# Analysis of microbial changes in the rhizosphere of unplanted and planted *Atractylodes lancea* via high-throughput sequencing

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**Research**

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## Abstract

# Background

*Atractylodes lancea* is a traditional Chinese medicine, which typically requires more than 3–4 years of continuous cropping to obtain the underground medicinal components. With continuous cropping years, the quality and yields of *A. lancea* medicinal materials decrease, while pests and diseases increase. These aspects are intimately correlated with rhizospheric microorganisms.

# Methods

This research paper employed high-throughput sequencing for its detection in soil that was cultivated for three years and never cultivated to clarify the relationship between the microbial diversity of the rhizosphere and continuous *A. lancea* cropping.

# Results

The rhizosphere microbial community was altered following the continuous cropping of *A. lancea*. The bacterial diversity and richness were observed to decrease, while the fungal community diversity increased, and richness decreased. The total OUTs of the soil bacteria and fungi of unplanted and planted *A. lancea* were 59.58% and 37.65%, respectively. At the phylum level, the relative abundance of Proteobacteria, Gemmatimonadetes, Acidobacteria and Chloroflexi decreased, whereas the relative abundance of Mortierellomycota increased. At the genus level, *Bradyrhizobium*, *Striaticonidium*, *Dactylonectria*, *Sphingomonas*, *Burkholderiaceae*, *Rhodanobacter*, *Arthrobacter*, *Scleroderma*, *Mortierella* and *Penicillium* were significantly different between the two sample groups.

# Conclusions

Our results revealed that following the cultivation of *A. lancea*, the rhizospheric microbial community was altered. This study preliminarily determined the

## Background

*A. lancea* is a traditional bulk medicinal material of China, which is derived from the dried rhizomes of *Atractylodes lancea* (Thunb.) DC. and *Atraetylodes chinensis* (DC.) Koidz. [1]. It is primarily distributed across Jiangsu, Shanxi, Hebei, as well as Inner Mongolia Provinces in China. As it has the anti-inflammatory, anti-cancer effects and possesses beneficial pharmacological efficacy in the treatment of digestive system diseases and rheumatism, lowering blood sugar and more, it has been used extensively in traditional Chinese medicine [2].

Studies have revealed that long-term monocultures can reduce crop yields and adversely affect quality [3]. Most of the plants that contain medicinal rhizomes have continuous cropping obstacles, for example, *Panax notoginseng (Burk.)F.H.Chen* and *Panax ginseng C. A. Mey.* [4]. Long-term continuous cropping seriously impacts the physical and chemical properties of soil, as well as the composition of soil microbial communities. Existing researches have indicated that the continuous cropping difficulties in soils are primarily caused by changes in the structures of biological flora, aggravation of infectious diseases and insect pests, as well as the allergic autotoxicity of medicinal plants [5]. Among these, the modification of biological flora plays a very important role, as soil microorganisms affect crop growth. Soil microorganisms can decompose organic matter, release various nutrients, affect plant growth, enhance stress resistance and overall adaptability [6]. For example, *Proteus*, *Scleroderma*, and *Actinomyces* are associated with disease suppression. Changes in soil resident microorganisms can reflect changes in soil quality. Furthermore, different types of soil and vegetation can generate variable different biological flora, which is related to the complexity of soil microbial interactions, including those of microorganisms with soil and plants [7]. The beneficial interactions between the host and its microbiome are responsible for maintaining the health of the entire organism. Disease is often associated with microbial abnormalities [8–9], and microbial diversity has been identified as a key factor in the prevention of disease. *A. lancea* is a perennial plant, and continuous cropping has become a major challenge that affects its cultivation. In practice, it was found that the effects of continuous cropping was mainly manifeste in terms of poor growth, reduced quality, and increased occurrences of pests and diseases [10]. During the continuous *A. lancea* planting process, the above situation will also appear, resulting in the instability of yields and quality. However, there are no reports that articulate changes to the microbial community following the continuous cropping of *A. lancea*. Therefore, an analysis of the microbial species and community structures of the soil after the cultivation of *A. lancea* will assist with the provision of certain data that support the resolution the issues associated with continuous cropping of *A. lancea*.

The analysis of the rhizospheric soil microorganisms of *A. lancea* encompassed traditional research methods including plate culture, biomarker, biochemical, molecular biological methods and more [11]. However, these research methods have several limitations for the study of microorganisms, for instance, the types of identified microorganisms are often incomplete, whereas the methods involved are quite complex. Compared with traditional research methods, high-throughput sequencing is efficient, accurate, and widely employed in various disciplines [12]. The use of high-throughput sequencing to investigate the rhizospheric microorganisms of *A. lancea* provided an opportunity for the further analysis of important bacteria involved in the continuous cropping challenges of *A. lancea*. For this paper, high-throughput sequencing technology was employed to amplify and sequence the microbial flora in the rhizosphere of *A. lancea* and soil samples that were not planted with *A. lancea*. The relationship between the continuous cropping of *A. lancea* and soil microorganisms is discussed to provide a specific reference for a potential solution to the continuous cropping of *A. lancea*.

# Material And Methods

# Soil samples and reagents

The experiment was conducted in Xialiang Town, Zhashui County, Shaanxi Province, China (109 ° 10'52.57 "E, 33 ° 39'52.704" N). Rhizosphere soil samples of *A. lancea* at different growth stages, up to three years as well as soils that were never planted with *A. lancea* were collected. These two soil samples were designated ZX3 and ZXCK, respectively. When a soil sample was extracted, we divided the plot involved into three sub plots. Each plot was 10 m wide x 10 m long, and the plots were 5 m apart. In each plot, we collected five rhizosphere soils in an S-shape at a collection depth of 15–30 cm. Subsequent to extracting samples, they were transferred to the lab in sterilized self-sealing bags at a low temperature. The root system and soil mass were then removed and stored in a -refrigerator at -80℃ after sieving for future use.

# Extraction And Purification Of Soil Genomic DNA

A soil genomic DNA extraction kit (Tiangen Biochemical Technology Company.) was used to extract the genome of the sample, and the DNA was extracted according to the manufacturer's instructions. An agarose gel was prepared and electrophoresed (25 V, 8 h), after which the gel containing the band that was located was removed, and the DNA was retrieved using a DNA recovery kit. A UV spectrophotometer (Nanodrop 2000) was employed to determine the DNA concentration, OD260 and OD280 values. The OD260/OD280 values should be between 1.7–1.9, and stored at -20 °C following normal testing.

# PCR Amplification

The extracted DNA samples were diluted to an appropriate level at 1.0 ng. μL-1 according to the concentration, following which the diluted DNA was employed as a template to amplify the gene sequences of rRNA and ITS rRNA using specific primers with Barcode. The primer of the 16SV4 region was 515F-806R, whereas, the primer of the ITS1 region was ITS1F-ITS2, and the primer of the ITS2 region was ITS2-3F-ITS2-4R.

The PCR products were purified, while 2% agarose gel was used for electrophoresis detection. The mixing of the same quantities proceeded according to the PCR product concentration. After thorough mixing, a 1 × TAE concentration of 2% agarose gel was used for electrophoresis to purify the PCR products.

# Library Construction And Sequencing

An Ion Plus Fragment Library Kit 48 rxns library construction kit from Thermofisher was employed to construct the library, which was quantified by Qubit and qualified for library detection. Subsequently, IonS5TMXL was used for sequencing.

# Data analysis

Sequencing data processing Cutadapt was initially employed to perform low-quality partial cuts on the reads, followed by separating the sample data from the reads obtained according to Barcode, and then cutting the Barcode and primer sequences to obtain the original data (Raw Reads). The above-mentioned Reads were processed for removing the chimera sequence [13]. The Reads sequence was compared with the species annotation database to detect the chimera sequence https://github.com/torognes/vsearch/ [14], and finally the chimera sequence was removed to obtain the final valid data (Clean Reads).

