Alleviating Continuous Monocropping Obstacle in Melon: Biological Elimination of Phenolic Acid

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Research Article

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Abstract

Background: Melon (*Cucumis melo* L.) is one of the most important fruit crops grown in China. However, the yield and quality of melon have significantly declined under continuous cropping. Phenolic acids are believed to be associated with the continuous monocropping obstacle (CMO) and can influence plant microbe interactions. Coumaric acid (CA) is one of the major phenolic acids found in melon root exudates. The objectives of this study were to estimate the elimination of CA by the soil bacterium K3 as well as its effects on mitigating melon CMO. CA degradation was investigated by monitoring the CA retained in the growth medium using high performance liquid chromatography (HPLC). The effects of CA and K3 on rhizosphere soil microbial communities were investigated by the spread plate method and Illumina MiSeq sequencing. Furthermore, the effects of CA and K3 on melon seedling growth were measured under potted conditions. The changes in soil enzymes and fruit quality under K3 amendment were examined in a greenhouse experiment.

Result: The results suggest that the addition of CA had the same result as the CMO, such as deterioration of the microbial community and slower growth of melon plants. HPLC and microbial analysis showed that K3 had a pronounced ability to decompose CA and could improve the soil microbial community environment. Soil inoculation with K3 agent could significantly improve the fruit quality of melon.

Conclusion: Our results show that the effects of K3 in the soil are reflected by changes in populations and diversity of soil microbes and suggest that deterioration of microbial communities in soil might be associated with the growth constraint of melon in continuous monoculture systems.

Background

Intensive farming practice exacerbates the continuous monocropping obstacle (CMO) of crops, and successive cropping of conspecifics renders soil conditions less suitable for their growth[1]. Potent allelochemicals, phenolic acids are believed to be associated with CMO and have a negative influence on microbial communities and soil functions [2]. Phenolic acids from root exudates have been identified in many plants, such as tobacco[3], peanuts [4], wheat, and watermelon[5, 6] .

Minor changes in the composition and quantity of root exudates (such as phenolic acids) can cause significant changes in microbial populations in the rhizosphere.. Phenolic acids have an important effect on the growth of plant pathogens, which has been confirmed by many studies. Benzoic acid (0.1 $mmol{\cdot}L^{-1}$) could promote the mycelial growth and sporulation of the peanut root rot pathogen *Fusarium* sp. *in vitro* [7]. The secretion of p-hydroxybenzoic acid, ferulic acid, and cinnamic acid from the roots of watermelon stimulated *Fusarium* wilt pathogen *Fusarium oxysporum F*. sp. *niveum* spore germination and growth[5]. In addition, root exudates from many crops have been shown to be potentially autotoxic. High levels of phenolic acids (150 $\mu g{\cdot}g^{-1}$ and 75 $\mu g{\cdot}g^{-1}$) significantly inhibited the growth of peanut plants (such as plant height, root length, and fresh weight) [4]. The addition of phenolic compounds to soil significantly reduced the leaf area, plant height and dry weight of cucumber. A similar phenomenon

has also appeared in melons. With the increase of the continuous cropping period of melons, the phenolic acid content of the rhizosphere soil and the incidence of fusarium wilt increased significantly, and the growth rate of melons decreased significantly [8].

Phenolic acid secreted by roots could not only impact the microbial biodiversity and abundance of rhizosphere soil but also manipulate biological and physical interactions between plants and soil microorganisms[9]. The soil microbial community is thought to be responsible for biological processes needed to maintain a healthy soil and suppress plant diseases[10], such as mineral nutrition cycling, organic matter turnover, soil structure formation, and toxin removal[11]. High microbial diversity and appropriate composition play a pivotal role in maintaining soil health and promoting plant growth by preventing pathogen invasion and establishment[12], antagonizing pathogen growth, competing with pathogens for nutrients[13], and modulating the host immunity[14]. In addition, a higher proportion of soil bacteria indicates better soil quality and higher soil nutrient content.

It has been illustrated that biocontrol bacteria could adjust the ratio between soil bacteria and fungi[15]. The rhizosphere microbial community structure that was destroyed by continuous inoculation was able to be repaired with biocontrol agents, as shown by the number of bacteria in rhizosphere soil, which increased markedly under biocontrol applications compared with a control (CK), but the density of pathogen and fungal increased dramatically in the rhizosphere of CK plants [16]. It has been found that microbial community structure was ameliorated by bio-organic fertilizer, which was fermented from *Paenibacillus polymyxa*, *Bacillus subtilis*, *Penicillium* sp., and *Aspergillus* sp. [8]. Furthermore, consistent overall effects were observed with the biocontrol amendments *Rhizoctonia solani* and *Trichoderma virens* on continuous cropping soil of potato, including increased bacterial population [17].

Many studies have shown that allelochemicals and autotoxins are easily degraded by microbes[18]. Thus, the effects of a vast diversity of microorganisms on the fate of plant phenolic compounds (and other potential allelochemicals) found within the soil should not be underestimated. However, under continuous cropping, relatively little is known about the detailed effects of phenolic acids on inhibiting the growth of soil bacteria. There is still no experimental evidence for the ability of biocontrol bacteria to decompose phenolic acids.

Consecutive monoculture of melon (*Cucumis melo* L.) is widespread in China and responsible for a continuous decline in fruit yield and quality and increased crop susceptibility to diseases[8]. In this study, we assessed the amelioration of K3 on continuous cropping soil of melon and melon plants grown in both pots and in field conditions. Previous studies on melon root exudates have found that coumaric acid (CA) is the main phenolic acid in melon root exudates [19]. We determined the changes of soil microbes after the amendment of K3 and CA. The elimination of CA by K3 was also measured. The overall goal of this study was to understand the mechanism behind the ability of K3 to overcome the obstacles of the continuous cropping of melon.

Results

Effects of CA on soil microorganisms and melon growth

The addition of CA solutions had strong effects on these soil microorganisms (Table 1). In the MB medium, fungal populations were significantly higher than those in the CK, whereas the bacterial counts were not significantly different compared with CK. The bacteria-to-fungi ratio in CAG treatment was 57.80% lower than that in CK group. In the soil matrix, the addition of CA leads to an increase in the number of bacteria in the soil, and a decrease in the number of fungi. There is also a significant decrease in the ratio of bacteria to fungi due to the addition of CA in the soil, but the amplitude is lower than in the MB medium.

Table.1 Effects of exogenously applied CA on culturable soil microorganisms

Treatments	Counts of bacteria lg(cfu·ml^{-1})	Counts of fungi lg(cfu·ml^{-1})	lg(bacteria-fungi ratio)
CK	7.161 ± 0.021a	5.686 ± 0.019b	1.443a
CAG	7.034 ± 0.113a	6.432 ± 0.024a	0.609b
CK1	6.812 ± 0.047a	5.159 ± 0.064a	1.652a
CAG1	6.725 ± 0.046a	5.185 ± 0.016a	1.541b
CK, soil microorganisms in MB medium; CAG, soil microorganisms in MBC medium; CK1, soil microorganisms in soil with distilled water; CAG1, soil microorganisms in soil with CA solution.			

