The Effects of *Suillus Luteus* Inoculation on Rhizospheric Fungal Community Diversity and Structure of *Pinus Massoniana* in Mining Area

Peiyi Yu (✉ 702487008@qq.com)

Central South University of Forestry and Technology <https://orcid.org/0000-0001-5236-3987>

Chen Ning

Central South University of Forestry and Technology Changsha Campus: Central South University of Forestry and Technology

Jingzhen Chen

Hunan Academy of Forestry

Fan Zhu

Central South University of Forestry and Technology Changsha Campus: Central South University of Forestry and Technology

Airong Shen

Hunan Academy of Forestry

Wenbin Zeng

Central South University of Forestry and Technology Changsha Campus: Central South University of Forestry and Technology

Lijuan Jiang

Central South University of Forestry and Technology Changsha Campus: Central South University of Forestry and Technology

Research article

Keywords: Heavy metals, Fungal community, plant's rhizosphere, *Suillus luteus*, *Pinus massoniana*, ectomycorrhizal fungi, 18S rRNA amplicon sequencing

Posted Date: October 9th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-87130/v1>

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Abstract

Background

As important decomposers and plant symbionts, soil fungal communities play a major role in remediating heavy metal polluted soils. However, diversity and structure of fungal communities generally remain unclear in mining area. This study aimed to assess the rhizospheric fungal community composition of masson's pine (*Pinus massoniana*) in lead-zinc mining area of Suxian district, Hunan Province, China. The experiment was treated as three ways: masson's pine inoculated with or without *Suillus luteus* and bulk soil without plant as control.

Results

The results showed that the inoculation of ectomycorrhizal fungi could enlarge the plants' capability to absorb heavy metals and secrete soil enzymes. The richness and diversity of fungi in rhizospheric soil were significantly higher than bulk soil ($p \leq 0.05$), but no obvious difference between rhizospheric soils inoculated with and without ectomycorrhizal (ECM) fungi while the community structure was changed. The rhizospheric fungi belong to 6 phylum, 25 classes, 65 orders, 115 families and 150 genera and the dominant phyla were Chytridiomycota (50.49%), Ascomycota (38.54%), and Basidiomycota (9.02%). By using LEfSe and heatmap, the relative abundance of *Suillus*, *Paraglomus*, *Agaricus*, and *Tulasnella* were the highest with ECM fungi inoculation. Redundant analysis (RDA) showed that the community structure significantly changed with ECM fungi inoculation, which was significantly related to soil water content, carbon nitrogen ratio, bulk density, available potassium, and soil enzymes.

Conclusions

All together, the inoculation with ECM fungi may change the inhabit environment of microorganisms and the dominant fungi in soil, which provided a screening of keystone species in the heavy metal-contaminated mining area.

Background

Mining activities lead to the accumulation of heavy metals such as lead (Pb), zinc (Zn), cadmium (Cd), arsenic (As), mercury (Hg), etc. in the soil, creating inhospitable environments and endangering the dynamics of the ecosystem that most microorganisms are difficult to survive [1, 2]. Moreover, heavy metals accumulated in living organisms involving human beings that along the trophic chain and interfere with their normal metabolism, such as soil respiration, microbial growth, and plant photosynthesis [3]. Therefore, the interactions between soil organisms and heavy metal polluted environment have become an issue of international relevance [1].

As a matter of fact, mining areas are mostly inadaptation for seed germination and plant growth, not only for the poisonousness of high concentration of heavy metals, but also the unfavorable soil

physicochemistry property, such as poor nutrients, high level of soil salinization, high clay mineral, low soil ventilation and moisture [4], which greatly restrict the growth and physiological activity of plants in the polluted area, and eventually affects the transport process and efficiency of heavy metals [5]. Ectomycorrhizal (ECM) symbioses, which forms between special groups of fungi and plant's roots, are essential for enlarging the absorption area of host plant roots [6], binding soil particles together, and promoting the absorption of water and nutrient elements [7]. Moreover, in the highly polluted mining areas, ECM fungi can help their host to adapt to heavy-metal polluted soils by immobilization of heavy metals in plants' roots and limit the transfer of heavy metals to the shoot of plants [8, 9]. Shi et al. [10] showed that *Pinus thunbergii* inoculated with *Pisolithus* sp1 combined remediation system had higher tolerance index, and the total accumulated chromium (Cr) were 2.83–27.75 fold higher than non-ectomycorrhizal (NM) seedlings in pot experiments. As one of 'early stage' ECM fungi that commonly coexist in pioneer coniferous species [11], *Suillus* species have also proved to be independently evolving in resistance to excessive concentrations of heavy metals, such as zinc (Zn) and cadmium (Cd) [12, 13, 14, 15]. However, how those 'early-stage' ECM fungal species help the hosts to adapt to heavy metal polluted areas and shape the rhizospheric environment remains unclear.

