Molecular Characterization of Distinct Fungal Communities in the Soil of a Rare Rarth Mining Area

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Abstract

The exploitation of ion absorbed rare earth elements (REEs) has caused serious ecological destruction and environmental pollution. Effect on soil fungal structure and diversity caused by mining activities are usually ignored, although fungi are one of the most important components in soil ecosystems. In the present research, quantitative polymerase chain reaction (qPCR) and high-throughput Illumina MiSeq sequencing were conducted to characterize fungal community composition and structure in soil of a rare earth mining area after *in-situ* leaching. Statistical analyses, Network and FUNGuild were used to conduct in-depth analysis. *Ascomycota, Basidiomycota, Glomeromycota* and *unclassified fungi* were the most abundant phyla in the mining soils. Organic matter, TC and TN contents, but not pH or REEs contents, were the vital factors to determine soil fungal abundances and diversities. Fungal community structures were stable after leaching practice, but nutrition contents significantly and positively contributed to fungal abundances and diversities. Fungi could mediate the interaction between species to enhance their ability to resist the harsh environment of REEs toxicity or ammonium caused by *in-situ* leaching practice. Saprotroph in phyla *Ascomycota* and *Basidiomycota* were the dominant fungal trophic mode in the mining soils, and they played a critical role in nutrient cycling, transformation processes and reducing metal toxicity. Symbiotrophs of phyla *Glomeromycota* contributed to soil aggregation and slowing down nutrient losses after *in-situ* leaching practice.

Introduction

Rare earth elements (REEs) were an important component of the development of modern science and technology. Increasing demand for rare earth resources in various industries had resulted in excessive exploitation of rare earth mines. The largest ion-absorbed REEs deposit in the world is in Jiangxi province, accounting for more than half of the total production in China. While, excessive mining activities and neglected environmental managements caused serious ecological destruction and environmental pollution [1].

Nowadays, *in-situ* leaching is the dominating technology for extraction REEs from ion-absorbed REEs deposits, given that there is less topsoil removed and the process can be performed *in-situ*. The leaching solution is generally 3–5% ammonium sulfate ($(NH_4)_2SO_4$), and the leaching process takes 150–400 days [2]. During the leaching process, the injection holes are drilled across REEs deposits, with a diameter of 0.2 m and a distance of 1–2 m between each hole. Due to the lack of effective anti-seepage measure and imperfect solution collection system, abundant NH_4^+-N and rare earth ions infiltrate into the mining soil and surrounding regions, and then become the serious pollution source, which not only results in mining soil acidification and surface vegetation destroying, but also makes restoration more difficult.

Besides the macroscopic ecological degradation in mining areas, the soil properties and soil microorganisms underwent relatively great changes. Excessive ammonium and REEs accumulated in mining soil, and organic matter might be washed away along with the extraction and leaching process. Previous study reported that total rare earth elements (TREEs) and ammonium were the strongest

predictors of the bacterial community structure in the mining area, and bacterial and archaeal abundances were significantly and negatively correlated with ionic REEs [3]. As one of the most abundant living organisms on the earth, fungi are vital participants in the degradation of organic matters and mineral components, element cycle, and adsorption and fixation of metal ions [4, 5], which are indispensable for regulating the soil environment. Simultaneously, the process of fungi decomposing plant residues was a key step to release inorganic nutrients and convert them into soil organic matter, which was a prerequisite for the improvement of soil physicochemical properties and nutrient capacity [6]. Some fungi, such as arbuscular mycorrhizal fungi (AMF), could increase soil microbial biomass and activities to promote plants growth [7, 8]. Consequently, fungi should be beneficial to the improvement of soil quality, plant growth and phytoremediation in mining area. However, we know little about the diversity and function of fungal groups in REEs mining soil. It is not yet clear how fungal communities are influenced or reshaped by anthropogenic mining activities, and whether *in-situ* leaching practice would affect fundamental environmental factors of fungal communities.

Thus, we conduct a comprehensive examination of fungal communities in soil of a REEs mining area after *in-situ* leaching practice using qPCR and MiSeq sequencing technique. We characterized the distribution of fungal communities to evaluate the correlations between fungi communities and *in-situ* leaching as well as other soil environmental factors in mining area, and then gain insight into fungal potential interactions and functions by network and FUNGuild. This study will provide an ecological basis for the influence of REEs exploitation on mining ecosystem, and expand our understanding of microbial ecology in a natural environment subjected to pollution.

Materials And Methods

Study site and Sample collection

Six sampling sites were randomly selected in Longnan County, Jiangxi Province, China. Six topsoil samples (0–5 cm, marked as LK1_1, LK2_1, LK3_1, LK4_1, LK5_1, and LK6_1) and six deep layer soil samples (50–60 cm, marked as LK1_2, LK2_2, LK3_2, LK4_2, LK5_2, and LK6_2) were both collected at each site by a stainless steel core soil sampler. A mountain topsoil sample (0–5 cm) labelled as CKS, and a farmland topsoil sample (0–5 cm) labelled as CKT were used as reference soils. Soil physicochemical properties, including pH, electrical conductivity (EC), moisture content, organic matter, total C (TC), total N (TN), nitrate, ammonium, ion adsorption REEs and total rare earth elements (TREEs), were analyzed. Details of the experimental site description, sampling process and analytical methods of soil physicochemical properties have been described in our previous research [3].

Soil DNA extraction and quantitative PCR

Soil DNA was extracted from frozen soil samples by using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA), and quantified by spectrophotometry (NanoDrop 2000 Spectrophotometer, Thermo Scientific, USA). The fungal ITS gene copy number was determined in triplicates via qPCR in an ABI 7500 Fast (Thermo Fisher, USA) with the primer pair of ITS1F/ITS2F. Each PCR reaction contained 2 μL

extracted soil DNA, 16.4 μL SYBR Green qPCR Master Mix, and 0.8 μL 5 μM forward and reverse primers (each). qPCR programme run started at 95°C for 5 min for an initial denaturation, and then continued with 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min. The amplification was confirmed with a melting curve and visualized by 1.5% agarose gel electrophoresis. Standard curves were constructed with a 10-fold serial dilution of known copy numbers of plasmids harbouring the target gene.

