Soil Microbial Communities Altered by Titanium Ions in Different Agroecosystems of Pitaya and Grape

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Abstract

Titanium ions can significantly promote plant growth, but it is unclear whether the application of titanium ions to plants has any effect on the soil microbial community. In this study, we conducted field surveys to determine the effect of titanium ions on soil microbial communities of the pitaya and grape plantations in Panxi area by performing full-length 16S rRNA gene and ITS amplicon sequencing using PacBio Sequel. The results showed that the application of titanium ions significantly altered the composition and structure of soil microbiota. Root irrigation with titanium ions in pitaya garden, the diversity of soil fungi was significantly reduced. Although there was no statistically significant difference, bacterial diversity also declined. While, the foliar spray of titanium ions on grapes greatly reduced the soil microbial diversity. Moreover, the soil microbiota had a core of conserved taxa, and their relative abundances were significantly altered by titanium ions. Moreover, titanium ions enhanced the cooccurrence relationships and probably improved the stability of the soil microbial community. Our results highlight the different responses of bacterial and fungal communities to titanium ions and sites and provides a basis for the application of titanium ions in plant farming.

Introduction

Global agriculture has to increase food production to cope with rapid population growth, while reducing the use of factory fertilizers and chemical pesticides [1, 2]. For improving agricultural production, and establishing sustainable and eco-friendly agriculture, some beneficial methods have been explored, such as applying new agricultural growth regulators, keeping soil micro-ecological balance and developing probiotics, etc. [3–5].

Microbes are closely related to plants and play key roles [6]. Microbes can improve soil by decomposing organic matter and fixing nitrogen, and had many beneficial effects on plant, such as promoting the absorption of the main nutrients from the soil (nitrogen, phosphorus and potassium, etc.), secreting plant hormones to promote plant growth, and producing metabolites to help plants resist biotic and abiotic stresses [7–9]. However, despite their importance to plants, our knowledge of microbes is still very limited [10, 11]. To harness agricultural microbes for food security, it is necessary to better understand how agricultural systems (crops and tillage practices) interact to form microbial communities [9, 12, 13].

Titanium is a transition metal, mainly in the form of ilmenite and rutile, widely distributed in the crust and lithosphere. Some researchers have evaluated the effects of titanium ions on plant growth and development [14]. Titanium ions can promote the crop growth and development by stimulating some enzyme activities, increasing chlorophyll content and photosynthesis [15]. Moreover, it was found that Nanoparticle TiO₂ promoted plant growth by affecting microRNA expression [16]. And it can extend the vase life of cut flowers [17]. However, titanium ions act on soil microbial community remains unclear.

Pitaya (*Hylocereus polyrhizus* sp.) is a novelty fruit with high nutrient content and drought tolerance, which is planted in tropical or subtropical areas [18, 19]. Pitaya is a landmark product and one of the

supports of agricultural industry in Panzhihua City, Sichuan Province, China. Due to the suitable climate, the pitaya in Panzhihua area is less affected by diseases, freezing and typhoons compared with other producing areas in China.

Crimson Seedless grape (*Vitis vinifera* L.) is popular with consumers all over the world because of its unique crisp and sweet taste [20, 21]. As also, it is a landmark product in Xichang area, which is a suitable area for the grape variety planting, with high planting density, high yield and good economic benefits. Xichang is adjacent to Panzhihua, they are usually collectively referred to as Panxi region in China.

In this study, we conducted field surveys to detect whether the application of titanium ions to crops would affect soil microbial composition and diversity by PacBio Sequel full-length amplicon sequencing analysis. Our research aims to explain the role of titanium ions in promoting plant growth from the perspective of soil microorganisms, so as to further understand its application in agriculture.

Materials And Methods

Site description and experimental design

This study was conducted in the Panxi region, Panzhihua and Xichang cities, Sichuan Province, Southwest of China. Panzhihua has a humid subtropical monsoon climate and Xichang has tropical plateau monsoon climate. The two places are characterized by abundant sunshine, abundant rain and concentrated precipitation. The average annual sunshine duration is about 2,500 hours and the average annual precipitation is 1000 mm.

