and fungal (b) phyla in soils under different treatments. Relative abundances (%) of Top 10 main bacterial (c) and fungal (d) genera in soils under different treatments. Different lowercase letters indicate significant differences at  $P < 0.05$ . The treatments are denoted as follows: CK for untreated soil, CK+Fe for soil with added iron mineral, SB for soil with added sugarcane bagasse, SB+Fe for soil with added sugarcane bagasse and iron mineral, PP for soil with added potato peel, and PP+Fe for soil with added potato peel and iron mineral. The soils in treatments of SB, SB+Fe, PP, and PP+Fe were flooded and mulched with plastic film

### Potential fungal plant pathogen

A total of 312,956 high-quality sequences were identified as potential fungal plant pathogens among the rhizosphere fungi, affiliated with 72 genera (Fig. 5a). The top six predominant taxa were *Plectosphaerella*, *Fusarium*, *Mycochlamys*, *Acremonium*, *Thermomyces*, and *Pseudopithomyces* in all treatments, accounting for 6.1%, 5.0%, 4.4%,

1.9%, 1.2%, and 1.1%, respectively. The abundances of potential fungal plant pathogens in RSD treatments were significantly lower than those in treatments without RSD. Furthermore, the highest abundance of potential fungal plant pathogens was observed in CK, while PP and PP+Fe had the lowest pathogen abundance, with relative abundances ranging from 4.1% to 47.3% (Fig. 5a). There was no significant difference in the Shannon diversity index of soil fungal pathogens among all treatments (Supplemental Fig. S2). PERMANOVA analysis based on Bray-Curtis distances showed that the overall distribution patterns of potential fungal plant pathogens in different treatments were significantly different ( $P < 0.01$ ) (Fig. 5b). The first and second principal coordinate axes explained 31.05% and 20.01% of the total variance, respectively.

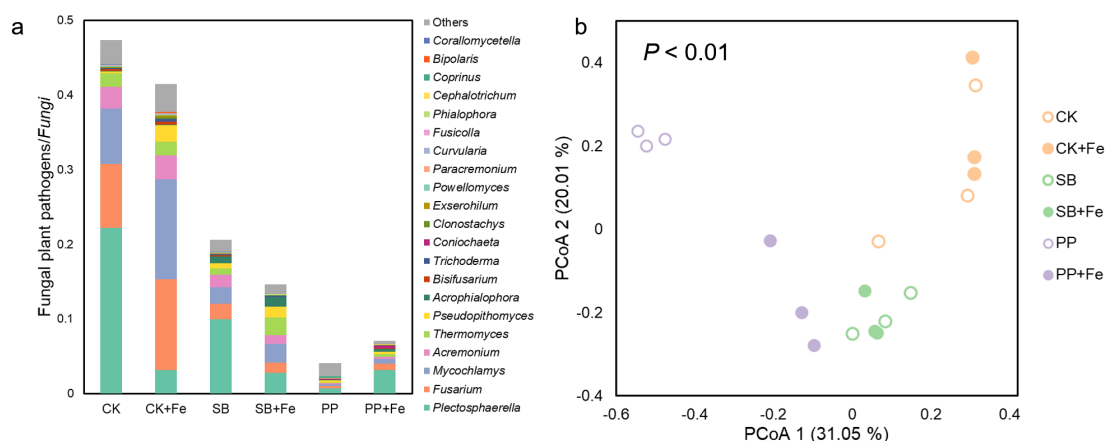


**Fig.5** Taxonomic profiles of potential fungal plant pathogens in different treatments. (a) the relative abundance of fungal plant pathogens in soils under different treatments. (b) PCoA plot of fungal plant pathogen communities at ASV level based on Bray–Curtis distances grouped by treatments. The treatments are denoted as follows: CK for untreated soil, CK+Fe for soil with added iron mineral, SB for soil with added sugarcane bagasse, SB+Fe for soil with added sugarcane bagasse and iron mineral, PP for soil with added potato peel, and PP+Fe for soil with added potato peel and iron mineral. The soils in treatments of SB, SB+Fe, PP, and PP+Fe were flooded and mulched with plastic film