Illumina MiSeq sequencing of soil samples

The extracted DNA of each sample was used to amplify with the primer pair SSU0817F/1196R for Illumina sequencing analysis, and the reverse primer labelled with a 12-bp barcode unique for pooling multiple samples in one run of MiSeq sequencing. PCR was performed to amplify 2 μL template DNA in a 20 μL reaction volumes, containing 2 μL 10×Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL of each primer at 5μM, 0.2 μL TaKaRa rTaq Polymerase, 0.2 μL BSA, and sterile double-distilled H_2O. PCR conditions: denaturation at 95°C for 5 min, then 40 cycles (95°C for 30s, 55°C for 30 s, 72°C for 40 s), and a final extension at 72°C for 10 min. The amplifications were checked in a 1.5% agarose gel electrophoresis, and then purified using an AxyPrep DNA Gel Extraction Kit (Axygen, US). The purified amplicons were pooled in equimolar, and then sequenced on Illumina MiSeq platform (paired-end 2×250 bp mode) at Shanghai Majorbio Bio-pharm Technology Co. Ltd. The raw sequences have been deposited in the NCBI Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) as BioProject SRP140551.

Bioinformatics and statistical analysis

The raw sequences were demultiplexed and again quality controlled to remove low-quality sequences. Singletons were removed before downstream analysis, and high-quality sequences were chosen at 97% identity of phylotypes by USEARCH v7.0, including chimaera detection.

The representative sequences were taxonomically determined on Silva database (release 132) using RDP classifier v2.11 with a 70% confidence threshold. Alpha diversity estimator (sobs, Chao1, ACE, Shannon and Simpson indices) were calculated by Mothur version v.1.30.2 to compare the complexity of species diversity. Principal coordinate analysis (PCoA) was calculated based on unweighted-unifrac-full-tree distance metrics using the R package [9]. Canonical correlation analysis (CCA) was analyzed to determine which environmental factors were most related to fungal community structure, using Canoco program for Windows 4.5 (Biometrics, the Netherlands). Heatmap was calculated using the pheatmap package in R, using pearson correlation coefficient to display the relationship between the most abundant fungal class and soil physicochemical properties. The association networks of the fungal communities were performed by NetworkX [10]. To identify different functional groups within fungal communities and link their relative abundance to particular ecological function, FUNGuild was used to taxonomically parse fungal sequences into three ecologically relevant trophic modes – saprotrophs, symbiotrophs and pathotrophs. These three modes were further subdivided into specific guilds that comprised fungi with similar lifestyle modes (e.g. saprotrophs, mycorrhizae, pathogens) [11].

Independent samples *t*-test and Pearson/Spearman rank correlation analysis were analyzed using SPSS (v 22.0). Statistical significance was determined at the confidence level of 0.05. In order to describe the strength of the relevant magnitude, effect size was determined by Cohen's *d* [12, 13].

Results

Soil physicochemical properties

The physicochemical properties of 14 soil samples have been analyzed in our previous research, as well as the REEs contents [3]. In brief, the mining soils were strongly acidic (4.75 ± 0.33, mean ± s.d.), and the reference soils were neutral (7.35 ± 0.63). Contents of NH_4^+-N and NO_3^--N varied greatly in the mining area. Organic matter (3.15–10.9%), ionic element Y (7.34–297.30 mg/kg) and ionic REEs (14.62-604.96 mg/kg) showed that contents of soil samples in mining soil were significantly higher than the reference soil samples. All the soil physicochemical properties were shown in Supplementary Table S1. The concentrations of individual REEs in all samples were also summarized in Table S2 and S3.

Fungal abundance and community structural diversity

Table 1 shown the abundances of fungal ITS genes in all the soil samples. The abundances of fungi in the mining area varied from 3.04×10^5 to 9.59×10^8 copies per gram soil. Fungal ITS genes in reference soil samples were heavily outnumbered those of the mining soil samples, except LK3-1, and the differences were significant ($p < 0.05$).

Table 1
Abundances of fungal ITS genes in soil samples.

Sample	Fungal ITS gene $\times 10^5$ copies/g soil
LK1_1	73.2 ± 7.45fg
LK1_2	23.9 ± 1.81fg
LK2_1	n
LK2_2	3.04 ± 0.64g
LK3_1	9590 ± 66.5a
LK3_2	161 ± 1.87ef
LK4_1	261 ± 36.7e
LK4_2	n
LK5_1	n
LK5_2	86.9 ± 2.37fg
LK6_1	508 ± 75.5d
LK6_2	n
CKS	1598 ± 72.1c
CKT	2824 ± 188b
Data are average value ± standard deviation; n, significant interference by nonspecific amplifications.	
Different letters in a single column represent significant differences ($p < 0.05$).	

Across all fourteen samples, 412,023 sequences of the fungal 18S rRNA gene were remained after quality trimming, which were clustered into 340 phylotypes. Among them, 309 phylotypes represented 357,568 sequences were identified in the twelve mining soil samples. In order to correct the sampling effort, the minimal sequencing number of 17,139 was randomly selected in each sample and then used for subsequent community analysis. Fungal community relative abundance estimator (ACE), the richness index (sobs and Chao1) and diversity estimators (Shannon, Simpson) were compared for different depths. Predicted abundance and richness using Sobs, ACE and Chao1 estimators showed that the fungal community abundance and richness of the mining topsoil were significantly greater than those of the deep layer soil (Supplementary Table S4 and S5).

The fungal phyla with high relative abundance were *Ascomycota*, *Basidiomycota*, *Bicosoecida*, *Glomeromycota*, and *Platyhelminthes*. No significant taxonomic differences were observed between mining soils and reference soils, but the proportion of each dominant fungal phylum varied significantly.

The majority of fungal sequences belonged to *Ascomycota* and *Basidiomycota*, which accounted for 52.73% and 24.61% of all the mining soil fungal sequences. *Ascomycota* was the most abundant phylum of the two reference soil samples, and accounted for 74.64% and 92.00%, respectively. *Basidiomycota* were more abundant in mining soils than that in reference soils (Fig. 1). A certain proportion of the obtained fungal 16S rRNA gene sequences (accounting for 0.723–33.59%) could not be related to known fungal phyla. *Glomeromycota* were more abundant in deep layer soil than that in topsoil, and the effect size of the difference was large, although it was not significant ($d > 0.8$, Supplementary Table S5).

