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Effects of continuous cropping of *Pinellia ternata* (Thunb.) Breit. on soil physicochemical properties, enzyme activities, microbial communities and functional genes

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Abstract

Background: *Pinellia ternata* (Thunb.) Breit. is a commonly used herb in traditional Chinese medicine, and the main raw material of various Chinese patent medicines. Continuous cropping obstacle (CCO) is the main factor leading to the decline of crop yields and quality.

Methods: Metagenomics sequencing technology was used to analyze the microbial community and functional genes of continuous cropping (CC) and control (CK) soils of *P. ternata*. In addition, differences in physicochemical properties, enzyme activities, microbial community composition and the abundance of functional genes in CC and CK were evaluated, as well as the relationship between these factors and CCO.

Results: Results indicated that CC of *P. ternata* led to the decline of rhizosphere soil pH, nutrient imbalance and enzyme activity reduction. Metagenomic analysis indicated that CC also changed the composition of the microbial community, causing an increase in the relative abundance of pathogenic microorganisms such as *Fusarium*, *Klebsiella oxytoca* and *Pectobacterium carotovorum* in the *P. ternata* rhizosphere. The relative abundance of potentially beneficial *Burkholderia* and *Bradyrhizobium* was recorded to decrease. Results also showed that there were considerable differences in CC and CK about the abundances of functional genes related to soil enzymes and the degradation of *P. ternata* allelochemicals, as well as the microbial groups which they belong. These results clarified the effects of CC on the microbial community structure and functional genes of soil. In addition, *Burkholderia* and *Bradyrhizobium* might play important roles in enhancing soil fertility and reducing the toxicity of phenolic acids in rhizosphere soil.

Conclusions: CC of *P. ternata* changed the physicochemical properties, microbial community and functional genes of rhizosphere soil. *Burkholderia* and *Bradyrhizobium* for enhancing soil fertility and reducing the toxicity of phenolic acids might be potentially beneficial. These results provide theoretical guidance for bioremediation of CCO soil of *P. ternata* and other staple crops.

Keywords: *Pinellia ternata*, Continuous cropping obstacle, Enzyme activity, Microbial community, Allelochemical degradation, Functional gene

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Background

Pinellia ternata (Thunb.) Breit. (genus: *Pinellia*; Family: Araceae) is a slightly poisonous and pungent perennial herb whose dry tuber is often used in medicine [1]. In addition, *P. ternata* is also one of the commonly used Chinese herbal medicines in traditional Chinese medicine (TCM). Its tuber has unique pharmacological characteristics, such as effects of drying dampness and expectoration, anti-obesity, antipyresis and hemostasis [2, 3]. The utilization frequency of *P. ternata* among 588 kinds of Chinese medicine prescriptions ranks the 22nd. The market demand and price for this herb is increasing [4]. Continuous cropping obstacles (CCO) indicates that when the same crop or closely related crops are continuously planted on the same land, crop diseases and pests are exacerbated and crop yield and quality decline, even under normal cultivation management measures. Since 70% of root-type Chinese medicinal materials have varying degrees of CCO [5], it poses a severe challenge to the cultivation of medicinal plants [6]. The CC of *P. ternata* will not only cause aggravation of pests and diseases, soil salinization and acidification, but also reduce the photosynthetic performance of leaves [7] and the activity of antioxidant enzymes of the plant, lead to membrane lipid peroxidation damage [8, 9], ultimately resulting in the yield and quality decline of *P. ternata*. Investigations have shown that changes in soil physicochemical properties, nutrient decline, soil microbial community structure imbalance, accumulation of soil-borne pathogens and phenolic acid allelochemicals can all result in CCO [10–12]. Even under normal management practices, the yield of *P. ternata* after 2–4 years of continuous cropping (CC) is generally reduced by 80–90% [13]. A common method to overcome CCO in *P. ternata* production is to incorporate crop rotation. However, long rotation periods and the reduction of cultivation area greatly reduce the utilization rate of *P. ternata* land resources, thereby seriously restricting the sustainable development of the *P. ternata* industry.

To date, the cause of CCO in *P. ternata* and mitigation measures are rarely reported. He et al. [10, 13] found that CC of *P. ternata* changed the physicochemical properties of the soil and the structure of the microbial community, causing serious degradation of soil fertility and a decrease in the diversity of the rhizosphere soil microbial community. However, the relationship between CCO of *P. ternata* and microbial functions is not clear. In addition, the interaction between microbial community and soil physicochemical properties needs to be further studied. Previous findings showed vanillin, vanillic acid, protocatechuic acid, syringaldehyde, syringic acid, gallic acid, ferulic acid, benzofuran, chlorogenic acid, etc.,

have significant inhibitory effects on the growth of *P. ternata* seedlings. Therefore, these compounds are considered as main allelochemicals autotoxins of *P. ternata* [9, 14]. In addition, these allelochemicals autotoxins are also the main substances that cause CCO in other staple crops, such as *Arachis hypogaea* [15, 16], *Glycine max* [17], and *Radix pseudostellariae* [18]. Plants can release allelochemicals into the environment through a variety of pathways, for instance volatilization, leaching, residue decomposition and root secretion [11]. However, studies on the biodegradation of these allelochemicals in CC soil of *P. ternata* and the selective shaping of the rhizosphere microflora of *P. ternata* as well as how to jointly affect the growth and development of *P. ternata* have not been reported yet. At present, the main measures to mitigate CCO of *P. ternata* include rotation, intercropping and soil sterilization [10, 13, 19]. These measures, however, have disadvantages, including long rotation periods, low land resource utilization rates and high costs. Although bioremediation may provide new ways to solve CCO, it is still lacking on screening the microorganisms for beneficial to soil and allelochemicals degradation, constructing bioremediation microbial inoculants and studying the effect and mechanism of bioremediation CC soil of *P. ternata*. In addition, with the continuous development of omics technology, metagenomics technology has been widely used in functional genomic analysis of various environmental samples, such as soil samples [20, 21] and compost samples [22]. In this study, we took CC soil and unplanted soil (CK) of *P. ternata* as the research objects. Combining the physicochemical and metagenomic data, we analyzed the differences in physicochemical properties, enzyme activities and microbial community composition, as well as the abundances of functional genes related to soil enzyme and the degradation of the main allelochemicals of *P. ternata*. It is expected to expand our understanding on the relationship between these factors and *P. ternata* CCO, providing theoretical guidance to mitigate *P. ternata* CCO.