OTU clustering and species annotation: Using Uparse software [15] to cluster the Clean Reads of all samples, the sequences were clustered into OTUs with an identity coefficient of > 97%, while simultaneously selecting representative sequences of OTUs. Species annotation analysis (set threshold is 0.8~1) was performed to obtain taxonomic information. Multi-sequence alignment was performed using MUSCLE software [18] (Version 3.8.31), and the homogenization was performed with the smallest quantity of data in each sample as the standard for subsequent sample diversity analysis (Alpha, Beta).

Alpha diversity analysis: The Shannon index was calculated using Qiime software (Version 1.9.1), and the analysis of differences between groups of Alpha diversity indices was performed using R software (Version 2.15.3). Parametric and non-parametric tests were performed respectively.

## Results

# Dilution curve

The bacterial and fungal dilution curves for the three replicates of samples ZX3 and ZXCK are shown in Fig. 1. As the dilution factor increased, the curve became flatter, which indicated that further sequencing would result in only a small number of new species (OTUs). As the number of bacterial and fungal sequences in the rhizospheric soil samples of *A. lancea* increased, the dilution curve gradually revealed a flat trends, as the sequence results essentially covered all species information in the sample.

# Diversity Analysis

# OTUs classification

The statistics on bacterial and fungi OTUs revealed that the bacteria detected in the two groups of samples covered 37 phyla, 187 families, and 395 genera. The number of OTUs detected in ZX3 was 3,696, whereas the number of OTUs detected in ZXCK was 3,849. The fungi detected in the two groups of samples covered 15 phyla, 190 families, and 284 genera. The number of OTUs detected in ZX3 was 2,429, and the number of OTUs detected in ZXCK was 2,354 (Table 2).

Table 1
Species statistics of different classification levels of soil bacterial and fungal microbial communities. **ZX3** and **ZXCK** represent the rhizospheres from the consecutive three-years cultured soils, and those that had never been planted with *A. lancea* respectively.

| Microbe | Sample name | Kingdom | Phylum | Class | Order | Family | Genus | Species | OUTs |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria | ZX3 | 3 | 37 | 48 | 105 | 186 | 394 | 259 | 3696 |
| | ZXCK | 2 | 37 | 49 | 105 | 187 | 395 | 246 | 3849 |
| Fungi | ZX3 | 2 | 15 | 44 | 98 | 190 | 284 | 305 | 2429 |
| | ZXCK | 2 | 15 | 41 | 85 | 170 | 284 | 286 | 2354 |

Species annotations were made on bacterial and fungal OTUs, and it was found that species could be identified at different taxonomic levels such as phylum, class, order, family, genus, and species. It can be seen from the table that in the two groups of samples, the OUTs detected by bacteria were 3,696 and 3,849, whereas the OUTs detected by fungi were 2,429 and 2,354 respectively. Compared to the ZXCK sample, the OUTs of the bacteria in ZX3 decreased, whereas the OUTs of the fungi increased.

A Venn diagram can intuitively reflect the difference and overlap of the composition of the soil bacterial communities OUTs between the two groups of samples.

As can be seen from Fig. 2a, the number of unique OTUs of ZX3 and ZXCK was 765 and 950, whereas the number of OTUs shared by ZX3 and ZXCK samples was 2,528, and the shared OUTs occupied 59.58% of the total OUTs. It can also be seen from Fig. 2b that the number of OTUs shared by the ZX3 and ZXCK samples was 1,185, and the shared OUTs occupied 37.65% of the total OUTs. The number of unique ZX3 OTUs was 1,050, unique ZXCK OTUs was 912.

Table 2
Alpha diversity index table for bacteria and fungi. The Chao1 index and Ace index represent the community richness, whereas the diversity is represented by the Shannon index and the Simpson index. **ZX3** and **ZXCK** represent the rhizosphere soils from the consecutive three-years cultures and those that had never been planted with *A. lancea*, respectively.

| Microbe | Sample name | Observed species | Shannon | Simpson | Chao1 | Ace |
|---|---|---|---|---|---|---|
| Bacteria | ZX3 | 1969.33 | 8.82 | 0.993479 | 2103.02 | 2135.13 |
| | ZXCK | 2160 | 9.12 | 0.994796 | 2312.6 | 2323.52 |
| Fungi | ZX3 | 1175 | 6.25 | 0.944552 | 1272.3 | 1237.16 |
| | ZXCK | 1158.33 | 6.16 | 0.934756 | 1281.4 | 1237.32 |

# Alpha Diversity Analysis

It can be seen from the bacteria Alpha diversity index table (Table 2), that the Chao1 index of the experimental group ZX3 was 2,103.02 and the Ace index was 2,135.13, which were both lower than ZXCK. Simultaneously, the Shannon index and Simpson index of ZX3 were lower than ZXCK.

The fungi diversity index revealed that the Chao1 index and Ace index of the experimental ZX3 group were lower than those of the control group ZXCK, while the Shannon and Simpson indices of the experimental group ZX3 were higher than that of the ZXCK control group.

# Relative Abundance Analysis

The bacterial species abundance was analyzed at the Phylum classification level (Fig. 3), which showed the top ten species with a relative abundance of bacterial species, which included Proteobacteria, Actinobacteria, Acidobacteria Gemmatimonadetes, Chloroflexi, Bacteroidetes, Verrucobacterium, Firmicutes, Thaumarchaeota and Latescibacteri. The relative abundance of these 10 species in all samples was ≥ 0.7%. The relative abundance ratios of Proteobacteria in samples ZX3 and ZXCK were 38.139% and 38.715%, whereas the relative abundance ratios of Actinobacteria in the ZX3 and ZXCK samples were 21.085% and 18.020%. The relative abundance ratios of Acidobacteria in the ZX3 and ZXCK samples were 20.196% and 22.334%. The relative abundance of the three strains in both samples was > 18%; however, compared to ZX3 and ZXCK, the abundance of the two strains of Proteobacteria and Acidobacteria declined, while the relative abundance of Actinomycota increased.

The species abundance of fungi was analyzed at the level of phylum classification, as shown in Fig. 3, which revealed the relative abundance of bacterial species. They were Ascomycota, Mortierellomycota, Basidiomycota, Mucoromycota, Chytridiomycota, Zoopagomycota, Rhizopusmycota, Glomeromycota, Aphelidiomycota and Neocallimastigomycota. The relative abundance ratios of Ascomycota in the ZX3 and ZXCK samples were 29.602% and 41.530%, whereas the relative abundance ratios of Mortierellomycota in the ZX3 and ZXCK samples were 37.170% and 21.669%. The relative abundance of Basidiomycota in the ZX3 and ZXCK samples were 2.704% and 18.893%. Compared with ZXCK, the relative abundance of Ascomycota and Basidiomycota decreased, and the relative abundance of Mortierellomycota increased. The relative abundances of the three were significantly different in ZX3 and ZXCK.

The bacterial composition of cultivated and uncultivated *A. lancea* soils was significantly different (Fig. 4). The PCoA ranking showed the changes in the bacterial community in the soil prior to and following the planting of *A. lancea* (Fig. 4a). The first principal component (41.6% contribution) and second principal component axis (22.46% contribution) distinguished the bacterial communities in the two groups of samples. Moreover, the distribution of bacterial communities varied for different samples. In particular, the difference between the soil samples planted with *A. lancea* and those without was more obvious (Fig. 4b).

Fungal compositions also differed prior to and following the cultivation of *A. lancea*, which showed variations based on PCoA ordination analyses (Fig. 5a). The first principal component (41.11% contribution) and second principal component axes (28.39% contribution) differentiated the fungal composition of soils planted with *A. lancea* and never planted with *A. lancea.* Furthermore, the distribution of fungal communities varied prior to and following the cultivation of *A. lancea* (Fig. 5b).