## Relationships of soil physicochemical properties, microbial community and *Fritillaria* yield and quality

The Mantel test demonstrated significant correlations ( $P < 0.05$ ) between the microbial community and soil physicochemical properties, as well as *Fritillaria* quality (Fig. 6 and Supplemental Table S4). Soluble urease enzyme (SUE) and potassium (K) were the key factors influencing rhizosphere bacteria, while soil pH, electrical conductivity (EC), soil organic matter (SOM), K, SUE, and microbial activity were the crucial factors for rhizosphere fungi (Fig. 6). Total carbon (TC), total nitrogen (TN), calcium (Ca), ammonium ( $\text{NH}_4^+$ ), and nitrate ( $\text{NO}_3^-$ ) concentrations were not significantly correlated with the microbial community. *Bacillus* was positively associated with SUE, K, the yield, and quality of *Fritillaria*, and the relative abundances of *Microvirga*, *Azotobacter*, and *Chaetomium* ( $P < 0.05$ ). The relative abundance of *Chaetomium* was positively correlated with SUE, the contents of K and Ca, *Fritillaria* yield, and *Fritillaria* quality, including both peimine and peiminine ( $P < 0.05$ ). Furthermore, the bacterial and fungal Shannon diversity, *Bacillus*, *SC-I-84*, *Nitrospira*, *Microvirga*, *Acrophialophora*, *Chaetomium*, *Mortierella*, *Fusarium*, *Cladosporium*, the contents of K, microbial activity, and *Fritillaria* yield were significantly associated ( $P < 0.05$ ) with potential fungal plant pathogens (Fig. 6). The relative abundance of potential fungal plant pathogens was negatively correlated with *Aspergillus*, *Chaetomium*, soil pH, TC, TN, SOM, microbial activity, and *Fritillaria* yield, but positively correlated with soil EC, *Nitrospira*, *Fusarium*, *Cladosporium*, and *Plectosphaerella* (Supplemental Fig. S3). The relative abundance of *Fusarium* was positively associated with the relative abundances of *Cladosporium*, *Podospira*, *Botryotrichum*, *Mycoclamys*, *Mortierella*, *Pseudopithomyces*, *Acrophialophora*, and *Nitrospira*, while microbial activity, *Fritillaria* yield, and the relative abundances of *Microvirga*, *Azotobacter*, *Chaetomium* were negatively correlated with *Fusarium* ( $P < 0.05$ ). Notably, fungal Shannon diversity, microbial activity, the contents of K, and the genera of *Bacillus*, *Microvirga*, *Azotobacter*, *Talaromyces*, *Chaetomium*, *Mortierella*, *Clonostachys* were the main factors affecting the *Fritillaria*

quality (Fig. 6). *Bacillus*, *Microvirga*, *Azotobacter*, *Talaromyces*, *Chaetomium*, and microbial activity were positively correlated with both peimine and peiminine ( $P < 0.05$ ).