Titanium ions preparation: Anti-hydrolysis stabilized ionic titanium (ASIT, Patent No. PCT/US8308840B2) was provided by Tigrow (Tianjin) Science and Technology Ltd. It is same as we used in the previous study (Li et al, 2018) and the inventory concentration of titanium is 4000 mg/L.

The pitaya (*Hylocereus polyrhizus* sp. var. Panxi Dadi-red), grow on a farm in rural areas of Panzhihua City (101°72'E, 26°59'N), which was planted in 2014. In this farm, the soil traits and the management methods are the same. Four times of root irrigation with diluted titanium ions solution (concentration 6-8mg/L) were performed (named as HpTi) within a year, respectively before winter (2018) and next year after the beginning of spring, flowering stage, and fruit growth stage (2019). The adjacent control group was irrigated with water at the same times (HpCon), respectively.

The vine (*Vitis vinifera* L. var. crimson seedless grape), planted in 2018, grow on a farm (102°14'E, 27°45'N) of Xichang city with the same soil characteristics and the same managements. Different from pitaya, the treatment group (VvTi) were foliar sprayed with titanium ions (concentration 3-4 mg/L) four times a year, after winter pruning (2018) and next year after germination, flowering, initial fruiting (2019). Distinguishingly, the control group (VvCon) were foliar sprayed with same amount of water.

Sample collection

Each group was sampled with six repeats, each repeat was randomly selected at 5x5m point, and the 10-20 cm soil layers were taken. We adopted the three-point sampling method, briefly triplicate soil samples from each point were taken and mixed them as a repetition. For each point 6 equally spaced repetitions were performed, for a total of 24 samples. All the soil samples were collected in November, 2019. Fresh soil samples were sieved with 2 mm mesh to remove rocks, plant residues and impurities, and then divided into two parts. One part was for DNA extraction immediately, and the other was stored at -80°C.

DNA extraction and amplicon sequencing

Total DNA was extracted from 0.5 g of each soil sample using the PowerSoil® DNA Isolation Kit according to the instructions of the kit (MO BIO Laboratories Inc., CA, USA). The isolated DNA is diluted ten times to SynergyHTX enzyme marker (Gene Company Limited) to check the quality and concentration of DNA.

The microbial diversity is mainly carried out basing on the 16S rRNA region for bacteria and on the internal transcriptional region (ITS region) for fungi. The total genomic DNA was amplified using primers specific to bacterial 16S full-length primers 27F and 1492R (27F: AGRGTTTGATYNTGGCTCAG and 1492R: TASGGHTACCTTGTTASGACTT) or the fungal ITS full-length primers ITS1 and ITS4 (ITS1: F-CTTGGTCATTTAGAGGAAGTAA and ITS4: R-TCCTCCGCTTATTATTATGC). Meanwhile, for each sample, the forward and reverse primers are tailed with the sample-specific PacBio barcode sequence for multi-channel sequencing. Barcode primers were chosen to reduce chimerism formation [22].

PCR products were quantified with ImageJ according to the electrophoresis results, and were recovered and purified with 0.8X magnetic beads. High-through sequencing was performed using the PacBio Sequel platform at Biomarker Technologies Company (Beijing, China). PacBio sequence data for 16S rRNA gene and ITS are available in the NCBI Sequence Read Archive (SRA) under the BioProject ID PRJNA699417.

Data processing

CCS (Circular Consensus Sequencing) sequence was obtained by correcting the original subreads (SMRT link, version 8.0). Then, Lima v1.7.0 software was used to identify different samples according to barcode, and Cutadapt V2.7 (error rate 0.2) was used to identify forward primer and reverse primer, and CCS sequence without primer was discarded. Finally, CCS sequence length was filtered, and sequences that did not meet the length threshold (16S: 1200bp-1650bp; ITS: 300bp-1000bp) were discarded.

