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Variations in microbial community and ciprofloxacin removal in rhizospheric soils between two cultivars of *Brassica parachinensis* L.

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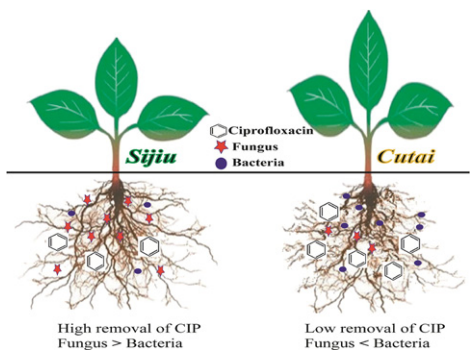
HIGHLIGHTS

- The microbial community in rhizosphere of the two cultivars varied significantly.
- *Spirochaeta* and *Trichosporon* might play a key role in CIP degradation.
- Variation in *Trichosporon* between the two cultivars led to different CIP removal.
- Carbon substrate utilization differed in rhizospheric microbes of the two cultivars.

GRAPHICAL ABSTRACT



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ABSTRACT

Ciprofloxacin (CIP) is one of most used quinolone antibiotics detected frequently in agricultural soils and vegetables. In the present study, variations in microbial community and CIP removal in rhizospheric soils between two cultivars of *Brassica parachinensis* L. that accumulate higher and lower CIP (*Sijiu* and *Cutai*, respectively) were investigated under CIP stress (0 mg/kg in CK, 2.94 mg/kg in T1, and 67.11 mg/kg in T2). The removal rates of CIP in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai*, with a significant difference in T2 (48.7% > 39.4%, $P < 0.05$). The pyrosequencing of 16S rRNA and ITS gene indicated that the microbial diversity and community structure in rhizospheric soils of the two cultivars varied significantly. *Spirochaeta* and *Trichosporon* might be associated with CIP degradation, and higher relative abundances of *Trichosporon* in rhizoshperic soils of cultivar *Sijiu* might be responsible for higher CIP removal. Fourteen bacterial genera and ten fungal genera were screened as potential biomarkers for CIP removal process. The community level physiological profiling in rhizospheric soils of the two cultivars under CIP stress differed significantly, and more C substrates that favored CIP removal were observed in rhizoshperic soils of cultivar *Sijiu*. Our results demonstrate that variations in microbial community and the utilization of C substrates played important roles in differring the CIP removal in rhizospheric soils between the two cultivars.

1. Introduction

Antibiotics are intensively used as human and veterinary antimicrobials worldwide (Karci and Balcioglu, 2009). A great number of

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antibiotics have been released into the environment from the excretion of human and animals, the discharge of aquaculture, and the effluent of wastewater treatment plants, etc. (Andreu et al., 2007; Boxall, 2008; Kimosop et al., 2016 and Wu et al., 2016). They are widely detected in wastewater, sewage sludge (biosolid), surface water, soil, and vegetables (Dorival-Garcia et al., 2013; Jia et al., 2012; Wu et al., 2015; Li et al., 2014; Xu et al., 2015; Miller et al., 2016). With the increasing use of livestock manure, biosolids and wastewater, antibiotic levels in agricultural soil are elevated (Pan et al., 2014; Prosser and Sibley, 2015; Riemenschneider et al., 2016). We have detected antibiotics in the soils from both conventional and organic farms (Li et al., 2011; Wu et al., 2014; Xiang et al., 2016). Antibiotics in soils can be taken up and accumulated by crops, leading to a food safety risk (Li et al., 2014; Pan et al., 2014; Christou et al., 2017). The ubiquitous occurrence of antibiotics in soils and crops as well as their resistance can pose a potential risk to health and the environment (Knapp et al., 2010; Lau et al., 2017; Zhu et al., 2013).

Many adverse effects of antibiotics on soil microbe have been reported (Girardi et al., 2011; Reichel et al., 2014; Cleary et al., 2016; Wepking et al., 2017). Ciprofloxacin (CIP), one of most used quinolone antibiotics (Ferber, 2002), could affect soil microbial communities and activities (Girardi et al., 2011). Nevertheless, previous studies focused on the effect of antibiotics on soil microbial community in the absence of plants (Girardi et al., 2011; Liao et al., 2016; Cleary et al., 2016; Wepking et al., 2017), very few studies reported the changes of microbial community in rhizospheric soils exposed to antibiotics (Lin et al., 2016; Reichel et al., 2015).

Removal of antibiotics from agricultural soil is very important to elevate the soil quality and to ensure the safety of agricultural product. Nevertheless, chemical remediation such as antibiotic degradation by nanoparticle or laccase oxidation (Darwish et al., 2016; Ding et al., 2016; Pan and Chu, 2016) might be high-cost and impractical for a large-scale agricultural soil contaminated by antibiotics. Bioremediation, especially for phyto-rhizoremediation based on beneficial plant-microbe interactions, is considered as an effective "green technology" to enhance the degradation of antibiotics in soil (Mathews and Reinhold, 2013). CIP in soil could be mineralized and detoxified to allow further biodegradation by microorganisms (Girardi et al., 2011), and meanwhile bacterial community structure displayed a profound shift during CIP biodegradation (Liao et al., 2016). Plants exert a biostimulation activity toward rhizospheric microbial communities and improve the degradation of organic contaminants in soil (Feng et al., 2017; Musilova et al., 2016). However, no study has reported phyto-rhizoremediation of antibiotic contaminated soil. Our previous study showed that the removal rates of CIP in soil growing various cultivars of Chinese flowering cabbage (*Brassica parachinensis* L.) varied significantly (Data unpublished). Unfortunately, the variations in microbial community in rhizospheric soils of different plants exposed CIP and their effects on CIP removal remain unknown.

In the present study, based on our previous research result (Data unpublished), two cultivars of *Brassica parachinensis* L. with higher and lower CIP accumulation, namely cultivar *Sijiu* and cultivar *Cutai*, respectively, were selected for a pot experiment. The objectives of this study are: 1) to investigate the removal of CIP in rhizoshperic soils of the two cultivars, 2) to illuminate differences in microbial community and metabolism in rhizospheric soils of the two cultivars, and 3) to reveal the effects of rhizospheric microbiota of the two cultivars on CIP dissipation from soil. The results are conducive to promoting the potential application of *Brassica parachinensis* L. to simultaneous remediation of CIP contaminated soil and safe production of agricultural crops.

2. Materials and methods

2.1. Experimental design

CIP (purity > 98%) was purchased from Dr. Ehrenstorfer - Schäfers (Augsburg, Germany). The seeds of cultivars *Sijiu* and *Cutai* were purchased from Vegetable Research Institute, Guangdong Academy of Agricultural Sciences, China. The soil used for pot experiment was collected from an experimental farm, Guangzhou (no CIP was detected). It is air-dried and sieved (2 mm) for use. The main properties of the soil are as follows: organic matter 3.8%, cation exchange capacity 8.9 cmol/kg, total nitrogen 3.2×10^3 mg/kg, total phosphorus 8.3×10^2 mg/kg, total potassium 2.82×10^4 mg/kg, pH 6.8, and clay 11.2% (dry weight).