Materials and methods

Soil sampling

The sampling site, Zhongchengzhai Village, Yilong County, Sichuan Province (30°11′–31°39′ N, 106°14′–106°52′ E, China; 445 m.a.s.l), is located in the transitional zone between the northern Sichuan Basin and the hills in central Sichuan. The climate in this area is dominated by a temperate subtropical humid monsoon climate with an average annual temperature of 15.08 °C and a mean annual rainfall of 130.68 mm. The annual sunshine duration was 1000–1400 h. Two type of soil samples are continuous *P. ternata* cropping soil (CC) for 2 years and unplanted soil (CK), the CC sampling area is

about 100 m away from that of CK. In addition, CC and CK had the same plantation history, and the previously planting crops including peanuts, rape and corn, before *P. ternata* was planted in CC soil. In the second year of continuous cropping, the yield of *P. ternata* was 50% lower than that of the first year, and serious diseases and pests occurred. The soil was sampled in the 'five-point sampling' trajectory on a sampling area of 20 m × 20 m. For each type of soil, five sampling sublocations (5 m × 5 m) were located at the four corners and the center of the sampling region. In each sublocation, the rhizosphere soil of 5 *P. ternata* plants (about 3 m apart from each other) was sampled using the method of Edwards [23]. The 3–5 mm soil around the *P. ternata* tubers (5–15 cm below the topsoil), together with the soil on the tubers, were collected as the rhizosphere soil.

The soil samples were collected using a shovel and placed in sterile plastic bags after removing the topsoil and were immediately placed on ice for transport to the laboratory within 5 h. Once the samples had reached the laboratory, the soils from the five sampling sublocations were mixed by method of coning and quartering as one type of soil sample. A portion of samples were stored at 4 °C for the determination of soil physicochemical properties and enzyme activities. The remaining samples were stored at –80 °C for high-throughput sequencing (HTS) analysis.

Physicochemical analysis

Soil pH of the water extract (soil sample:deionized water = 1:5, m/v) was determined using a pH meter (PB-10, Sartorius, Germany). P and K contents in soil samples were determined using an inductively coupled plasma optical emission spectrometer (ICP Optima 8300, PerkinElmer, USA) [24]. Total N and organic matter were determined using an elemental analyzer (vario MACRO cube, Elementar, Germany) [25]. The enzyme activity of catalase (CAT) was measured by ultraviolet spectrophotometry [26], the enzyme activity of polyphenol oxidase (PPO) was measured using pyrogallol colorimetry, the enzyme activity of sucrase (Suc) was measured using 3,5-dinitrosalicylic acid colorimetry and the enzyme activity of urease (Ure) was measured using indophenol blue colorimetry (Nanodrop 2000C, Thermo USA) [27]. Total phenolic acid (TPA) content was obtained using the Folin–Ciocalteu colorimetric method (Nanodrop 2000C, Thermo USA) [28]. Each measurement was done in triplicates.

DNA extraction and HTS analysis

Total DNA from soil samples was extracted using an E.Z.N.A. Soil DNA Extraction Kit (D5625-02, Omega, USA). The extracted DNA was broken into short

fragments using the Whole Genome Shotgun (WGS) strategy. A library of inserted fragments with appropriate lengths was constructed. And then the paired-end (PE) sequencing was performed on the Illumina HiSeq HTS platform. After sequencing, quality control on generated raw data was undertaken using FastQC (including GC content distribution, distribution of base quality and sequence average mass), and Cutadapt [29] was used to screen effective sequences. The MetaGeneMark was then used to predict functional genes [30]. The Lowest Common Ancestor (LCA) algorithm [31] in MEGAN (MetaGenome Analyzer) [32] software was adopted to undertake annotation and abundance analysis for species.

Statistical analysis

Data analysis and plotting were performed using R [33]. The significant difference of soil nutrients and enzyme activities between the two groups was analyzed by Student's *t*-test. And the correlation between enzyme activity and nutrients content was analyzed by SPSS25. The SciPy library of Python software [34] was used to obtain statistical analysis results of the differential abundance significance of species in CC and CK soils. The MetagenomeSeq package was used to analyze the significantly different species between the two samples. The hclust and ggtree in R were used to analyze the relationship between functional genes and microbial communities [35].

Sequence accession number

Sequence data have been deposited in National Center for Biotechnology Information database and the accession numbers is PRJNA730399.

Results and discussion

Physicochemical properties and enzyme activities

Physicochemical properties of the rhizosphere soil, as well as enzyme activities, in CC and CK soil (Table 1). Soil pH recorded a significant decline after CC of *P. ternata* and is consistent with the results of He et al. [10, 36]. This result is probably associated to a decrease in the ability of soil to absorb the exchanging cations after CC of *P. ternata* [37]. The microorganisms then gradually converted plant litter in the soil into organic acids, such as protocatechuic acid, resulting in soil acidification [36]. The acidified soil is more suitable for the development of fungal communities, which will promote the proliferation of soil-borne pathogens [38] and increase the incidence of soil-borne pathogens [39]. It is believed that the decline of soil nutrients is an important factor resulting in CCO [13]. But it is worth noting that in this study, the nutrient contents (except total P), available N, available P, total K, and available K in CC were significantly higher than those in CK (Table 1). Similar phenomena that some

nutrient elements are actually increased in CC soil have been found in *R. pseudostellariae* and *Coptis chinensis* [40, 41]. Although the reason for this change is unclear, it suggests that CCO may be caused by more than a loss of soil nutrients. Soil enzymes are involved in the cycle of soil elements and the development of soil fertility [12], which can be used as important indicators to evaluate soil quality and soil ecosystem stability [42, 43]. Ure, CAT, PPO and Suc activities of CK were significantly higher than those of CC ($p < 0.01$). This might be related to the increase of allelochemicals such as TPA caused by CC (Table 1), which could inhibit the enzyme activity of soil microorganisms [44]. Correlation coefficients between soil enzyme activities and nutrient contents (except total P) indicate that soil Ure, CAT, PPO and Suc were all negatively correlated with organic matter, total N, total K, available P and other nutrients (Table 2). Therefore, it is speculated that CC caused the decline of soil enzyme activities, and further lead to the different changes of the contents and proportions of various nutrients in the soil after CC, resulting a certain degree of soil nutrient imbalance [45]. This result also indicates that *P. ternata* CCO might be caused by the imbalance of soil nutrients. In addition, the reduction of soil enzyme activity might lead to the decline of soil quality and ecosystem stability [42], and increase the accumulation of toxic substances in the rhizosphere soil of *P. ternata* [40]. These changes will cause deterioration of the microenvironment of the rhizosphere soil of *P. ternata*, thus reducing crop yield and quality.