Usearch software was used to cluster Tags at 97% similarity level, obtain operational

taxonomic unit (OTU), and conduct taxonomic annotation of OTU based on Taxonomy database of Silva 132 (bacteria) and UNITE 8.1 (fungi). Then, the statistics of the community composition of each sample was made at various classification levels (phylum, class, order, family, genus, species), and generate species abundance tables by using QIIME software.

The alpha diversity index of each sample was evaluated using Mothur (Version V. 1.30) software. Using Mothur software and R Language tools, the Shannon diversity Index dilution curve was drawn according to the Shannon index of each sample at different sequencing depths. The index reflecting the microbial diversity of each sample.

Principal coordinate analysis (PCoA) was performed using the OmicShare tools (<http://www.omicshare.com/tools>). Upset plot analysis of OTUs was performed using the OmicStudio tools at <https://www.omicstudio.cn/tool>.

Network analysis

For the in-depth characterize the effect of titanium application on occurrence and co-exclusion patterns of soil bacterial and fungal, we performed Spearman rank correlations (R version 3.6.1) on the relative abundance of OTUs at all groups ($\rho > 0.8$, $p\text{-value} < 0.001$). All networks were visualized with the *Fruchterman-Reingold* layout permutations in *igraph*. Correlation analysis was performed using the OmicStudio tools at <https://www.omicstudio.cn/tool/62>.

Statistical analyses

Wilcoxon test was used to determine the significant differences in alpha diversity among different groups. A permutation multivariate analysis of variance (PERMANOVA) was performed based on different matrices to determine whether beta diversity differed significantly between groups. Differences in the relative abundance of phyla and family/genera were corrected by one-way ANOVA and Storey-FDR multiple tested for P values. P value less than or equal to 0.05 indicated significant difference.

Results

Overview of the soil microbiome

For each sample, six repetitions were collected, for a total number of 24 samples in four groups. A total of 170,115 and 192,199 CCS sequences were obtained from the 16S rRNA gene and ITS sequencing of the four groups of samples by the PacBio Sequel, respectively. Of them, 143646 and 183025 effective CCS were obtained after identified PacBio barcode sequences, quality-filtering, and removing chimera, respectively. Each sample sequence numbers of bacteria ranged from 3,790 to 7,058 with a median of 6,684, and the average read length was 1,457 bp (median of 1,457 bp). Each fungal sample sequences ranged from 7,057 to 7,831 with a median of 7,669, and the average read length was 631 bp (median 620 bp).

These CCS sequences were divided into 1,624 bacterial OTUs and 1,080 fungal OTUs. Each sample contained 424 to 885 bacterial OTUs and 214 to 450 fungal OTUs. Of the 1,624 bacterial OTUs, 644 were shared among the four samples, 153 were specific to pitaya groups (Hp) and 217 were specific to grape soils (Vv) (Fig. 1a). There were 194 common fungal OTUs were found in all the four soils, meanwhile 193 in the soil planted pitaya and 82 in grape soils (Fig. 1b).

Microbiota diversity is conditioned by site, cultivation and titanium ions

The Shannon and Chao1 indexes of bacteria ranged from 7.71 to 8.89 and 635.58 to 1152.52, while those of fungi ranged from 5.40 to 7.34 and 217 to 476, respectively (Fig. 2). In the pitaya soil, there were no significant differences ($p>0.05$) in the Shannon and Chao1 indexes of bacterial communities between the HpCon and HpTi groups (Fig. 2a). However, the Shannon indexes of fungal communities of HpCon (Fig. 2b) were higher than that of HpTi ($p<0.05$). Moreover, in the grape soil, the Shannon indexes of bacterial communities and Chao1 indexes of fungal communities were significantly decreased after using titanium ($p<0.05$).

The PCoA (Fig. 3) showed that the soil community were distinct. The four groups were separated, and the repetitions of each group clustered together. The difference between communities were significant ($p=0.001$) in both bacterial and fungal as PERMANOVA showed. In addition, the two agroecosystems of pitaya and grape were separated and the distance within a same site was relatively close. For subsequent analyses, the data for using titanium ions in pitaya and grape were discussed separately.