# Differential Species Analysis

The differences in bacteria were shown in Fig. 6, where we can see that *Bradyrhizobium* and *Arthrobacter* had the most significant species difference between the two groups of samples, where in the ZX3 sample, the *Bradyrhizobium* had a higher population average. The differences between *Cyanobacteria*, *Sporichthya*, *Candidatus Koribacter*, *Jatrophihabitans* and *Pseudonocardia* were obvious in the two groups of samples.

An analysis of fungal differential species revealed that *Dactylonectria* was significantly different between the two groups of samples. *Aspergillus*, *Humicola* and *Striaticonidium* exhibited significant differences between the two groups of samples.

The ZX3 and ZXCK samples were selected for analysis in the top ten bacterial subordinate levels. The total bacterial abundance of these 10 genera accounted for 73.2% and 76.2% of all detected bacterial levels of the genus level, covering a large portion of the species. The genus name on the abscissa was plotted, as well as the percentage of bacterial species abundance as the ordinate (Fig. 7a). As can be seen from the figure, the richness of *Sphingomonas*, *Solibacter*, *Rhodanobacter*, *Bryobacter* and *Haliangium* increased in the ZXCK sample, whereas *Burkholderiaceae*, *Arthrobacter*, *Gammaproteobacteria*, *Bradyrhizobium*, and *Streptomyces* declined in abundance. Among these, the proportion of *Sphingomonas* in the two groups of samples was 4.6% and 5.7%, *Burkholderiaceae* was 6.3% and 1.0%, *Rhodanobacter* was 3.0% and 2.1%, and the genus of Arthrobacter was 4.5% and 1.7%. The four genera had obvious differences between the two groups of samples.

According to the cluster heat map of the dominant bacteria genera (Fig. 7b), there were some differences in the species of different groups in the same sample; however, the differences between the ZX3 and ZXCK samples were more obvious. Among these, the abundance of *Gammaproteobacteria* and *Streptomyces* were higher in the ZX3 sample, while the abundance of *Rhodanobacter* was higher in the ZXCK sample.

The top ten fungal subordinate levels in the ZX3 and ZXCK samples were selected for analysis (Fig. 8a). The total abundance of the fungal in the ten genera accounted for 67.1% and 50.4% of the fungi species in all detected genera. It can be seen from the figure that the abundance of *Mortierella* and *Pseudogymnoascus* in the ZXCK sample decreased, whereas the abundance of *Scleroderma*, *Fusarium*, *Penicillium*, *Metarhizium*, *Aspergillus*, *Trichocladium* and *Epicoccum* increased. Among these, the proportions of *Scleroderma* in the ZX3 and ZXCK samples were 0.001% and 14.2%, *Mortierella* was 14.2%

and 4.0%, and *Penicillium* was 1.9% and 6.7%, respectively. The fungi of the three genera differed significantly between the two groups of samples.

According to the cluster heat map of the dominant fungi genus (Fig. 8b), *Pseudogymnoascus* and *Mortierella* were more abundant in the ZX3 sample, whereas the abundance of *Epicoccum* and *Scleroderma* were higher in the ZXCK sample.

# Analysis And Discussion

Continuous cropping is a major issue for cultivation of *A. lancea*., which seriously affects the quality of *A. lancea*. It is generally acknowledged that the problems associated with *A. lancea* continuous cropping are intimately associated with rhizosphereic microorganisms. In terms of changes in soil microbial community structures, plant allelopathy and autotoxicity can alter the structures of microorganisms in the soil, which affects the growth of plants [19]. Studies have shown that the root exudates of *C. citratus*, *A. conyzoides* and *B. Pilosa* can decrease the germination rate, root length of seedlings, and seedling heights of radish, rice, and cucumber [20]. The allelopathic substance lycopene in tomato has inhibitory effect on other plants, fungi and insects. Researchers have also discovered that there is an allelopathy phenomenon in *A. lancea*, which often promotes the increase or decrease in the number of one or more types of microorganisms, and can changes microbial structures [21]. Soil microorganisms play a critical role in the prevention of plant diseases. Once a plant is attacked by root pathogens, it can employ microbial aggregates in the soil to prevent infection [22]. An increasing number of studies have shown that diseases involved in different plant growth processes are closely related to rhizosphere microorganisms [23–25]. Following continuous long-term cultivation of plants such as cotton, the soil microbial communities that support many plants will change, thus affecting their growth [26]. Therefore, changes in the populations of rhizospheric microorganisms and their community structures are the most likely reasons for the continuous problems with *A. lancea*.

Following an analysis of the dilution curves of bacteria and fungi, we found that sequencing results can essentially cover all species data in the sample; thus, it was feasible to employ this method to treat samples to reflect the diversity of microorganisms. The six curves of the bacterial dilution curve and the fungal dilution curve did not overlap, which indicated that there were certain differences in the microbial species between different soil samples. The analysis of OUTs and Alpha diversity revealed that following the planting of *A. lancea*, the soil microbial communities changed, the bacterial flora richness was greater than the fungal richness, and the bacteria in the rhizosphere microbial community of the two samples were dominant. Further, the species similarity of bacteria was greater than that of fungi, and the fungi exhibited more significant changes following continuous cropping. After continuous cropping, the diversity and richness of the bacterial communities were reduced; however the richness of the fungi communities was reduced, and the diversity of fungi was increased. Microbiological research by Lanping on soil for planting *A. lancea* indicated that the microbial community structures of bacteria and fungi in the soil rhizosphere for two consecutive years of *A. lancea* were lower than that of the annual samples,

which was similar to the results of our study [27]. This also occurred in plants such as ginseng and peanuts, which both showed a trend of increasing fungi diversity [28–29]; thus, it was speculated that changes in soil resident fungi are one of the main reasons behind the continuous cropping issues.

Through abundance analysis, it was found that the relative abundances of Ascomycota, Mortierellomycota and Basidiomycota were significantly different between the two groups of samples. The relative abundance of Basidiomycota decreased, whereas the relative abundance of Mortierellomycota increased. Studies have shown that Ascomycota and Basidiomycota are important soil resident decomposers, where most Ascomycota are saprophytic bacteria that can decompose many types of recalcitrant organic matter, which play an important role in nutrient cycling [30]. Following continuous cropping, the abundance of Ascomycota and Basidiomycota decreased, and the nutrient cycling in the rhizosphere was affected, which resulted in a decline in the quality of *A. lancea* medicinal materials. Mortierellomycota is mostly saprophytic in soil, a few of which are the mycorrhizal fungi of forest trees. Their abundance was observed to increase, which was presumed to be related to the continuous cropping of *A. lancea*. The differences in bacterial were analyzed, and it was found that *Bradyrhizobium* and *Arthrobacter* were significantly different between the two groups of samples. *Bradyrhizobium* is a rhizobium of the class *Proteobacteria* [31], which can form a symbiotic relationship with host plants and fix the free nitrogen in the ambient atmosphere into forms that host organisms can use, such as ammonia ($NH_3$) or ammonium ($NH_{4+}$), which are intimately related to plant growth [32]. The analysis of different species of fungi revealed that *Dactylonectria* was significantly different between the two groups of samples. Researchers found that *Dactylonectria* fungi are primarily related to plant diseases [33–35]. The change of *Dactylonectria* fungi may be related to the occurrence of pests and diseases of *A. lancea*.

Further analysis of the species in the two groups of samples at the subordinate level revealed that *Sphingomonas*, *Burkholderiaceae*, *Rhodanobacter*, *Arthrobacter*, *Scleroderma*, *Mortierella* and *Penicillium* were significantly different between the two groups of samples. It was inferred that the continuous cropping issues of *A. lancea* radix were related to changes in the bacterial and fungal community structures described above. Research has revealed that *Sphingomonas* has the capacity to degrade cellulose, and the inhibition of glucose on cellulose is eliminated through its absorption and use [36], and the species of *Penicillium* are closely related to fruit decay. Simultaneously, studies also have shown that the complex repair of *Penicillium* and biochar can reduce the content of effective arsenic, while improving the microbial environment in arsenic-contaminated soil, showing good remediation performance for arsenic-contaminated soils [37–38].