The theoretical concentrations of CIP in soil for pot experiment were set according to the realistic environmental concentrations, differential responses of microbial community, and plant growth. Generally, the residual concentrations of CIP in agricultural soil were a few mg/kg or less (Hu et al., 2010; Wu et al., 2014; Zhang et al., 2016), but up to dozens of mg/kg was observed in the soil applied with pig manure (Wang, 2014). Thus, the theoretical concentrations of CIP in soil for pot experiment were set at 0 mg/kg (CK), 5 mg/kg (T1), and 80 mg/kg (T2). The artificial CIP polluted soil was made as follows: 20 kg of soil (2 mm, 10% of the experimental soil used) spiked with CIP aqueous solution was mixed thoroughly, then the soil was evenly mixed with another 180 kg of original soil (2 mm, 90% of the total soil amount). Four kilogram of the polluted soil was packed into each ceramic pot (23 cm inner diameter at top, 15 cm inner diameter at bottom, 25 cm height). Generally, unlike the aged one, the bioavailability and toxicity of CIP that was freshly spiked soil are higher, but they were reduced with the mineralization or degradation of CIP in soil (Girardi et al., 2011). CIP that was freshly spiked in the soil growing plants degraded fast at beginning (1–14 days), thereafter it degraded gradually (15–56 days) (Xiao et al., 2012). For better simulating CIP in the field soil and reducing the bioavailability and toxicity of freshly spiked CIP, the soil artificially polluted with CIP was aged for 15 days in the dark after adding 800 mL deionized water (the actual initial concentrations of CIP were measured at 0 mg/kg in CK, 2.94 ± 0.26 mg/kg in T1, and 67.1 ± 11.8 mg/kg in T2). Then, the seeds of cultivars *Sijiu* and *Cutai* were sowed separately in pots in a glasshouse with natural light condition in Jinan University, Guangzhou. The pots were arranged in a completely randomized block design with three replicates, and irrigated with deionized water to keep 50% ~ 70% of water-holding capacity every day. The unplanted pots received the same conditions as the planted ones.

The samples of rhizospheric soil from the two cultivars were collected at flowering stage (52 days after planting) by extracting roots from soils and removing excess bulk soil with gentle shaking. At the same time, the soil samples were also collected from the treatments without plant. Some of the soil samples were analyzed immediately by Biolog EcoPlate™, and the rest were stored immediately at −80 °C for DNA extraction and freeze-dried for CIP analysis, respectively. The extraction and quantification of CIP in soil by high performance liquid chromatography–tandem mass spectrometry system (HPLC-MS/MS, Alliance 1100 of HPLC, AB4000QTRAP of MS, Agilent) were conducted based on the methods described by previous studies (Wu et al., 2014). The quantitative analysis was based on a nine-point calibration curve (ranging from 0.5×10^{-3} to 1.00 mg/L for CIP). Recovery tests were performed by spiking CIP standard solutions at three concentrations of 0.05, 0.25, and 1.00 mg/kg, the mean recoveries were 82.3%, 92.5% and 88.6%, respectively. The limits of quantification ranged from 0.006×10^{-3} to 0.013×10^{-3} mg/kg. The detailed procedures of sample extraction, cleanup, and HPLC-MS/MS analysis were presented in Supplementary Information.

2.2. Microbial community-level physiological profiling determination

Metabolism of microbes in rhizospheric soils of the two cultivars was studied by community-level physiological profiling (CLPP) using Biolog EcoPlate™ technique that has been demonstrated to be effective at evaluating microbial physiological metabolic characteristics in microbial communities (Garland and Mills, 1991; Garland, 1997). The Biolog EcoPlate (21,124 Cabot Blvd. Hayward, CA 94545, USA) contains three

sets of 31 different carbon sources (Table S1). The procedure for Biolog EcoPlate™ assay is described in web of Microbial Community Analysis with EcoPlates™ (http://www.biolog.com/products-static/microbial_community_literature.php).

2.3. Illumina high-throughput sequencing

The soil samples were subjected to DNA extraction using E.Z.N.A. Soil DNA kits (Omega Bio-Tek, Norcross, GA, USA). The V5 + V6 region of 16S rRNA genes of bacteria and ITS region of fungi were amplified using the following primers: Forward 5′-CCAGGGTTGCGCTCGTTG-3′ and Reverse 5′- AACMGGATTAGATACCCKG-3′, Forward 5′- CCGCAT CGATGAAGAACGCAGC -3′ and Reverse 5′- TCCTCCGCTTATT GATATGC -3′, respectively.

The obtained sequence sequenced by Illumina was assembled using the Flash software (http://www.genomics.jhu.edu/software/FLASH/index.shtml). The Qiime (version 1.8.0, http://qiime.org/) and Mothur (version 1.35.1, http://www.mothur.org/) were used to obtain high-quality sequences for subsequent analysis. Then the high-quality sequences were classified and blasted to operational taxonomic units (OTUs). Species abundance statistics (e.g., Chao1, Shannon, Simpson and observed species), and principal coordinate analysis (PCoA) were carried out based on the taxonomic and abundance information of OTUs. Furthermore, LEfSe (version 1.0) was used to detect differentially abundant taxa for biomarker discovery using the online Galaxy workflow frame work (http://huttenhower.sph.harvard.edu/galaxy/), the threshold on the logarithmic linear discriminant analysis (LDA) score for discriminative features was set to 2.0.

2.4. Statistical analysis

Two-way ANOVA and Duncan's multiple range test ($\alpha = 0.05$) analyses were conducted using SPSS Statistics 19. Pearson's correlation coefficients (r) were calculated based on the relative abundance of genus and the removal rate of CIP in soil.

3. Results

3.1. Difference in removal rate of CIP in rhizospheric soils of the two cultivars

The removal rates of CIP in rhizospheric soils of the two cultivars varied to different degrees (68.6–78.7% at T1, 37.1–48.7% at T2, respectively) (Fig. 1). They were all higher compared with those of CIP in unplanted soils, with significant difference for both cultivars in T1, and cultivar *Sijiu* in T2 ($P < 0.05$). It is worth noting that the removal rates of CIP in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai*, with significant difference in T2 (48.7% > 39.4%, $P < 0.05$). These results indicated that the growth of the two cultivars could greatly promote the removal of CIP in rhizospheric soils, especially for cultivar *Sijiu*.

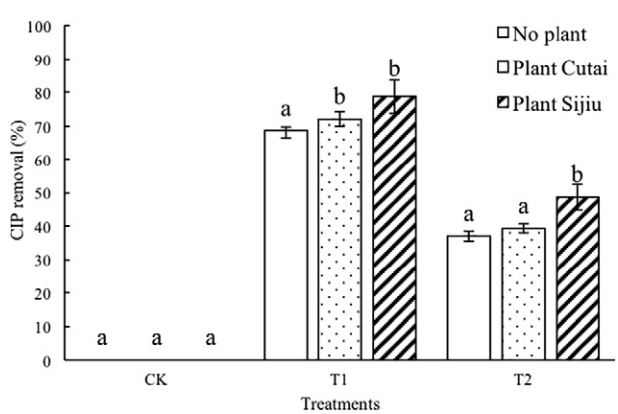

Fig. 1. Removal rates of CIP in soils ($P < 0.05$) Note: removal rates were calculated based on the formula: $(C_0–C)/C_0 \times 100$. C_0 is the actual initial concentration of CIP, C is the residual concentration of CIP at day 52.

3.2. Difference in rhizospheric bacterial community of the two cultivars

After denoising and quality control processing, a total of 613,433 bacterial sequence reads for bacteria were obtained from 18 samples. OTU assignment that was conducted at the 97% sequence similarity level resulted in 118,173 OTUs. The result showed that the total number of sequences and OTUs of cultivar *Sijiu* were higher than those of cultivar *Cutai* in CK and T1, but the opposite result was observed in T2 ($P < 0.05$) (Table 1). The α-diversity indices including Chao 1, Observed species, Shannon, and Simpson were recorded in this study (Table 1). The mean α-diversity of bacteria in rhizospheric soils of cultivar *Sijiu* was higher than that of cultivar *Cutai*, and significant difference was found in CK ($P < 0.05$). The α-diversity of bacteria in rhizospheric soils of cultivar *Sijiu* in various treatments generally kept unvaried, but significant increase was observed for cultivar *Cutai* in T2 compared with CK. These results implied that the bacterial community diversity, richness, and evenness in rhizosphere of cultivar *Sijiu* were better maintained, which was conducive to removing more CIP in soil.