In addition, compared with CK, the TPA content of CC soil increased by 47.2% (Table 1), indicating that CC of *P. ternata* resulted in an accumulation of phenolic acid allelochemicals in the rhizosphere environment. The accumulation of phenolic acid allelochemicals is also an important factor causing *P. ternata* CCO [46, 47]. Li et al. [48] believed that while allelochemicals released into the soil were decomposed and transformed by microorganisms, they could promote changes in rhizosphere microflora, thereby jointly affecting crop growth and development.

Composition of soil microbial community

HTS generated 55,258,098 and 45,942,465 clean reads from CC and CK samples, respectively. High-quality reads were clustered to 609,406 Operational Taxonomic Unit (OTU) from all samples using a sequence similarity > 97%, which could be divided into 64 phyla, 125 classes, 275 orders, 530 families and 1431 genera (remove unclassified and unidentified). Species composition and the relative abundance in CC and CK soils are shown in Fig. 1.

Species composition and the relative abundance in CC and CK soils at the phylum level (Fig. 1a) indicate that

Table 1 Physicochemical properties and enzyme activities of CC and CK soils

Item	CK	CC
pH	7.630 ± 0.012	7.080 ± 0.021***
Total N (g kg ⁻¹)	1.086 ± 0.009	1.157 ± 0.009
Available N (mg kg ⁻¹)	22.167 ± 1.65	28.00 ± 0.000*
Organic C (g kg ⁻¹)	4.199 ± 0.019	4.873 ± 0.007
Total P (g kg ⁻¹)	0.381 ± 0.001	0.356 ± 0.007*
Available P (mg kg ⁻¹)	32.500 ± 0.500	72.133 ± 1.050***
Total K (g kg ⁻¹)	2.792 ± 0.062	3.423 ± 0.113**
Available K (mg kg ⁻¹)	94.333 ± 1.427	184.000 ± 1.414***
TPA (μg g ⁻¹)	34.900 ± 0.000	51.368 ± 0.751**
Ure (μg (g h) ⁻¹)	24.528 ± 0.739	3.656 ± 0.508***
CAT (mg (g 20 min) ⁻¹)	0.382 ± 0.013	0.361 ± 0.014***
PPO (μg (g h) ⁻¹)	103.733 ± 4.745	55.289 ± 1.663**
Suc (mg (g d) ⁻¹)	4.127 ± 0.189	2.657 ± 0.046**

CC continuous cropping, CK control check, Ure urease, CAT catalase, PPO polyphenol oxidase, Suc sucrose, TPA total phenolic acid

Asterisks in the same row indicate significant difference test by Student's *t*-test (**, $p < 0.05$; ***, $p < 0.01$; ****, $p < 0.001$)

Proteobacteria was dominant in both CC and CK (average abundance was 69.6% ± 0.7%), followed by Actinobacteria (8.0% ± 0.7%). This result is consistent with the findings of He et al. [13]. Differential abundance analysis of microorganisms indicated that the abundance of Cyanobacteria in CC significantly increased, while that of Nitrospirae and Crenarchaeota markedly decreased. The species composition and the relative abundance distribution in CC and CK soils at the genus level are shown in Fig. 1b. The top 10 genera in relative abundance in CC samples were: *Gemmatirosa* (3.1%), *Sphingomonas* (2.9%), *Bradyrhizobium* (2.5%), *Burkholderia* (2.4%), *Pseudomonas* (1.9%), *Ramlibacter* (1.5%), *Luteitalea* (1.4%), *Phenylobacterium* (1.3%), *Microcoleus* (1.2%) and *Variovorax* (1.1%). In CK samples, the top 10 genera in relative abundances were *Burkholderia* (3.3%), *Bradyrhizobium* (2.2%), *Gemmatirosa* (2.1%), *Luteitalea* (2.1%), *Pseudomonas* (1.7%), *Sphingomonas* (1.4%), *Streptomyces* (1.3%), *Variovorax* (1.1%), *Sorangium* (1.1%) and *Anaeromyxobacter* (1.1%). Differential abundance analysis of species showed that the abundances of *Microcoleus*, *Phenylobacterium* and *Sphingomonas* in CC obviously increased, while that of *Nitrospira* notably decreased.

CC of *P. ternata* caused changes in the microbial community composition of rhizosphere soil. The relative abundance of Cyanobacteria in CC and CK soil was 1.8% and 0.3%, respectively. This phenomenon might be due to the continuous accumulation of pathogens (such as *Fusarium*) in the rhizosphere caused by CC, which inhibited the growth of beneficial microorganisms in the soil

Table 2 Spearman correlation between enzyme activity and nutrients content

Spearman (r)	Organic C	Total N	Total P	Total K	Available P	Available K	Available N
Ure	-0.600	-0.400	0.600	-0.500	-0.600	-0.205	-0.289
CAT	-0.949	-0.632	0.812*	-0.986**	-0.872	-0.632	-0.845*
PPO	-0.400	-1.000**	0.657	-0.657	-0.700	-0.928**	-0.926**
Suc	-0.400	-0.400	0.829*	-0.657	-0.700	-0.841*	-0.741