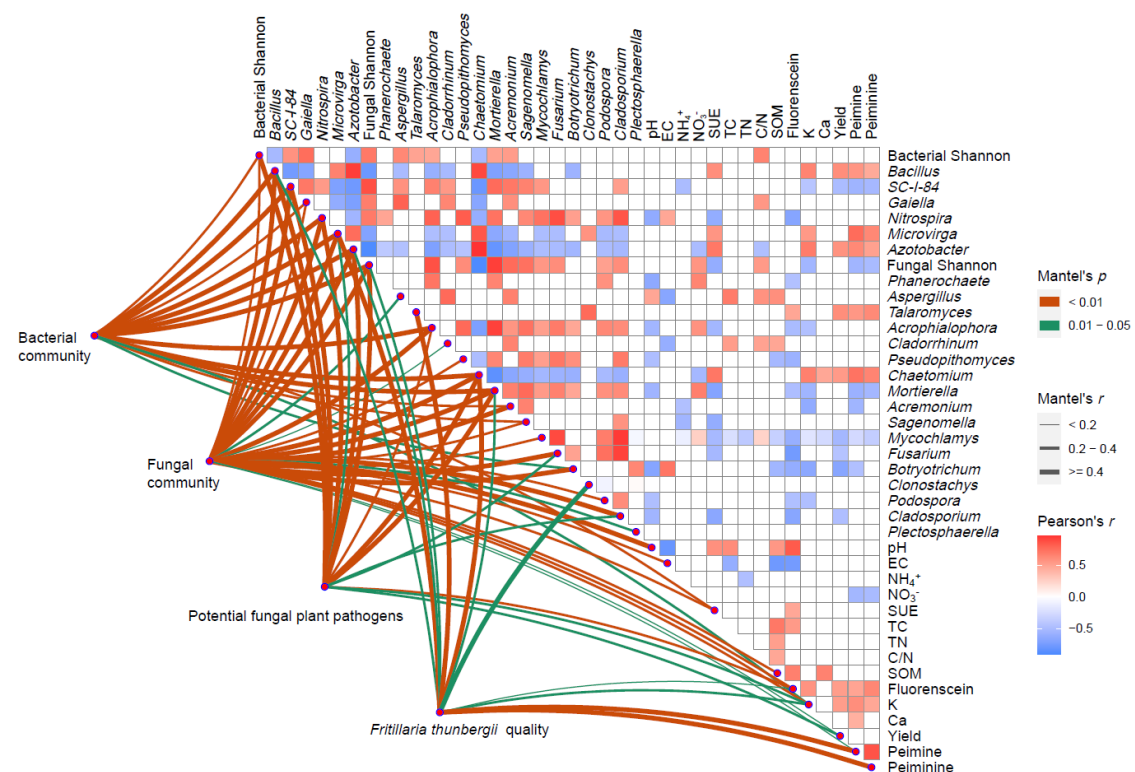


**Fig.6** Paired comparison of environmental factors, bacterial community, fungal community, potential fungal plant pathogens, *Fritillaria* yield, and quality with a color gradient representing significant Pearson's correlation coefficient. The width of the edge corresponds to the distance dependence of Mantel's  $r$  statistic, and the statistical significance based on 9,999 permutations represents the color of the edge. The magnitude of Mantel's  $r$  indicates the strength of the correlation. The color of the connecting line indicates the correlation between different community and environmental factors. The significance level is \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ , blank indicates a nonsignificant ( $P > 0.05$ ) correlation

## Discussion

### RSD with potato peel and Fe significantly improved soil properties

Soil pH, EC, OM, nutritional elements, and microbial activities are vital properties that

may be applied to assess soil fertility and health as well as plant development (Lemaire et al. 2019). In this study, we found that RSD can significantly improve soil properties. Compared with the treatments without RSD, RSD significantly increased soil pH (Fig. 1a). This is consistent with other investigations, primarily due to the utilization of  $H^+$  during denitrification and other redox processes under anaerobic circumstances (Huang et al. 2016; Ji et al. 2022). Continuous cropping causes soil acidification, which further results in an absence of crucial elements notably K and Ca (You et al. 2015). Compared to CK, the RSD-related treatments with potato peel dramatically improved the content of K and Ca in soil, primarily due to the abundant nutrients in potatoes (Table. S1). In addition to soil acidification, excessive fertilization and continuous cropping can also cause soil salinization, and further damage crop production (Huang et al. 2016). We found RSD-related treatments considerably reduced the soil salinity (EC), maybe as a result of microbial activity that converted the soil ions (e.g.,  $NO_3^-$ ,  $SO_4^{2-}$ ) into gases (e.g.,  $N_2$ ,  $N_2O$ , and  $H_2S$ ) (Fig. 1b) (Zhao et al. 2018). Besides, the lowest content of soil  $NO_3^-$  presented in the PP+Fe treatment (Fig. 1c) may be a result of the effortless decomposition of the potato peel and the higher microbial activities induced by a sufficient supply of iron mineral (Lopes et al. 2020). Notably, SOM is one of the most significant indicators of soil quality contributing to soil fertility, water retention, and biodiversity (Ondrasek et al. 2019). Our results showed that PP, PP+Fe, SB, and SB+Fe treatments significantly increased SOM compared to the CK treatment (Fig. 1d), which may be a direct result of the anaerobic decomposition of potato peel or sugarcane bagasse (Meng et al. 2019). Additionally, one of the key indicators of soil function is soil microbial activity, which contributes to organic matter degradation and soil energy flow (Liu et al. 2019). Compared to the non-RSD (CK, CK+Fe) treatments, the soil microbial activity was observed to be significantly ( $P < 0.05$ ) increased in the PP, PP+Fe, SB, and SB+Fe treatments, where the PP+Fe treatment demonstrated the highest impact (Fig. 1e). Considering the crucial role of microorganisms in the decomposition of organic substrates, nutrient cycling, and other chemical transformations in soil, the useable organic material stimulates soil microorganisms to utilize the C or N and other nutrients from it as an