Fungi are known as an important component of belowground community closely related with plant root and rhizospheric environment [16]. It is reported that fungi can form mycorrhizal symbiosis with more than 90% of vascular plants [17] and support by up to 20% net primary productivity through their nutrition metabolism [18] in different forest ecosystems. Soil fungi are also the key decomposers in forest ecosystem, which play an important role in the turnover of soil carbon pool and nitrogen source [19] and provide key ecosystem functions in soil processes [20]. As Yuan et al. [21] mentioned, as an important decomposer, some special soil fungi communities could better adapt to their habitat environment and decompose the resistant compounds to absorb heavy metal elements more rapidly in the origin place than out of its habitat environment, which showed a great of "home-field advantage" in field experiment of fenced and grazed grassland. Besides, fungi can significantly affect the aboveground vegetation, including the growth of plant biomass [22], resistance against extreme circumstances like drought habitats [23], and heavy metals biodegradation and transportation [24, 25]. Previous studies showed that the majorities of fungi, such as Ascomycetes, Basidiomycetes, and Zygomycetes were adapt at resisting the toxic of heavy metals via co-metabolism pathways [26]. However, fungi are easily affected by surrounding soil micro-environment, for example, the diversity of plant's rhizosphere, soil composition properties and carbon nitrogen ratio (C/N), organic matter (OM), nitrogen (N), pH and soil water content (SWC), which lead to the variation of fungal community structures [27]. Besides, the interspecific interaction among the fungal groups can also influence the fungal community structures [28, 29]. Those changes can highly impact the fungal community succession, which directs to the biochemical cycling functions associated with different fungal guild [30]. Thus, a major challenge is to disentangle the effects of heavy metal pollution from environmental covariation (e.g. soil nutrients) on host-rhizospheric fungi interactions.

In this study, we determined the rhizospheric fungal community of masson's pine with and without ECM fungi (*Suillus luteus*) inoculation in heavy metals (Pb/Zn) polluted soils by high throughput sequencing

method on 18S rRNA region. We aimed to (1) determine how *S. luteus* inoculation influences the fungal diversity and composition in masson's pine rhizospheric soils, and (2) assess the fungal communities under host-mycorrhizal association covaried with soil environment in a mine-tailing site.

Results

Heavy metals and enzymatic activities

Heavy metal and enzymatic activities were determined for soil samples from each sample (Table 1). The concentrations of heavy metals in the rhizospheric soil with ectomycorrhizal fungi inoculation were below Hunan background (Pb, 29.7mg/kg; Zn, 94.4mg/kg), while other treatments were above Hunan background. The heavy metal concentration of Pb ranged from 64.27 to 125.33 mg/kg, and Zn ranged from 27.47 to 233.40 mg/kg (Table 1). The concentration of Pb and Zn in the rhizospheric soil inoculated with (WE) or without ectomycorrhizal fungi (NE) was much more lower than that of bulk soil (BS) ($p \leq 0.05$), however, there was no obvious difference of Pb between the rhizospheric soil in WE and NE treatments ($p \geq 0.05$). All enzymatic activities in the rhizospheric soil of WE treatment was greater than those of BS treatment ($p \leq 0.05$) (Table 1). The activities of CAT and SAC in the rhizospheric soil of NE treatment was similar to that in WE treatment, while activities of AKP and URE showed a weakly mounting trend compared with that of bulk soil (BS) ($p \geq 0.05$). These results indicated that plant's root system could reduce the concentration of Pb and Zn, promote the secreting of soil enzymes to better absorb nutrients, and inoculation of ectomycorrhizal fungi further enlarged the plants' capabilities to absorb heavy metals and secrete soil enzymes.

Rarefaction measurement

A total of 442,621 quality-filtered gene sequences were obtained (ranging from 43,062 to 60,946 sequences) in 9 samples, with an average length of 401 bp. These OTU belong to 6 phylum, 25 classes, 65 orders, 115 families and 150 genera. The result showed that the rarefaction curve of species diversity tended to smooth gradually with the increase of the sequence of samples (Fig. 2). Additionally, either WE or NE treatment showed an increase of species richness compared with that of treatment without host plants (BS).