In soil containing exogenous CA, slow growth of melon plants was observed compared with that of CK1 (Fig. S1, Table 2). The plant height, shoot fresh weight and root fresh weight in the CAG1 treatment were reduced by 31.84%, 33.97%, and 40.91%, respectively, compared with CK1. A lower blade number and root length were also observed in the CAG1 treatment, equivalent to a decrease of 8.77% and 19.13%, compared with CK1 respectively. The addition of CA resulted in significantly lower root vigor of muskmelon seedlings than the control group CK1 (Table 2).

Table 2
Defense enzymes of melon seedling leaves and root vitality in response to CA

Treaments	Plant height (cm)	Blades number	Root length (cm/plant)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Root vitality (mg•g^{-1}•h^{-1})
CK1	26.38 ± 2.9a	5.7 ± 0.a	14.90 ± 2.7a	3.088 ± 0.67a	0.308 ± 0.04a	0.821 ± 0.14a
CAG1	17.98 ± 1.8b	5.2 ± 0.b	12.05 ± 0.9a	2.039 ± 0.49b	0.182 ± 0.02b	0.349 ± 0.05b
CK1, potting soil with the addition of distilled water; CAG1, potting soil with the addition of CA solution. Values are mean ± standard error (n = 6); different lowercase letters among treatments indicate significant difference (P< 0.05).						

The decomposition of CA by K3

The colony morphology of K3 on MBC agar is shown in Fig. 1a. Colonies growing on MBC showed normal growth. The amount of K3 and the CA concentration in the fermentation broth are shown in Fig. 1b. After incubation for 18 h, the counts of K3 increased from 3.196 to 5.858 lg CFU·mL^{-1} while the concentration of CA decreased from 61.318 μg·mL^{-1} to 37.858 μg·mL^{-1}. The decomposition rate of CA was 1.303 μg·mL^{-1}·h^{-1}.

Effect of K3 on the microbial community in the continuous cropping soil of melon

The Effect Of K3 On The Soil Microbial Composition

For bacteria, the number of sequences per sample ranged from 99,654 to 127,418, with an average of 113,636 ± 14,516 reads. The coverage ranged between 99.22% and 99.28%, demonstrating that most of the bacterial taxa in the soil samples were detected (Table S1). At the phylum level, we focused on phyla with a relative abundance (RA) of over 1.0% (Fig. 2a). Overall, the dominant phyla across all samples were *Proteobacteria* (31.50–44.63%), *Actinobacteria* (10.31–17.05%), *Acidobacteria* (10.70–17.29%), *Bacteroidetes* (7.60–12.60%), *Chloroflexi* (2.96–5.77%), *Gemmatimonadetes* (2.88–4.72%), *Verrucomicrobia* (3.06–3.27%), *Planctomycetes* (1.62–2.69%), and *Firmicutes* (1.16–4.21%). Compared with non-continuous soil (CK0), *Actinobacteria* abundances in CK1 and T2 significantly ($P < 0.05$) decreased by 39.56% and 35.31%, respectively, and the relative abundances of *Planctomycetes* significantly ($P < 0.05$) increased by 65.74% and 43.83%. The differences between CK1 and CK0 did not reach statistical significance in other dominant bacteria. The relative abundances of *Proteobacteria* and *Bacteroidetes* in the T2 treatment significantly ($P < 0.05$) increased by 29.76% and 65.72% compared with CK1, respectively. However lower relative abundances of *Acidobacteria*, *Gemmatimonadetes*, and *Planctomycetes* were observed in the T2 treatment, equivalent to a decrease of 38.13%, 35.50%, and 13.22% compared with CK1, respectively (Fig. 2a).

An average of 194,944 ± 25,340 fungal sequence reads per sample was obtained. The coverage data showed that 99.22–99.28% of the fungal taxa in the soil samples were detected (Table S1). At the phylum level, the fungal community was dominated by members of *Ascomycota* and *Zygomycota* (Fig. 2b). The most noticeable difference between continuous cropping soil (CK1, T2) and non-continuous soil (CK0) was that the relative abundances of *Glomeromycota* were significantly ($P < 0.05$) reduced by 99.88% and 99.92%, respectively, while *Zygomycota* abundances were significantly ($P < 0.05$) increased by 2.89 and 2.74 times, respectively. The abundances of *Ascomycota* and *Basidiomycota* in the T2 treatment differed significantly ($P < 0.05$) from that of CK1, equivalent to a 1.52-fold increase and a 92.53% decrease, respectively. Thus, the *Ascomycota* community was markedly promoted, whereas the *Basidiomycota* community was inhibited in the presence of K3 (Fig. 2b). At the genus level, the dominant fungi *Mortierella*, *Conocybe*, *Chaetomium*, and *Fusarium* showed a significant change are listed in Table S2. *Conocybe* were not found in non-continuous soil (CK0), but were detected in the CK1 and T2 treatment, whereas the relative abundance of *Mortierella* increased significantly ($P < 0.05$) in CK1 and T2.

Compared with CK1, *Chaetomium* significantly ($P < 0.05$) increased by 6.49 times, whereas *Fusarium* significantly decreased by 84.61% in the T2 treatment.

The Effect Of K3 On The Soil Microbial Diversity

In general, the OTUs of bacteria and fungi in continuous cropping soil (CK and T2) were lower than those of healthy soil (CK0) (Fig. 3). After the T treatment, the bacterial OTUs of continuous soil increased, and the OTUs affiliated to fungi decreased. The unique bacterial OTUs treated by T2 were higher than those of CK1 by 90.00%. Moreover, higher Chao1 and ACE indices of the T2 treatment were shown than those under CK1 (Table 3), indicating that the abundance of bacterial species in continuous soil increased greatly after treatment with K3. At the same time, the Simpson index in the T2 treatment increased compared with that of CK1, showing that there was a clear ecological advantage of the dominant species in the bacterial community, and thus the community diversity was reduced. The shared OTUs of CK1 and T2 fungi accounted for 68.47% and 78.94% of the respective OTUs, separately, indicating that the fungal composition was different between the two treatments. After the K3 treatment, its characteristic OTUs decreased from 31.53% (CK1) to 21.06% (T2). Decreases in the Chao 1 and ACE index were observed, indicating that the fungi changed from peak abundance state (CK1) to low abundance state (T2) (Table 3).

From the perspective of fungal community diversity, the Simpson index of the T2 treatment was significantly ($P < 0.05$) lower than that of CK1, demonstrating that the dominant species in CK1 played key roles in the community resulting in a lower community diversity. The prevalence of the dominant species in the fungal community was decreased in the T2 treatment increasing the diversity of fungal communities.

Table.3 Community richness and Community diversity

Treatments	Bacteria			Fungus		
	Chao 1	ACE	Simpson	Chao 1	ACE	Simpson
CK0	4740.6b	4618.4c	0.001a	988.4a	987.2a	0.038c
CK1	5049.8a	4954.8b	0.001a	859.3a	848.3a	0.144a
T2	5141.4a	5076.1a	0.002a	811.9b	816.9a	0.089b

CK0, control treatment with healthy soil; CK1, control treatment with monocropping soil; T2, High concentration of K3 inoculated treatment with monocropping soil (3 g· kg^{-1} soil). Different lowercase letters among treatments indicate significant difference ($P < 0.05$).