This study revealed that soil microorganisms changed following the planting *A. lancea*, which signified that the continuous cropping issues of A. lancea had an intimate relationship with soil microorganisms. Future experiments should further validate the roles of bacteria and fungi with significant differences in the two groups of continuously cropped A. lancea, and the relationship between the rhizosphere of A. lancea rhizomes, important specific species of bacteria and fungi, and the kinetics of microbial change. Studies have confirmed that inoculation with AV fungi has a positive impact on the growth of functionally

symbiotic plants [39]. Further, endophytic actinomycetes and arbuscular mycorrhizal fungi, AMF, and chitosan assist with changing the community structure of rhizospheric microorganisms of A. lancea, and affect the physical and chemical properties of soil [40–41]. In further studies regarding the continuous cropping of A. lancea, AV fungi, endogenous actinomycetes and arbuscular mycorrhizal fungi, AMF, and chitosan might be employed to address continuous cropping obstacles.

## Conclusion

In summary, following the continuous cultivation of *A. lancea*, the soil microbial communities in the rhizosphere were altered, and overall, showed a decline in bacterial diversity and richness. Fungal community diversity increased, while the richness declined. Individual fungi and bacteria exhibited significant differences after the planting of *A. lancea*. Our work provides a certain perspective for understanding the occurrence of continuous cropping obstacles in *A. lancea* and points out a potential direction for resolving the problems of continuous cropping for *A. lancea.*

## Declarations

# Availability of data and materials

Not applicable.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All authors consent to the publication of this manuscript. Neither the article nor portions of it have been previously published elsewhere.

### Competing interests

All authors declare no conflicts of interest

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# Authors’ contributions

YX, JFN, ZZW designed this study. XQW, LJC did some experiments. YX drafted the manuscript. JFN and ZMD contributed to the revision of the manuscript. All authors read and endorse the final draft.

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# References

1. National Pharmacopoeia Committee. Pharmacopoeia of People's Republic of China. Part 1. Beijing: Chemical Industry Press; 2015. p. 161.
2. Xie J, Peng F, Yu L, Peng C. Pharmacological effects of medicinal components of *Atractylodes lancea*. (Thunb.) DC Chinese Medicine. 2018;13:59.
3. Bennett AJ, Bending GD, Chindler C, Hilton S, Mills P. Meeting the demand for crop production: the challenge of yield decline in crops grown in short rotations. Biol Rev Camb Philos Soc. 2012;87(1):52–71.
4. Jian ZY, Wang WQ, Meng L, Zhang ZL. Research progress of continuous cropping obstacles of ginseng medicinal plants. Modern Chinese Medicine. 2008;10(6):3–5.
5. Zhou F, Cao G, li J, Zhao Z, Tang L, Huang T. Advances in the Mechanism of Continuous Cropping Disorders of Chinese Medicinal Plants and the Mitigation Measures. Journal of Mountain Agriculture Biology. 2019;38(3):67–72.
6. Berg G, Köberl M, Rybakova D, Müller H, Grosch R, Smalla K. Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS microbiology ecology. 2017;93 (5).
7. Garbeva P, van Veen JA, van Elsas JD. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annual Review of Phytopathology. 2004;(420:243–70.
8. Souto XC, Pellissier F. Feedback mechanism in the chemical ecology of plants: role of soil microorganisms. Chemical Ecology of Plants. 2002;89–97.
9. Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol. 2009;68:1–13.
10. Study on Continuous Cropping Obstacle and Control Strategy of Medicinal Plants
Wang XY. Study on Continuous Cropping Obstacle and Control Strategy of Medicinal Plants. 2017 3rd International Conference on Economics, Social Science, Arts, Education and Management Engineering (ESSAEME 2017). Hangzhou, China Dec. 11–12, 2017.
11. Bridge P, Spooner B. Soil fungi: diversity and detection. Plant and Soil. 2001; (232):147–154.

12. Besser J, Carleton HA, Gerner-Smidt P, Lindsey RL, Trees E. Next-generation sequencing technologies and their application to the study and control of bacterial infections. Clinical Microbiology and Infection. 2018;(4):335–341.
13. Kathrin PA, Wemheuer B, Daniel R, Meinicke P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics. 2015;31(17):2882–4.
14. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet Journal. 2011;17(1).
15. Haas BJ, Gevers D, Earl AM. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res. 2011;21(3):494–504.
16. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8.
17. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–7.
18. Quast C, Pruesse E, Yilmaz P. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(D1):590–6.
19. Jacek K, Jan K. Response of the bacterial community to root ex-udates in soil polluted with heavy metals assessed by molecular andcultural approaches. Soil Biol Biochem. 2000;32:1405.
20. Zeng RS, Luo SM. Allelopathic effects of root exudates of cymbopogon citratus, Ageratum conyzoides and Bidens pilosa. Journal of South China Agricultural University. 1996;17(2):119–20.
21. Guo LP, Huang LQ, Jiang YX, Zhu YG, Chen BD, Zeng Y, Fu GF, Fu MH. Bioactivity of extracts from Rhizoma and Rhizosphere soil of Cultivated *Atractylodes lancea DC*. and Identification of their Allelopathic compounds. Acta ecologica sinica. 2006;26(2):528–35.
22. Mendes R, Kruijt M, Bruijn I. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science. 2011;332(6033):1097–100.
23. Wang W, Zhang DY, Wen H, Wang Q, Peng C, Gao J. Soil fungal biodiversity and pathogen identification of rotten disease in Aconitum carmichaelii (Fuzi) roots. PloS one. 2018;13(10):e0205891.
24. Jiang JH, Song Z, Yang XT, Mao ZQ, Nie XH, Guo H, Peng XW. Microbial community analysis of apple rhizosphere around Bohai Gulf. Scientific reports. 2017;7:8918.
25. Wu Lk, Wu HM, Chen J, Wang JY, Lin WX. Microbial community structure and its temporal changes in *Rehmannia glutinosa* rhizospheric soils monocultured for different years. European journal of soil. 2016;72:1–5.
26. Zhang W, Du Y. Analysis of the succession of structure of the bacteria community in soil from long-term continuous cotton cropping in Xinjiang using high-throughput sequencing. Arch Microbiol. 2018;200:653–62.
27. Guo LP, Huang LQ, Jiang YX, Cheng ML, Dong M, Zeng Y. Change of microbial community in rhizoma sphere of cultivated *Atractylodes lancea*. China Jouranl of Chinese materal medcial. 2007;

(12):1131–1133.