energy source, thereby supporting the development of microorganisms and increasing microbial activities (Momma et al. 2013; Zhao et al. 2018). Although sugarcane bagasse has been proven to be an effective RSD substrate in previous studies, potato peel waste demonstrated a significantly better effect on the improvement of soil properties in this study. Notably, the RSD treatments accompanied with iron mineral demonstrated better effects on the improvement of soil microbial activity (Fig. 1e). A probable reason for this may be that iron mineral is a scarcer resource and a competing element for soil microorganisms (Xiang et al. 2022). The microbial metabolism process of iron reduction also improves the availability of organic C to microorganisms (Chen et al. 2020). Thus, the addition of iron could significantly mitigate this competition and promote microorganism growth. All the results demonstrated that the application of RSD, especially with potato peel and Fe, can effectively mitigate soil deterioration induced by continuous cropping of *Fritillaria*.

#### **RSD with potato peel remarkably enriched beneficial microbes while reduced the relative abundance of potential plant pathogens**

Rhizosphere microbial community plays an important role in nutrient cycling, plant development, and disease prevention (Sterkenburg et al. 2015). Our results suggest that different RSD treatments lead to significant variations in bacterial and fungal community structures (Fig. 3 and 4). Furthermore, the PP and PP+Fe treatments significantly decreased fungal diversity and altered the bacterial and fungal community composition compared to the CK treatment (Fig. 3). The relative abundance of *Firmicutes* was significantly higher in the PP and PP+Fe treatments compared to other treatments, primarily due to the ability of *Firmicutes* to decompose carbohydrate, protein, and other macromolecule organics in potato peels during the RSD (Yang et al. 2022). *Ascomycota* was the predominated fungal phyla, and its relative abundance was significantly increased in the PP+Fe treatment compared to other treatments (Fig. 4b). *Ascomycota* is known to thrive in habitats with low nutrient availability as an oligotrophic fungal group (Yang et

al. 2019) and has a significant impact on the degradation of organic materials in rhizosphere soil and soil fertility (Schoch et al. 2009). Therefore, the substantial enrichment of *Ascomycota* in the PP+Fe treatment is consistent with the notable improvement in soil properties in this treatment. Moreover, most potential fungal plant pathogens were detected from the treatments without RSD, among which *Plectosphaerella*, *Fusarium*, and *Mycoclhamys* were the top three predominant pathogenic fungi (Fig. 5a). Members of these genera have been identified as biomarkers for diseased rhizosphere soils and have frequently caused plant disease and impaired crop development, thus revealing the significance of RSD in the elimination of plant pathogens (Desjardins et al. 2000; Su et al. 2017; Wang et al. 2022). The lowest abundance of pathogens was observed in PP and PP+Fe treatments, while *Bacillus*, *Azotobacter*, *Microvirga*, and *Chaetomium* were significantly enriched in these two treatments (Fig. 4 and 5). Bacterial species of *Bacillus*, *Azotobacter*, and *Microvirga* genera have been commonly acknowledged as important plant growth promoters by nitrogen fixation, producing phytohormones and enzymes, promoting inorganic phosphate solubilization, and prompting induced-systematic resistance in plants (Jiménez-Gómez et al. 2019; Nehra and Choudhary 2015; Shafi et al. 2017). Members of *Chaetomium* are known to decompose complex polysaccharides by producing cellulase, which might be crucial for substrate degradation (Hakulinen et al. 2010). The application of potato peel significantly stimulated their growth. Additionally, *Chaetomium* species have been proven to be prospective antagonists of several soilborne and seed-borne plant pathogens due to their ability to produce antifungal substances, such as tetrahydrofuran (Joong-Hyeop et al. 2005). Hence, *Bacillus*, *Azotobacter*, *Microvirga*, and *Chaetomium*, as the beneficial genera receiving increasing attention, may be crucial for soil disinfestation (Huang et al. 2016; Zhao et al. 2018). Therefore, the enrichment of these prospective antagonists in RSD treatments may suppress the growth of potential plant pathogens during cultivation which is expected to increase due to continuous monocropping. The underlying mechanisms of suppression include inducing systemic resistance, competition for nutrients, production of lytic enzymes and antibiotics, physical barrier formation, and

biofilm inhibition (Mendes et al. 2013; Ab Rahman et al. 2018). Furthermore, it has been reported that plant pathogens have a competitive advantage in obtaining iron, thus, suppressing the growth of other organisms due to iron deficiency (Gu et al. 2020). Therefore, RSD with iron mineral significantly promoted the growth of potential antagonists, which then reduced the number of prospective infections from soilborne pathogens (Xiang et al. 2022). Accordingly, the application of RSD, especially potato peel and Fe, can significantly improve the soil microbial community structure by increasing beneficial microbes and decreasing harmful fungi caused by *Fritillaria* continuous cropping.

