

The rotation of *Phallus impudicus* benefits the soil microenvironment via improving the soil microbiome structure and soil properties

Wang Yanhong

Guizhou University of Traditional Chinese Medicine

Xu Jiao

Guizhou University of Traditional Chinese Medicine

Yuan Qingsong

Guizhou University of Traditional Chinese Medicine

Guo Lanping

China Academy of Chinese Medical Sciences

Xiao Chenghong

Guizhou University of Traditional Chinese Medicine

Yang Changgui

Guizhou University of Traditional Chinese Medicine

Li Liangyuan

Guizhou University of Traditional Chinese Medicine

Jiang Weike

Guizhou University of Traditional Chinese Medicine

Zhou Tao (✉ taozhou88@163.com)

Guizhou University of Traditional Chinese Medicine

Research Article

Keywords: traditional Chinese medicine, soil nutrient, soil microbial community, soil humus composition, *Armillaria mellea*

Posted Date: March 8th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2645237/v1>

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Abstract

Background: Rhizosphere microbiome play vital roles in soil nutrient cycling and plant growth, their diversity and composition are commonly influenced by soil management systems. Currently, however, the specific dynamic of microbial community in cultivation of *Gastrodia elata* and rotation of *Phallus impudicus* remains unknown. To narrow this knowledge gap, soil physicochemical properties and soil microbial community in the rhizosphere soil of *G. elata* cultivation and rotation of *P. impudicus* management were analyzed and compared.

Results: The results revealed that the cultivation of *G. elata* and rotation of *P. impudicus* both increased the diversity, complexity, and stability of the soil microbiome structure and reshaped the soil microbiota composition. In addition, the cultivation of *P. impudicus* reversed the *G. elata*-induced decrease in soil pH and nutrient levels, increased soil humification and improved humus composition. The RDA result indicated that the soil microbial community strongly correlates with soil pH and exchangeable Ca/Mg ratio, indicating that soil nutrient levels play dominant roles in soil microbial community improvement in this rotation management system.

Conclusions: Our findings provide insights into microbiome assembly and soil physicochemical properties in cultivation of *G. elata* and rotation of *P. impudicus*, rotation affected soil microorganisms mainly through changes in soil nutrient profile and might have potential benefits in next growing season. In addition, our results highlight the importance of rotation management during artificial cultivation of traditional Chinese medicine for sustainable development.

Background

Soil microenvironment comprises abundant and complex soil microorganisms [1], their diversity and composition play dominant roles in maintaining soil functions through involvement in the turnover of soil nutrient [2, 3]. In general, land utilization is a major factor affecting soil microbiological indicators [4, 5]. A better understanding of how soil microbial community and soil properties respond to planting strategy has a potential contribution to improvement of agronomic practices and optimization of microorganisms for agricultural sustainability [6].

Rotation practice, for now has become more and more popular in the world. Compared with a monoculture system, an rotation system has obvious advantages in improving soil structure, rhizosphere microbial communities, and nutrient bioavailability [7]. Numerous studies addressing how rotation management alter soil microbial biomass and/or activities to gain a better understanding of assessment of planting strategies. For instance, the rotation of wheat with *Pinellia ternate* was reported to have positive impacts on soil microbial diversity and structure [8]. However, rotation management can also result in different microbial communities and lead to reduced yield in harvest [9]. This is likely due, in large part, to the selection of rotating plant, for that soil microbial community would differ among crop species [10, 11]. Therefore, it is necessary to evaluate changes of soil microbial and physiochemical

indicators to assess whether the planting strategies are appropriate under certain circumstances like rotation management.

G. elata has been traditionally used in Oriental countries for its therapeutic benefits. It is widely applied in traditional Chinese medicine (TCM) to treat various neurological diseases, such as vertigo, general paralysis, and tetanus [12, 13]. This Orchidaceae has abandoned photosynthetic genes and lives symbiosis with non-photosynthetic fungi [14], obtaining its carbon entirely from *A. mellea*. Normally, *A. mellea* influence plant growth in the next growing season as a result of competition with other microbes associated with secondary metabolites [15, 16]. To date, the artificial cultivation of *G. elata* has been last for 6 decades since the 1960s [17], it is time to explore a promising avenue for the sustainable development of *G. elata* industrial. Based on techniques and biological attributes, farmers choose a Phallaceae fungi, namely *P. impudicus* to rotate with *G. elata*. It was reported that *P. impudicus* is capable of decomposing substrate and have been shown to be responsible to nutrient allocation, uptake and storage [18]. To our knowledge, this is the first measurement of soil microenvironment changes in this agronomic rotation practice. The objectives of this work were to reveal the: 1) variation of microbial community under different planting strategies, i.e. cultivation of *G. elata* and rotation of *P. impudicus*, and 2) relationships between soil microbial community and soil properties in the context of rotation management.

Methods

Experiment site and soil samples collection

The trial site was located in Dafang County (105°55' E, 27°13' N, 1500 m a.s.l.), Bijie, Guizhou Province, one of the major cultivation areas of *G. elata* in China. The climate is subtropical humid monsoon with an average temperature of 12.5°C and mean precipitation of 1,100 mm. The soil type in this region is yellow soil or “Zheltosol” [19]. The rotation began with *G. elata* planting in October, 2016 and harvesting in November, 2017 followed by immediate planting of *P. impudicus*, then *P. impudicus* was harvested in November, 2018. The experiment set three replicates of each treatment, detailed planting and sampling information is described below:

To ensure the uniform, the trial was performed in a single field site that was newly reclaimed. Field holes, each 100 cm × 30 cm × 30–50 cm (length × width × depth), were established in 2016. To plant *G. elata*, 5 pieces of chestnut wood that were fully infected with *A. mellea* were placed on the bottom of each hole, and 1 kg juvenile tubers of *G. elata* were set evenly on the chestnut wood. Then, covered them with twigs and leaves infected with *A. mellea* to maintain humidity at the growing season. Finally, soils were filled back to the holes and covered with straws or leaves [20].