All the sequences obtained from the 18 samples were classified into 14 different domains (abundance ≥ 0.2%) (Fig. 2a). Besides the *Archaea* (which was assigned to *Euryarchaeota*), the bacteria were mainly assigned to *Actinobacteria*, *Bacteroidetes*, *Proteobacteria*, and TM7 (Table S2). The relative abundances of TM7 in cultivar *Sijiu* were significantly lower than those in cultivar *Cutai* (13.85% < 24.34% in CK, 11.03% < 18.63% in T1, and 12.92% < 22.34% in T2). Further investigation at the order level (Fig. 2b, Table S3), significant differences in *Methanosarcinales*, *Roseiflexales*, and EW055 in CK, T1, and T2 were found in rhizosphere soils between cultivar *Cutai* and cultivar *Sijiu*.

Considering the bacterial genus composition (Fig. 2c, Table S4), it can be found that the relative abundances of some genera including *Spirochaeta* ($r = 0.51$), *Geobacter* ($r = -0.49$), and *Gallionella* ($r = -0.55$) were significantly correlated with the removal of CIP in rhizospheric soils of both cultivars (Table 2). For example, significantly positive correlationship between the relative abundances of genus *Spirochaeta* and the removal rates of CIP was observed, and the relative abundances of this genus in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai*. Two-way ANONA analysis showed that the relative abundance of *Spirochaeta* was significantly affected by plant cultivars ($P < 0.01$) and the CIP concentrations ($P < 0.05$). The negative correlations between the relative abundances of *Geobacter* and *Gallionella* and removal rates of CIP were recorded. The relative abundances of the genera *Geobacter* and *Gallionella* in rhizospheric soils of cultivar *Sijiu* were always higher than those of cultivar *Cutai*.

Significant difference in the community compositions of bacteria in rhizospheric soils between the two cultivars are shown in a cladogram representation performed by LEfSe (Fig. 3). There were seven significantly different families, with the enrichment of *Sphingobacteriaceae* in T2 of cultivar *Cutai*, *Ardenscatenaceae* and *Gaiellaceae* in CK of cultivar *Sijiu*, *Bryobacteraceae*, *Sanguibacteraceae*, and *Methanomicrobiaceae* in T1 of cultivar *Sijiu*, and *Holophagaceae* in T2 of cultivar *Sijiu*. The bacterial community composition was also significantly different at the genus level, with fourteen significant genera in different treatments of the two cultivars, i.e., *Saprospira* in CK of cultivar *Cutai*, *Saccharopolyspora* in T1 of cultivar *Cutai*, and *Sphingobacterium* in T2 of cultivar *Cutai*, and as well as *Ardenscatena*, *Caloramator*, *Cellulomonas* and *Leucobacter* in CK of cultivar *Sijiu*, *Nonomuraea*, *Rummeliibacillus*, *Pedobacter*, *Methanoculleus*, and *Clostridium* in T1 of cultivar *Sijiu*, *Geothrix* and *Paludibacter* in T2 of cultivar *Sijiu*. These differentially abundant taxa can be considered as potential bacterial biomarkers serving as functional bacteria for the removal of CIP in agriculture soil.

Table 1
Diversity of bacterial and fungal community in rhizospheric soils of the two cultivars.

Treatment		Sequences	OTUs	Chao 1[1]	Observed_species[2]	Shannon[3]	Simpson[4]
Bacteria							
CK	*Cutai*	29,514 ± 5900 a[5]	5247 ± 215 a	12,869 ± 1318 a	4454 ± 478 a	8.827 ± 0.294 a	0.015 ± 0.001 c
	Sijiu	29,970 ± 6993 a	6311 ± 1103 ab	14,956 ± 208 bc	5236 ± 113 b	9.614 ± 0.142 b	0.008 ± 0.002 a
T1	*Cutai*	35,018 ± 7247 ab	6513 ± 817 ab	13,711 ± 665 ab	4895 ± 185 ab	9.402 ± 0.187 b	0.008 ± 0.002 a
	Sijiu	35,940 ± 3109 ab	7283 ± 155 b	15,223 ± 598 c	5329 ± 204 b	9.631 ± 0.204 b	0.007 ± 0.002 a
T2	*Cutai*	42,711 ± 6133 b	7652 ± 635 b	14,584 ± 166 bc	4980 ± 97 b	9.290 ± 0.134 b	0.011 ± 0.002 bc
	Sijiu	31,325 ± 6882 ab	6386 ± 800 ab	14,998 ± 592 bc	5159 ± 260 b	9.621 ± 0.179 b	0.006 ± 0.001 a
F value[6]	*Cultivar*	1.154	0.209	13.939**	12.782**	17.332**	14.197**
	Concentration	2.032	3.627	2.052	1.628	2.799	3.823*
Fungi							
CK	*Cutai*	33,944 ± 4920 ab	380 ± 25 a	543 ± 50 a	336 ± 27 a	2.052 ± 0.196 a	0.538 ± 0.092 c
	Sijiu	32,052 ± 7364 a	422 ± 69 a	668 ± 38 b	381 ± 24 ab	2.440 ± 0.288 ab	0.437 ± 0.105 bc
T1	*Cutai*	38,340 ± 1947 ab	461 ± 21 a	690 ± 59 b	382 ± 21 ab	2.271 ± 0.243 a	0.519 ± 0.036 c
	Sijiu	36,144 ± 3668 ab	454 ± 17 a	692 ± 30 b	390 ± 6 b	2.838 ± 0.074 bc	0.321 ± 0.034 ab
T2	*Cutai*	45,328 ± 11,971 b	446 ± 53 a	605 ± 110 ab	350 ± 31 ab	2.696 ± 0.205 bc	0.337 ± 0.087 ab
	Sijiu	32,648 ± 5003 ab	440 ± 37 a	683 ± 51 b	394 ± 30 b	2.947 ± 0.284 c	0.284 ± 0.074 a
F value	*Cultivar*	3.069	0.275	5.063*	7.646*	14.668**	10.013**
	Concentration	1.243	3.217	2.674	1.866	10.054**	7.769**

[1] Chao 1: richness estimator.
[2] Observed_species: number of OUT.
[3] Shannon: diversity index that characterizes species diversity.
[4] Simpson: diversity index that characterizes species diversity.
[5] Mean ± SD ($n = 3$). Data with the same letter(s) in the same column are not significantly different ($P > 0.05$).
[6] F value: Differences carried out based on two-way ANOVA.
* Significantly different ($P < 0.05$).
** Significantly different ($P < 0.01$).

To gain insight into similarities of the bacterial community in rhizospheric soils of the two cultivars, PCoA of beta diversity analysis was performed based on the Weighted Unifrac distances and the taxonomic and abundance information of OTUs (Franke-Whittle et al., 2015). As shown in Fig. S1, PC1 explained 34.24% of the variation observed, and PC2 explained 31.00% of the variation. Firstly, a clustering of cultivar *Sijiu* and cultivar *Cutai* significantly separated. Secondly, bacterial communities of cultivar *Cutai* in T1 and T2 that met closely were greatly separated from those in CK. However, bacterial communities of cultivar *Sijiu* in T1 and T2 separated well. These results suggested that the bacterial communities in rhizospheric soils of cultivar *Sijiu* were significantly different from those of cultivar *Cutai*.