The actual effect of K3 on continuous cropping of melon

Amelioration on CMO of melon seedlings by K3 addition in pots

All of the biomass and agronomic traits of seedlings in CK1 showed significantly lower values than in other treatment groups (Fig. S2, Table 4). The plant height, blade number, root length, and fresh weight of shoots and roots in the CK1 treatment groups differed significantly ($P < 0.05$) from those of CK0 resulting in decreases of 51.54%, 18.18%, 85.51%, 60.14%, and 50.00%, respectively.

Table 4
Melon seedling growth in response to K3 inoculation

Treatments	Plant height (cm)	Blades number	Root length (cm/plant)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)
CK0	13.00 ± 1.37b	7.7 ± 0.5a	20.70 ± 2.55a	1.48 ± 0.33a	0.04 ± 0.00a
CK1	6.30 ± 0.27c	6.3 ± 0.5b	3.00 ± 0.87b	0.59 ± 0.07c	0.02 ± 0.00c
T1	12.00 ± 0.87b	7.3 ± 0.8a	10.30 ± 1.84b	0.91 ± 0.11b	0.04 ± 0.01b
T2	16.50 ± 3.00a	7.7 ± 0.8a	7.73 ± 0.68b	1.42 ± 0.30a	0.05 ± 0.00a

CK0, control treatment with healthy soil; CK1, control treatment with monocropping soil; T1, low concentration of K3 inoculated treatment with monocropping soil (1 g· kg^{-1} soil). T2, High concentration of K3 inoculated treatment with monocropping soil (3 g· kg^{-1} soil).

After inoculation of K3 agent to the soil (T1 and T2 treatments) better growth of melon plants was observed compared with CK1, especially in the T2 treatment. In the T1 treatment, the plant height, the blade number, and the fresh weight of shoot and root significantly ($P < 0.05$) decreased by 51.54%, 15.87%, 35.16%, and 50.00%, respectively (Table 4). A significant ($P < 0.05$) increase of the plant weight and blade number was observed in the T2 treatment (Fig. S2), reaching to 1.62-fold and 1.55-fold respectively. The root length in the T2 treatment did not differ significantly ($P > 0.05$) from the CK treatment, resulting in a 1.58-fold increase. The fresh weight of shoots and roots in the T2 treatment significantly ($P < 0.05$) improved by 1.41-fold and 1.50-fold, respectively, relative to the CK1 treatment group (Table 4).

Effect of K3 on rhizosphere microorganism quantity of melon in the greenhouse

Microbial densities, including bacteria and fungi, are shown in Fig. 4. The number of soil bacteria peaked in April and subsequently exhibited a downward trend as the melon plants grew. The densities of soil fungi gradually increased from February to April, and after a drop to the lowest values in June, they rebounded in July. The levels of soil bacteria significantly ($P < 0.05$) increased during the whole growth period of melon after amendment with K3 agent, except in May. The densities of soil fungi in the K3 treatment were lower than those of CK, although they did not significantly decrease except for in

February. In the root zone soil of melon, the bacteria-to-fungi ratio was higher for the K3 treatment than the CK treatment.

Improvement in soil enzyme activities by K3 under continuous cropping conditions

The soil enzyme activity in the K3T treatment increased substantially compared with CK in the whole melon growing period (Fig. 4). The sucrose activity (Fig. 4a) of the K3-inoculated soil was significantly ($P < 0.05$) higher in February, April, and May, equivalent to increases of 96.11%, 234.15%, and 54.16% compared with that of the CK, respectively. However, no significant difference was observed in other months. Higher urease activity was observed in the K3T treatment group. The urease activities (Fig. 4b) in the K3T treatment were significantly ($P < 0.05$) increased by 20.06%, 8.58%, and 18.62% in April, June, and July, respectively, compared to that of the CK. Neutral phosphatase activity (Fig. 4c) showed increases with melon growth, with the lowest and highest values appearing in February and June, respectively. Significant ($P < 0.05$) increases in neutral phosphatase activity of 9.84%, 10.31%, and 14.12% were found in April, May, and June, respectively, after inoculation with K3, compared with CK. Increases in PPO activity (Fig. 4d) were parabolic and reached a maximum in April, and the PPO activity in the K3T treatment was significantly ($P < 0.05$) higher than that of CK in April, resulting in a 43.77% increase.

Improvement effect of K3 on melon fruit quality in greenhouse

Soil inoculation with K3 agent could significantly improve the fruit quality of melon (Table 5). Single fruit weight markedly increased by 10.19%, although this increase was not significant ($P > 0.05$) compared with CK. The amount of soluble sugar, soluble solids, and vitamin C were significantly ($P < 0.05$) increased by 20.26%, 21.21%, and 10.14%, respectively, compared with those of the CK. The NO_3-N in fruits was significantly ($P < 0.05$) reduced by 50.86% under the K3T treatment compared with that of the CK.

Table 5
Fruit quality of melon in response to K3 infection

Treatments	Single fruit weight	Soluble sugar	Soluble protein	Soluble solids	Vitamin C	NO_3-N
	(g)	(mg/g)	(mg/100 g)	(%0	(mg/100 g)	(mg/g)
CK	346.7 ± 15.9a	73.1 ± 0.3b	8.0 ± 1.2a	13.8 ± 1.8b	86.9 ± 0.4b	2.4 ± 0.3a
K3T	382.0 ± 27.1a	87.9 ± 0.9a	10.1 ± 0.4a	16.7 ± 1.4a	95.7 ± 0.9a	1.2 ± 0.2b

CK, control treatment; K3T, K3 inoculated treatment (200 g· m^{-2} soil).

Discussion

Due to the high complexity of rhizosphere, the catabolism of root exudates and its stimulating effect on microbial activity were studied by evaluating the role of a single low molecular weight organic substance in a simple system [20, 21]. The results showed that phenolic compounds in root exudates were effective regulators for microbial assembly in rhizosphere [22]. In this study, the allelopathic role of CA on soil microorganisms and the elimination of CA by K3 were explored, which will help to elucidate the mechanisms underlying the problem of natural regeneration failure in melon fields and could guide us toward more optimal approaches of soil disease control during continuous monoculture.

Soil microorganisms are ubiquitous, play important functions related to soil fertility, play a key role in plant growth and health, and provide the first line of defense against root infections by soil pathogens [23, 24]. The change of soil microbial community structure may lead to the change of microbial function performed by the community, which will affect the growth of plants [25, 26]. Therefore, the deterioration of soil bacterial communities causes poor plant performance in continuous planting systems.

Based on cultivation-dependent methods, previous researches have proved that phenolic acid can change the number of culturable soil microorganisms [27]. In addition, compared with other types of compounds, root exudates rich in phenolic compounds have a more significant impact on the soil microbial community[28, 22]. According to reports, in some cases, phenolic acid from root exudates can inhibit or promote the biochemical and physiological processes of microorganisms. Microbial metabolism is an important determinant of the degree and duration of phenolic acids in soil[18].