On harvest, topsoil, leaves and bulk soil were carefully removed, then soil adhering to the rhizo zone of *G. elata* that was referred to rhizosphere soil was collected and sieved (2 mm), then thoroughly homogenized (refer to GE treatment). The soils were divided into 3 portions, one portion with

approximately 2 g was immediately frozen in liquid N and kept at -80°C until DNA extraction. One portion for the measurement of soil ammonium-nitrogen ($\text{NH}_4^{+}\text{-N}$) and nitrate-nitrogen ($\text{NO}_3^{-}\text{-N}$) was stored at 4°C , and the remaining soil was air dried prior to determination of physicochemical properties.

P. impudicus was planted in November, 2017 after the *G. elata* harvest with a sowing density of 1.5 kg strains per hole and harvested in November of the following year. After harvest, rhizosphere soil surrounding *P. impudicus* was sampled as describe above (refer to PI treatment). Control soil samples also collected from barren land at this moment (refer to CK treatment).

Measurements

After mixing 2 g of air-dried soil in 10 mL of deionized water and letting this mixture settle for 30 min, we measured the pH of each sample using a PHS-25 pH meter (Mettler Toledo, Giessen, Germany). Organic carbon (TOC) content was analyzed using the potassium dichromate heating oxidation-volumetric method, while total nitrogen (TN) content was measured using the Kjeldahl method. Ammonium-nitrogen ($\text{NH}_4^{+}\text{-N}$) and nitrate-nitrogen ($\text{NO}_3^{-}\text{-N}$) were extracted with fresh soils using 2 M potassium chloride (1:10), and their concentrations were measured using a continuous-flow analyzer (San++, Skalar, Netherlands).

Available manganese, zinc, and copper were extracted using diethylene triamine pentaacetic acid-triethanolamine-calcium chloride (DTPA-TEA- CaCl_2 , pH 7.3), while exchangeable potassium, calcium, and magnesium were extracted using ammonium acetate (pH 7.0). Contents of available elements and exchangeable cations were measured using atomic absorption spectrophotometry (AA-6880, Shimadzu, Japan). Humus was extracted using a mixture of 0.1 M sodium pyrophosphate and 0.1 M sodium hydroxide, and humus content was determined using the potassium dichromate heating oxidation-volumetric method.

Soil Microbiome Sequencing

Microbial DNA was extracted using the E.Z.N.A.® soil DNA Kit (Omega Bio-tech, Norcross, GA, U.S.). The 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), and the ITS rRNA gene were amplified with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') by thermos cyclor PCR system (GeneAmp 9700, ABI, USA). PCR products were isolated and extracted from a 2% agarose gel, then purified. Further, the purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Bioinformatics And Statistical Analysis

The significance in a diversity of soil microbiome and relative abundance at the genus level among different soil samples was tested using the one-way analysis of variance (SPSS 20.0, IBM, Chicago, IL, USA) and Duncan's multiple range test at a 0.05 significant level. Based on the calculated Bray-Curtis distance, principal coordinate analysis (PCoA) and hierarchical clustering analysis (HCA) was performed to investigate the variation of microbial community structure among treatments. To assess the difference in the microbial community composition variations, permutational multivariate ANOVA (PERMANOVA) was conducted using the *anosim* function in the R software package *vegan* (Version 2.4-0) [21]. The venn diagrams were constructed to visualize shared and unique OTUs among samples. Based on the pairwise spearman method and correlation coefficient with threshold value $r \geq 0.5$, the co-occurrence network analysis of bacterial and fungal which relative abundance were ranked among top 50 were calculated respectively at $P < 0.05$ significant level. Then the network was visualized by Cytoscape software (Version 3.7.1) [22]. The RDA analysis were performed using the *vegan* package (Version 2.4-0) [21] in R version 3.1.2 for Windows [23]. The changes of relative abundance in bacterial and fungal genera were conducted with one-way ANOVA and Duncan's multiple range test ($P < 0.05$), dominant genera with significant changes are shown.

We performed one-way ANOVA to assess the significance of differences in soil biochemistry, available elements, exchangeable cations and humus variations among different soil samples. Differences associated with $P < 0.05$ based on Duncan's multiple range test were defined as significant. All analyses were performed using SPSS 20.0 (IBM, Chicago, IL, USA).

Results

Effect of *G. elata* cultivation and rotation management on soil physiochemical properties

All soil samples, including control, were acidic (Table 1). In general, CK (soil collected from barren land) had the lowest pH value, cultivation resulted in the increase in soil pH value in GE (soil adhering to the rhizo zone of *G. elata*) and PI (rhizosphere soil surrounding *P. impudicus*) treatments. A similar trend was also found in soil available elements and exchangeable cations, such as available manganese and copper, soil exchangeable potassium and magnesium (Figure S1). There was no significant difference ($P > 0.05$) in TOC and $\text{NH}_4^+\text{-N}$ concentrations between CK and GE, the highest TOC and $\text{NH}_4^+\text{-N}$ concentrations was found in PI sample. However, GE had significantly higher ($P < 0.05$) $\text{NO}_3^-\text{-N}$ concentration than CK and PI samples. Total N content did not differ significantly among CK, GE and PI (Table 1).