Significant differences in relative abundances of typical groups at class level were observed between mining soil and reference soil (Fig. S1). *Agaricomycetes* (2.28–91.14%) were the most abundant fungi in the mining soils, and it was dominant in mining soil sample LK6-2, but this fungal class merely represented 8.46% and 0.56% in two reference soil samples, respectively. In contrast, *Sordariomycetes* (52.03–76.65%) was significantly more abundant in the reference soils than that in mining soils. The PCoA clearly grouped the fungal communities according to different soil depths, although a certain similarity was observed between mining topsoil and deep layer soil. The first two axes (PC1 and PC2) explained 42.47 and 17.39%, respectively, of the total variance in fungal species (Fig. 2).

Fungal community links to soil properties

In the mining soils, the correlations between soil physicochemical properties and fungal abundances were conducted by Pearson rank correlation analysis (Supplementary Table S6). The fungal abundances were significantly and positively correlated with NH_4^+-N, NO_3^--N, organic matter, TC and TN content. No significant correlations could be found between fungal abundances and pH, EC, ionic REEs or TREEs contents (all $p > 0.05$).

Spearman rank correlation analysis was reflected which environmental factors contributed to the variation in fungal community diversity (Supplementary Table S7). Organic matter, TC and TN contents were shown to be significantly and positively correlated with fungal abundance and richness indices (ACE, Chao 1 and Sobs). Moreover, TC and TN significantly and positively contributed to fungal community diversity (Shannon index). While, none of the estimator indices exhibited any significant correlation with pH, NH_4^+-N, NO_3^--N, ionic REEs or TREEs (all $p > 0.05$).

In-situ leaching mining changes environmental characteristics and microbial community structures. The interference of leaching on microbial communities may primarily be regulate by soil geochemical properties. Therefore, associations between the fungal community structures and soil properties were investigated. CCA revealed that the fungal community structures were mediated by soil physicochemical properties (Fig. 3). Axis1 and axis2 explained 16.47% and 13.31% of the total variation. However, none of the environmental factors significantly contributed to the fungi-soil property relationship. A correlation heatmap explored the relationships between soil physicochemical properties and relative abundances of specific lineages at the class level (Fig. 4). Significantly positive correlations were observed between relative abundance of *Sordariomycetes* and TC, TN, NH_4^+-N, and NO_3^--N contents. Organic matter, NO_3^--

N, TC and TN contents extremely significantly and positively affected relative abundances of *Tremellomycetes* and *Eurotiomycetes.*

Association network and FUNGuild analysis

The resulting soil fungal network (Fig. 5, Supplementary Table S8) was analyzed with the first 100 dominant phylotypes, representing 97.8% of the mining soil total sequences. The average neighbours number of the network was 11.78, and the phylotypes belonging to *Ascomycota* (OTU62) and *unclassified_Fungi* (OTU243) both presented 30 neighbours. Betweenness centrality (BC), closeness centrality (CC) and degree centrality (DC) were calculated to describe the importance of species-nodes within the network, and the restrictive conditions of BC > 0.05, CC > 0.45 and DC > 0.18 were optionally determined. Then, four phylotypes (OTU243, OTU62, OTU237, and OTU43) belonging to phyla *Ascomycota*, *Basidiomycota*, and *unclassified fungi*, were selected to represent the putative cornerstone of the fungal network structure. *Ascomycota* was the most abundant phylum in the network. There were twelve phylotypes related to class *Sordariomycete*s phylum *Ascomycota*. *Agaricomycetes* in phylum *Basidiomycota* had fifteen phylotypes. Thirty-one phylotypes of the network with two keystone species affiliated to the *unclassified fungi*, and they were all represented by unclassified phyla groups. *Sordariomycete*s and *Agaricomycetes* were considered as cornerstone classes.

We used the FUNGuild tools to better understand the important roles that fungi played in mining soils (Fig. 6). According to the ecologically relevant trophic modes, *unclassified fungi* comprised 67.44% of all the sequences in mining soils. Saprotrophs, symbiotrophs and pathotrophs accounted for 19.89%, 5.93% and 1.22%, respectively. Saprotroph-pathotroph-symbiotroph accounted for 5.15%, and the relative abundance of pathotroph-symbiotrophs was the least (0.35%). There were no significant differences in ecological functional compositions between topsoil and deep layer soil. Compared with reference soils, there were more AMF and ectomycorrhizal fungi (EMF), less endophyte in the mining soils.

Discussion

Soil properties influencing fungal community composition and diversity

Leaching solution represented an important source of exogenous pollution in the mining area, and the leaching process resulted in soil acidification and accumulation of leaching solution and REEs [2]. Previous studies found that soil pH was not only closely related to fungal diversities [14, 15], but also demonstrated an extremely significant relationship with fungal abundance [16]. So, pH was considered to be one of the dominating factors in determining the soil fungal community distribution [17, 18]. While, in mining soils of this study, pH exerted little impact on fungal community composition, abundance, and diversity (Fig. 3, Fig. 4, Supplementary Table S6 and S7). Such inconsistency could be due to the mining soil pH fluctuating within a small range from 4.27 to 5.19, and fungi are relatively stable in this strongly acidic soil. On the other hand, fungal species have a wider growth pH optimum, suggesting a more

obtuse response of fungi to environmental pH changes [17]. Therefore, pH level of acidic rare earth miming soil is not the major factor affecting fungal community composition and diversity.

Metals are known to alter soil ecosystem diversity, structure and function. Studies have reported the negative effects of heavy metals on fungal growth and reproduction [19]. A decrease in fungal radial growth after exposure to Cd, Cu, Zn and Ni has been observed [20]. Contents of Cr, Zn and Cd were strongly correlated with the diversity and structure of soil fungal community [21, 22]. However, neither ionic REEs nor TREEs displayed a significant influence on fungal richness, diversity, and abundance, although the contents of both ionic REEs and TREEs in mining soil were abundant (Fig. 3, Supplementary Table S6 and S7). One likely explanation is that the fungal community in the mining soil has adapted to the long-term stress of REEs, for this reason, they were not sensitive to soil REEs.