3.3. Difference in rhizospheric fungal community of the two cultivars

After quality control processing and denoising, 655,368 fungal sequences were included in analyses from 18 samples. OTU assignment that was conducted at the 97% sequence similarity level resulted in 7808 OTUs. The total number of reads and OTUs were not significantly different between the two cultivars in all treatments ($P > 0.05$). Similar to bacteria, the mean α-diversity of fungus in rhizospheric soils of cultivar *Sijiu* was higher compared with that of cultivar *Cutai* (Table 1), with significant differences in Shannon and Simpson index in T1 and Chao 1 index in CK. Generally, the α-diversity including Shannon and Simpson of fungus in rhizospheric soils of the two cultivars increased with increasing CIP level, with significant difference between T2 and CK. These results implied that the fungal community diversity, richness, and evenness in rhizosphere of cultivar *Sijiu* performed better, which was conducive to removing more CIP in soil.

All of the sequences obtained from the 18 samples were classified into 4 different domains (abundance ≥ 0.2%) (Fig. 4a). Besides the dominant groups (which were unidentified, >50%), the remainders were mainly assigned to *Ascomycota*, *Basidiomycota*, and *Chytridiomycota* (Table S5). As major fungal phylum, *Ascomycota* showed no significant differences among all treatments, implying that *Ascomycota* played a limited role in differring CIP removal between the two cultivars. In T1, the relative abundance of *Basidiomycota* in rhizospheric soil of cultivar *Sijiu* was significantly higher than that of cultivar *Cutai* (21.95% > 3.07%, $P < 0.05$), but *Chytridiomycota* was just the opposite (0.56% < 4.99%, $P < 0.05$). At the order level (Fig. 4b, Table S6), the relative abundance of *Pezizales* in rhizospheric soils of cultivar *Sijiu* was significant lower than that of cultivar *Cutai* in CK and T2 (5.91% < 12.04%, and 4.48% < 14.11%, $P < 0.05$). However, *Trichosporonales* and *Agaricales* were the opposite in T1 (17.96% > 1.25% and 3.73% > 1.51%, $P < 0.05$).

Fungal community composition at the genus level was conducted in 10 dominated genera (Fig. 4c, Table S7). *Trichosporon* (r = 0.50) was observed to positively influence the removal rate of CIP in rhizospheric soils (Table 2). The relative abundances of *Trichosporon* in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai*, and the relative abundances of this genus in rhizospheric soils of the two cultivars improved with increasing CIP level, with significant difference in cultivar *Cutai* between T2 and CK (14.94% > 0.72%, $P < 0.05$). Two-way ANONA analysis showed that the relative abundance of *Trichosporon* was significantly affected by plant cultivars ($P < 0.01$) and CIP concentrations ($P < 0.05$). On the other hand, *Rhizophlyctis* (r = −0.50) had significantly negative correlationship with the removal rate of CIP in rhizospheric soil of the two cultivars. The relative abundances of this genus in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai* in CK and T2, but on the contrary in T1 (4.96% < 0.46%, $P < 0.05$). Meanwhile, its relative abundance in rhizospheric soil of cultivar *Cutai* in T1 was significantly higher than that in T2. However, which was the opposite in cultivar *Sijiu*.

The significantly different fungal taxa in rhizospheric soils of the two cultivars were shown in Fig. 5. There were five significantly different families, with the enrichment of *Boletaceae* in T2 of cultivar *Cutai*, as well as *Rhizophlyctidaceae* in CK of cultivar *Sijiu*, *Chaetomiaceae* in T1 of cultivar *Sijiu*, *Basidiobolaceae* and *Trichosporonaceae* in T2 of cultivar *Sijiu*. At the genus level, there were ten significantly different genera in rhizospheric soils of the two cultivars, including *Plectania* in CK of cultivar *Cutai*, *Trichoderma* in T1 of cultivar *Cutai*, *Dactylellina*, *Phoma*, and *Peyronellaea* in T2 of cultivar *Cutai*, as well as *Rhizophlyctis* in CK of cultivar *Sijiu*, *Scutellinia*, *Sporobolomyces*, and *Trichocladium* in T1 of cultivar *Sijiu*, and *Trichosporon* in T2 of cultivar *Sijiu*. These differentially abundant taxa can also be considered as potential fungal biomarkers serving as functional funguses for the removal of CIP in soil.

PCoA analysis of fungal community was shown in Fig. S2. PC1 explained 57.54% of the variation observed, and PC2 explained 15.33% of the variation. The fungal communities in rhizospheric soils of cultivar

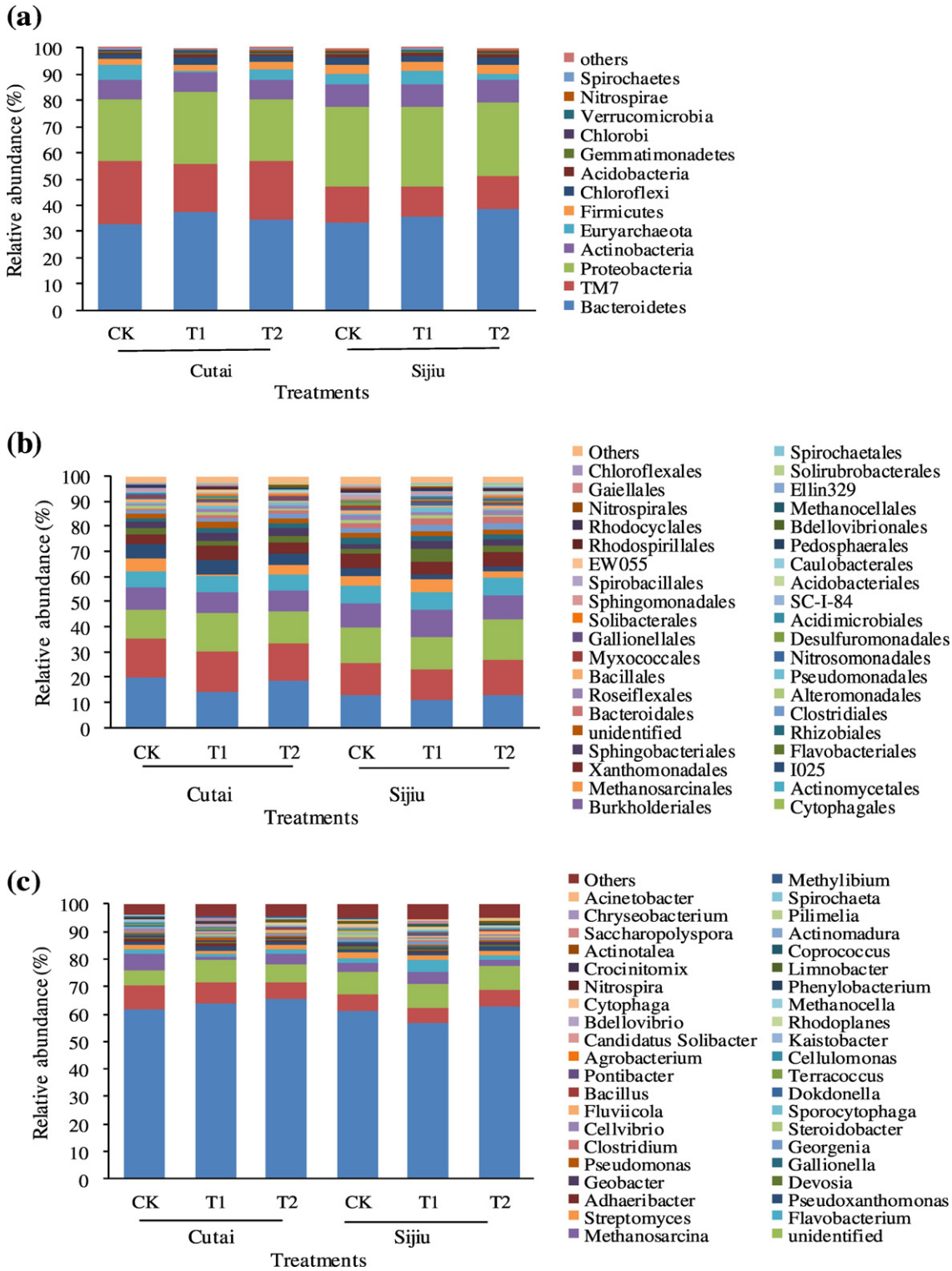

Fig. 2. Comparison of bacterial community in rhizospheric soils of the two cultivars at the phylum (a), order (b), and genus (c) level (abundance ≥ 0.2%).