Table 1

Changes of soil properties. Values are mean $\pm$ SE (n = 3). Lowercases a, b, and c in table indicate significant differences between treatments, analyzed by one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

Soil categories	pH	TOC	TN	NH ₄ ⁺ -N	NO ₃ ⁻ -N
		g/kg		mg/kg	
CK	4.44 $\pm$ 0.10 c	18.3 $\pm$ 3.00 b	1.63 $\pm$ 0.73 a	19.2 $\pm$ 7.79 b	8.48 $\pm$ 2.12 b
GE	5.76 $\pm$ 0.03 a	9.57 $\pm$ 0.74 b	2.50 $\pm$ 0.44 a	7.65 $\pm$ 0.35 b	22.7 $\pm$ 2.90 a
PI	4.88 $\pm$ 0.02 b	85.2 $\pm$ 4.62 a	0.86 $\pm$ 0.34 a	70.5 $\pm$ 6.36 a	13.4 $\pm$ 2.27 b

The highest humification, total carbon content in humus, ratio of humic to fulvic acid, as well as carbon content in humin, humic acid, and fulvic acid was found in PI sample. Conversely, CK and GE showed no significant difference in humus composition and properties (Table S1).

Soil microbial α - and β -diversity responses to *G. elata* cultivation and rotation management

We performed α - and β -diversity analyses to investigate the soil microbial community changes. The Chao 1 indice and Shannon index of the bacterial and fungal community were higher in GE and PI, compared with that in CK (Table 2). There was no significant difference in the richness of microbiota community between GE and PI (as indicated by Chao 1 index). In contrast, the Shannon index of bacterial and fungal communities in GE was significantly higher ($P < 0.05$) than that in CK and PI. These results imply that either cultivation of *G. elata* or rotation of *P. impudicus* would greatly influence the soil microbial diversity.

Table 2

Diversity indices in different soil samples. Lowercases a, b and c in table show significantly differences between different soils (CK GE and PI), determined by one-way ANOVA and Duncan's multiple range test ($P < 0.05$).

Soil categories	Chao 1 indice	Shannon index	Chao 1 indice	Shannon index
	Bacterial community		Fungal community	
CK	1133 $\pm$ 13.7 b	5.44 $\pm$ 0.02 c	743 $\pm$ 40.7 b	3.91 $\pm$ 0.02 c
GE	1502 $\pm$ 32.1 a	6.00 $\pm$ 0.05 a	1412 $\pm$ 59.0 a	4.86 $\pm$ 0.02 a
PI	1516 $\pm$ 24.6 a	5.89 $\pm$ 0.01 b	1249 $\pm$ 44.4 a	4.69 $\pm$ 0.03 b

Overall, the Bray-Curtis PCoA analysis showed that the bacterial community and fungal community structure in GE and PI were greatly separated from CK (Fig. 1A and C), which indicated that *G. elata* cultivation and rotation of *P. impudicus* had significant impacts ($P < 0.05$) on soil microbial community structure. The HCA result showed that soil microbiota was well separated and varied dramatically in different soil samples (Fig. 1B and D). In addition, the Venn diagram showed that the numbers of peculiar OTU in GE and PI were much higher than that in CK (Fig. 2A and B), especially in the soil fungal

community. These results indicated that soil microbiome became more diverse and complex in soil associated with *G. elata* and *P. impudicus*, compared with that in barren land.

The cultivation of *G. elata* and rotation management improved the complexity and stability of the soil microbial structure

The network of bacterial and fungal community were constructed respectively to describe the complexity and stability of soil microbiome structure in all soil samples (Fig. 3). The network average path length in bacteria and fungi were 1.88 and 1.83, and the network diameter in these was 4.00. The nodes in the co-occurrence network of bacteria and fungi were 50 and 49 and were assigned to eleven bacterial phyla, and five fungal phyla, the edges of bacteria and fungi were 438 and 411, respectively.

In this rotation process, *Proteobacteria* genera (18 nodes) had more complex correlation with PI than other bacterial phyla (Fig. 3A). Genera *Variibacter*, *norank_f__HSB_OF53-F07* and *norank_f__Caulobacteraceae* all interacted with 26 genera, fungus *Sporothrix* interacted with 20 genera, including 8 positive and 12 negative interaction genera (Fig. 3B), indicating their important roles to shape soil microbial community in rotation of *P. impudicus*.

The cultivation of *G. elata* and rotation management reshaped the soil microbiota composition

At genus level, relative abundances of microbes were analyzed and compared to evaluate the variations of bacterial and fungal community composition to different planting pattern. For bacterial genera (Fig. 4A), the relative abundance of *Bacillus* and *norank_o__Gaiellales* significantly increased ($P < 0.05$), while the relative abundance of *Acidibacter*, *Acidothermus*, *norank_c__JG37-AG-4*, *norank_f__Acidobacteriaceae__Subgroup_1*, *norank_f__DA111*, *norank_f__ODP1230B8.23*, and *norank_f__YNPFFP1* significantly decreased ($P < 0.05$) in GE compared to CK. Interestingly, PI helped to increase the relative abundance of genera which GE decreased before. Besides, the relative abundances of some genera, like *Bryobacter*, *Burkholderia-Paraburkholderia*, *Candidatus_Solicaber* and *Roseiarcus* showed no significant difference between GE and CK, however, the relative abundances of these genera in PI were significantly increased ($P < 0.05$) compare with GE and CK.