Different concentrations of phenolic acids have different effects on microbial growth[29].According to reports, in some cases, phenolic acid from root exudates can inhibit or promote the biochemical and physiological processes of microorganisms [30]. Benzoic acid (BA) amendment of the soil was found to significantly reduce the ratio of bacteria to fungi[7]. Continuous application of p-coumaric acid to the rhizosphere of cucumber has the same result[31]. Qu's research has shown that bacterial diversities decreased with the increasing concentration of BA and 3-phenylpropanoic acid[32]. The addition of 4 $\mu g \cdot kg^{-1}$ BA or 8 $\mu g \cdot kg^{-1}$ 3-phenylpropanoic acid phenols significantly ($P < 0.05$) reduced the soil microorganism density and soil microbial diversity, and extrapolated that BA and 3-phenylpropanoic acid destroyed the balance between the bacteria and fungi, and led to the accumulation of pathogenic microbes and reduction of rhizosphere growth promoting bacteria [3]. In this study, adding CA to the liquid medium under co-cultivation conditions significantly increased the number of soil fungi, reduced the number of soil bacteria, and reduced the ratio of bacteria to fungi, which is consistent with previous research results.

Under field conditions, phenolic acid can be continuously produced and secreted into the surrounding soil by roots. This could reinforce the effects of the microbial utilization of phenolic acid in the rhizosphere and lead to a shift in soil microbial communities[7]. Since the fields are continuously monocultured, plant root exudates and the identical regimes of the fields could lead to disruption of soil microbial community composition and structure[33]. The application of microbial agents has been found to be an efficient method to improve the deteriorated soil microbial environment caused by continuous cropping. Previous

research found that after the application of effective microbial agents pathogens decreased while the antagonists increased. Moreover, the abundance of bacteria related to elemental cycling and plant growth promotion increased substantially[34].

Plant health in natural environments depends on interactions with complex and dynamic communities comprising macroorganisms and microorganisms[35, 36]. In the pot experiment of the current study, which explored the improved effect of different K3 application dosages on continuous soil, the results obtained by plate count showed that the densities of soil bacteria markedly increased after amendment with K3 agent; as a result, the bacteria-to-fungi ratio increased. The same trend was observed in the experiments of greenhouse and liquid medium co-culture. To gain further insight into the effect of treatment on root microbial diversity, we performed β- and α-diversity analyses. The OTU distribution (β-diversity) analysis showed that the bacterial OTUs increased and the fungal OTUs decreased in the continuous monocropping soil treated by K3 (T2 treatment). In the T2 treatment, the diversity of bacteria increased, accompanied by a decrease in the fungal community compared with CK1. To pinpoint differences within the rhizosphere microbiota, the microbial diversity within each sample (α-diversity) was calculated. This calculation showed that bacterial communities in the T2 treatment were significantly more diverse and complex than those in CK1 and that the Simpson diversity for the T2 treatment was significantly lower than that of CK1. One interpretation of our results is that the addition of K3 caused some bacteria with ecological advantages to proliferate and thus the original dominant fungus lost its advantage. From the perspective of community structure, the relative abundance of the dominant composition of bacteria and fungi changed substantially at the phylum level; thus, K3 amendment of soil strongly stimulated or inhibited specific bacterial and fungal phyla.

In regard to bacterial phyla, most studies have shown that the relative abundance of *Acidobacteria* is markedly negatively correlated with soil pH[37]. The results of this study showed that the addition of K3 reduced the relative abundance of *Acidobacteria*, which suggests that the K3 decomposed the phenolic acid in the continuous monocropping soil to increase the soil pH, thus reducing the abundance of *Acidobacteria*, which are adapted to the acidic environment. *Proteobacteria* and *Bacteroidetes* have been reported to be more abundant in the rhizosphere of the microbial remediation soil than diseased soil[38], whereas, *Proteobacteria* can suppress pathogens [15]. This indicates that the relative abundance of *Proteobacteria* is positively correlated with soil health.

For the fungal community, further analysis at the genus level revealed that *Mortierella* was highly enriched in the CK1 and T2 treatment, indicating that they grow better in the continuous cropping melon soil. Interestingly, the relative abundance of *Fusarium* belonging to the *Ascomycota* significantly decreased by 84.61% under the T2 treatment, whereas *Chaetomium* significantly increased by about 6.49-fold. *Chaetomium* spp. is widely distributed in natural soil and has potential biocontrol effects on many plant pathogens and nematodes[39]. In contrast, *Fusarium*, in particular, harbors notorious pathogenic plant fungi with a wide variety of hosts and infection strategies[40]. The present results indicate that the fungal environment for plant growth was optimized after K3 treatment. From this perspective, we can speculate that K3 as a biocontrol agent and acts as a soil guard to maintain a healthy soil microbial

structure by promoting the growth of beneficial bacteria and inhibiting the propagation of pathogenic fungi.

Biodegradation as a means of biological control usually depends upon the oxidative activities of microorganisms and has played an important role in eliminating environmental compounds [41]. Inoculation of beneficial microorganisms could degrade phenolic allelochemicals as an energy source, and detoxify these substances[42]. It therefore represents a promising way to reduce the accumulation of phenolic acid and alleviate their stimulation for soil pathogenic fungus growth. Nevertheless, biodegradation efficiency, persistence, and competitiveness of the microbial agents in soil restrict their applications. Therefore, screening appropriate microorganisms for soil phenolic acid degradation may be necessary for a successful control of soil-borne pathogens in continuously cropped soils. Some Gram-positive species, such as *Bacillus pumilus* [43], *B. subtilis* [44], and *Lactobacillus plantarum* [45] have the genes encoding phenolic acid decarboxylase (PAD). In *B. subtilis*, several phenolic acids specifically induce expression of PAD, which converts these antimicrobial compounds into vinyl derivatives [46]. However, there are few studies on the effect of beneficial bacteria on the decomposition of phenolic acid in continuously cropped soil. In the present study, K3 could grow on MBC medium, which implied that K3 could degrade CA as an energy source. Further HPLC experiments were performed to quantify the content of CA, and we found that the concentration of CA decreased from 61.318 $\mu g \cdot mL^{-1}$ at the beginning to 37.858 $\mu g \cdot mL^{-1}$ after incubated for 18 h, with a corresponding increase in K3 counts. The decomposition rate of CA was 1.303 $\mu g \cdot mL^{-1} \cdot h^{-1}$. This result indicates that K3 has a high decomposition ability for CA.

Many experiments have shown that exogenous phenolic acids can inhibit plant growth. Consistent with previous findings, the pot experiment of the current study also showed that exogenous CA caused apparent effects of seedling growth, including biomass reduction and changes in horticultural characteristics. Treatment with the K3 agent, mitigated the hindering effects of CA on seedlings. This was because K3 has the ability to degrade CA in continuously cropped soil. This could be because, on the one hand, the PAD of K3 is expressed under CA induction, showing strong catabolic CA capacity, which avoids the interference effect of CA on the soil microbial community. On the other hand, K3 acts as a soil health guard, maintaining good soil microorganism community structure by inhibiting the growth of pathogenic fungi and promoting the growth of bacteria. As a result, the biomass of plants significantly increased and the plant height, root length and number of leaves increased substantially.