Diversity and richness

The richness (Chao1 and ACE) and diversity (Shannon) of soil fungal communities were analyzed among 9 samples of three different treatments (Table 2). The result showed that fungal diversity of plant rhizospheric (WE and NE treatments) soil fungal communities were significantly higher than BS treatment ($p \leq 0.05$), however, there was no significant difference between WE and NE treatments.

Variation in fungal community structure

NMDS demonstrated that the triplicate samples of each treatment were clearly clustered in one group (Fig. 3a). Multigroup comparison boxplot revealed that the difference of inter-group was significantly

great than intra-group ($p \leq 0.05$) (Fig. 3b). Moreover, we made a statistical analysis and Bonferroni-corrected test of the factors causing the inter-group differences and found that the treatment of ECM fungi inoculation was the main reason that caused the difference (Fig. 3b).

Phylum-level taxonomic affiliation of OTUs

The dominant phyla were Chytridiomycota (50.49%) and Ascomycota (38.54%) in three treatments (Fig. 4). In total, those dominant phyla accounted for 89.03 % of the fungal sequences. The relative abundance of Basidiomycota and Cryptomycota were greater than 1%, but less than 10%. One-Way ANOVA showed that the relative abundance of Basidiomycota and Chytridiomycota were significantly greater in the soil sample of WE than those of BS, while the relative abundance of Ascomycota was much lower in the soil sample of WE. Taken together, these results revealed a great change in fungal community at the soil inoculated with ECM fungi, which progressively differentiated the dominant phyla in the soil with ECM fungi inoculation from the soil inoculation without ECM fungi.

Responses of rhizospheric fungal community in the soil inoculated with ectomycorrhizal fungi

Another major purpose of our study was to screen out the keystone species by comparing fungal communities in different samples. LDA analysis revealed that significant variations in soil fungal communities from the level phylum down to the family (Fig. 5). The dominant fungal species existing in the soil inoculated with ECM fungi mainly were phylum of Ascomycota and Chytridiomycota. LEfSe results at genus level showed that *Agaricus*, *Melanotaenium*, *Suillus*, and *Yamadazyma* were the dominant groups for the treatments with ECM fungi inoculation; *Brunneosphaerella*, *Saccharomycopsis*, and *Spencermartinsiella* were the dominant groups in NE. Those compositional differences were further confirmed by the adonis PERMANOVA test (LDA scores of ≥ 2) that there were significant differences of fungal community composition among the soil samples of WE, NE, and BS (Weighted Unifrac $R^2 = 0.74$, $p \leq 0.001$; Un-weighted Unifrac $R^2 = 0.70$, $p \leq 0.001$). The top 50 most abundant fungus genera were selected according to their relative abundance information at genus level in each sample (Fig.6). Compared with BS treatment, *Paraglomus*, *Panaeolus*, *Suillus*, *Tulasnella*, *Umbelopsi*, and *Zoophagus* were clustered into one group that were significantly higher in WE treatment, and *Catenomyces*, *Fimicolochytrium*, *Irineochytrium*, and *Synchytrium* were significantly higher in NE treatment.

Relationship between fungal communities and environmental variables

Seven physicochemical variables (AN, AP, AK, C/N, bulk density, and soil water content) and four soil enzyme (urease, saccharase, catalase, and alkaline phosphatase) were selected for RDA biplot analysis (Fig. 7). The first axis of RDA explained 54 % of the variation, while the second axis explained 39.8%. Totally, these two axis explained 93.8% of the variations between soil factors and fungal community composition. The community structure of rhizospheric fungi changed positively when inoculated with ECM fungi, which was closely related to saccharase ($F=8.9$, $p=0.008$), urease ($F=6.739$, $p=0.006$), C/N ($F=6.036$, $p=0.016$), catalase ($F=6.002$, $p=0.01$), alkaline phosphatase ($F=5.638$, $p=0.024$), bulk density ($F=5.109$, $p=0.038$), and soil water content ($F=4.361$, $p=0.026$).

Discussion

Fungal diversity of rhizosphere

ECM fungi is a highly mutual symbiotic combination between mycorrhizal fungi and host plants [31]. This unique symbiotic relationship can help plants survive in adverse conditions, especially in the soil contaminated with heavy metals [9, 32]. In our previous study also found the ECM fungi enhanced the survival and growth of masson's pine in Pb/Zn tailing area [33]. In this current study, we used the Illumina MiSeq technique to investigate diversity and structure of rhizospheric fungal community at different inoculation treatments of masson's pine in mining area contaminated with Pb and Zn. The results indicated that the fungal diversity and richness in the rhizospheric soil of masson's pine were significantly higher than bulk soil. However, there was little difference between the soil with/without ECM fungi inoculation (Table 2), which illustrated that the difference of plant rhizospheric fungal diversity was mainly determined by "rhizosphere effect" of plant [26, 34]. Plants exchange the nutrients and water frequently with the surrounding environment of the root system during the growth process, which caused the micro-environments in the rhizosphere different from that in the non-rhizospheric soil. Those differences could result in the great discrepancy of the richness and diversity in the rhizospheric soil of the fungal groups.