As for fungal genera (Fig. 4B), the relative abundance of *Clavulinopsis*, *Russula*, *Sebacina*, *unclassified_f__Russulaceae*, and *unclassified_p__Ascomycota* were significantly decreased ($P < 0.05$) in GE and PI compared to CK. However, the relative abundance of *Mortierella* and *unclassified_k__Fungi* were greatly increased in GE compared with CK. No significant difference was found in relative abundance of *Chaetosphaeria*, *Chloridium*, *unclassified_c__Leotiomycetes*, and *unclassified_f__Russulaceae* between GE and CK, however, their abundances were greatly increased in PI sample.

Relationship Of Soil Properties And Microbial Community

The redundancy analysis (RDA) was performed to reveal the driving factors for developing soil bacterial and fungal communities on the genus level (Fig. 5). The result showed that bacterial community change

was correlated with soil variables: pH, Ca/Mg ratio and HA/FA ($P < 0.05$, Fig. 5A), and fungal community change was correlated with soil variables: pH, Ca/Mg ratio, C/N ratio and HA/FA ($P < 0.05$, Fig. 5B). The first two axes explained 87.51% and 90.43% of bacterial and fungal community variations, respectively, which suggested a remarkable correlation between microbial community and soil properties (soil pH, C/N ratio, Ca/Mg ratio and HA/FA). These results indicated that soil pH and other soil properties significantly impacted soil bacterial and fungal communities in *G. elata* cultivation or rotation of *P. impudicus*.

Discussion

The changes of soil microbial biodiversity and community distribution are sensitive to planting strategies

In this study, we evaluated the impact of planting strategies on soil microbial diversity and community structure. The α diversity revealed that the microbial (either bacterial or fungal) richness and biodiversity tended to be increased in fields where *G. elata* cultivation or rotation of *P. impudicus* were applied (Table 2). The more pronounced difference observed with Chao 1 indice in GE rather than PI may indicate that cultivation of *G. elata* and its symbiotic fungi *A. mellea* exerted more stimulation on soil organisms than *P. impudicus* did. The great changes of soil microbial structure among different soil categories is further confirmed with the result of β diversity, which implied great separation between cultivation of *G. elata* and rotation of *P. impudicus*.

Considering the relative abundance of soil microbes responded different to plants and planting strategies, we analyse the changes of specific members, especially those who have potential benefit in nutrient cycling. Specifically, the relative abundance of some potentially beneficial bacteria and fungi, such as *Candidatus_Solibacter* and *Penicillium*, significantly increased in rhizosphere soil surrounding *P. impudicus* compared with that in soil adhering to the rhizo zone of *G. elata* and control (Fig. 4). Members of *Candidatus_Solibacter* were reported to be related to the degradation of potential soil allelochemicals [24]. Although some species of *Penicillium* can cause crop diseases, most of them have been discovered as beneficial fungi, which can control plant diseases by inhibiting the growth of pathogenic organisms [25]. Besides, the relative abundance of other microbes, such as *Trichoderma*, also showed the same trend with *Candidatus_Solibacter* and *Penicillium*, which implied the sensitivity of these potentially beneficial fungi to planting strategy, especially rotation of *P. impudicus* in this rotation management.

Interestingly, the relative abundance of *Bacillus* significantly increased in the cultivation of *G. elata*, and sequentially decreased in the rotation of *P. impudicus* (Fig. 4), which was in accordance with the changes of NO_3^- -N (Table 1). This result was consistent with previous research which indicated that the relative abundance of genus *Bacillus* significantly decreased in tobacco-rice rotation cropping system [26]. As far as we know, *Bacillus* is capable to produce antibiotic substances and has the capacity to fix nitrogen [27]. Therefore, exudates of *A. mellea* presumably induced the enrichment of *Bacillus* in cultivation of *G. elata*, and stimulated it to solubilize nitrogen into available pattern. This seems to indicate a distribution of the

species of this genus and others like *norank_o_Gaiellales*, *unclassified_k_Fungi*, and *Mortierella* are in a particular trophic niche, and further influenced and characterized by plant variety in rotation system. The correlation between soil nutrient availability and these fungal species could have an ecological significance and be linked to the niches in rotation systems.

Soil Microbial Community Improvement Is Associated With Soil Properties

Soil properties, notably pH, is considered a major driver of determining the soil bacterial diversity and composition [28]. The RDA analysis suggested that soil pH integrated with soil nutrition had a strong correlation with microbial community changes in the cultivation of *G. elata* (Fig. 5). Elements such as potassium, zinc, magnesium, and calcium are known to provide substrate for soil microorganisms and are about to change the activity and composition of the microbial community [29]. In the cultivation of *G. elata*, soil available zinc, exchangeable potassium, calcium, and magnesium are observed to increase compared to that in barren land (Figure S1). The increase might be due to the symbiosis fungus *A. mellea*, for it has the ability to released such elements into the soil through rhizomorph exudate [30]. These available elements are supposed to be connections between soil pH and soil microorganisms to response to cultivation management.