28. Dong LL, Xu J, Zhang LJ, Yang J, Liao BS, Li XW, Chen SL. High-throughput sequencing technology reveals that continuous cropping of American ginseng results in changes in the microbial community in arable soil. Chinese Medicine. 2017;12:18.
29. Chen MN, Li X, ang QL, Chi XY, Pan LJ, Chen N, Yang Z, Wang T, Wang M, Yu SL. Soil eukaryotic microorganism succession as affected by continuous cropping of peanut—pathogenic and beneficial fungi were selected. PLoS ONE. 2012;7(7):e40659.
30. Beimforde C, Feldberg K, Nylinder S, Rikkinen J, Tuovila H, Dörfelt H, Gube M, Jackson DJ, Reitner J, Seyfullah LJ, Schmidt AR. Estimating the phanerozoic history of the ascomycota lineages: Combining fossil and molecular data. Mol Phylogenet Evol. 2014;78:386–98.
31. .Kalita M, Malek W. Genista tinctoria microsymbionts from Poland are new members of *Bradyrhizobium japonicum* bv. genistearum. Syst Appl Microbiol. 2010;33(5):252–9.
32. Probst CM, Ridgway HJ, Jaspers MV. Jones EE. Pathogenicity of *Ilyonectria liriodendri* and *Dactylonectria macrodidyma* propagules in grapevines. European Journal of Plant Pathology. 2019:1–17.
33. Agustí-Brisach C, Cabral A, González-Domínguez E, Pérez-Sierra A, León M, Abad-Campos P, García-Jiménez J, Oliveira H, Armengol J. Characterization of *Cylindrodendrum*, *Dactylonectria* and *Ilyonectria* isolates associated with loquat decline in Spain, with description of Cylindrodendrum alicantinum sp. nov. Eur J Plant Pathol. 2016;145(1):103–18.
34. Malapi-Wight M, Salgado-Salazar C, Demers J, Veltri D, Crouch JA. Draft genome sequence of Dactylonectria macrodydima, a plant pathogenic fungus in the Nectriaceae. Genome Announc. 2015;3(2):e00278-15.
35. VanInsberghe D, Maas KR, Cardenas E, Strachan CR, Hallam SJ, Mohn WW. Non-symbiotic *Bradyrhizobium* ecotypes dominate North American forest soils. The ISME Journal. 2015;9(11):2435–41.
36. Wang Z, Tao M, Fang C, Chen XY. Isolation, identification and characterization of a Sphingomonas sp.strain. Journal of Dalian. 2019;38(06):403–7.
37. Duan J, Li H, Ma X, Liang W, Li J, Gao Y. Study on the remediation of arsenic pollution in soil by the combination of *penicillium* and biochar. Acta Sci Circum. 2019;39(06):1999–2005.
38. Shanawaer S, Yu S, Maimaiti, Guo Q, Bai J. Identification of the Pathogen Causing Jujube Fruit Mildew (Part â□□)——Isolation and Identification of *Penicillium* fungus Causing Jujube Fruit Mildew. Xinjiang Agricultural Sciences. 2016;53(4):698–705.
39. Bainard LD, Koch AM, Gordon AM, Klironomos JN. Growth response of crops to soil microbial communities from conventional monocropping and tree-based intercropping systems. Plant and Soil 2012;363 (2013):345–356.
40. Ji CL, Tian MM, Ma JF, Jin HR. Advances in the researches on the effects of arbuscular mycorrhizal fungi on plant nutrition metabolism and growth effects. Journal of Zhejiang Normal University (Natural sciences). 2010;33(3):303–9.

41. Liang X, Tang M, Lu L, Zhao X, Dai C. Effects of three arbuscular mycorrhizal fungi (AMF) species on the growth, physiology, and major components of essential oil of *Atractylodes lancea*. Chin J Ecol. 2018;37(06):1871–9.

# Figures

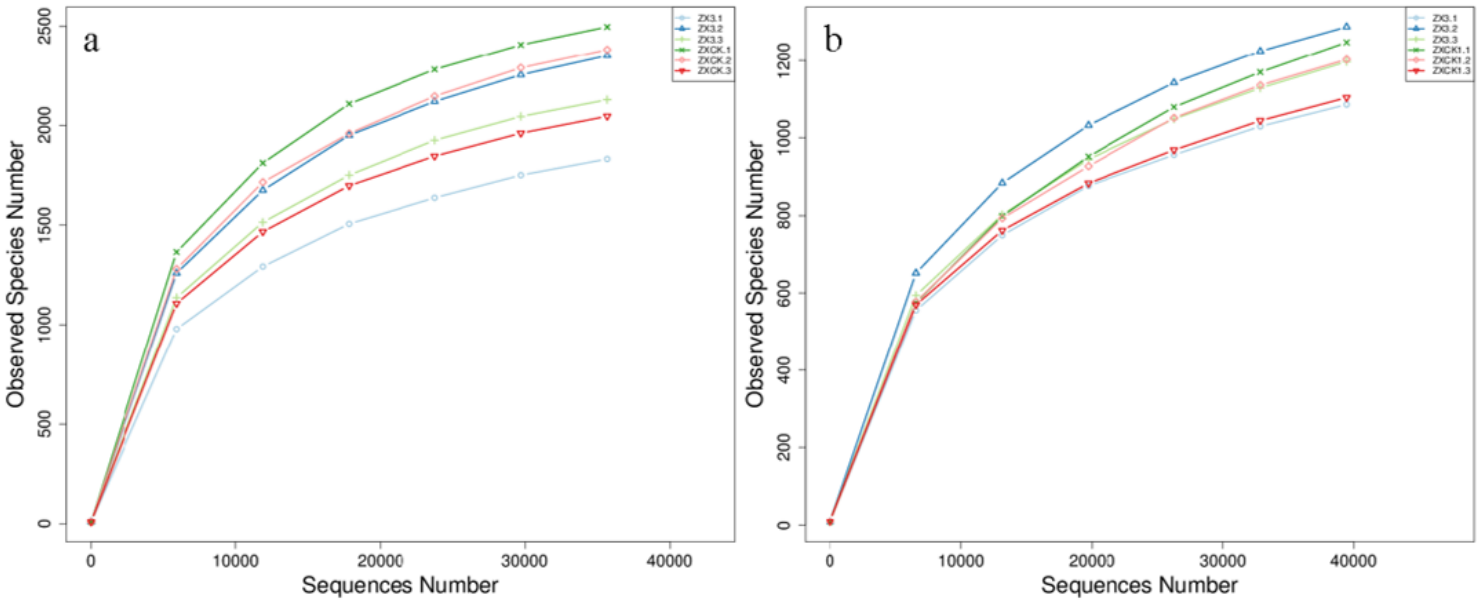
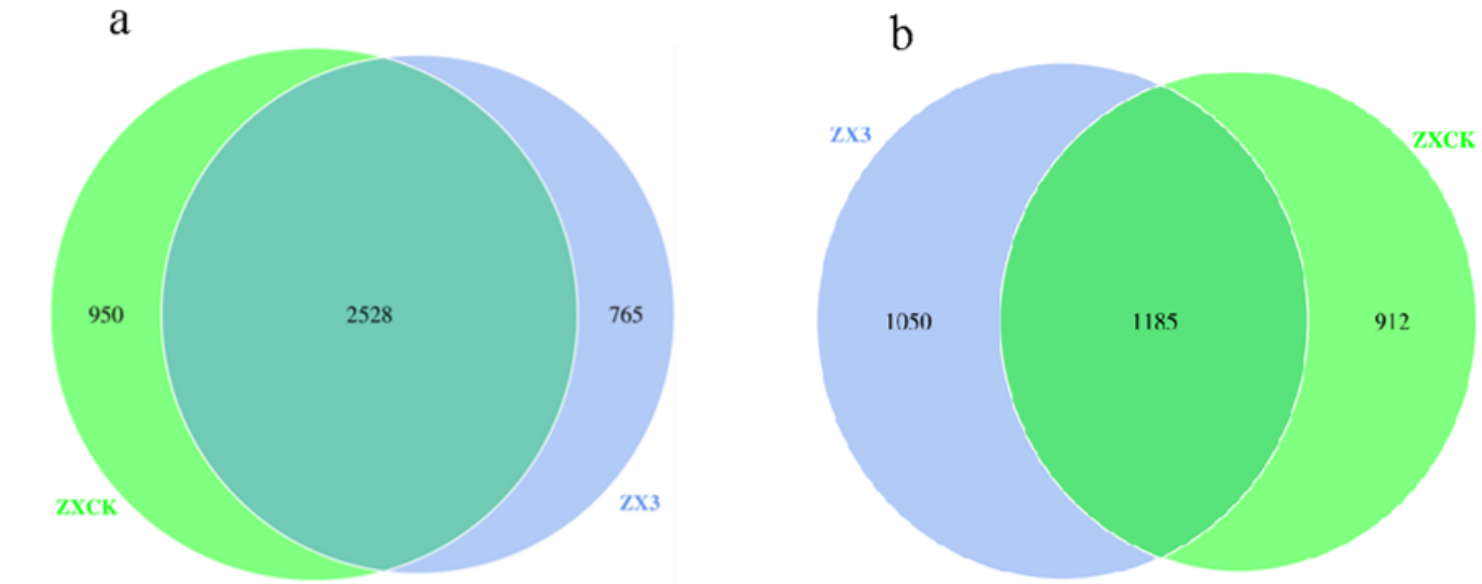