In this study, the fungal diversities and abundances exhibited significantly positive relationships with organic matter, TC and TN contents (Supplementary Table S6 and S7). Furthermore, several highly abundant fungal taxa were observed to have an extremely significant and positive correlation with organic matter, TC and TN (Fig. 4). These results suggested that nutrient elements contents were the dominant edaphic parameters in influencing the fungal community composition. This finding is consistent with the result that fungal community composition is often most closely associated with soil organic matter related characteristics, such as carbon and nutrient types, as well as soil quality [21, 23–25]. Because soil carbon and nitrogen are the fundamental energy sources and component elements for fungi, thus they could affect soil fungi distribution via determining the metabolism [26]. Although the soil nutrients in the mining soil were significantly related to the abundance and diversity of fungal communities, these environmental factors did not significantly contribute to the fungi-soil property relationship (Fig. 3). This may be because fungi are more likely to degrade lignocellulose from plant residues than bacteria and archaea, allowing them to first obtain nutrients from many of the relevant available substances [27].

Predicted functional profiles provided by the network and FUNGuild

Current knowledge about microbe in soil is mostly related to prokaryote, and the contents about fungal communities of various ecosystems are sketchy. Studying and understanding fungal communities is of paramount importance, since fungi not only comprise a major portion of biodiversity and biomass, but also play crucial roles in maintaining soil processes that affect the functions of soil ecosystem [28].

The network graph was built to reveal positive and negative relationships between phylotypes, and describe the co-occurrence patterns within different taxonomic lineage in the mining soil. *Ascomycot*a and *Basidiomycota* were the dominant fungal phyla in the mining soil and the network (Fig. 1 and Fig. 5). Both these two fungal phyla possess critical genes that can encode cellulose decomposition enzymes, promote carbon conversion processes [29, 30], and they play a critical role in nutrient cycling. Most *Ascomycota* are saprophytes, and they were dominant at early stages of the litter degradation process [31, 32]. The richest source of biocontrol fungi is the order *Hypocreales* (class *Sordariomycetes* phyla

Ascomycota), and species in this order are notable for their ability to derive nutrition from diverse nutrient sources [33]. A keystone phylotype of the network in the study belonged to this order. A previous study found that members of *Ascomycota* have a limited ability to degrade the recalcitrant lignin-containing litter material [34]. While, phylum *Basidiomycota* were the important fungi to the degradation of lignocellulosic organic matter [35], and they also participate in soil carbon transformation processes [36]. As the most abundant class in the mining soil, *Agaricomycetes* have been proved to be crucial decomposers, producing both hydrogen peroxide and enzymes, degrading complex plant compounds such as cellulose and lignin [37]. Previous studies had pointed out that at lower values of pH, the accumulation of organic matter in highly contaminated soil could offset the negative effects of metals to a certain extent, and reduce metal bioavailability [38, 39]. Therefore, *Ascomycot*a and *Basidiomycota* could not only stabilize REEs and reduce metal toxicity in the mining soils, they also could provide a significant amount of nutrients for plants and other microorganisms. In addition, some fungus had the ability to tolerate high concentrations of toxic heavy metals and form a variety of heavy metal oxalate deposits [40]. Coupled with the evidence that nearly all the members in the fungal network exhibited positive correlations with each other, fungi in the mining area could improve their adaptability to the adverse environment by regulating the interaction between species.

To the best of our knowledge, this is the first report addressing fungal functions in REEs mining soil. Among the trophic modes, saprotroph was the dominant fungal trophic mode in the mining soils (Fig. 6). It is generally reported that saprophytic fungi are the main decomposers of plant residues in soil, and they have important significance for nutrient cycling and organic matter decomposing [18, 41]. They can also convert organic carbon into available and effective forms [42], and produce a series of hydrolytic and oxidizing enzymes which could decompose carbohydrates and coordinate soil nutrients cycling [43, 44]. These results suggest that saprotrophs could provide nutrients to other microorganisms and plants, moreover, accumulate of organic matter in contaminated soil, and offset the negative effects of REEs to a certain extent in the mining soil.

Symbiotrophs also accounted for a certain proportion in the mining soils, which included AMF and EMF (Fig. 6). It has been proved that almost all plants can form a plant-fungus association (mycorrhizal symbiosis) that promotes the growth of plants, as well as the diversity and stability of microbial community [45]. Mycorrhizal fungi have been successfully applied in ecological restoration of mine sites [46]. AMF are known to improve plant nutrient uptake [45], and they also contribute to ecosystem functions maintenance, such as reducing nutrient losses and soil aggregation [46]. EMF are widely distributed plant symbiotic fungi, and they can utilize their extensive mycelial network to obtain carbon sources from plant roots and promote plants to absorb mineral nutrients [47]. Due to long-term stress of leaching solution, plants in the mining area need the help of symbiotrophs to resist the toxicity from high concertrations of ammonia and REEs; in exchange, fungi obtain more nutrients. This was why symbiotrophs were more common in the mining soils than those in reference soils. These fungi with symbiotropic functions in the mining soil were mainly dominated by *unclassified fungi* of phyla *Glomeromycota*. They should be conducive to slowing down the soil nutrients losses caused by *in-situ* leaching, promoting plant nutrient absorption and plant growth.

In addition, the *unclassified fungi* can not be ignored and are worthy of further studies, because they not only constitute a considerably high proportion in all the mining soil sequences (more than 60%) due to the limitations of the FUNGuild database, but also contributed to maintaining community structure of the network (Fig. 5 and Fig. 6).