The truth is the cultivation of *G. elata* and rotation of *P. impudicus* affected the abundances of a number of genera across several phyla, in which most of the genera were also associated with soil physiochemical properties, especially some soil taxa that were more sensitive to soil pH variations. For instance, *Acidothermus*, an acid-loving genus, its abundance initially decreased with the increase of pH in *G. elata* cultivation, then increased with the reduction of pH under rotaiton of *P. impudicus* (Fig. 4A, Table 1). Indicating that rotation of *P. impudicus* helped to improve the environment for acid-loving genera living. In other words, the rotation of *P. impudicus* can help maintain soil microbial composition by recovering soil nutrient equilibrium, which is also closely related to other soil microorganisms' activities [31].

Besides, we also found that the relative abundances of five bacterial groups, *Acidibacter*, *Bryobacter*, *Burkholderia-Paraburkholderia*, *Candidatus_Solibacter*, and *Roseiarcus*, four fungal groups, *Chaetosphaeria*, *Chloridium*, *unclassified_c_Leotiomyces*, and *unclassified_f_Thelephoraceae* had significantly higher relative abundance in soil associated with rotation of *P. impudicus* (Fig. 4), which consistent with the changes of soil TOC content and soil humus composition and properties among different treatments (Table 1 and S1). This result indicated that in addition to soil pH value, other soil parameters, such as soil C sources, also contributed to the microbial community compositions in this study. Demonstration of Sul et al [32] previous work also supported our discovery. They emphasized that TOC was essential in explaining the differences in the soil bacterial community structures in tropical agricultural soil [32]. These results strongly implied that the shift pattern of soil microbial community

composition and structure in soil associated with cultivation of *G. elata* and rotation of *P. impudicus* are related to soil physiochemical changes, which mediated by the rotation management.

Soil properties re-optimized by rotation of *P. impudicus* has potential benefits in maintaining plant health in the next *G. elata* cultivation season

We found that rotation of *P. impudicus* decreased soil pH, which cultivation of *G. elata* increased before (Table 1). The observed increase in pH in soil associated with *G. elata* might be caused by abundant and alkaline rhizomorph exudate released by *A. mellea* [30]. Regarding that *G. elata* is normally cultivated in acidic soils [33, 34], and the *A. mellea* rhizomorphs typically grow well at a depth of 30 cm in soil with pH 4.3–4.6 [35], the reduction in pH after rotation of *P. impudicus* is beneficial and it may help re-optimize the soil environment for *G. elata* and its symbiotic fungus *A. mellea* growth in the next *G. elata* cultivation season.

According to previous studies, *A. mellea* normally assimilates soil carbon and significantly reduces carbon content in humus, especially levels of fulvic acid to obtain its growth materials [36], that's can explain why a reduction (even though not statistically significant) in soil humus composition in *G. elata* cultivation was found at first (Table S1). However, *P. impudicus* was reported to have the ability to producing dense cross-linked networks of thick cords, and bonding soil particles into a coacervate [37]. Therefore, in our study, rotation of *P. impudicus* was observed to increase the ratio of humic to fulvic acid, degree of humification and TOC concentration (Table S1), which implies that soils where *P. impudicus* was cultivated after *G. elata* had greater carbon storage capacity than soils where only *G. elata* was planted. It is commonly accepted that high C storage is vital for soil health and long-term ecosystem sustainability [38]. Accordingly, rotation of *P. impudicus* help reverse *G. elata*-induced changes in pH value and C storage in soil, which may become more preferable for the growth of *G. elata* and its symbiosis fungus *A. mellea*.

Conclusion

By comparing the differences of soil physicochemical properties and soil microbial community in the rhizosphere soil of *G. elata* cultivation and rotation of *P. impudicus* management, we found that the impact of *G. elata* cultivation and rotation of *P. impudicus* on the abundance of a number of major bacterial and fungal genera was related to soil physiochemical properties, such as pH and other elements. Rotation of *P. impudicus* improves soil quality by reversing *G. elata*-induced changes in soil pH, nutrient levels, and humus composition, which may have a beneficial effect on plant health in following growing season. This study highlighted the importance of rotation management in artificial cultivation of TCM, and had deep implications in choosing plants as target rotation object.

Abbreviations

TCM: traditional Chinese medicine; CK: soil samples collected from barren land; GE: soil adhering to the rhizo zone of *G. elata*; PI: rhizosphere soil surrounding *P. impudicus*; OTUs: Operational taxonomic units.

Declarations

Acknowledgements

We sincerely thank Guizhou Wumeng Fungi Industry Co., Ltd. to supply the fields planting *G. elata* and *P. impudicus*.

Author Contributions

ZT, GL and JW designed this study. Samples collection, material preparation and measurements were performed by XJ, LL and YC. The statistical analysis was done by WY and XC, first draft of the manuscript was written by WY and YQ. All authors read and approved the final manuscript.

Funding

We wish to thank the National Natural Science Foundation of China (project No. 81960694 and 32060080) for buying *G. elata* juvenile tuber *P. impudicus* strains, the Guizhou Provincial Science and Technology projects (project No. Z.K. [2021] General 523) for founding experiment setup and the sample collection, the High-level Innovative Talents Program of the Guizhou Province of China (project No. Qian Ke He Platform and Talent [2018]5638-2) for 16S rRNA and ITS rRNA sequencing, and the Research Platform Team Project of the Education Department of Guizhou Province (project No. Qian Jiao Ji [2022]021) for cultivation of *A. mellea*, the Scientific and technological innovation project of China Academy of Chinese Medical Science (project No. CI2021B013) for traffic fees commuted to planting bases.

Availability of data and materials

Raw data of 16S rRNA gene and ITS rRNA amplicon sequences supporting the findings of the present study are available in the Sequence Read Archive of NCBI under BioProject accession PRJNA905858 and PRJNA905861, respectively.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹ Resource Institute for Chinese & Ethnic Materia Medica, Guizhou University of Traditional Chinese Medicine, Guiyang, China.