Soil is a complex and dynamic system that supports plant growth. Soil microorganisms are fundamental for soil health and provide ecosystem services that are essential for plant production [47]. In the continuous cropping soil environment, plant growth and development are restricted by microbial deterioration and represent a major constraint to sustainable agricultural production [48]. Beneficial microbes can directly inhibit pathogens by producing antimicrobial compounds. However, beneficial microbes can also inhibit pathogens indirectly by stimulating the plant's immune system, a phenomenon called induced systemic resistance [49]. Several researchers have reported the effects of beneficial bacteria for improving plant growth under a normal as well as a stressful environment [50]. Beneficial microbes optimized the rhizobacteria of peanut, which mitigated the CMO [34]. Similarly, bio-organic

fertilizers supported by *P. polymyx* and *B. subtilis* effectively suppressed *Fusarium* wilt disease in melon caused by continuous cropping [8]. Biocontrol bacteria could alleviate crop diseases effectively and ensure the normal growth of cucumber by inoculating *Bacillus* spp., *Bacillus amyloliquefaciens*, and other bacteria for disease regulation[51]. In line with previous research, the pot experiment in the current study was conducted with continuous monocropping soil treated by K3, in which horticultural characteristics and biomass of melon were significantly improved.

The soil enzymes, sucrose, urease, and neutral phosphatase play essential roles in the cycling of elements. PPO has frequently been reported to participate in plant defense against pests and pathogens[52]. It is generally believed that continuous cropping causes a decrease in soil enzyme activity. The results of the greenhouse experiment in the present study showed that the K3 agent significantly increased the activity of urease, neutral phosphatase, and PPO, especially increasing the sucrose activity. At the same time, the quality of melon fruit was greatly improved; the amount of soluble sugar, soluble solids, and vitamin C significantly increased, accompanied by significant reduction in NO_3-N. These results show that the inoculation of K3 can alleviate CMO effectively and improve melon growth.

The present study demonstrated that the K3 agent has a significant effect of alleviating melon CMO by decomposing phenolic acids and improving rhizosphere microbial communities. This was reflected by the increased biomass of seeding plants, the changed horticultural characteristics of melon, the increased soil enzyme activity, and the improved quality of fruit after inoculation with K3. The current study provides a theoretical and practical foundation for the use of K3 agent in other crops.

Material And Methods

Effects of CA on soil microorganisms and melon growth

Effects of CA on soil microorganisms

To determine the effect of CA on microbial (bacteria and fungi) abundance of the healthy soil (previous crops were wheat), 4 g of the healthy soil (fresh weight equivalent) was added to 36 mL of sterilized water and shaken on a rotary shaker at 28°C and 180 rpm for 30 min to create a soil suspension. MB (mineral basal medium consisting of 1.0 g NH_4NO_3, 0.5 g $MgSO{\cdot}7H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.5g NaCl, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, and 5 g glucose) liquid medium amended with 100 $\mu g{\cdot}mL^{-1}$ CA (CAT), and the MB liquid medium was taken as the CK. Two mL of the above prepared soil suspension was added to the above two different MB liquid mediums and co-cultured in a shaker at 28°C and 180 rpm for 18 h. Serial dilutions of fermentation liquor were made, and 0.1 mL aliquots were spread on the surface of the plates. Luria broth (LB) agar and Rose Bengal agar medium [53] were used to evaluate the population of bacteria and fungi, respectively. Plates were incubated at 25–30°C for 24–72 h. After culturing, colonies were counted and expressed as number per milliliter of medium.

Inhibitory effects of exogenous CA on the growth of melon seedlings

Melon seeds (*C. melo* L.) cultivar “Tianbao” were purchased from an agricultural market in Yangling, Shaanxi Province, China.All seeds were surface sterilized with 0.7% sodium hypochlorite solution for 5 min and rinsed three times in sterile distilled water. After the seeds were kept at 55°C for approximately 30 min, the they were germinated in the incubator at 37°C. When the sprouts emerged, the seeds were sown in the matrix soil. At the one-and-a-half-leaves stage, seedlings that showed consistent growth were selected to be transplanted to the pots, and one seeding was planted in each pot.

Pot experiments were carried out in a tissue culture room on the North Campus of Northwest A&F University from 8 June 2018 to 2 July 2018.To assess the induced toxicity of exogenous CA to melon seedlings, the experimental soil was collected from a field that had never been planted with melon (previous crops were wheat). The experiment was arranged in a completely randomized design with six replicates. A 20-mL aqueous solution of 10 $\mu g \cdot mL^{-1}$ CA was added to 500 g of soil (dry weight equivalent) and mixed to obtain the CAG1 treatment (400 $\mu g \cdot kg^{-1}$ soil CA). Soil with an equal volume of distilled water was used as the CK1. Soil moisture was maintained at 20% (absolute water content of soil) of the water holding capacity.

The number of soil bacteria and fungi was determined as above. At the seedling stage, an appropriate amount of water was added to the pots to loosen the soil. Six plants were uprooted, and the soil adhering to the melon seedling roots was shaken off slightly. The roots were rinsed carefully until there was no soil left, and the root surface was then dried with absorbent paper. This was followed by measurement of the fresh weight of shoots and roots. The plants were placed for drying in an oven for 15 min at 105°C and then placed at 70°C for 3 days. The dry weight of each plant, the length and width of the first true leaf, plant height, and root length were measured directly. Phenylalanine ammonia lyase (PAL) activity, peroxidase activity (POD), polyphenol oxidase (PPO) activity, root activity (TTC method) of melon seedling leaves were determined with reference to Cao Cuiling's method [54].

Decompositionof CA byK3

The *Bacillus* K3 used in this study was previously isolated and identified by our laboratory, Northwest A&F University (Yangling, China). The K3 agent was prepared by fermentation of K3; the number of effective bacteria was higher than 3.01×10^{10} $cfu \cdot g^{-1}$.

Single colony of K3 was picked and incubated on MBC (mineral basal medium with CA as the sole carbon source). The MBC medium consisted of 1.0 g NH_4NO_3, 0.5 g $MgSO{\cdot}7H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.5 g NaCl, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 15 g agar powder, and 1 L CA solution (100 $\mu g \cdot mL^{-1}$). A small number of K3 that were activated on LB medium were vertically spotted onto MBC agar media using an inoculation needle. Plates were incubated at 25–30°C for 192 h. The treatment was cultured four times.

In order to corroborate the ability of K3 to decompose CA quantitatively, the concentrations of CA in the MBC liquid medium were determined before and after inoculation of K3 for 18 h. The density of K3 was detected by the flat colony counting method before and after culture. The K3 bacterial solution activated

in the LB liquid medium was inoculated in MBC liquid medium and cultured (30°C, 180 rpm for 18 h on a shaker). The treatment was cultured in triplicate. Then, the culture was centrifuged to obtain the supernatant for detection by HPLC.

The samples were analyzed using 1525 high-performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) with a Cymmetry-C18 column (4.6 mm × 250 mm). The column temperature was maintained at 25°C. The injection volume was 10 μL. The UV detector wavelength was set at 280 nm. The flow velocity was 1 mL· min^{-1}. The mobile phase A was methanol and B was acetic acid (pH = 2.8). Separation of the sample was achieved by a gradient elution method to separate and shorten the retention time of the phenolic acid species. Elution conditions: 70% (0 min) → 50% (15 min) → 50% (7 min) → end (0 min).