Ure urease, CAT catalase, PPO polyphenol oxidase, Suc sucrose

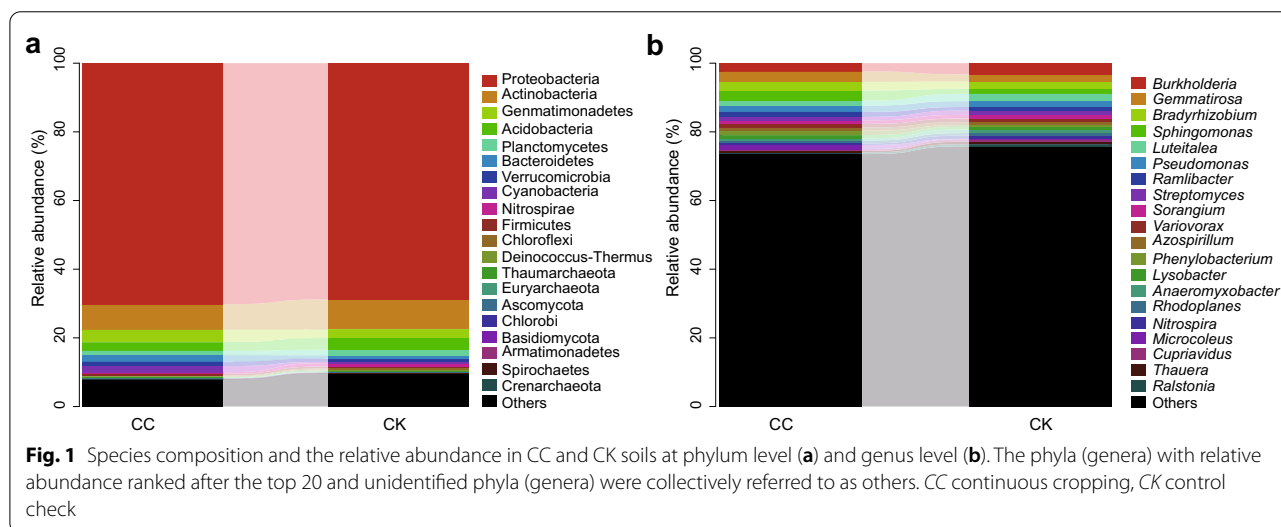
|r| ≥ 0.7: highly correlated; 0.4 ≤ |r| < 0.7: moderately correlated; 0.2 ≤ |r| < 0.4: weakly correlated; (**, p < 0.05; ****, p < 0.01)

and promoted the continuous reproduction of *Microcoleus* and *Streptophyta*. This could lead to the occurrence of root rot and soft rot [13]. Research by Hu et al. [49] showed that *Fusarium*, *Klebsiella oxytoca* and *Pectobacterium carotovorum* were the main pathogens in CC soil of *P. ternata*. Results from our analysis also found that the abundance of *Fusarium*, *K. oxytoca* and *P. carotovorum* in CC were 1.56-fold, 2.17-fold, 2.36-fold higher than CK, respectively. Many studies have shown that the disorder of the microbial flora mediated by allelochemicals plays a key role in CCO [50], and phenolic acid allelochemicals can promote the formation and germination of *Fusarium oxysporum* spores [51]. The phenomenon that CCO leads to an increase of pathogenic bacteria has also been reported in the continuous cropping of *R. pseudostellariae* [52].

Previous investigations have recorded that *Burkholderia* have the ability of dissolving soil P [53] and N fixation [54], as well as phenolic acid allelochemicals degradation [55, 56]. The previous findings indicated that *Burkholderia* plays an important role in enhancing soil fertility and reducing the toxicity of phenolic acid allelochemicals, possibly is a kind of potentially beneficial soil bacteria for *P. ternata* cultivation. In our study,

the abundance of *Burkholderia* was 3.3% in CK soil and 2.4% in CC soil, indicating that CC might inhibit some beneficial bacteria in *P. ternata* soil. *Sphingomonas* can use aromatic compounds as carbon and energy sources for growth and reproduction [57]. It has diverse degradation genes and highly active degrading enzymes, showing strong advantages in the degradation of aromatic compounds [58, 59]. In our study, the abundance of *Sphingomonas* in CC was 1.5% higher than that in CK, which was inferred to be related to the higher content of TPA in CC soil than that in CK (Table 1). These phenolic acid compounds might provide more carbon sources and energy sources for the growth and reproduction of *Sphingomonas*.

These results indicate that CC of *P. ternata* changed the composition of the microbial community in the rhizosphere soil, inhibited the growth of beneficial microorganisms, and promoted the growth and reproduction of soil-borne pathogens and other harmful microorganisms. These changes resulted in an unhealthy rhizosphere microbial community structure [9], leading to a decrease in yield and quality of *P. ternata*.



Analysis of functional genes and microorganisms to which they belong

The soil enzyme related

The abundance of functional genes related to soil enzyme and their microbial communities are shown in Fig. 2. Ure is a nickel-containing urea hydrolase that catalyzes the hydrolysis of urea to produce ammonia and carbon dioxide, which is beneficial to the absorption and utilization of plants. As Ure activity in CK was significantly higher than that in CC (Table 1), our results indicate that catabolism of urea in CK is greater than that in CC, consistent with lower N contents in the CK soil (Table 1). However, the abundances of functional genes related to Ure detected in CC, such as *ureC* (K01428), *ureB* (K01429), *ureA* (K01430) and *ureAB* (K14048), were all higher than those in CK (Fig. 2a). It can be seen from Fig. 2b that microorganism sources encoding these Ure related genes in CC and CK differed. *Thermobacillus* was a unique genus in CK, while the abundances of *Nitrospira*, *Rhodoplanes*, *Streptomyces*, *Thaurea* and *Methylobacterium* were higher in CK.

CAT could decompose hydrogen peroxide into water and molecular oxygen to protect cells from damage [60]. In our study, CAT activity of CK was higher than that of CC. In CC soil, the decrease in CAT activity might increase the accumulation of toxic substances in the rhizosphere soil, thereby causing adverse changes in the rhizosphere microenvironment, and then affecting growth and development of *P. ternata*. The abundance of *katE* (K03781), a functional gene encoding CAT, was higher in CC than in CK (Fig. 2a). As shown in Fig. 2c, *Brevundimonas* was a unique genus in CC, and the abundances of *Mesorhizobium*, *Roseomonas* and *Sorangium* were higher in CK.