Conclusion

In this study, fungal communities in REEs mining soil was analyzed for the first time. *Ascomycota*, *Basidiomycota*, *Glomeromycota and unclassified fungi* were the most abundant phyla, which might play critical roles in complex compounds degradation and soil nutrient transformation processes. Organic matter, TC and TN contents were not only the dominating factors to determine the soil fungal abundances and diversities, but also significantly related to distribution of several abundant fungal taxa. However, neither ionic REEs nor TREEs displayed a significant correlation with the fungal structure. It implied that fungi in the mining soil have adapted to the long-term stress of REEs, and the communities were now stable. The network analysis demonstrated that *Ascomycota* and *Basidiomycota* not only played an important role in nutrient cycling, and also contributed to reducing REEs toxicity. Fungi could enhance and adjust the interaction between species to improve their adaptability and to adapt to the harsh environment of REEs toxicity or ammonium caused by *in-situ* leaching mining. Furthermore, the functional prediction results suggested that saprophytic fungi played an important role in nutrient cycling and transformation processes. Symbiotrophs in phyla *Glomeromycota* contributed to slowing down soil nutrients losses caused by *in-situ* leaching, promoting plant nutrient absorption and plant growth. These findings would expand our knowledge of microbial ecology in REEs mining area, and provide important information for ecological risk assessments of mining areas.

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Authors' contributions JJ Liu and C Li conducted the experiments, analyzed data and wrote the manuscript. WD Ma conducted the experiments. W Liu and WX Wu critically revised the manuscripts of important knowledge content.

Declarations

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Conflict of interest The authors declare that they have no conflict of interests.

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Figures

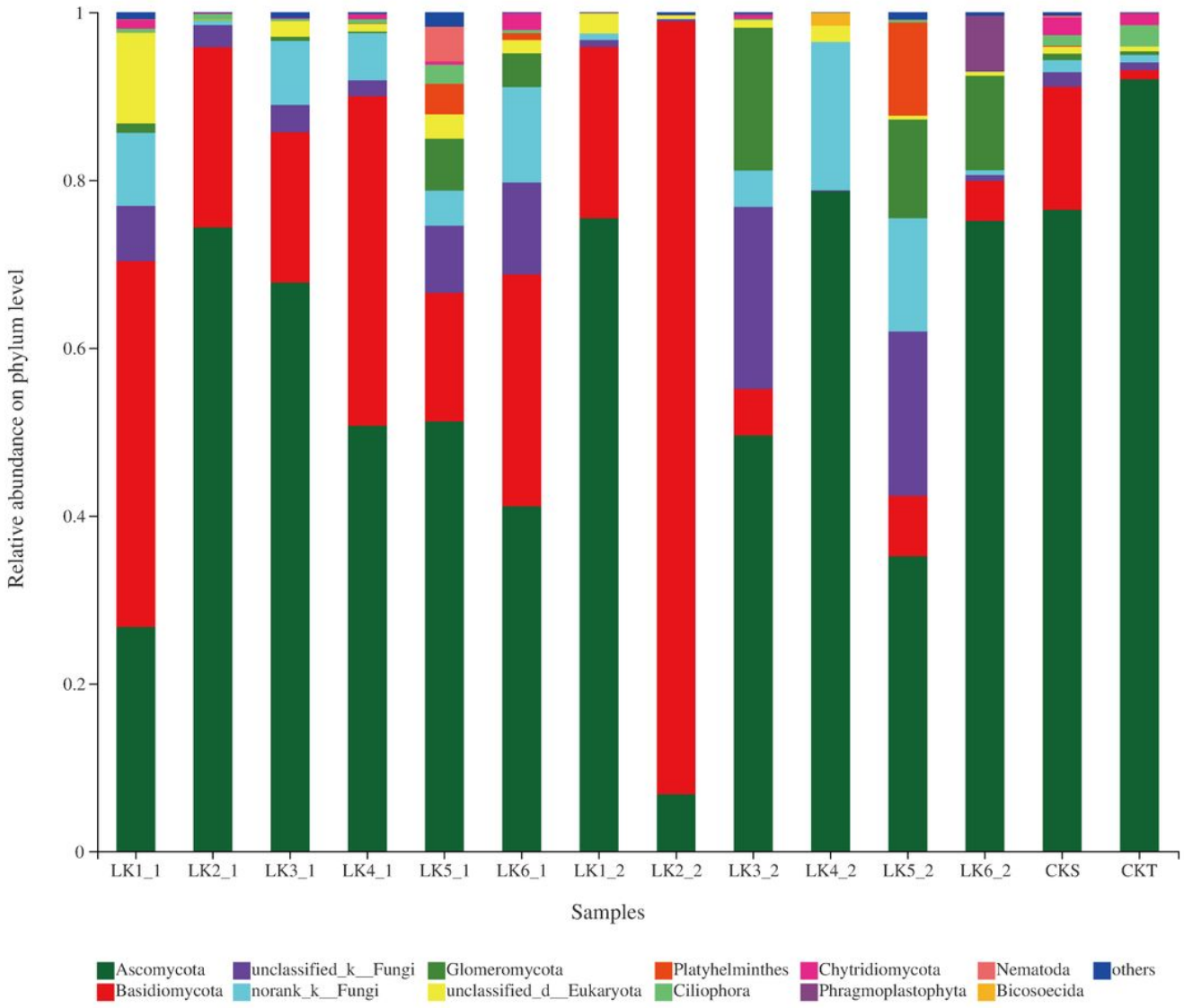

Figure 1

Relative abundance of fungal phyla in all the samples. Fungal clades with abundances > 1% are shown.