Previous studies showed that the fungal ergosterol content was much more higher in potato rhizospheric soil than soil without plants in the stage of growing season [35]. Besides, Thion et al. [26] found that the diversity of those fungi in the rhizospheric soil planted with alfalfa was significantly higher than that of non-rhizospheric soil of *in situ* experiment. Obviously, there are many factors that determine the relative abundance and diversity of the soil fungal communities, among them, the plants' root seems to changed soil microenvironment of physicochemical property, such as soil organic matter, water content, and soil enzymes [36], which greatly impact the growth and the number of fungi species indirectly [37]. Those results strongly support that the importance of plants and their "rhizosphere effects" was the main reason leading to the discrepancy of the diversity and structure of soil microorganism. Some other studies also showed that the ECM fungi inoculation would decrease the diversity of fungal community. According to Li et al. [38], the bacterial and fungal diversity reduced when *Pinus armandii* inoculated with *Tuber indicum* during early symbiotic stage as compared with non-ectomycorrhizal fungi soil. Compared with bulk soil, the great reduction of microbial diversity in the rhizosphere, was mainly attributed to large discrepancies between plant host species [34], or related to the surrounding sampling soil properties of the rhizosphere [37].

All together, our results revealed that even in a highly polluted mining area (Pb:131.25 mg/kg; Zn:262.67mg/kg), plants' rhizosphere had a protective effect on the growth of microbial abundance and diversity host in the plant when compared to the soil without plants, which maybe related to the reducing of the toxicity of heavy metal on plants' rhizosphere.

Indicators of the representative fungal genus

The difference in fungi community structure reflects the mycorrhiza and its metabolites, such as enzymes, extracellular polysaccharides, amino acids and growth hormone that have greatly effects on the surrounding environment. The differences in dominant populations may indicate that some mycorrhizal fungi can adapt to the specific environmental stress, especially in the rhizospheric soil polluted by heavy metals [8, 9]. Although there was little difference of rhizospheric fungal community diversity between WE and NE treatments, the community composition and dominant species of rhizospheric fungi were obviously different in the soil when inoculated with *Suillus* fungi (Fig.3, 4). Previous studies in forest ecosystem found that the phylum of Ascomycota and Basidiomycota were the dominant fungal communities either in rhizospheric soil or root samples of *Pinus armandii* with *Tuber indicum* inoculation [38]. By 454 pyrosequencing, Liu et al.[39] also found that the rhizosphere of Xinjiang jujube were mainly colonized by Ascomycota and Basidiomycota, whereas Chytridiomycota and Glomeromycota were probably restrained. However, in our research, besides Basidiomycota, there also a more abundant of Chytridiomycota was existed in the soil of ECM fungi inoculation compared with bulk soil, while the relative abundance of Ascomycota was low, which suggested that Basidiomycota and Chytridiomycota might closely related to the presence of ECM fungi (*Suillus luteus*) synthesis (Fig.5, 6). It also should be noted that fungal structure and composition were highly related with soils environment, and plant-fungus symbiont was undoubtedly shifted with soil pH, moisture, and soil nutrients, which indirectly determines fungal community composition [38].

In addition, our results showed that the relative abundance of *Suillus*, *Paraglomus*, *Agaricus*, *Tulasnella* and *Melanotaenium* were the functionality of abundant fungal genus in the WE (Fig.5). Some of these fungal genera are the important mycobiont with plants and enforce adaptability of plant for grow in stress conditions, such as metal-contaminated soils. Previous studies showed that *Pinus massoniana* seedlings were significantly resistant to heavy metals than non-mycorrhizal plants when inoculated with *S. luteus* [15]. Besides *Suillus luteus*, some mycorrhizal fungi (*Suillus bovinus*) inoculated plants (e.g. Pinaceae and Fagaceae) have been reported to develop tolerance to heavy metals such as Zn and Cd [12, 14], and improve the survival of plants on heavy metal-contaminated soils [13]. Others, such as *Paraglomus*, which was a common arbuscular mycorrhizal (AM) fungi and geographically widespread through locally sporadic in tilled agricultural soils [40], can increase growth performance of the peach seedlings and elevate the absorption of essential elements (K, Mg, Fe and Zn) for plants' growth and photosynthetic, and fix the heavy metal such as Cu^{2+} and Mn^{2+} to the root system under the potted conditions [41]; *Agaricus* species can also increase the inorganic nutrients uptake in the soil, thus accelerate the adaptation process of plant in the stressed environment [42]. These fungal mycobionts adhering to the roots of plants can form a huge network system underground (hartig network) [42] and release related antioxidant enzymes to acquire nutrients from organic compounds, such as chitin, melanin, and cellulose [43, 44]. The unique species adapted to these special environments would help plants survive against the environmental stress.