² National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China.

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Figures

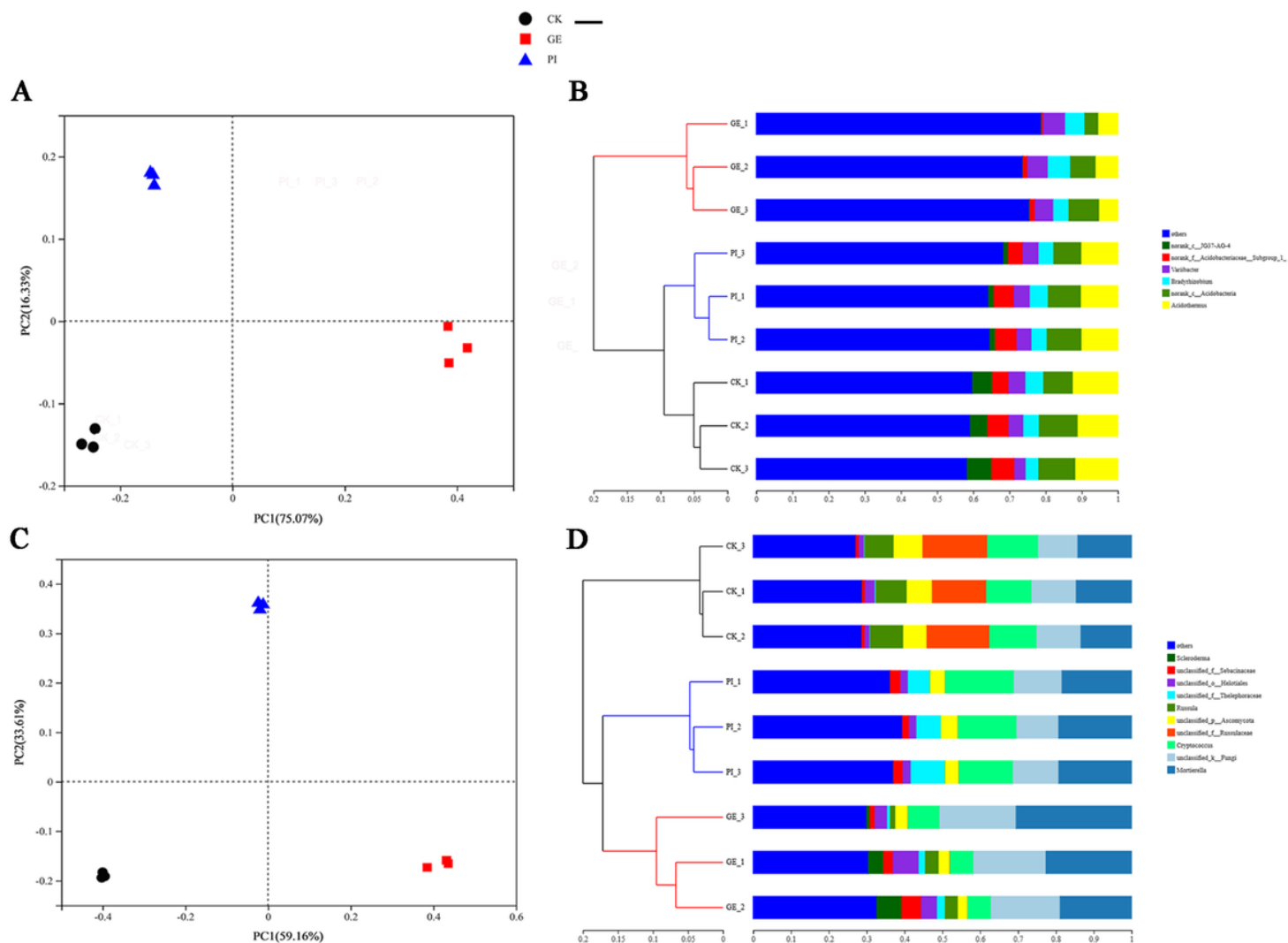


Figure 1

Principal coordinate analysis (PCoA) on OTU level and Hierarchical clustering analysis (HCA) on genus level, both were calculated based on the Bray-Curtis distance. A and B represent the PCoA analysis and HCA analysis of bacterial community; C and D represent the PCoA analysis and HCA analysis of fungal community. Abbreviation: CK, GE, and PI represent soil samples collected from barren land (control), soil adhering to the rhizo zone of *G. elata*, rhizosphere soil surrounding *P. impudicus*, respectively.