**RSD with potato peel waste significantly increased the yield and quality of *Fritillaria* by improving soil health and microbial composition**

In this study, we found that the PP+Fe treatment resulted in a 24.2% increase in *Fritillaria* yield and a 49.3% increase in peimisine content compared to the CK treatment (Fig. 2), though the content of peimisine in the *Fritillaria* bulb of the CK treatment also met the standard of Chinese Pharmacopoeia (2020 edition): the total amount of peimisine is required to be more than 0.08% (Fig. 2d). Additionally, the PP+Fe treatment exhibited the most responsive microbial community (in terms of their relative abundance), associated with the highest microbial activities and the lowest infestation rate and relative abundance of potential fungal plant pathogens (Fig. 1, 2, and 5). These results confirm that the conjunction of RSD with potato waste and iron mineral has the best remediation effect on the continuous cropping obstacle of *Fritillaria*. Moreover, *Fritillaria* yield was significantly positively correlated with microbial activity, K, and the relative abundance of *Bacillus*, *Azotobacter*, *Chaetomium*, and *Talaromyces* (Fig. 6). These genera are known to secrete antimicrobial substances that suppress numerous seed-borne pathogens (Kato et al. 2012). *Fritillaria* yield was negatively correlated with the relative abundance of potential fungal plant pathogens (Supplemental Fig. S3). Importantly, the mantel test indicated that *Bacillus*, *Microvirga*, *Azotobacter*, *Talaromyces*, *Chaetomium*, *Mortierella*,

*Clonostachys*, the content of K, and microbial activity were the main factors affecting *Fritillaria* quality (Fig. 6). *Bacillus*, *Azotobacter*, *Talaromyces*, *Chaetomium*, and *Clonostachys* are known to be effective biocontrol agents against several soilborne and seed-borne plant pathogens (Hakulinen et al. 2010; Kato et al. 2012; Lahlali and Peng 2014). Moreover, members of *Clonostachys* have been reported to inhibit *Botrytis cinerea* and *Fusarium oxysporum*, which can lead to serious economic losses, especially in *Fritillaria* (Lahlali and Peng 2014; Li et al. 2022).

Potassium is a crucial macronutrient for plants, which promotes root growth, proper seed development, higher yield and quality of fiber (Sattar et al. 2019). It is also essential for microorganisms, which play a vital role in various biological processes such as protein synthesis and enzyme activation (Read et al. 2006; Haro and Benito 2019). The correlation analysis showed a significant positive correlation between K content and microbial activity (Fig. 6). Soil microbial activity is an indicator of soil quality, and microorganisms can enhance their activity by utilizing organic materials to obtain nutrients and energy for their growth (Mendes et al. 2013). RSD treatment enhanced soil microbial enzymatic and metabolic activities, which may lead to an improvement in *Fritillaria* yield and quality by altering the accessible nutrients in the soil. Therefore, these findings indicate that the RSD with potato peel waste can promote *Fritillaria* yield and quality, primarily as a result of increased soil fertility and improvement of soil microbial composition.

### Author contribution

Y.Y., J.Z., P.W., G.L.D., and Y.G.Z. conceived and designed the investigation. Y.Y., P.W., and H.M.D. conducted the experiments. Y.Y. analyzed data and wrote the manuscript. J.Z., S.M.Y., Y.Y.Z., J.W.W., G.L.D., and Y.G.Z reviewed and edited the manuscript. All authors read and approved the manuscript.

### Declarations

## Ethical approval

This article does not contain any studies with human participants or animals performed by any authors.

## Conflict of interest

The authors declare no competing interests.

## Data availability

The original sequences have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject ID number PRJNA916813.

## Appendix A. E-Supplementary data

E-Supplementary data for this work can be found in the e-version of this paper online.

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