PPO participates in the conversion of aromatic compounds in soil organic components, oxidizing phenolic substances in the soil to quinones, and then condensing them into humic acid [61]. In this study, PPO activity of CK was significantly higher than that of CC, indicating that the conversion of aromatic compounds into other intermediate metabolites in CK was more efficient, which was consistent with the lower measured TPA content in CK (Table 1). However, the abundance of functional gene *yfiH* (K05810) encoding PPO in CC was higher than that in CK (Fig. 2a). Among them, *Corynebacterium*, *Nitrospira* and *Rhodanobacter* were unique genera in CK, and the abundances of *Marichromatium* and *Pseudomonas* were higher in CK (Fig. 2d).

Suc hydrolyzes high molecular weight sucrose molecules in the soil into glucose and fructose that can be absorbed and utilized by plants and microorganisms. Suc activity of CK in our study (Table 1) was significantly higher than that of CC, beneficial to the utilization of

soil carbon sources. Detected functional genes encoding Suc included *scaA* (K01193), *malL* (K01182) and *malZ* (K01187), and the abundances of these functional genes in CC were all higher than those in CK (Fig. 2a). The abundances of *Methylobacterium*, *Agromyces*, *Sorangium*, *Pseudomonas* and *Azoarcus* which contained these genes were higher in CK than that in CC (Fig. 2e).

We analyzed the abundance of top 5 microbial communities containing the functional genes related to soil enzyme. The results indicate that microorganisms with these functional genes mainly including *Burkholderia*, *Bradyrhizobium*, *Variovorax*, *Sphingomonas* and *Marichromatium* (Fig. 3). Moreover, the composition of these microbial communities in CC and CK recorded noticeable differences, further indicating that CC of *P. ternata* changed the microbial community structure of the rhizosphere soil.

Interestingly, the abundance of functional genes related to soil enzyme activity in CC was higher than that in CK (Fig. 2a), while measured activities of Ure, CAT, PPO and Suc in CK were all higher than those in CC (Table 1). It is speculated that the gene expression might be inhibited. RNA polymerase σ^H factor (encoding by *Spo0H* gene) is the main inhibitor of the expression of Ure [62], and the abundance of *Spo0H* in CC was 1.65-fold higher than CK. In addition, microorganisms producing Ure are reported mainly derived from, among others, *Streptomyces* and *Methylobacterium* [63, 64]. But their abundances reduced after CC of *P. ternata* (Fig. 2b). It is speculated that CC inhibited the expression of functional genes related to soil enzyme activity, reducing the abundance of enzyme producing microorganisms, thereby exhibiting lower enzyme activity in CC. More in-depth studies are needed about this phenomenon.

The allelochemical degradation related

Allelopathic autotoxicity means that plants release specific secondary metabolites into the environment, thereby causing harmful effects on the growth and development on themselves [65]. By summarizing the degradation pathways of the main allelochemicals of *P. ternata* (Fig. 4) [66–70], it could be seen that catechol and protocatechuic acid are two important intermediate metabolites.

Catechol 1,2-dioxygenase (C12O) is the key enzyme of the catechol ortho-cleavage pathway (Fig. 4), and the abundance of functional gene *catA* (K03381) encoding C12O was higher in CK than in CC (Fig. 5a), *Cupriavidus* was a unique genus in CC (Fig. 5b). Catechol 2,3-dioxygenase (C23O) was a key enzyme of the catechol meta-cleavage pathway. The abundances of genes encoding C23O in CC, *dmpB* (K00446) and *catE* (K07104), were higher than those in CK (Fig. 5a). Regardless of CK or CC, the abundance of C23O encoding gene was significantly