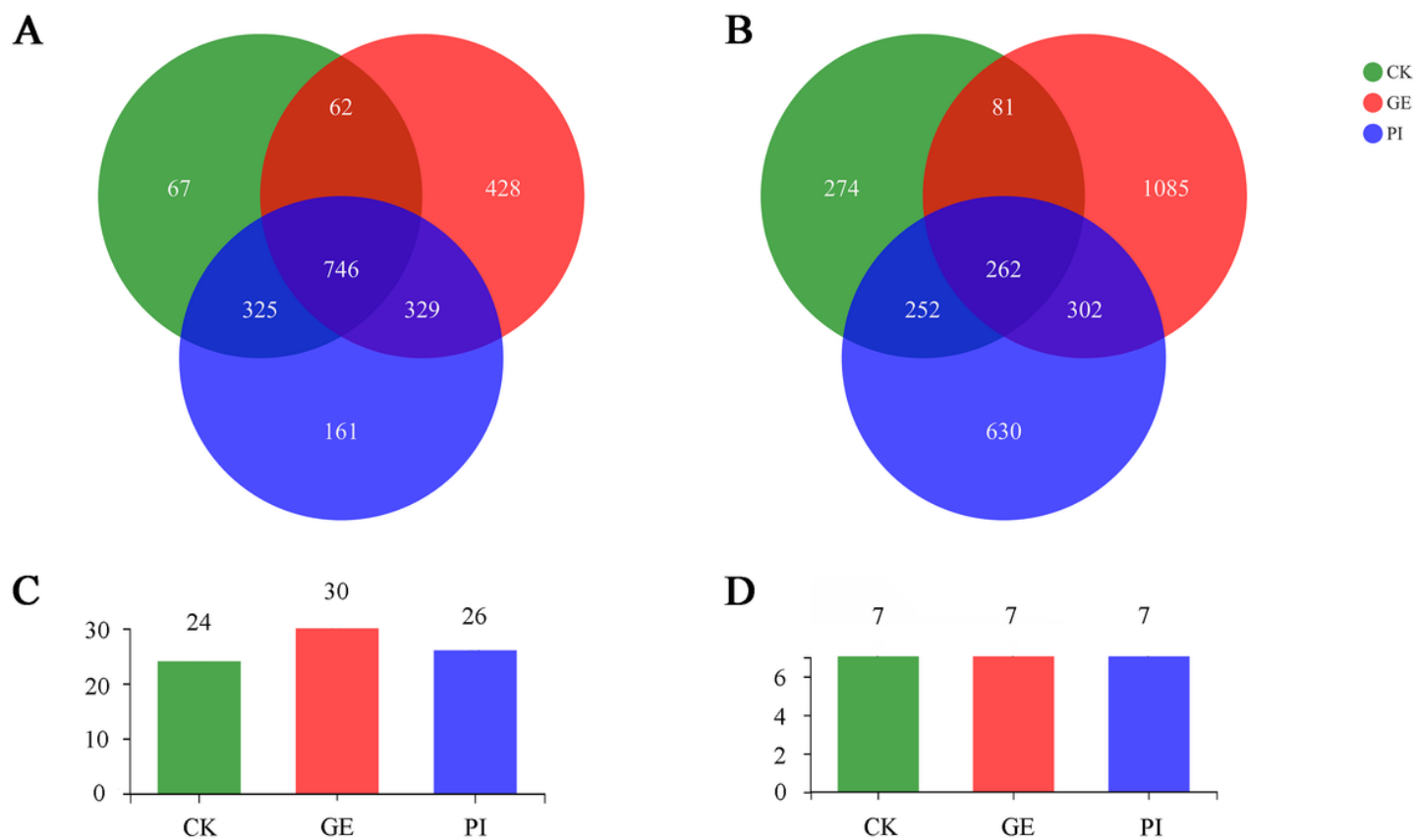


Figure 2

Shared OTUs in venn diagram of different soil samples. A and B, represent the venn diagram of bacterial community and fungal community on OTU level. C and D, represent the histogram of species numbers in different soil samples on phylum level. Abbreviation: CK, GE, and PI represent soil samples collected from barren land (control), soil adhering to the rhizo zone of *G. elata*, rhizosphere soil surrounding *P. impudicus*, respectively.