Effect of K3 on the microbial community in the continuous cropping soil of melon

Pot experiment design

This experiment was performed from September 15, to October 16, 2017, to explore the effect of different dosages of K3 on melon seedlings. Two different soils were used in this study. The first soil was collected from a field that had never been planted with melon and considered as a healthy potting soil (the previous crops were wheat). The second soil, had previously grown three seasons of melon successively and was galled from the melon root area (10–20 cm from the taproot), and applied as the continuous monocropping potting soil. Four treatments were included: CK0, 0.5 kg potting healthy soil; CK1, 0.5 kg continuous cropping potting soil; T1, 0.5 kg continuous cropping potting soil and 0.75 g K3 agent added to each pot with sufficient mixing; and T2, 0.5 kg continuous cropping potting soil and 1.5 g K3 agent added to each pot with sufficient mixing. The determination of plant biomass and agronomic traits is the same as 2.1.2.

DNA extraction and high-throughput sequencing

Galling rhizosphere soil of potted seedlings from root area (1–2 cm from taproot) was collected using a shovel. Soil samples were mixed thoroughly and transported in polyethylene tubes and immediately placed in a -80°C refrigerator. Soil total genomic DNA was extracted from 0.5 g soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) and dissolved into 50 μL sterile distilled water following the manufacturer's instructions. Extracted DNA concentration and quality were determined with a NanoDrop 2000 spectrophotometer (Thermo Fischer Scientific, Waltham, MA, USA). The bacterial and fungal libraries for MiSeq sequencing were constructed following previously described protocols. The gene-specific primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') were used to amplify the V4 region of bacterial 16S rRNA genes while primers ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS2 (5'-GCT GCG TTC ATC GAT GC-3') targeted the ITS1 region of the fungal internal transcribed spacer (ITS) [55]. Bacterial 16S rRNA and fungal ITS sequences were amplified using the ABI 2720 Thermal Cycler (Thermo Fisher Scientific). All amplifications were conducted in a 25 μL mixture including 12.5 μL of 2×KAPA HiFi HotStart ReadyMix,

the forward and reverse primers at 0.2 μM final concentration, 2.5 μL of template DNA (5 ng·μL^{-1}), and nuclease-free water up to 25 μL. The PCR conditions were 94°C for 2 min, followed by 25 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, with a final extension of 10 min at 72°C. The obtained PCR products were purified using Agencourt AMPure XPPCR Purification Beads (Beckman Coulter, Brea, CA, USA) and quantified by an Invitrogen Qubit3.0 spectrophotometer (Thermo Fisher Scientific). The purified amplicons were pooled in equimolar concentrations and employed for library construction. The final quality and concentration of the libraries were checked by Agilent 2100 Bioanalyzer Instruments (Agilent Technologies Inc., Santa Clara, CA, USA). All library preparation was performed using the Illumina MiSeq Benchtop Sequencer (Illumina, San Diego, CA, USA) platform at Genesky Biotechnologies Inc. (Shanghai, China).

Monitoring the effect of K3 on improving continuous cropping obstacles of melon throughout the growth cycle

Plastic greenhouse situated at Lingwan Village in Yangling (34°16'N, 108°0'E) was used for these experiments from 18 January to 2 July, 2017. The experiment consisted of two treatments: CK, the control treatment without K3 agent inoculation, and K3, the treatment with K3 agent inoculation (200 g·m^{-2} soil). All of the treatments were performed in triplicate.

Certain properties of the soil in the field were measured: pH, 7.34; organic carbon, 32.99 g·kg^{-1}, total nitrogen, 537.42 mg· kg^{-1}; available phosphorus, 48.86 mg·kg^{-1}; available potassium, 443.08 mg· kg^{-1}; NH_4-N, 16.31 mg· kg^{-1}; and NO_3-N, 24.34 mg· kg^{-1}.

To detect the microbial (bacteria and fungi) abundance in the rhizosphere soil, 2 g of the rhizosphere soil from the pot experiment was taken from each treatment. The rhizosphere soil of melon was obtained by collecting soil from the root area (10–20 cm from the taproot) of the greenhouse plant using a shovel. Soil samples were mixed thoroughly and transported in plastic bags to the laboratory. The method for determining the number of bacteria and fungi was the same as 2.1.1

After culturing, colonies were counted and expressed as number per gram (fresh weight) of soil.

Soil samples were collected from the top 20 cm of the soil surface in the greenhouse trial, air-dried, homogenized, and sieved to 100 mesh before analysis. The activity of sucrose, urease, neutral phosphatase, and polyphenol oxidase (PPO) of soil were measured following the method used by Gao[56].Single fruit quality was measured directly. The amount of soluble sugar was determined using the anthrone colorimetric method, soluble protein was evaluated using the Coomassie blue staining method, vitamin C was determined using the molybdenum blue colorimetric method, and nitrate nitrogen was measured by nitration of salicylic acid colorimetry. Soluble solids were assessed by a hand-held 2WAJ Abbe refractometer (Shanghai Optical Instrument Factory, Shanghai, China).

Statistical analysis

Microsoft Excel 2007 was employed to process the data. Statistical analysis was performed using SPSS 23.0 (SPSS, Inc., Chicago, IL, US). Comparisons among treatments were performed using one-way analysis of variance (ANOVA) or t tests in IBM, with significance at $P < 0.05$. MiSeq sequencing data analyses were conducted with packages in R. To estimate the taxonomic (operational taxonomic units (OTUs) and metagenomic species level and community diversity, the diversity index was calculated by “diversity” in vegan R package. All of the values are expressed as mean ± standard error.

Abbreviations

CMO: the continuous monocropping obstacle

CA: Coumaric acid

BA: Benzoic acid

LB: Luria broth

HPLC: high performance liquid chromatography

PAL: Phenylalanine ammonia lyase

POD: peroxidase

PPO: polyphenol oxidase

ANOVA: Analysis of variance

Declarations

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Authors’ contributions

HX and CC designed this research. HX, XY, LC, XM and NY carried out the experiments. HX and YK participated in the data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw data are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