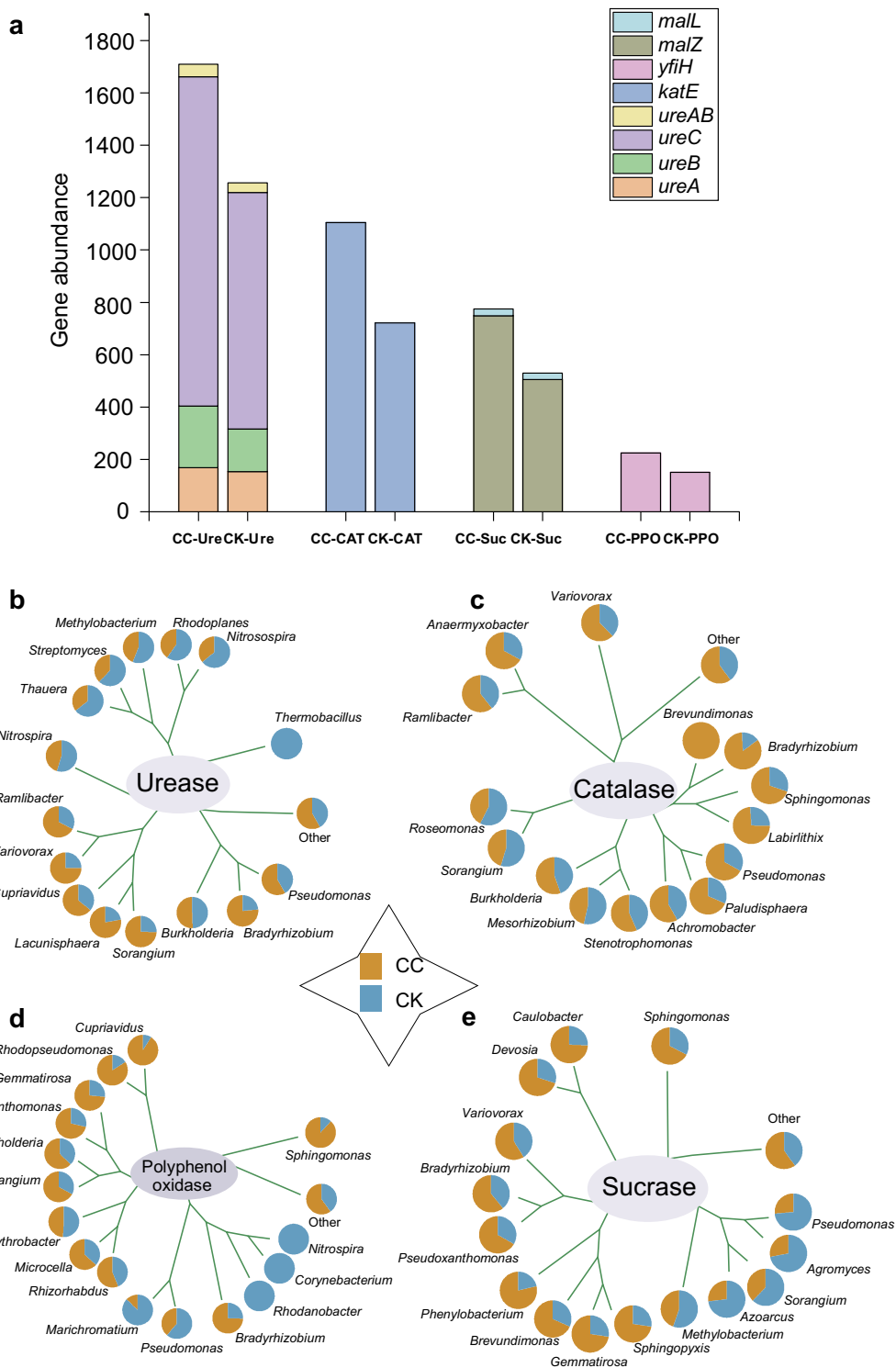


Fig. 2 The abundance of functional genes related to soil enzyme and the microorganisms to which they belong; **a** gene abundance; **b** Ure; **c** CAT; **d** PPO; **e** Suc; in **b–d**, the size of the different colors in each cake represents the proportion of the microorganism abundance of that species in CC and CK

higher than that of C12O. Therefore, we speculate that catechol mainly degraded phenolic acid allelochemicals through the meta-cleavage pathway in both CC and CK. As shown in Fig. 5c, *Pseudonocardia* and *Luteipulveratus* were unique genera in CC and CK samples, respectively. Protocatechuate 3,4-dioxygenase (P34O) was the key enzyme to degrade protocatechuic acid, and the abundances of functional genes *pcaG* (K00448) and *pcaH* (K00449) encoding P34O in CC were higher than those in CK (Fig. 5a). *Plantactinospira* was a unique genus in CC (Fig. 5d). The abundance of these allelochemicals degradation genes in CC is higher than that in CK, which may be related to the substrate induction caused by the higher concentration of TPA in CC, but it still needs further study and verification.

We also analyzed the abundance of top 5 microbial communities containing the functional genes related

to allelochemical degradation. The results indicate that microorganisms containing these functional genes were mainly *Burkholderia*, *Bradyrhizobium*, *Azoarcus*, *Gemmatirosa*, and *Cupriavidus* (Fig. 6). The abundances of the microorganisms to which each functional gene belonged significantly differed between CC and CK. *Burkholderia* and *Bradyrhizobium* were mainly enriched in CK, among which *Burkholderia* was also the genus with the highest abundance in CK (Fig. 1). They could also serve as potentially beneficial bacteria in the soil to enhance soil fertility [53, 71] and reducing the toxicity of allelochemicals [53]. Therefore, results from our study also could provide certain guidance for the screening of microorganisms which being beneficial to soil and degrading allelochemicals of *P. ternata*, further laying a foundation for bioremediation of the CCO soil of *P. ternata* and other staple crops.