Cutai in T1 and T2 obviously separated, while those of cultivar *Sijiu* in T1 and T2 clustered well. Noticeably, the distribution pattern of fungal communities is contrary to that of the bacterial communities as mentioned above, i.e., bacterial communities of cultivar *Cutai* in T1 and T2 clustered closely, while those of cultivar *Sijiu* separated greatly. These results suggested that the bacterial communities and the fungal communities in rhizospheric soils of the two cultivars adapted themselves to ClP stress with the opposite strategies.

3.4. Difference in microbial metabolism in rhizospheric soils of the two cultivars

The average well color development (AWCD) of the Biolog EcoPlate was used to represent the average microbial metabolic activity. As shown in Fig. S3, the microbial activity in the initial 24 h was very low, but increased quickly thereafter. After 216 h, the microbial activity reached a relatively stable phase. The microbial metabolic activity in

Table 2
The relative abundance of genera associated with CIP degradation and their correlation with removal rate of CIP.

Taxon	CK		T1		T2		F value[2]		r[3]
	Cutai	*Sijiu*	*Cutai*	*Sijiu*	*Cutai*	*Sijiu*	*Concentration*	*Cultivar*	
Geobacter	0.55 ± 0.31ab[1]	1.20 ± 0.42c	0.61 ± 0.02ab	0.78 ± 0.06bc	0.25 ± 0.04a	0.62 ± 0.30ab	4.57*	10.77**	−0.49
Gallionella	0.55 ± 0.24b	0.54 ± 0.35b	0.22 ± 0.06ab	0.30 ± 0.01ab	0.12 ± 0.02a	0.18 ± 0.03a	9.24**	0.33	−0.55
Spirochaeta	0.08 ± 0.03a	0.19 ± 0.05b	0.12 ± 0.03ab	0.20 ± 0.05b	0.22 ± 0.06b	0.21 ± 0.08b	3.51	4.84*	0.51
Rhizophlyctis	3.01 ± 1.62 ab	5.14 ± 2.62 b	4.96 ± 1.93 b	0.46 ± 0.31 a	0.78 ± 0.56 a	1.30 ± 0.59 a	3.06	0.37	−0.50
Trichosporon	0.72 ± 0.56 a	9.52 ± 8.11 ab	1.25 ± 0.27 ab	17.96 ± 11.62 b	14.96 ± 5.74 b	20.83 ± 2.98 b	6.09*	11.88**	0.50

[1] Mean ± SD ($n = 3$). Data with a same letter in the same rows are not significantly different ($P > 0.05$).
[2] F value: Differences carried out based on two-way ANOVA.
[3] r: Correlation coefficients between the relative abundance of genera and removal rate of CIP.
* Significantly different ($P < 0.05$).
** Significantly different ($P < 0.01$).

rhizospheric soils of cultivar *Sijiu* was lower compared to that of cultivar *Cutai*, with significant difference in CK ($P < 0.05$). In addition, the microbial metabolic activity in rhizospheric soils of cultivar *Cutai* decreased with increasing of CIP levels, with significant difference in T2 compared to CK and T1. However, the microbial metabolic activity in rhizospheric soil of cultivar *Sijiu* in T1 was significantly higher than that in CK and T2.

In order to investigate the effect of the two cultivars on the microbial CLPP, the optical density (OD) data of 31 carbon sources during 216 h of each treatment were subjected to PCA and analyzed. Two principal components (PC1 and PC2) were extracted following carbon sources (Fig. 6a–f, Table S9), which reflected the metabolic characteristics of the microbial communities. The carbon source utilization by microbes is obviously different in rhizospheric soils between the two cultivars, implying the variation in rhizospheric microbial communities.

The mean AWCD of 31 carbon sources also varied greatly (Table S10). Firstly, the microbes in rhizospheric soil of cultivar *Cutai* in CK preferred to utilize B2 (D-Xylose), C2 (i-Erythritol), F2 (D-Glucosaminic acid), G2 (Glucose-l-phosphate), H2 (D, L-α-Glycerol-phosphate), A3 (D-Galactonic acid γ-Lactone), E3 (γ-Hydroxy butyric acid), F3 (Itaconic acid), B4 (L-Asparagine), and C4 (2-Hydroxy benzoic acid), with weaker utilization of H4 (Putrescine) compared with that in cultivar *Sijiu*. Secondly, the microbes in rhizospheric soil of cultivar *Cutai* in T1 preferred to

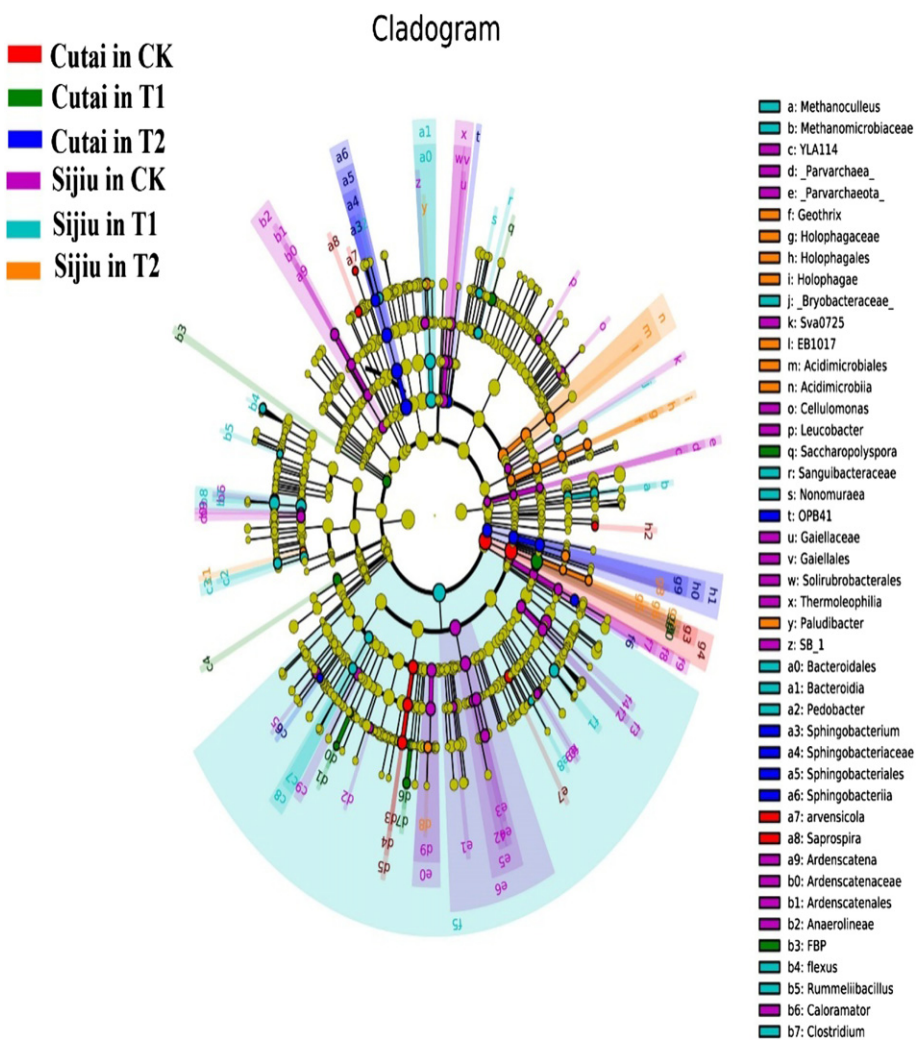

Fig. 3. Comparison of bacterial variations in rhizospheric soils of the two cultivars using the LEfSe online tool.