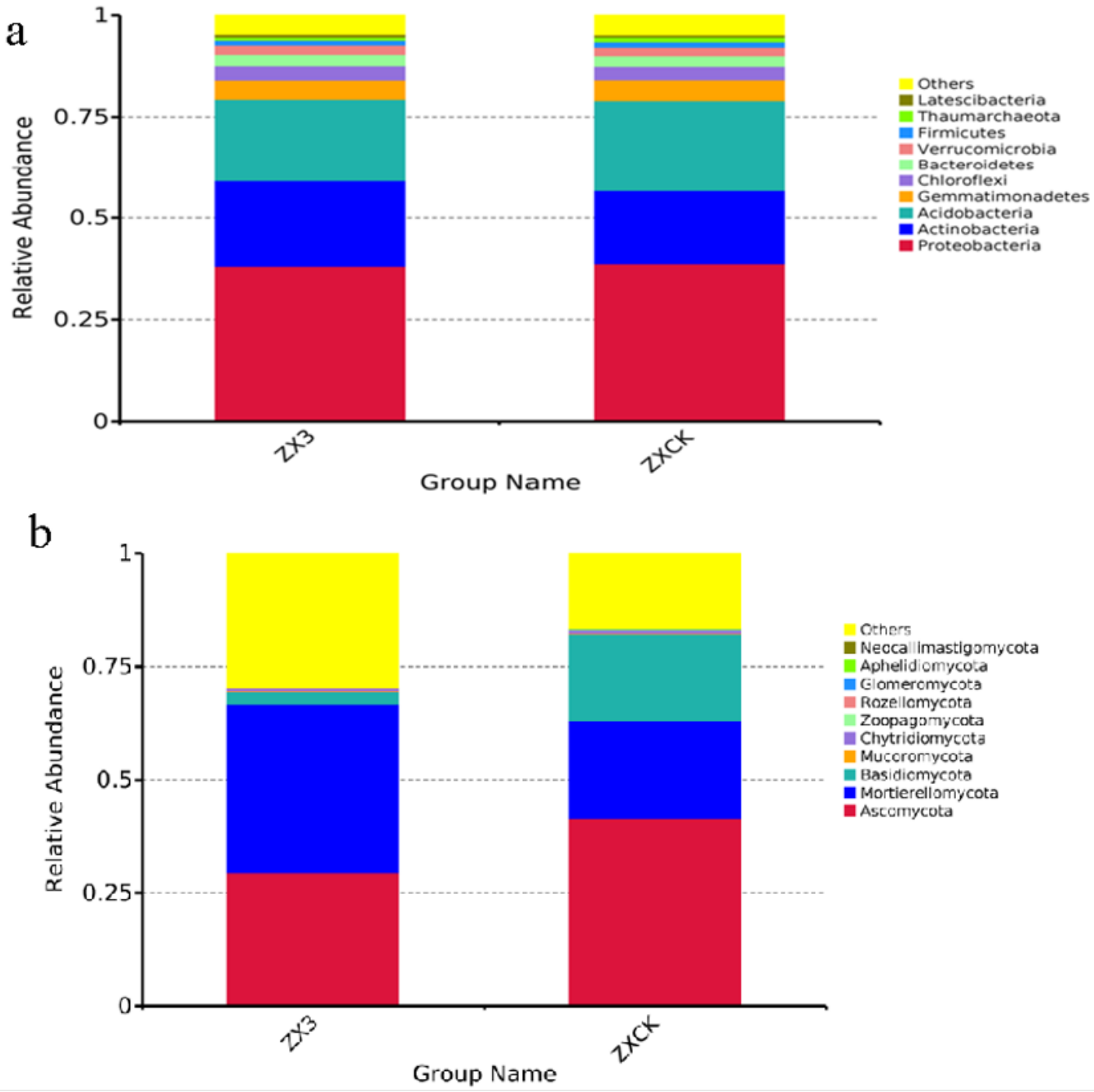

**Figure 1**

Rarefaction curves of microbial communities based on observed operational taxonomic units (OTUs) at the 97% similarity cut-off level for individual samples. The curve was constructed with the quantity of data extracted as the abscissa, and the corresponding number of bacterial and fungal species as the ordinate. ZX3 and ZXCK represent the rhizospheres from the consecutive three-years cultured soil, and soil that had never been planted with A. lancea, respectively. a – Bacteria; b – Fungi.



## Figure 2

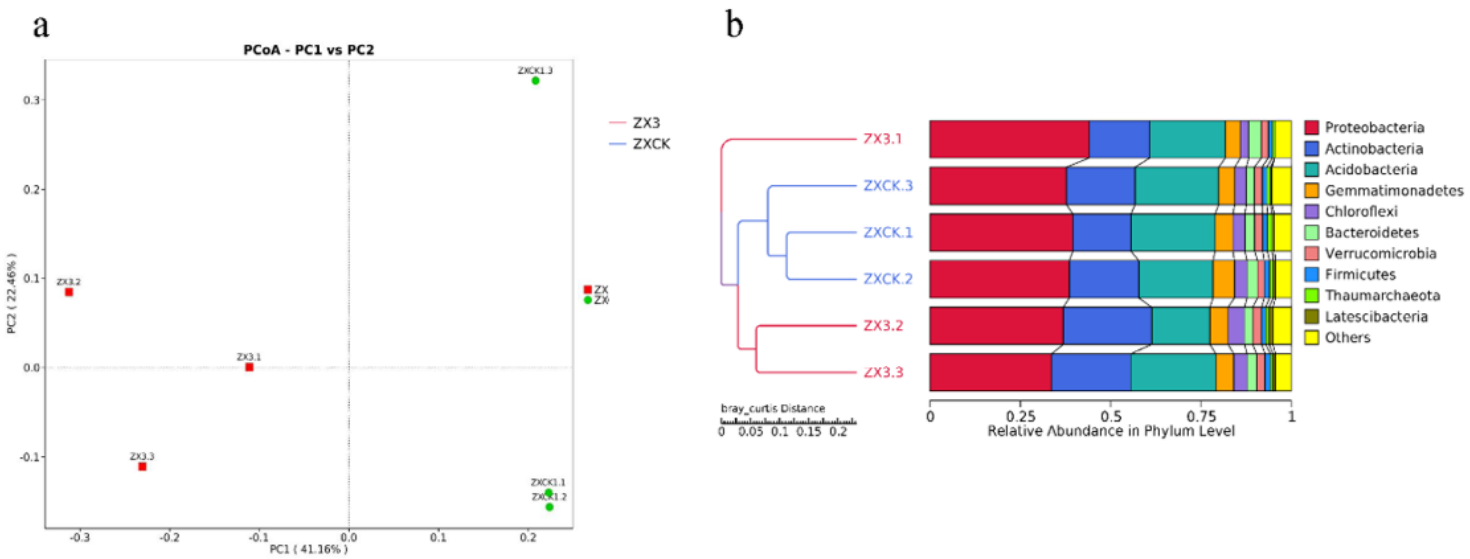
Venn diagrams created using OTUs show the number of OUTs that are unique to the sample and those OUTs shared between the samples. ZX3 and ZXCK represent the rhizosphere soils from the consecutive three-years cultures and soils that had never been planted with A. lancea respectively. a – Bacteria; b – Fungi