The microbiome structure is altered by location, cultivation and titanium ions

The change of bacterial community structure

The structure and richness of bacteria were intensely altered by titanium. In the pitaya soil, the bacterial communities were composed of 25 (HpCon) and 27 (HpTi) phyla, respectively. The seven most abundant bacterial phyla (Fig. 4a) were *Proteobacteria* (mean relative abundance: 39.2% in HpCon and 46.0% in HpTi), *Acidobacteria* (16.3% and 14.4%), *Bacteroidetes* (10.1% and 10.3%), *Planctomycetes* (12.5% and 8.2%), *Actinobacteria* (7.0% and 4.5%), *Verrucomicrobia* (5.7% and 5.8%) and *Gemmatimonadetes* (4.7% and 4.1%). These phyla represented 95.6% and 93.2% of sequences from HpCon and HpTi, respectively. The relative abundance of *Proteobacteria* and *Rokubacteria* (0.3% in HpCon and 2.3% in HpTi) were significantly higher and *Planctomycetes* were significantly lower in the soil using titanium ions ($p<0.05$).

Further classified in the family level, there were 24 families with average relative abundance $>1\%$ were identified in the pitaya soils, accounted for 70.8% and 63.6% in HpCon and HpTi respectively.

Chitinophagaceae (7.6% in HpCon and 8.5% in HpTi, belong to *Bacteroidetes*), *uncultured_bacterium_c_Subgroup_6* (8.0% and 6.6%, *Acidobacteria*), *Rhodanobacteraceae* (0.6% and 9.4%, *Proteobacteria*), *Nitrosomonadaceae* (4.9% and 3.8%, *Proteobacteria*) and *Gemmatimonadaceae* (4.4% and 3.9%, *Gemmatimonadetes*) were the most dominant bacterial families (Fig. 5a). The relative abundance of *Rhodanobacteraceae* were dramatically ($p<0.001$) higher in the applied-titanium soil.

For the soil samples planted grape, at the phyla level, all the sequences were classified into 27 phyla. Distribution of the 10 most abundant phyla were showed in Fig. 4a. *Proteobacteria* (45.6% in VvCon and 45.6% in VvTi) was the most abundant phyla, followed by *Acidobacteria* (14.2% and 10.8%), *Planctomycetes* (11.1% and 10.3%), *Gemmatimonadetes* (8.1% and 12.7%) and *Bacteroidetes* (8.1% and

6.6%). The abundances of *Acidobacteria* were significantly lower in VvTi than that in VvCon, conversely *Gemmatimonadetes* showed higher abundances after using titanium ($p<0.05$).

As for the family level in grape soils, there were 232 families were identified, although 94 of which were uncultured. Bacterial communities were dominated by *uncultured_bacterium_c_Subgroup_6* (9.9% in VvCon and 7.9% in VvTi), *Nitrosomonadaceae* (9.0% and 13.5%) and *Gemmatimonadaceae* (5.2% and 9.8%). Fellow 20 dominated family with mean relative abundance $>1\%$ accounted for 64.6% and 66.2% of the sequences in VvCon and VvTi respectively. The abundances of *Nitrosomonadaceae* and *Gemmatimonadaceae* were remarkably higher in the titanium-applied soil than that control group ($p<0.01$). In contrast, the abundance of *Chitinophagaceae* (3.7% and 1.8%, *Bacteroidetes*), *Comamonadaceae* (1.7% and 0.6%, *Proteobacteria*), *Xanthomonadaceae* (3.3% and 1.3%, *Proteobacteria*) and *TRA3-20* (3.7% and 2.5%, *Proteobacteria*) were substantially lower in the titanium-applied soil ($p<0.05$).