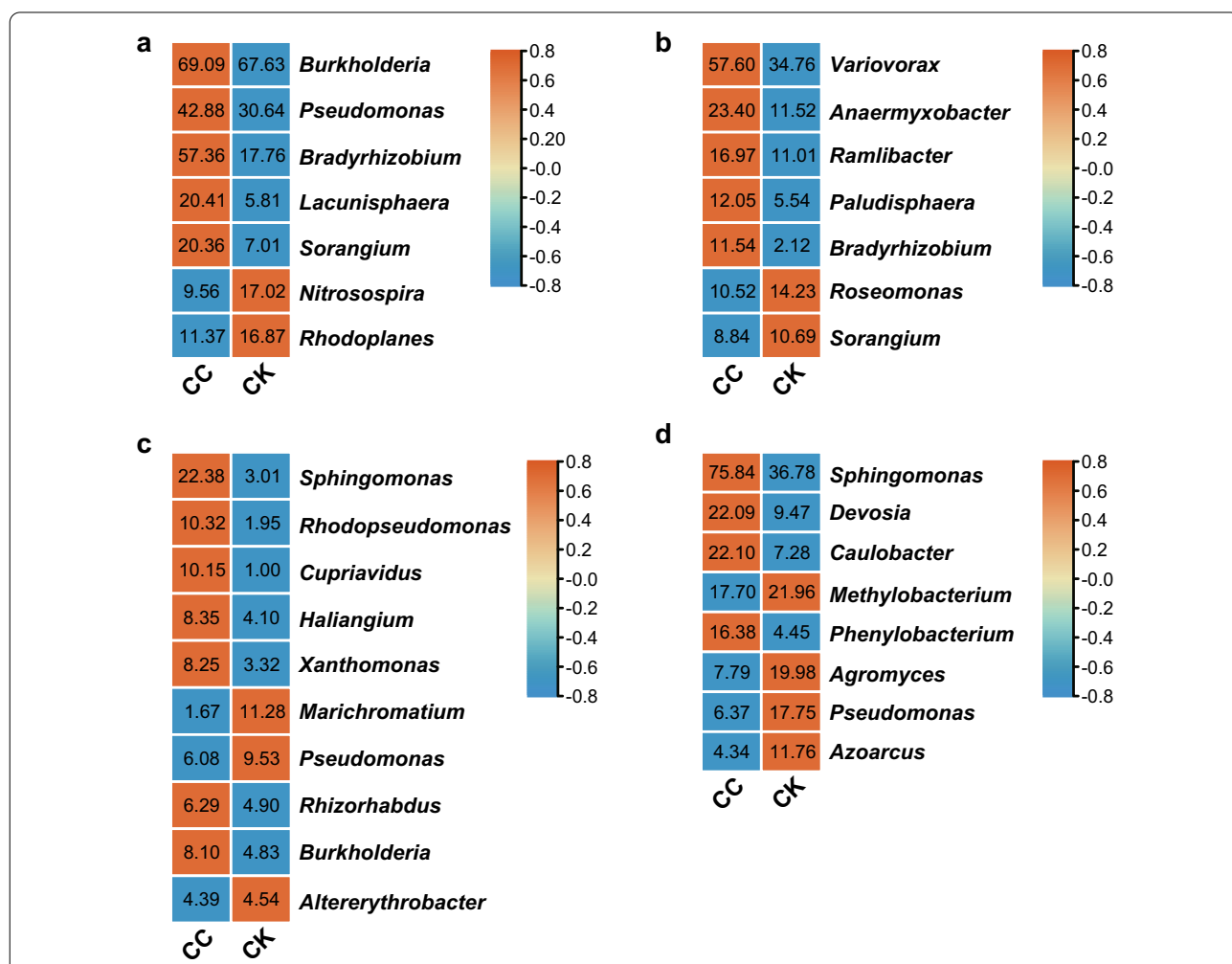
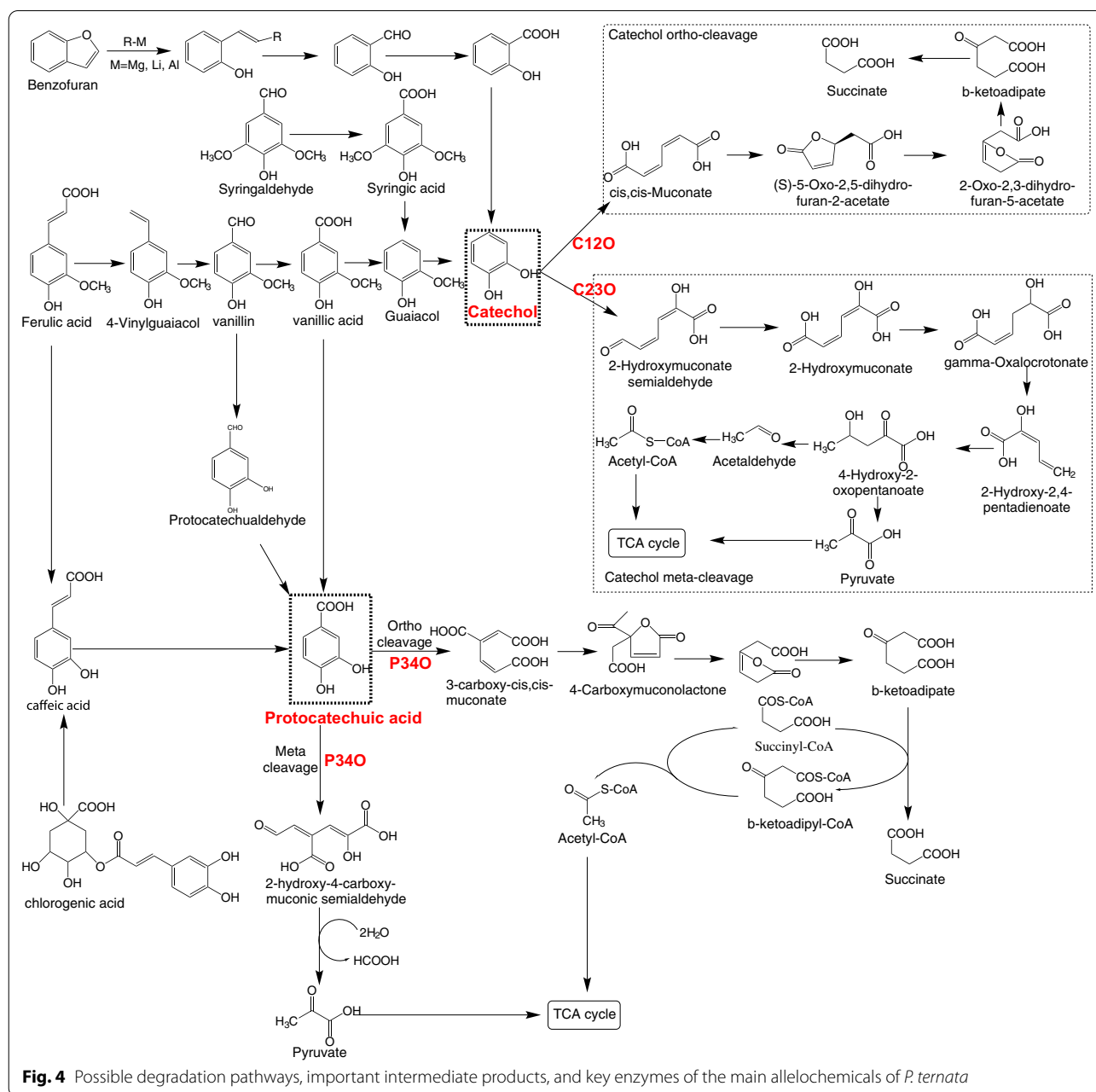