## Figure 3

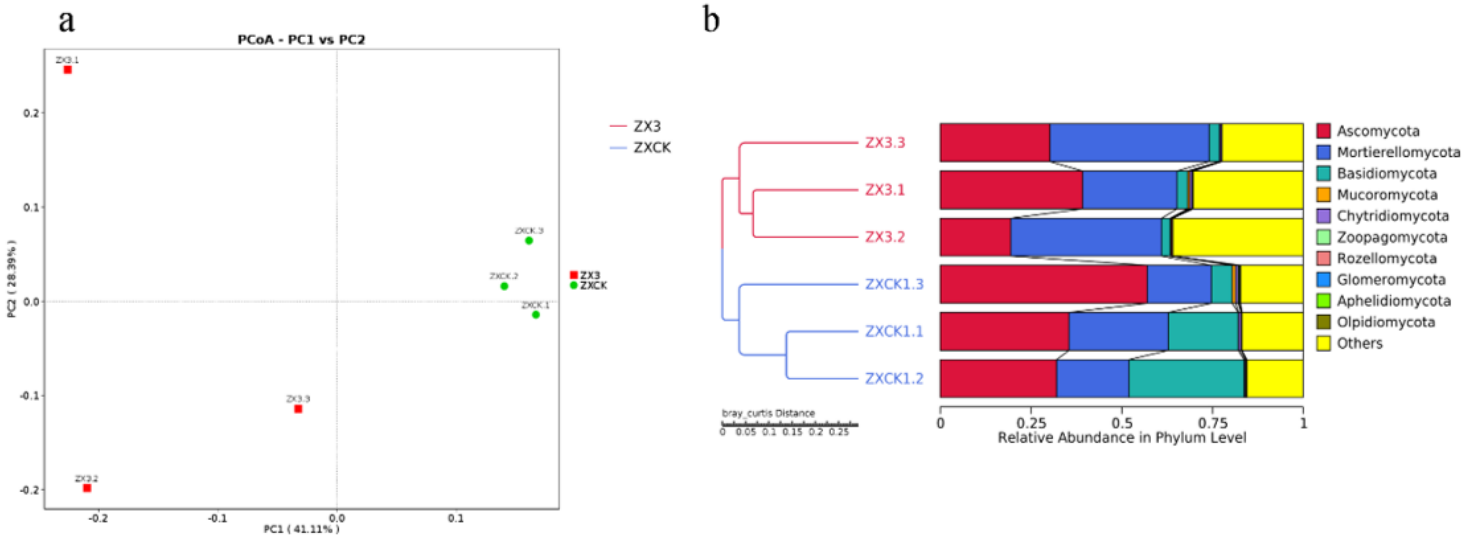
Relative abundances of the top 10 microbial Phylum level in the two different soil samples. ZX3 and ZXCK represent the rhizospheres from the consecutive three-years cultures and those that had never been

planted with A. lancea, respectively. a – Bacteria; b – Fungi



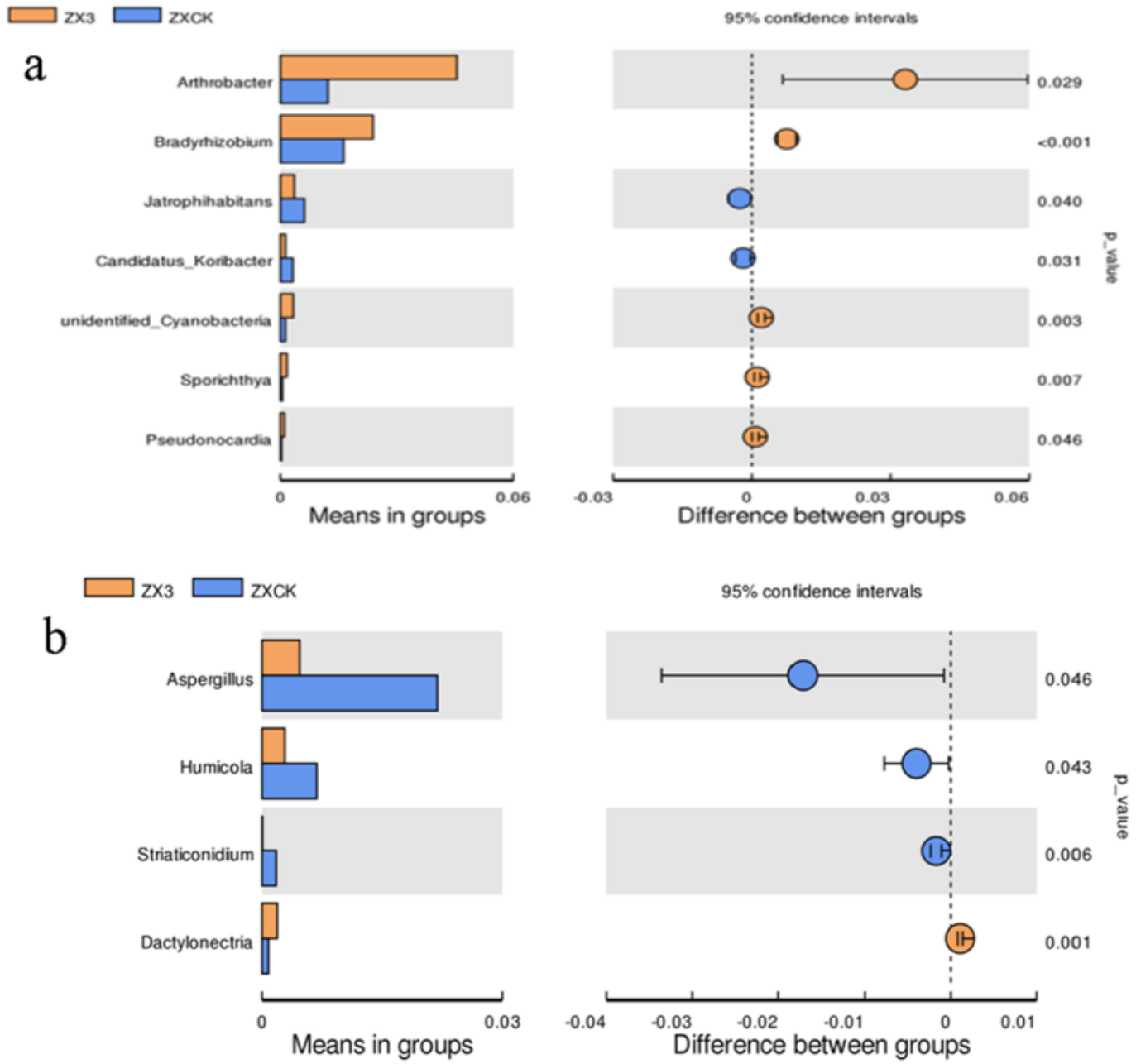

## Figure 4

Procinple coordinate analysis (PCoA) (a) and hierarchical clustering (b) of bacterial communities based on an unweighted pair-group method with arithmetic mean (UPGMA) clustering analyses at phylum level for the different soil samples. ZX3 refers to rhizosphere soil that has been planted with A. lancea for three years, whereas ZXCK refers to soil that was never been planted with A. lancea.