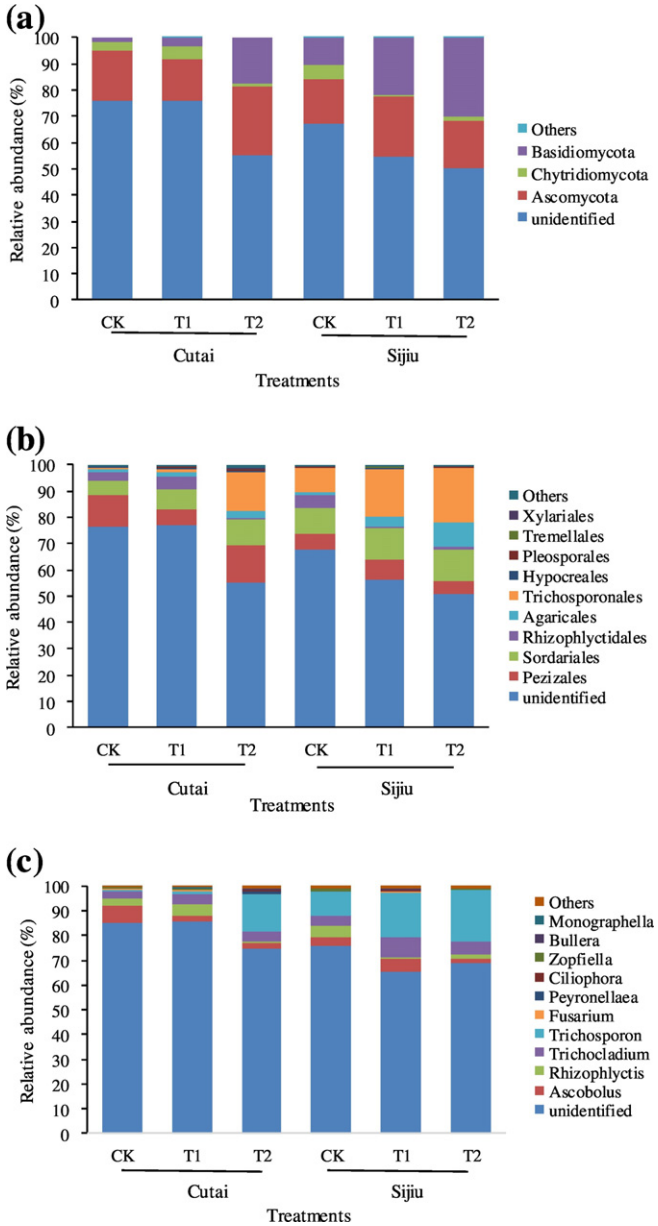

Fig. 4. Comparison of fungal community in rhizospheric soils of the two cultivars at the phylum (a), order (b), and genus (c) level (abundance ≥ 0.2%).

utilize C1 (Tween 40), B2 (D-Xylose), F2 (D-Glucosaminic acid), G2 (Glucose-l-phosphate), E3 (γ-Hydroxy butyric acid), and F3 (Itaconic acid), with weaker utilization of E1 (α-Cyclodextrin), C2 (i-Erythritol), and F4 (Glycyl-L-glutamic acid) compared with that in cultivar *Sijiu*. Thirdly, the microbes in rhizospheric soil of cultivar *Cutai* in T2 preferred to utilize E1 (α-Cyclodextrin), F1 (Glycogen), A2 (β-Methyl-D-glucoside), D2 (D-Mannitol), G2 (Glucose-l-phosphate), H2 (D, L-α-Glycerol-phosphate), B4 (L-Asparagine), E4 (L-Threonine), and F4 (Glycyl-L-glutamic acid), with weaker utilization of C1 (Tween 40), D1 (Tween 80), B3 (D-Galacturonic acid), F3 (Itaconic acid), A4 (L-Arginine), D4 (L-Serine), G4 (Phenyethylamine), and H4 (Putrescine) compared with that in cultivar *Sijiu*. Thus, it is obvious that the carbon source utilization of microbes in rhizospheric soils of cultivar *Sijiu* and cultivar *Cutai* presented selective specificity, leading to a significant variation in the removal of CIP in rhizospheric soils between the two cultivars. A higher utilization of C substrates including C1 (Tween 40), D1 (Tween 80), B3 (D-Galacturonic acid), F3 (Itaconic acid), A4 (L-Arginine), D4 (L-Serine), G4 (Phenyethylamine), and H4 (Putrescine) by rhizospheric microbes of cultivar *Sijiu* favored a higher CIP removal in soil.

4. Discussion

4.1. Effects of rhizospheric bacterial community on CIP dissipation

The removal of CIP from soil was mainly attributed to the biodegradation. Because the uptake of CIP by *Brassica parachinensis* L. accounted for <2% of the actual initial amount in the soils (Data unpublished), and the abiotic loss of CIP such as leaching, volatilization, photodegradation could be negligible (no leachate occurred through entire experiment, and the henry's law constant of CIP was 5.09×10^{-19} (SRC PhysProp Database, 2006)). As shown in Fig. 1, removal rates of CIP in soil were much higher in the presence of plants, especially for cultivar *Sijiu*, suggesting that the interaction of rhizospheric microbes and the used plants could contribute to the removal of CIP to varying degrees.

It is well known that the biodegradation of organic contaminants in soil is affected by microorganism, plants, and their complex interactions (Vergani et al., 2017). The degrading of polycyclic aromatic hydrocarbons (PAHs) in soil was enhanced by selectively stimulating growth of PAH-degrading populations in rhizosphere in the presence of sunflower, and a dramatic shift in PAH-degrading bacterial community structure was observed (Tejeda-Agredano et al., 2013). Many studies reported that the soil microbial community structures were strongly affected by various cultivars of some plants (e.g., maize and potato) (Aira et al., 2010; Doornbos et al., 2012; Inceoglu et al., 2013; Marques et al., 2014). In the present study, an obvious shift of bacterial community structure (Fig. S1) indicated that cultivar *Sijiu* and cultivar *Cutai* had different effects on bacterial community structures in rhizospheric soils.

Dissipation of CIP in soil might be related with some special bacterial phylum. Liao et al. (2016) reported that the CIP-degrading bacterial community was mainly composed of classes *Gammaproteobacteria*, *Bacteroidia*, and *Betaproteobacteria*. In the present study, the relative abundance of *Proteobacteria* (including *Gammaproteobacteria* and *Betaproteobacteria*) was the second highest among the bacterial phylum (Table S3), which might contribute to the CIP dissipation from rhizospheric soil.