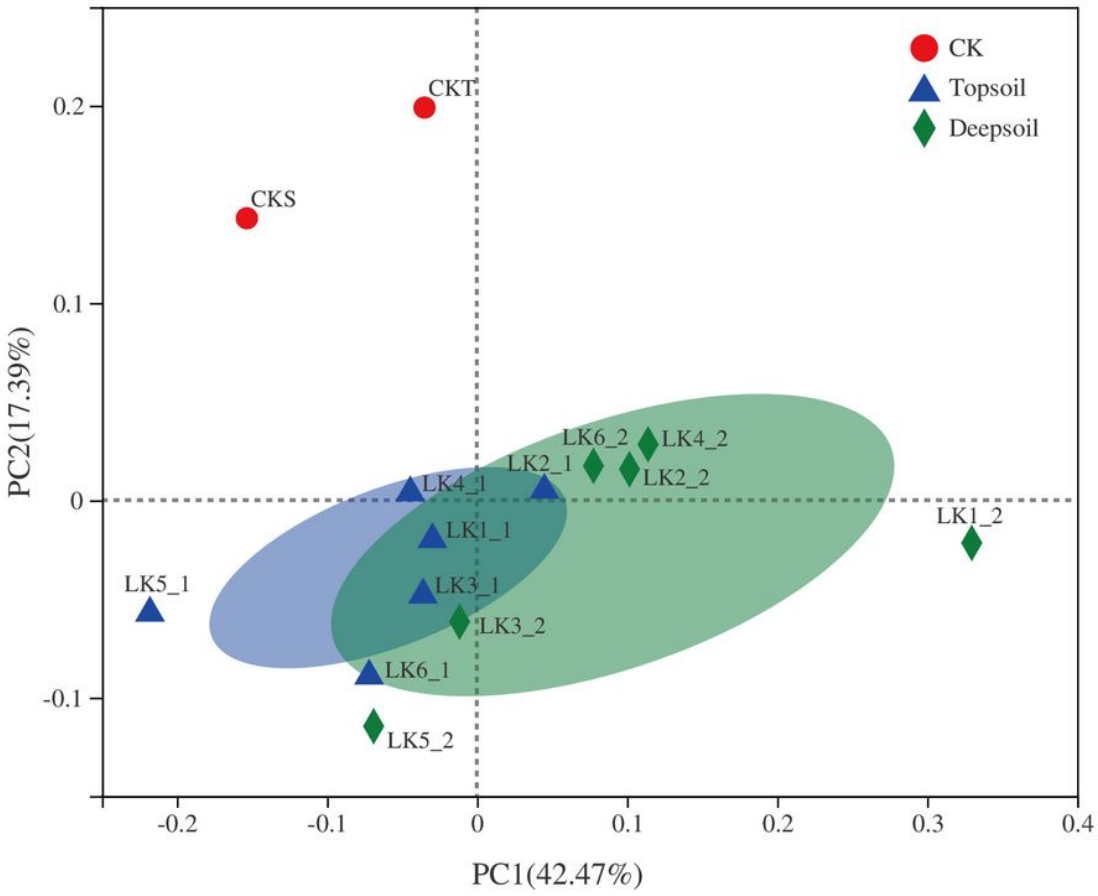

Figure 2

PCoA analysis of the soil fungal communities. The values of axes 1 and 2 are the percentages that can be explained by the corresponding axis. Topsoil, topsoil in mining area; Deepsoil, deep layer soil in mining area; CK, CKS and CKT.

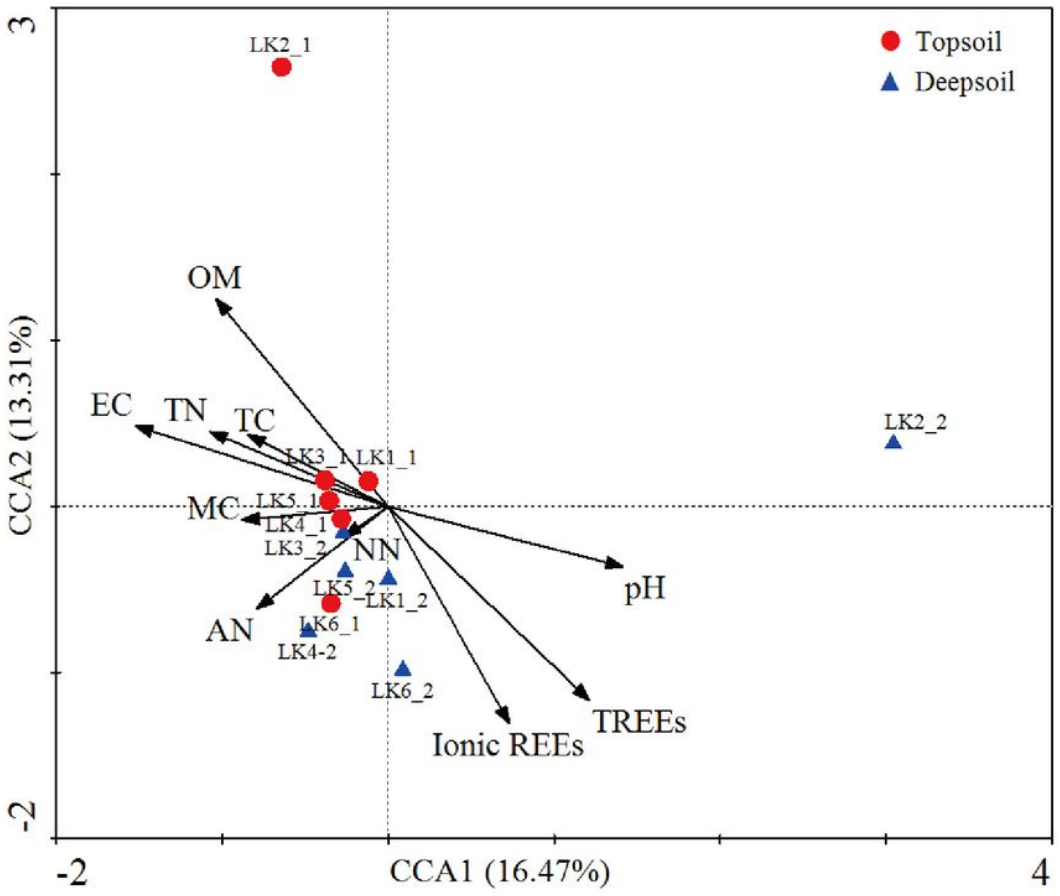

Figure 3

Correlations between mining soil properties and the fungal community structure determined by CCA. Topsoil, topsoil in mining area; Deepsoil, deep layer soil in mining area. Ten soil properties were taken into consideration (as shown in Table S1 and Table S3): MC, moisture content; OM, organic matter; NN, nitrate nitrogen; AN, ammonium nitrogen; TC, total C; TN, total N; EC, electrical conductivity; TREEs, total rare earth elements; Ionic REEs, ionic rare earth elements.