The change of fungal community structure

In the pitaya-planted soil, a total of 13 fungal phyla were identified. The most important phyla accounted for 98.3% of the sequences in each group, including *Ascomycota* (51.9% in HpCon and 56.7% in HpTi), *Basidiomycota* (23.5% and 12.3%), *Mortierellomycota* (11.0% and 14.7%), *Chytridiomycota* (2.7% and 9.0%), *Glomeromycota* (8.6% and 2.9%), and *Rozellomycota* (0.6% and 2.8%). The relative abundance of *Chytridiomycota* increased significantly ($p<0.05$) and the *Basidiomycota* and *Glomeromycota* decreased significantly ($p<0.05$) in the titanium-applied soil.

At the genus level, a total of 237 genera were identified, with 39 genera having a relative abundance greater than 1% in at least one sample, while 22.3% of sequences could not be classified to unknown genera. Furthermore, the dominant genera of fungal communities were *Mortierella* (10.8% in HpCon and 14.5% in HpTi, belong to *Mortierellomycota*), *Fusarium* (12.8% and 7.3%, *Ascomycota*), *Penicillium* (2.6% and 11%, *Ascomycota*) and *Thanatephorus* (5.7% and 3.4%, *Basidiomycota*). The relative abundance of *Penicillium* and *Cladosporium* (1.0% and 3.1%, *Ascomycota*) dramatically increased response to titanium ions, in contrast, the relative abundance of *Fusarium* and *Purpureocillium* (3.5% and 0.99%, *Ascomycota*) significantly decreased ($p<0.05$).

In the grape-planted soil, the fungal community were composed of 11 phyla and other unclassified phyla (3.1%~4.1%) (Fig. 5). The five most abundant phyla were *Ascomycota* (51.4% in VvCon and 34.2% in VvTi), *Basidiomycota* (21.2% and 19.1%), *Mortierellomycota* (16.6% and 25.0%), *Chytridiomycota* (2.8% and 14.4%), and *Glomeromycota* (3.5% and 3.1%). The relative abundance of *Chytridiomycota* and *Rozellomycota* were significantly higher in the soil using titanium ions ($p<0.05$) and the abundance of *Ascomycota* and *Basidiomycota* were significantly lower ($p<0.05$).

As for the genus level of fungi, about 69.8% to 82.0% of sequences were classified into 222 genera, and the 10 most abundant genera are shown in Fig. 4. The communities were dominated by *Mortierella* (16.0% in VvCon and 25.0% in VvTi), *Fusarium* (7.0% and 7.9%), *Botryotrichum* (7.0% and 1.3%),

Ascomycota), *Leucoagaricus* (0.22% and 6.4%), *Colletotrichum* (4.4% and 1.7%), *Wallemia* (5.2% and 0) and *Cladosporium* (2.2% and 1.5%). The relative abundance of *Leucoagaricus* and *Penicillium* (0.7% and 1.6%) were elevated significantly in the titanium-applied soil and the *Dactylonectria* (3.3% and 0.14%) were reduced ($p < 0.001$).

Titanium ions enhanced the cooccurrence relationships among the bacteria and fungi

The co-occurrence and co-exclusion network of OTUs in bacteria and fungi in soil community were analyzed (Fig. 6). We found that the co-occurrence was dominant in the interaction network. Fungi with high abundance were important nodes in the interaction network, including *Ascomycota*, *Glomeromycota*, *Chytridimycota* and *Basidiomycota*, etc. Co-occurrence relationship exists not only in the interior of high abundance phylum, but also between high abundance and other low abundance phylum, as well as between bacteria and fungi. In pitaya soil interaction network, the co-occurrence relationship of HpCon accounted for 67%, while that of HpTi accounted for 81%. As for grape soils, the proportion of lines representing co-occurrence increased from 67% to 70% after titanium application.

Discussion

To study soil microbial diversity, community composition and interaction network, we analyzed 24 soil samples from a Xichang vineyard and a Panzhihua pitaya orchard by the PacBio full-length 16S rRNA gene and ITS amplicons sequencing.