The genus of *Spirochaeta* that was positively correlated with the removal of CIP (Table 1) could secrete seven glycoside hydrolases for plant biomass degradation (Schiefner et al., 2016), and it was a dominating bacterium for lignocelluloses hydrolysis (Pandit et al., 2016). The relative abundances of *Spirochaeta* in rhizospheric soils of the two cultivars increased with increasing CIP levels (from 0.08% to 0.22%), and the differences between the two cultivars were not significant. Thus, although *Spirochaeta* might be a bacterial candidate for CIP degradation, it was less likely to play an important role in differring CIP removal in rhizoshperic soils between the two cultivars.

In contrast, two genera of *Geobacter* and *Gallionella* were negatively correlated with the removal of CIP in rhizospheric soil (Table 1). It is reported that *Geobacter* has a diversity of extracellular electron transfer mechanisms (Tan et al., 2016), which could lead to oxidoreduction of pollutant (Lu et al., 2016). Although the relative abundances of *Geobacter* in rhizosphere soils of the two cultivars in T2 decreased compared to CK, their relative abundances in rhizospheric soils of cultivar *Sijiu* were always higher than those of cultivar *Cutai*, suggesting that cultivar *Sijiu* could maintain the biological activities of this genus better than cultivar *Cutai*. *Gallionella* is classified as an autotrophic bacterium that uses CO_2 for its carbon source (Hallberg and Tai, 2014). The relative abundances of *Gallionella* in rhizospheric soils of the two cultivars decreased with increasing CIP levels, with significant difference in T2 compared with CK. On the other hand, the relative abundances of this genus in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai* in T1 and T2, demonstrating that the cultivar *Sijiu* could retain the autotrophic activities of this genus better than cultivar *Cutai*.

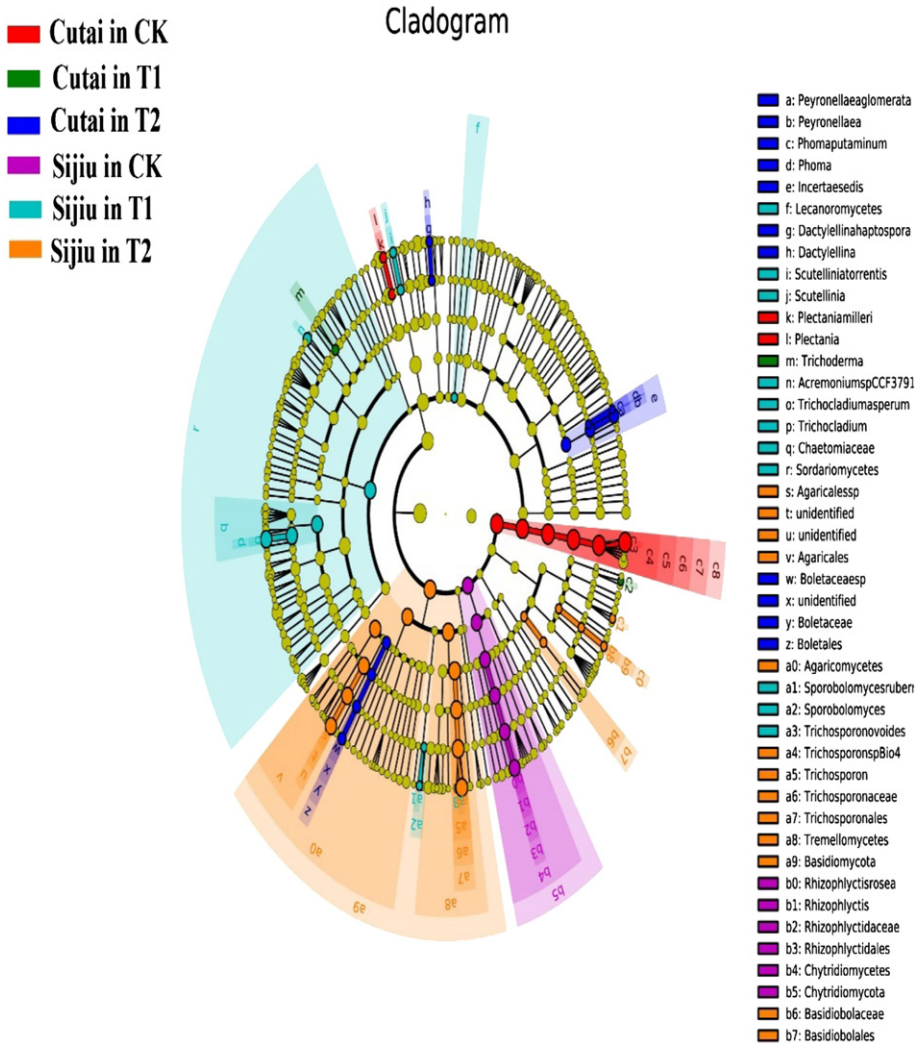

Fig. 5. Comparison of fungal variations in rhizospheric soils of the two cultivars using the LEfSe online tool.

In addition, the specific functional roles of fourteen significant genera screened by LEfSe (Fig. 3) might be considered as potential biomarkers of bacterial activities under the varied concentrations of CIP exposure. Certain of genera (*Leucobacter*) might have link with the CIP dissipation (Liao et al., 2016). These speculations should be investigated in future study to find out the specific mechanism of CIP removal in rhizospheric soils of the cultivars *Sijiu* and *Cutai*.

4.2. Effects of rhizospheric fungal community on CIP dissipation

Similar to the bacterial communities, the fungal communities in rhizospheric soils between cultivar *Sijiu* and cultivar *Cutai* were significantly different (Fig. S2). On the fungal composition, because >50% groups were unidentified, the numbers of phylum, order and genus were fewer.

CIP could be degraded by some fungal strains, e.g., *Penicillium notatum*, *Aspergillus fumigatus*, *Penicillium frequentans* and *Penicillium expansum* (Zhang et al., 2012), white rot fungus *Pleurotus ostreatus* (Singh et al., 2017). In the present study, only one fungal genus, *Trichosporon* had significantly positive influence on removal of CIP (Table 2). There are reports that *Trichosporon* has higher degradation of organic matter. For example, *Trichosporon cutaneum* could utilize simultaneously glucose, xylose, and arabinose as carbon sources (Chen et al., 2016; Qi et al., 2016). Moreover, *Trichosporon asahii* B1 isolated from a petroleum-polluted sediment could degrade the branched aromatic hydrocarbons (Thi et al., 2016). More important, *Trichosporon mycotoxinivorans* XPY-10 could efficiently degrade tetracycline antibiotics (Huang et al., 2016). CIP is an N-heterocyclic organic compound which can be degraded as the energy source by fungus (Prieto et al., 2011). In the present study, the relative abundances of *Trichosporon* in rhizospheric soils of the two cultivars increased with increasing CIP levels, and the relative abundances of *Trichosporon* in rhizospheric soils of cultivar *Sijiu* were higher than those of cultivar *Cutai*, in line with the trend in the removal rates of CIP in rhizospheric soils between the two cultivars. These results showed that *Trichosporon* might be a potential CIP-degrading fungus in rhizospheric soils of *Brassica parachinensis* L., and the variation in its relative abundances between the two cultivars might play an important role in differing the removal of CIP in rhizospheric soils. Although *Spirochaeta* and *Trichosporon* that were positively related with CIP removal might be associated with CIP degradation, the relative abundance of *Spirochaeta* was much lower among bacterial genera compared with that of *Trichosporon* in fungal community. In this case fungal community should have higher impact on CIP dissipation. Firstly, the amount of *Trichosporon* was higher than that of *Spirochaeta* (for example, *Sijiu* in T2). Secondly, some reports showed that the degradative capabilities of fungi to CIP were stronger than bacteria (Singh et al., 2017; Blánquez et al., 2016). Of course, the specific function and interaction with CIP of this fungus need to be further investigated in the following work.