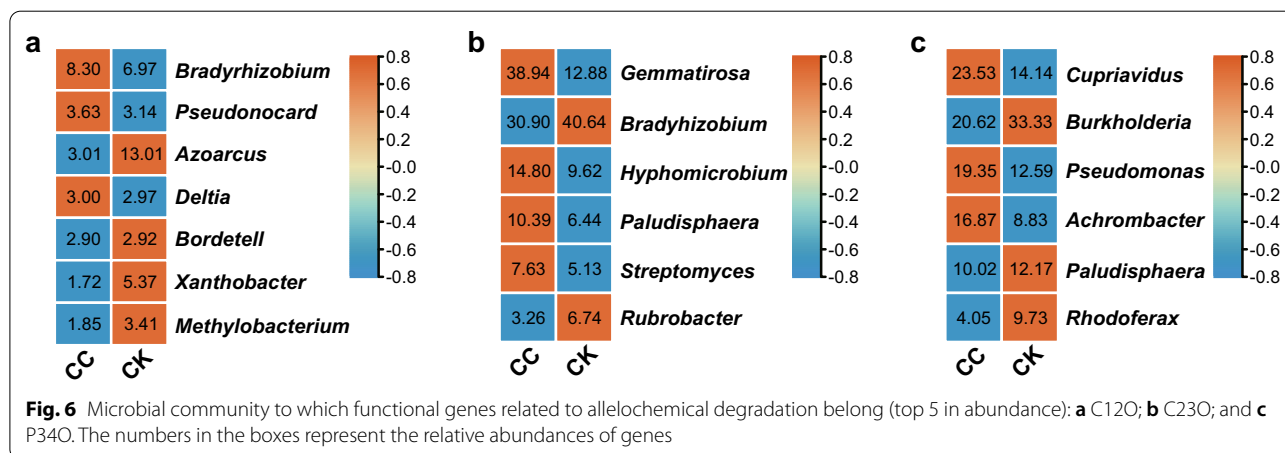
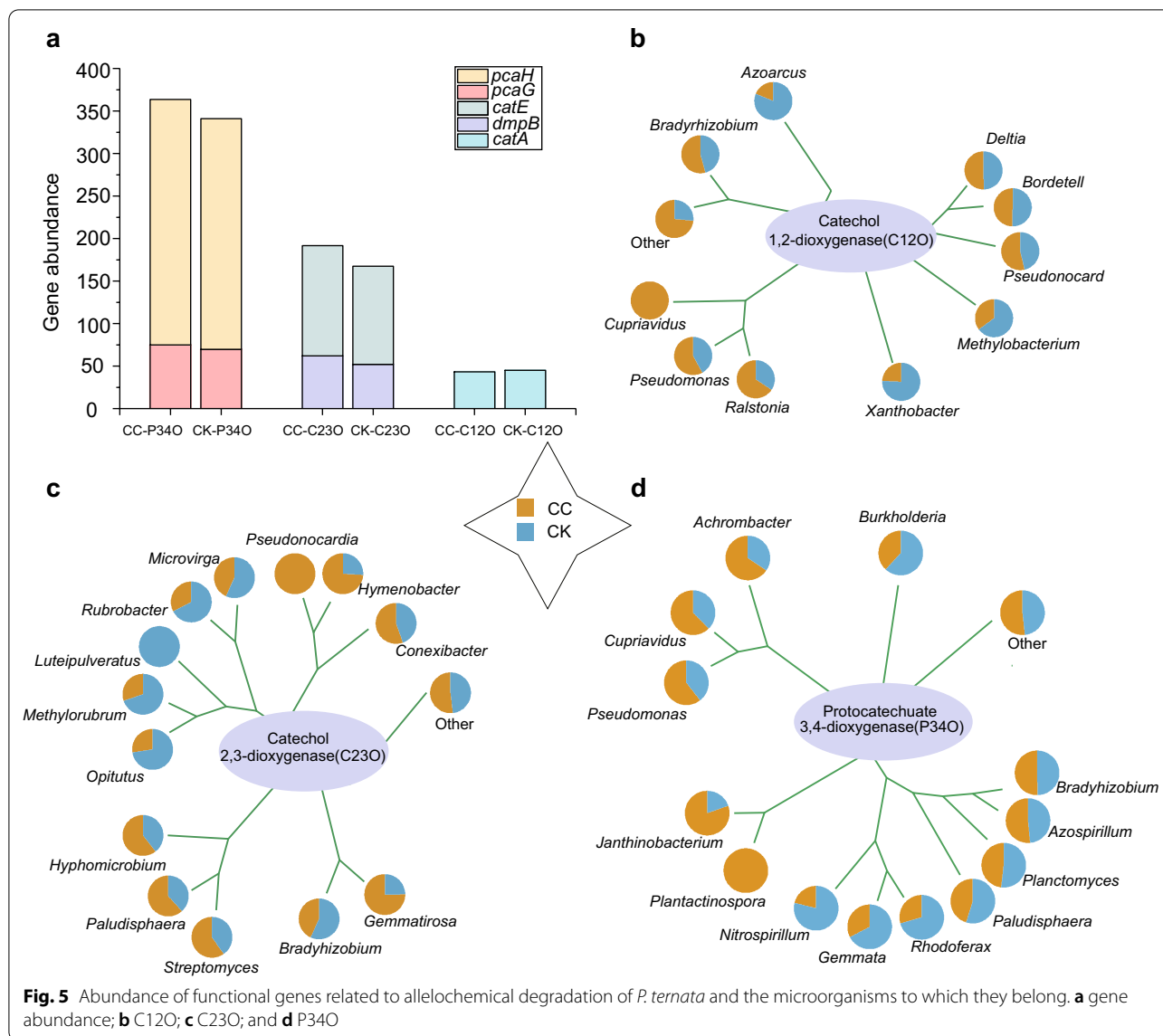
Fig. 3 Microorganisms to which functional genes related to soil enzyme belong (top 5 in abundance): **a** Ure, **b** CAT, **c** PPO, and **d** Suc. The numbers in the boxes represent the relative abundances of genes



Conclusions

Findings from our investigation indicate that CC of *P. ternata* changed the physicochemical properties, enzyme activities and the microbial community structure of rhizosphere soil. The decrease in soil enzyme activity caused by CC resulted in a soil nutrient imbalance. It also led to an increase in relative abundance of pathogenic bacteria in the *P. ternata* rhizosphere, and the decrease in the content of potentially beneficial bacteria. In addition, CC also caused adverse effects on functional genes related to soil enzyme and

allelochemical degradation, as well as the microbial communities to which they belonged. Results also indicated that *Burkholderia* and *Bradyrhizobium* might be potentially beneficial soil bacteria. Our findings increase our understanding of CCO of *P. ternata*, providing certain guidance to exploit resources such as soil beneficial bacteria and degrading bacteria of allelochemicals. These results provided a theoretical basis for the further development of microbial remediation for CCO soil of *P. ternata* and other staple crops. Further attention should be paid to the interactions between



P. ternata plants and their secondary metabolites with soil and microbial communities.

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Authors' contributions

YZ: investigation, methodology, software, visualization, writing—original draft. X–MQ: methodology, software, writing—review and editing. X–PT: software, visualization, writing—review and editing. TY: investigation, visualization, writing—review and editing. RD: writing—review and editing. JH: conceptualization, funding acquisition, project administration, resources, supervision, writing—original draft, writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All the data are included in this manuscript.

Declarations

Ethics approval and consent to participate

This study does not involve any human, animal or endangered species.

Consent for publication

All co-authors have seen and agreed on the contents of the manuscript, and there is no financial interest to report.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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