Full-length amplicon sequencing has been considered to improve the species-level resolution compared with the short-read sequencing [23, 24]. However, due to the limited existing research results and databases, long-read sequence may make it difficult for many uncultured microorganisms to determine their taxa in a complex system. In this study, the sequences classified as uncultured bacteria accounted for more than half (51.8%) at the genus level and 78.7% at the species level. As for fungi, the unclassified-sequences occupied 21.1% at the genus level and 41.4% at the species level. In soil, microorganisms are complex and diversity, exiting a large number of unidentified microbes. Even so, compared with the short-read of 16S rRNA gene and ITS amplicon sequencing, the taxonomic resolution is more accurate. We have reason to believe that with the improvement of the accuracy and high-resolution algorithms, as well as the amplification of database, full-length amplicon sequencing technology will be as a potential tool to discriminate closely related species [22].

Titanium ions has plant growth regulating activity, which has been widely used in agriculture as fertilizer, but its effect on soil microbes is still a mystery. In our previous study, we determined titanium ions affected gene expression of *Medicago sativa*, and found titanium ions can improve plant adaptability to the environment (Wei et al., 2019). And we also found that titanium ions can prolong the longevity of fresh cut flowers by inhibiting bacteria [17]. Therefore, we speculated that titanium ions may also affect soil microorganisms.

Our findings presented that microbiota diversity was conditioned by titanium ions. In the two regions, the Shannon and Chao1 index of bacteria and fungi in the titanium ions treatment groups were both lower than those in the control groups, indicating that titanium ions reduced the species diversity and uniformity. This is consistent with our previous findings that titanium ions inhibit bacteria and reduce the diversity in vase solutions[17]. Although titanium ions inhibited the existence of some microorganisms, the dominant microbes were richened obviously. Similar to our findings, sorghum rhizosphere effects have been found to reduce soil bacterial diversity by recruiting specific bacterial species [25].

Nitrosomonadaceae were richness both in the two type of soils. Interestingly, we detected that the Nitrosomonadaceae taxa were mainly distributed of Ellin6067 and MND1 at the genus level. The two genera were abundant in pitaya soil, while only MND1 enriched in grape soil. They are nitrifying types of bacteria which metabolize ammonia (NH_3) to nitrate (NO_3^-), thereby obtaining energy for themselves and providing nitrogen to plants [26, 27]. Titanium ions enriched *Nitrosomonadaceae* remarkably in the VvTi compared with VvCon, indicating that titanium ions are probably profitable to improving soil nitrogen. In addition, the relative abundance of the dominant fungus *Mortierella* was significantly increased by titanium application. *Mortierella* has been reported to be effective as a biocompound for remediation of heavy metals in soil [28]. And *Mortierella* may be a potential probiotic involved in regulating the rhizosphere microbial community of plants [29]. Thus, the increase of the potential probiotics may be beneficial to plants when the use of titanium ions.

In the pitaya planting soils, the number of fungi *Fusarium* in the titanium ions treatment group (HpTi) was reduced at the genus level compared with the control group (HpCon). *Fusarium* is generally considered a potential plant pathogen, which can cause wilt disease in many kinds of crops, such as cotton, watermelon, mango and banana, leading to great yield reduction and economic losses worldwide [30, 31]. The inhibition of titanium ions on *Fusarium* may be a reason for reducing the susceptibility of crops to related pathogens. We also had investigated the growth status and disease resistance of two kinds of plants. It was found that plants treated with titanium ions had significantly increased resistance to a variety of pathogens, and the application of microbicides was reduced by about half compared with the control (data not show). Consequently, the application of titanium ions can be evaluated as safe for agriculture.

Further analysis of the microbial interaction network indicated that fungi were the important network nodes in soil microbial associations and may play a vital role in recruitment. It is speculated that titanium ions affect the whole community by changing the abundance of key microorganisms in the network. In a complex network microbiome, microorganisms cooperate to provide each other with key nutrients needed for growth and survival. This co-occurrence plays an important role in the stability of microbial ecosystem. Titanium ions promote the co-occurrence in the network, which may make the soil microecological structure more stable. It should be emphasized that the interaction networks visualize co-occurrence and co-exclusion between taxa, but do not necessarily reflect the direct interaction between taxa. Nevertheless, we can use the networks as a useful tool to explore whether the key nodes or

abundant species directly affect other microbial members [12]. Furthermore, this could make us better understand the specific mechanism of the complex microbial community.