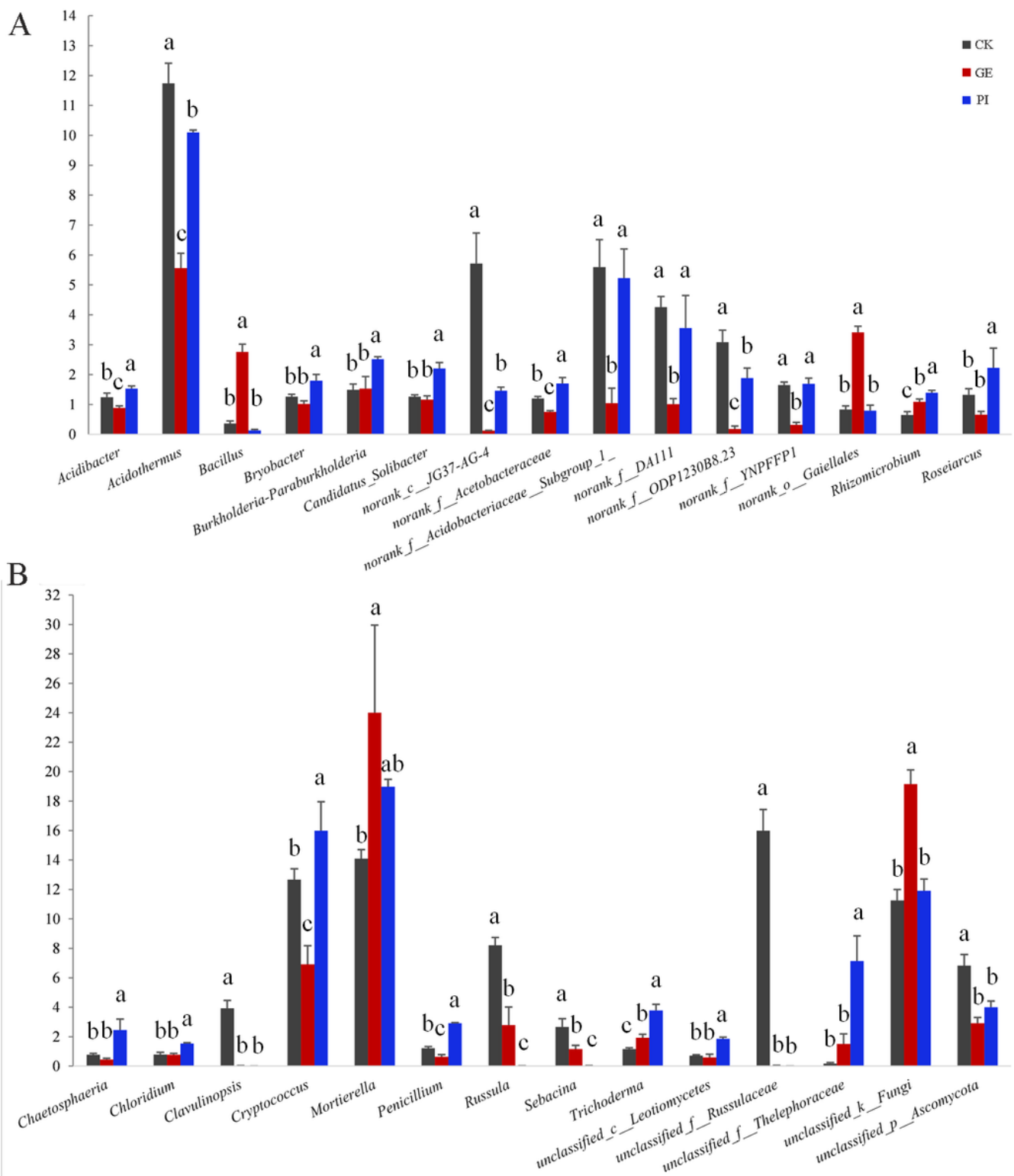


Figure 4

Relative abundances of microbial genera that showed significant differences among samples. A, B represent soil bacterial and fungal communities. Abbreviation: CK, GE, and PI represent soil samples collected from barren land (control), soil adhering to the rhizo zone of *G. elata*, rhizosphere soil surrounding *P. impudicus*, respectively. Lowercases a, b, and c in figures show significantly differences

between different soils (CK, GE and PI), determined by one-way ANOVA and Duncan's multiple range test ($P<0.05$).

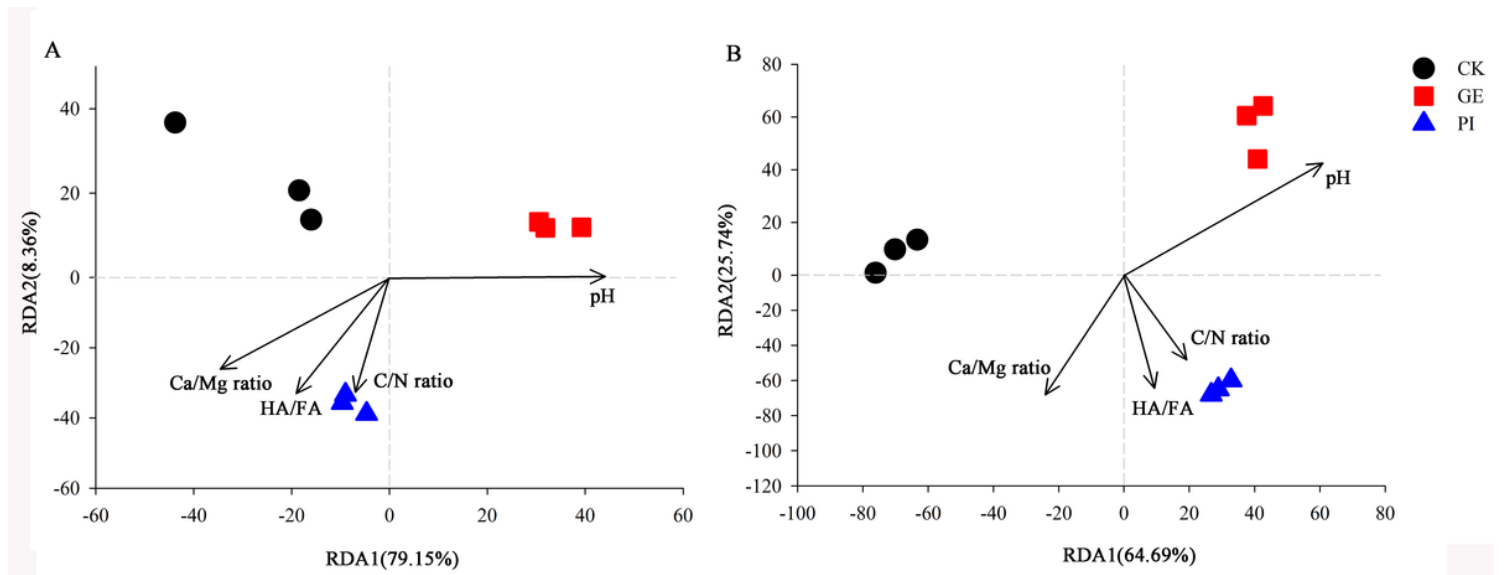


Figure 5

Redundancy analysis (RDA) of MiSeq data (symbols) and environmental characteristics (arrows). A, B represent soil bacterial and fungal communities. Abbreviation: CK, GE, and PI represent soil samples collected from barren land (control), soil adhering to the rhizo zone of *G. elata*, rhizosphere soil surrounding *P. impudicus*, respectively.

Supplementary Files

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