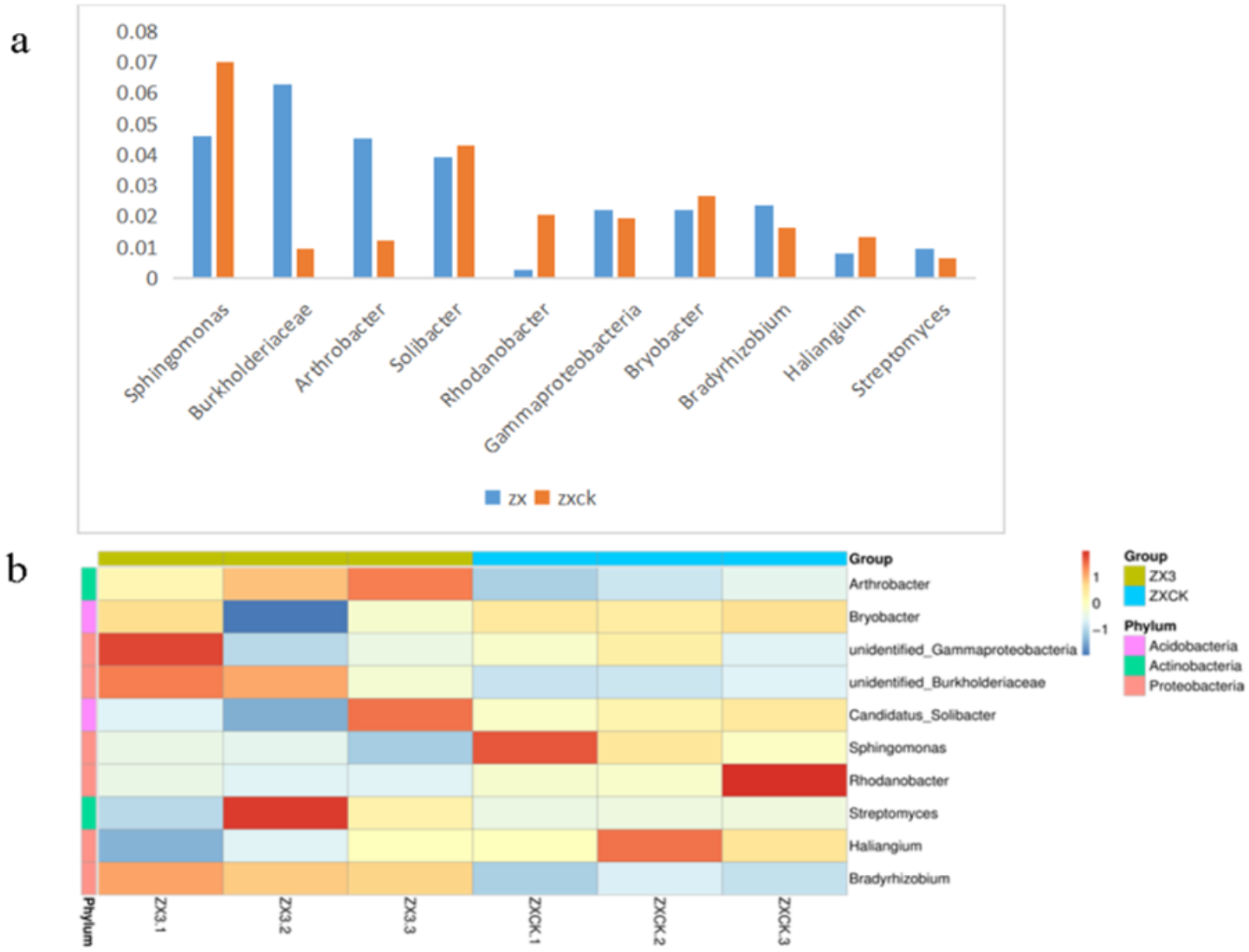

## Figure 5

Principal coordinate analysis (PCoA) (a) and hierarchical clustering (b) of fungal communities based on an unweighted pair-group method with arithmetic mean (UPGMA) clustering analyses at phylum level for the different soil samples. ZX3 refers to rhizosphere soil that was planted with A. lancea for three years, and ZXCK refers to soil that has never been planted with A. lancea.



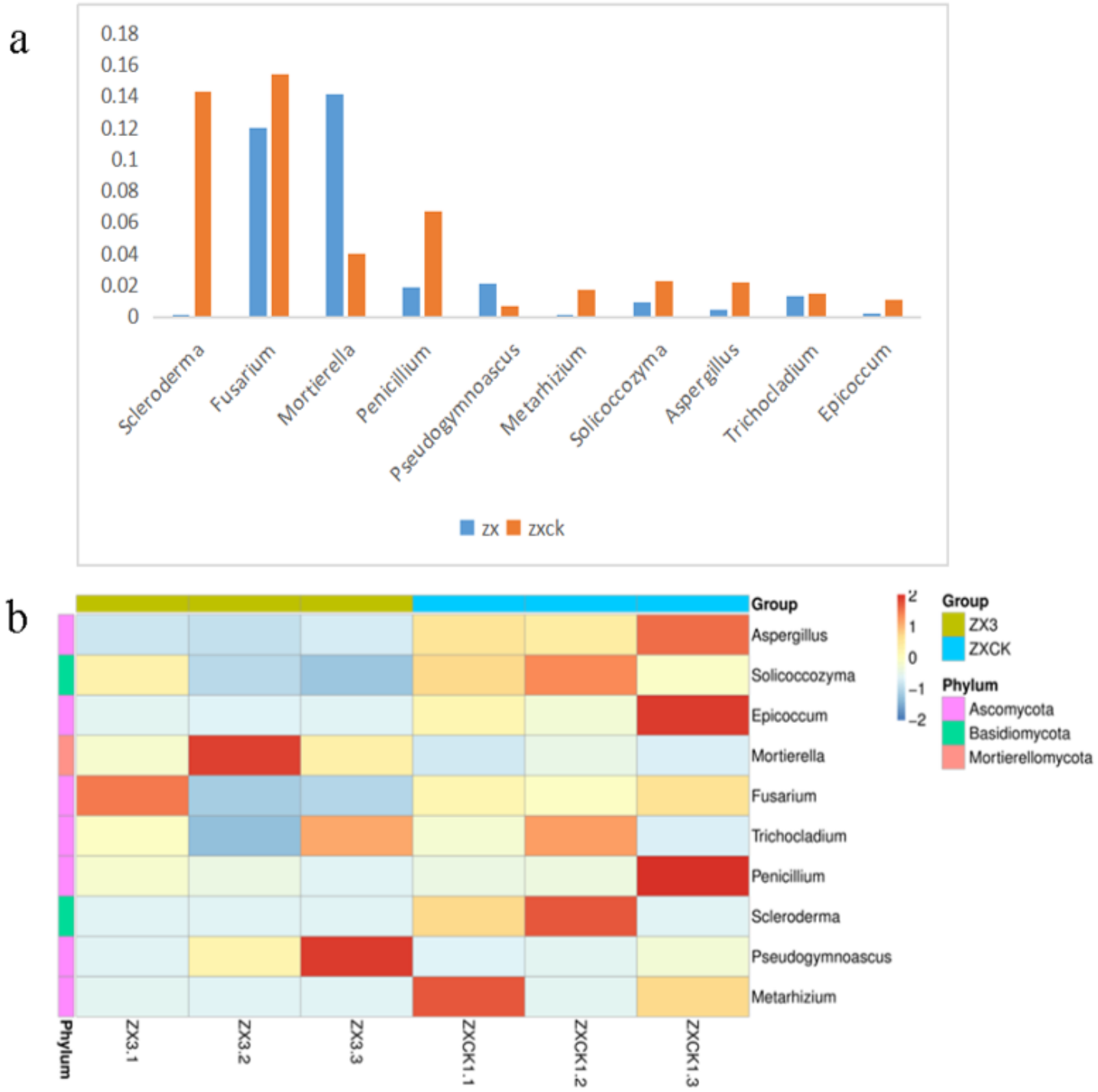

## Figure 6

Species difference analysis chart showing species differences between various samples. R-value> 0, indicates significant differences between groups; R-value <0, indicating that differences within groups were greater than differences between groups. ZX3 refers to rhizosphere soil that has been planted with A. lancea for three years, and ZXCK refers to soil that was never been planted with A. lancea. a – Bacteria; b – Fungi




## Figure 7

Analysis bacterial abundance among the top ten bacterial subordinate levels(a), the abscissa indicated the genus name, the ordinate indicated the relative proportion of bacteria. Heat map analysis of the top 10 most abundant genera in the different samples (b). ZX3 refers to rhizosphere soil that was planted with A. lancea for three years, and ZXCK refers to soil that was never been planted with A. lancea.




## Figure 8

Analysis of bacterial abundance in the top ten fungal genus levels (a), the abscissa indicates the genus name, and the ordinate indicates the relative proportion of bacteria. The heat map analyzed the top 10 most abundant genera in different samples. ZX3 refers to rhizosphere soil that was planted with A. lancea for three years, and ZXCK refers to soil that was been planted with A. lancea.