Meanwhile, from the results of beta diversity analysis, it can be revealed that the distance between microbial communities of different plants in different areas is larger compared with titanium ions treatment. Presumably, the differences of geographic location and cultivation were the important driver of soil microbiome structure [32]. Moreover, the effect of titanium ions on the community varied with different application method, so the change in microbiome did not show convergence. Because of the traits of the plant itself, the leaves of pitaya are not suitable for foliar spraying, so the method of root irrigation was selected. The root irrigation exhibited a greater impact on soil microbial community, probably due to direct contact with titanium ions.

Conclusions

In conclusion, the four groups of soil planted with pitaya or grape in Panxi area shared a core of taxa, including bacteria of *uncultured_bacterium_c_Subgroup_6*, *Nitrosomonadaceae* and *Chitinophagaceae* and fungi of *Mortierella*, *Fusarium* and *Leucoagaricus*, but the relative abundance of these taxa was different in each soil. The differences in soil and microbial community structure between pitaya and grape come from environment and plant itself. The use of titanium ions altered the soil communities of bacteria and fungi in difference agroecosystems. Titanium ions inhibited or enriched some microorganisms, especially some high-abundant taxa. Although the diversity reduced and the structure of communities altered, titanium ions enhanced the cooccurrence relationships and improved the stability of the community. The soil microbial interaction network may provide direction for further research. However, it is still a major challenge to understand the specific association mechanism between complex soil microorganisms and agricultural management. Our research explored the change of soil microorganisms by titanium ions application through full-length amplicons sequencing, and provided basis for further agricultural applications.

Declarations

Author Contributions

Yuan He designed and performed most of the experiments, analyzed data, drafted, and revised the manuscript. Xin-Yi Hou, Cai-Xia Li and Yan Wang participated in preparing and treating samples. Xin-Rong Ma conceived and designed research plan and experiments and supervised, critically revised, and complemented the writing. All authors read and approved the final manuscript.

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Conflict of Interest

The authors state that they have no competing financial interests or personal relationships.

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Figures

Figure 1

Upset and Venn plot of bacterial (a) and fungal (b) communities of the four group of soil samples. Upset plot consists of three parts: the horizontal bars on the left-hand side which shows number of OTUs, the exclusive intersections set (vertically connected filled dark circles) in the center of the plot, and the top vertical bar chart which shows the intersection numbers. Different colors in the bars correspond to four different groups of soil samples.

Figure 2

Alpha diversity of the soil samples. Chao1 and Shannon indices of bacteria (a) and fungi (b) are presented. Group differences were determined (Wilcoxon rank-sum test, with significance noted on the figure, $p < 0.05$).

Figure 3

Bata diversity of bacterial (a) and fungal (b) communities. The beta diversity was based on the Bray–Curtis distance of OTUs.

Figure 4

Bacterial (a) and fungal (b) communities of soil planted pitaya and grape.

Figure 5

Relative bacterial and fungal abundance histogram of soil microbial exposed to titanium treatments and control. (a: family for Hp, b: family for Vv, c: genus for Hp, d: genus for Vv). The number of asterisks indicates significant differences between treatments according to a one-way ANOVA and FDR (False Discovery Rate) adjustment ($p < 0.05$): * $0.01 < P \leq 0.05$; ** $0.001 < P \leq 0.01$; *** $P \leq 0.001$.

Figure 6

Bacterial and fungal co-occurrence and co-exclusion networks as affected by titanium-applied in pitaya and grape agroecosystems. Nodes represent individual OTUs; edges represent significant positive (pink) and negative (green) Spearman correlations ($\rho > 0.6$, $P < 0.001$). Node size is positively correlated with degree (the number of edges attached to a node). (a: HpCon left, HpTi right; b: VvCon left, VvTi right)