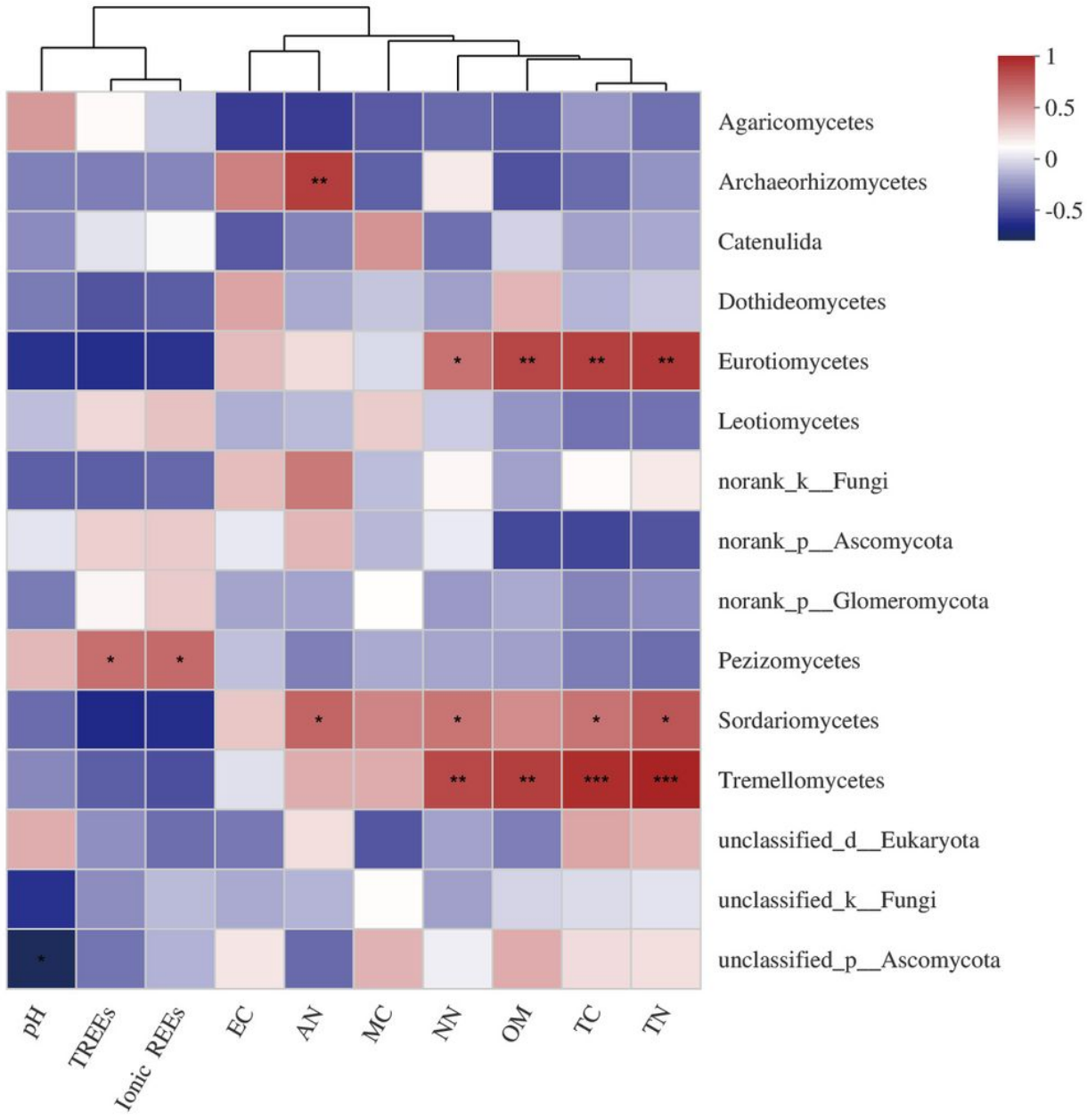

Figure 4

The heatmap of the correlation between the mining soil properties and the top fifteen fungal phyla. R values are plotted in different colors; the right side of the legend shows the color range and the corresponding R values. Significant values are shown as: * $p < 0.05$; ** $0.001 < p < 0.05$; *** $p < 0.0001$. Ten soil properties were taken into consideration (as shown in Table 1 and Table S3): MC, moisture content; OM, organic matter; NN, nitrate nitrogen; AN, ammonium nitrogen; TC, total C; TN, total N; EC, electrical conductivity; TREEs, total rare earth elements; Ionic REEs, ionic rare earth elements.