On the contrary, the relative abundances of *Rhizophlyctis* were negatively correlated with the removal rates of CIP in rhizospheric soils of the two cultivars. But *Rhizophlyctis rosea* was found to be a major

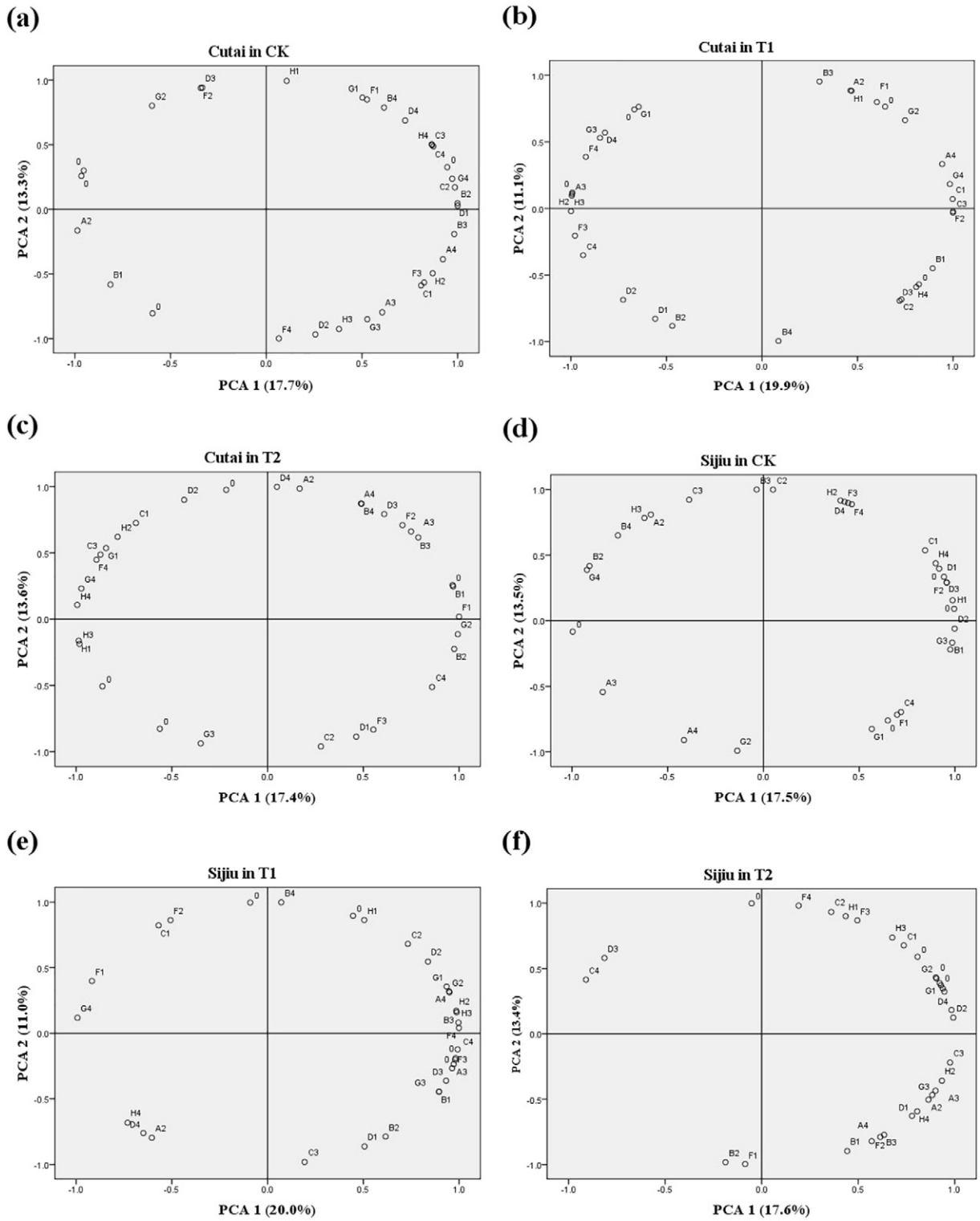

Fig. 6. Biology EcoPlate assay results of rhizospheric microbes of the two cultivars: (a)–(f) principal component analysis on microbial metabolism.

PAH-degrading fungus (Wang et al., 2013). The trend in the relative abundances of *Rhizophlyctis* in rhizospheric soils of cultivar *Sijiu* with increasing CIP levels was opposite to that of cultivar *Cutai*, suggesting that the activity of *Rhizophlyctis* was maintained in different ways between the two cultivars. At the same time, the specific functional roles of 10 potential fungal biomarkers including *Plectania*, *Trichoderma*, *Dactylellina*, *Phoma*, *Peyronellaea*, *Rhizophlyctis*, *Scutellinia*, *Sporobolomyces*, *Trichocladium*, and *Trichosporon* in rhizospheric soils of the two cultivars should be explored in future study (Fig. 5) so that the difference in CIP dissipation in rhizospheric soils between the cultivar *Sijiu* and cultivar *Cutai* could be better understood.

4.3. Effects of microbial activity on CIP dissipation

In the present study, the microbial metabolic activities in rhizospheric soils of cultivar *Sijiu* were weaker than those of cultivar *Cutai* (Fig. S3), which was the opposite in the trend in removal rates of CIP in rhizospheric soils of the two cultivars. Yan et al. (2008) reported

that the effects of plant species on rhizospheric microbes were driven by differential utilization of C substrates. These C substrates are representatives of C compounds produced in plants and released to soil as root exudates (Campbell et al., 1997). Likewise, in the present study, the utilization of C substrates by the microbes in rhizospheric soils of the two cultivars was plant cultivar and CIP concentration-dependent (Fig. 6a–f, Tables S8 and S9). Plants can change their root exudation in response to various environmental stresses, resulting in a large impact on the rhizospheric microbes (Doornbos et al., 2012; Tejeda-Agredano et al., 2013). For examples, root exudates of ryegrass exposed to phenanthrene improved bacterial diversity and modified phenanthrene-degrading bacterial population (Cebron et al., 2011). Root exudates of sunflower influenced the soil microbes and thus resulted in an increased degradation of xenobiotics (Tejeda-Agredano et al., 2013). Therefore, we believe that CIP as C substrates took part in the carbon source utilization in the rhizospheric microbial community of the two cultivars. Although the microbial metabolic activities in rhizospheric soil of cultivar *Sijiu* were weaker than those of cultivar *Cutai*, cultivar *Sijiu* might change its root exudates to induce rhizospheric microbe to prefer to utilize CIP, resulting in higher CIP removal in rhizospheric soils of cultivar *Sijiu*. This should be further investigated by other experiment.

5. Conclusion

Our results provide evidence of the importance of the cultivar-specific for CIP removal between the two cultivars of *Brassica parachinensis* L. More importantly, our results indicate that variations in rhizospheric bacterial and fungal community, and selective specificity to C substrate utilization played greater roles in differing CIP removal in rhizospheric soils between the two cultivars. Meanwhile, the CIP-degrading candidates and the dominant groups deserve further investigation into their potential beneficial role in CIP removal. Further innovative studies are required to reveal the affecting factors of the rhizospheric microbial community driving CIP removal. Such insights could provide renewed sense of how plant-microorganism combined bioremediation will be conducted to remediate CIP contaminated soil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.06.040.

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