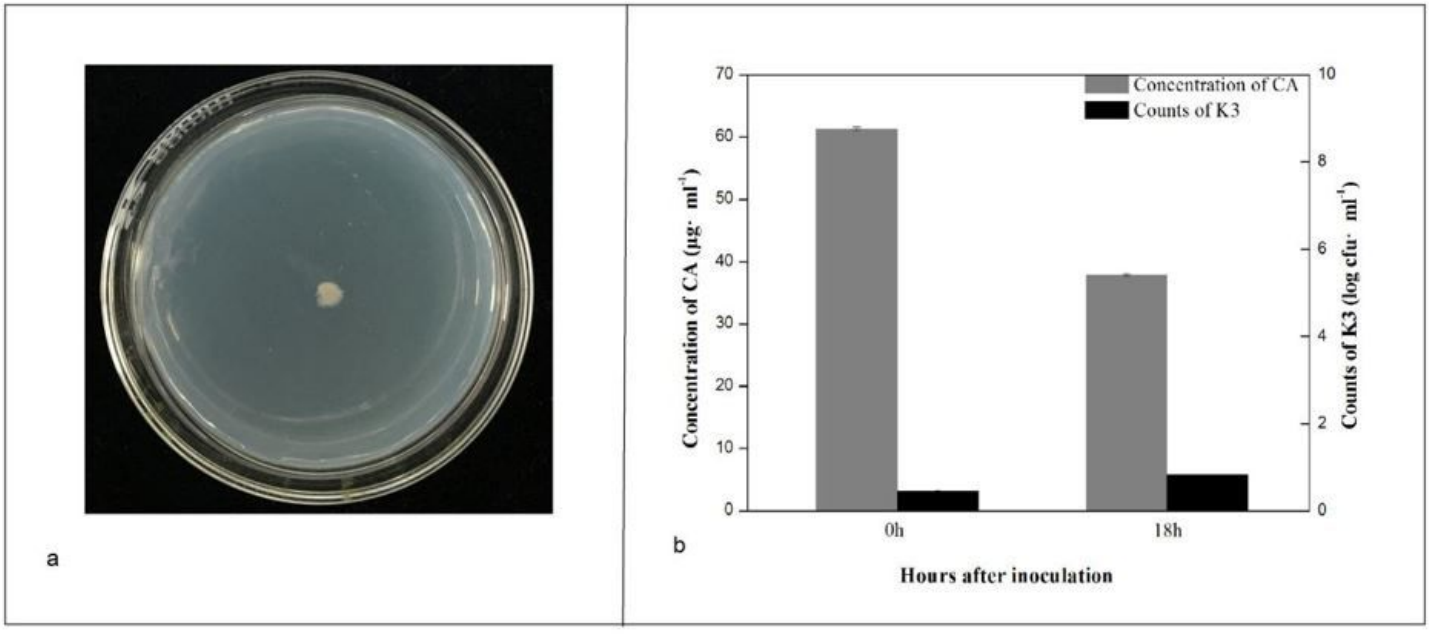

Figure 1

The decomposition of CA by K3. a, Colony morphology of K3 on MBC agar media after 5 days inoculation. b, Counts of K3 and concentration of CA in the liquid medium after inoculation of K3 for 0 h

and 18 h.

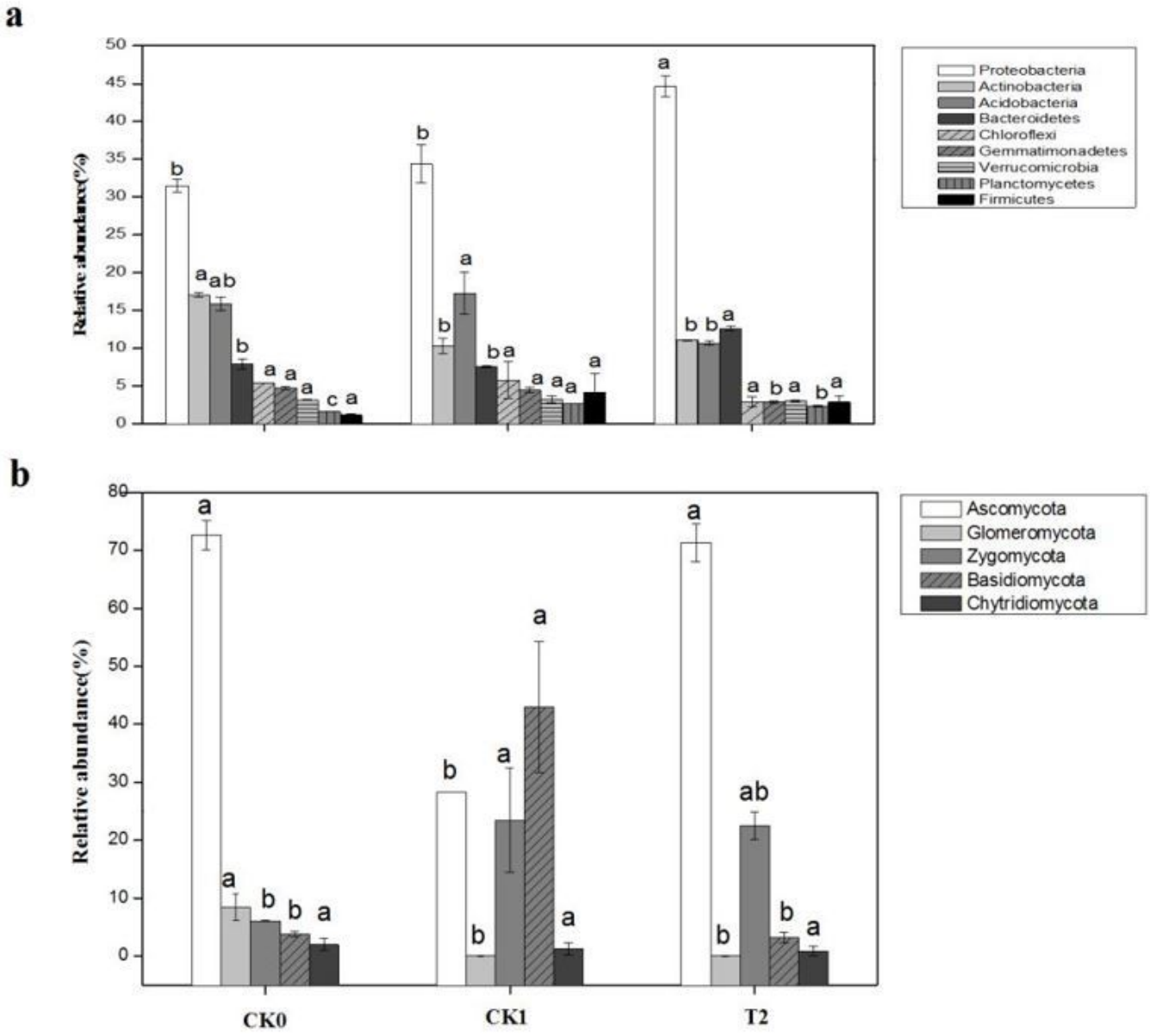

Figure 2

The average distribution of the bacteria (a) and fungal (b) community at the phylum level in the soil. Different lowercase letters among treatments indicate significant difference ($P < 0.05$). CK0, control treatment with healthy soil; CK1, control treatment with monocropping soil; T2, High concentration of K3 inoculated treatment with monocropping soil (3 g· kg−1 soil).