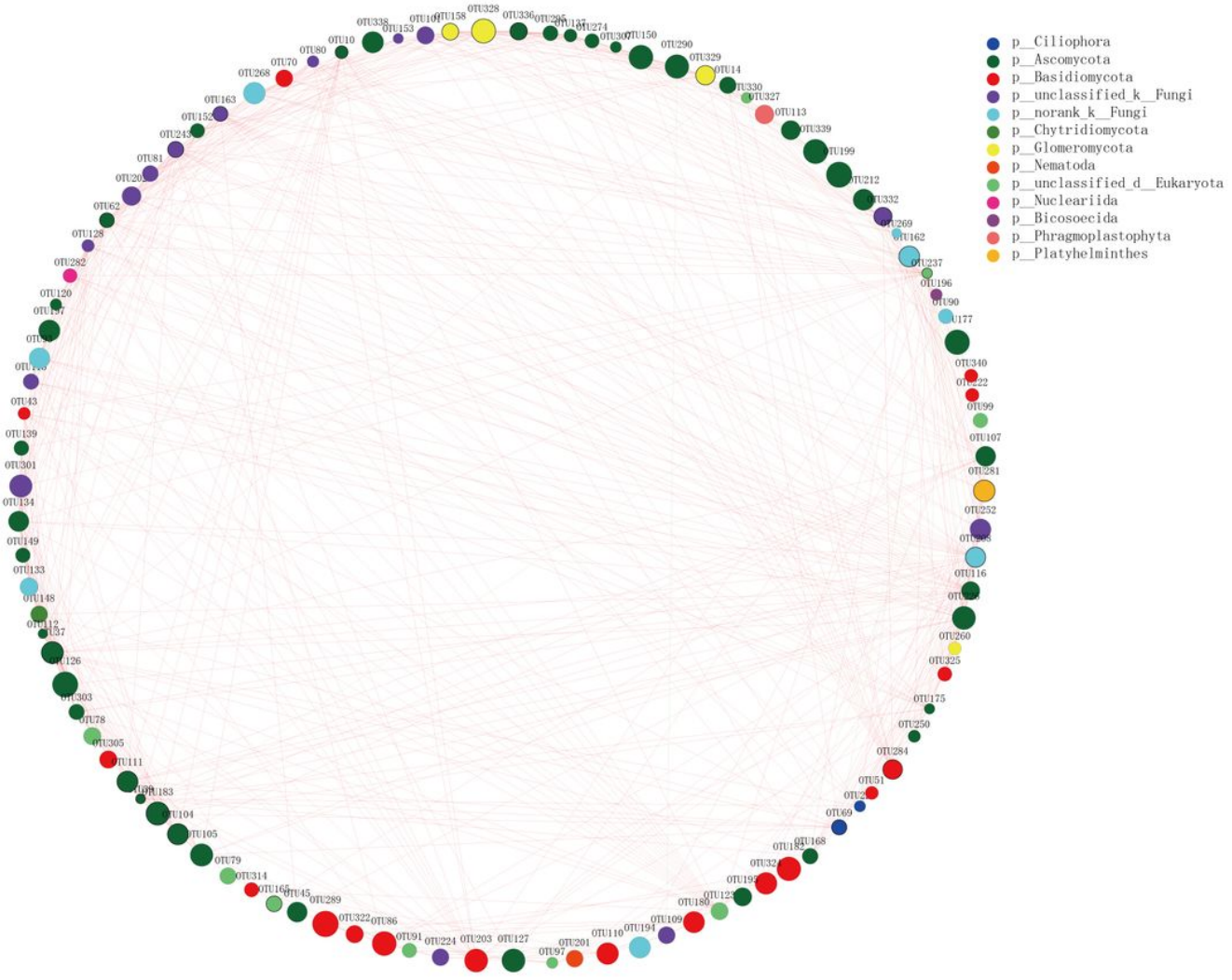

Figure 5

Fungal network diagram of the 100 most abundant phylotypes in the mining soil. Table S8 show the full taxonomy for all the phylotypes within the networks.

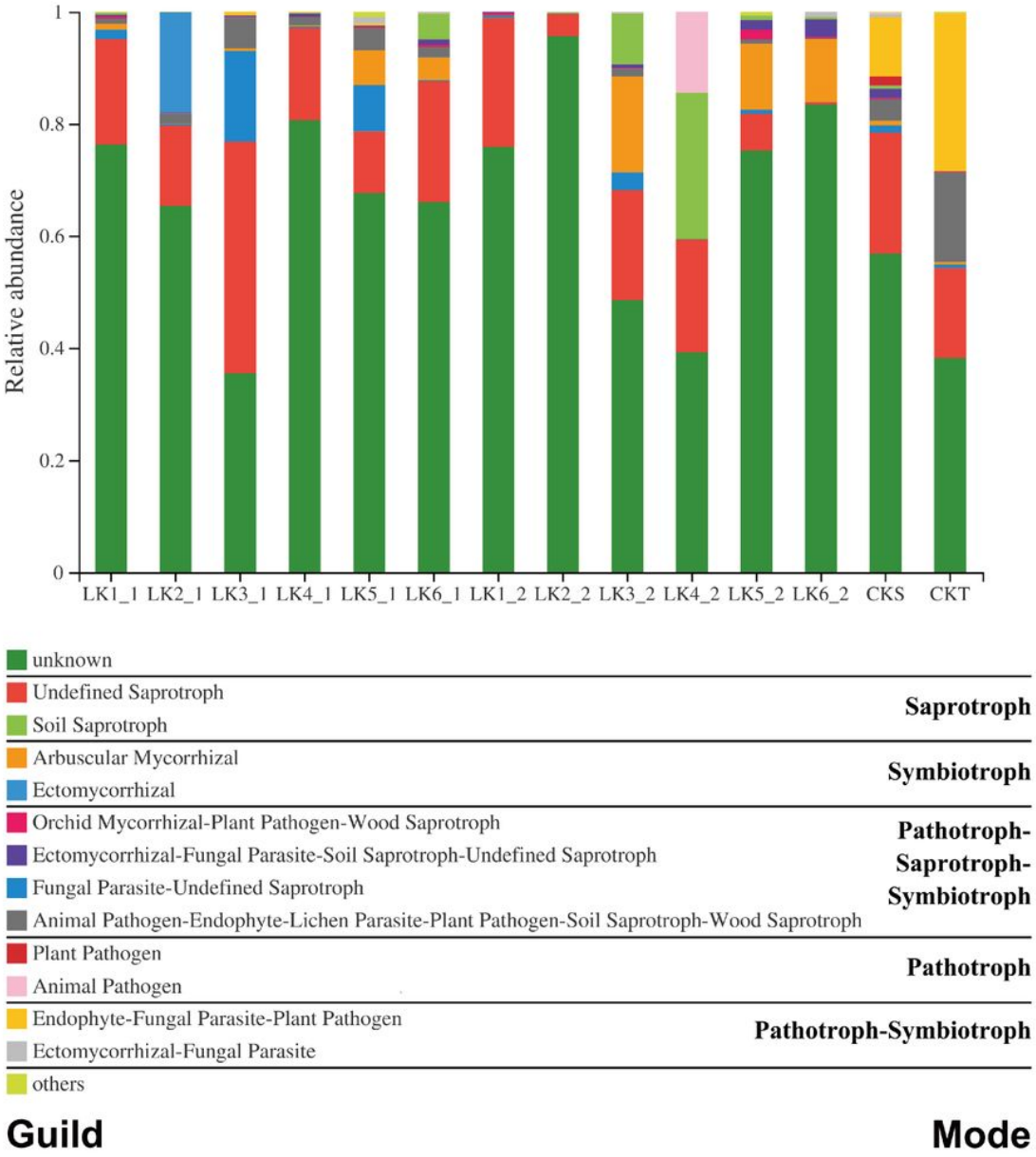

Figure 6

Compositions of fungal functional group (guild) inferred by FUNGuild.

Supplementary Files

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- Fig.S1Relativeabundanceonclasslevel.jpg
- FigureScaption.docx

- SupplementaryTable.docx