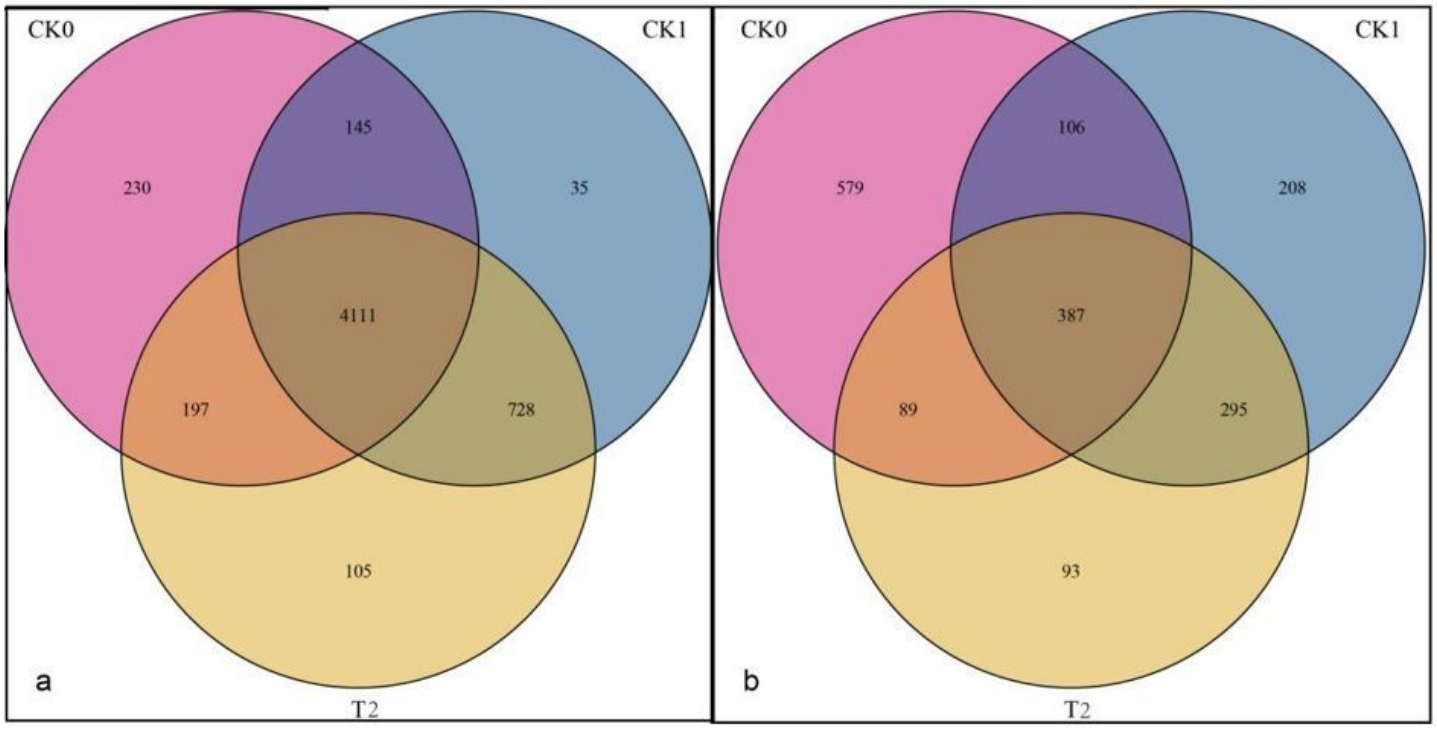

Figure 3

OTU distribution Venn diagram. a, Bacteria; b, Fungi. CK0, control treatment with healthy soil; CK1, control treatment with monocropping soil; T2, High concentration of K3 inoculated treatment with monocropping soil (3 g· kg−1 soil). Different sample groups are represented by different colors, and the overlapping areas of different color circles indicated the number of common species.

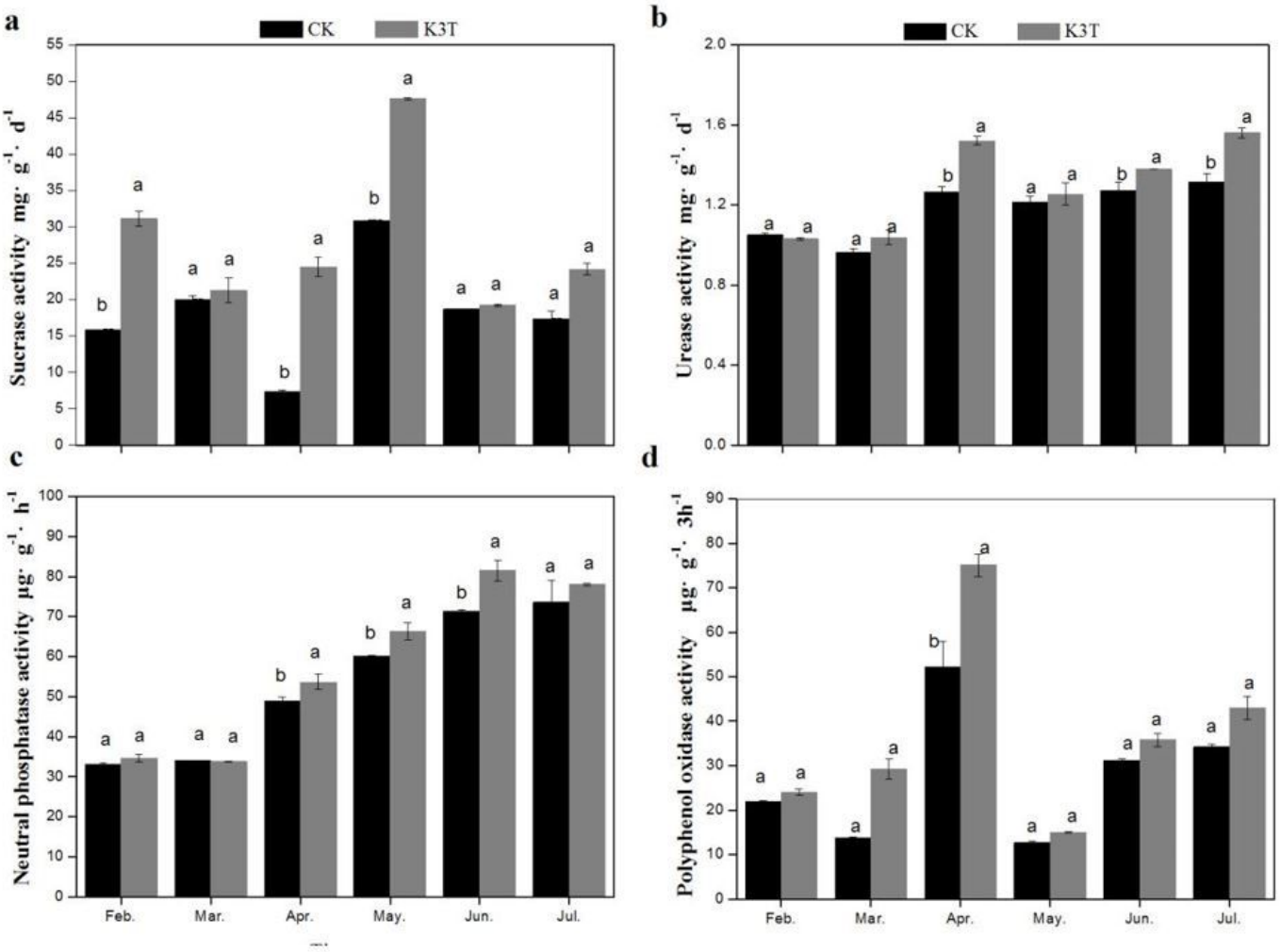

Figure 4

Effect of K3 on soil enzyme activity. Different lowercase letters among treatments indicate significant difference ($P < 0.05$). CK, control treatment; K3T, K3 inoculated treatment (200 g· m-2 soil). (a) Effect of K3 on soil Sucrase activity, (b) Effect of K3 on soil Urease activity, (c) Effect of K3 on soil Neutral phosphatase activity, (d) Effect of K3 on soil Polyphenol oxidase activity

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