Relationships between fungal community structure and environmental variation

Soil processes may be primarily affected by interaction among surrounding environmental factors, structure of soil microbial communities and their reciprocal relationship [45]. Alternatively, these mutual interrelationships have significant impact on the processes of ecosystem through feedback mechanism. Fungal communities act as a “bridge” between the plants, the soil environmental condition and their relationship [46], which play a vital role in driving nutrient and carbon cycling processes [19].

Numerous studies have indicated that soil physicochemical properties, such as pH, soil C:N, and soil water content could shape fungal communities composition and structure in the plant's rhizosphere [47], especially, in those heavy metal soils [48]. Our result showed that the environmental variables of soil water content, C/N, and bulk density were important factors determining the composition and diversity of soil fungi (Fig.7). Previous studies also indicated that fungal symbionts formed an important interface between the soil and the plant's rhizosphere that greatly affected soil physicochemical properties and microbial community structure in the rhizospheric soil [43], which was consistent with our results. As such, the existence of ECM fungi interact with the rhizospheric environment of host plant could thus in return affect the abundance and ECM could influence fungal community via altering physicochemical properties in the heavy metal polluted mining tails.

Soil extracellular enzyme is a kind of bioactive substance with catalytic ability, which decomposed and released by microorganisms and plants. Soil fungi play an important part in enzyme secretion that related with nutrient absorption and mobilization, such as nitrogen and/or phosphorus [19, 49]. Among these fungal communities, groups of ECM fungi are usually form symbiotic relationship with the roots of the plant [50], especially, on the condition of nutrient limitation, ECM fungi could receive carbohydrates from their plant host to power nutrient transformation [51] and soil enzyme expression [19]. Here, in our research, by combining 18S rDNA sequencing with soil enzyme analyses, we demonstrated relationships between fungal community structures and soil enzyme activities with ECM fungi inoculated in the masson's pine (Fig. 7). Obviously, ECM fungi had a major influence on soil enzyme activities during processes of restoration (Table 1). According to Burke et al. [50], ECM fungi were positively associated with the soil phosphate content and some soil enzymes of phosphatase (organic P degradation) and N-acetyl- β -glucosaminidase (NAG; organic N and chitin degradation) in field experiment of northern hardwood forest. Other researches also showed that some certain ECM fungi, such as the genera *Cortinarius* and *Russula*, were significantly associated with the activities of enzymes of lignin-degrading Mn-peroxidase [51] and plant cell wall-degrading in deeper layer of humus [52] in mineralizing nitrogen and phosphorus for the mutualistic symbiotic system [7], which was consistent with our research.

It is noteworthy that the discrepancy of diversity in plant's rhizosphere fungal community might be determined by “rhizospheric effect” of plant, while the dominated keystone fungal species chosen by *Suillus-Pinus* symbionts showed a more effective way in establishing of soil microenvironment system and promoting the growth of plants in heavy metal polluted mining tails.

Conclusions

In this study, we determined the composition and structure of rhizospheric fungal communities in plants' rhizospheric micro-environment with/without ECM fungi (*Suillus luteus*) inoculation in heavy metal contaminated mining area. The results indicated that plant's root system could reduce the concentration of Pb and Zn, and inoculation of ectomycorrhizal fungi further enlarged the plants' capability to absorb heavy metals and secrete soil enzymes. The fungal community diversity of masson's pine rhizospheric fungi were significantly higher than bulk soil but little difference was found between with/without *Suillus* fungi inoculation treatments, which could mainly be determined by "rhizosphere effect" of plant. However, the dominant species and interspecific relationship of rhizospheric fungi with/without *Suillus*-inoculation changed significantly, especially for the fungal groups (e.g. *Suillus*, *Paraglomus*, *Agaricus*, and *Tulasnella*) that could promote plant absorption of water and mineral elements and protect plant against heavy metal contaminated stress. Soil physicochemical properties of SWC, C/N, bulk density also importantly determined the fungal community structure and thus inversely influenced the ecological functions, such as soil enzyme. In all, the inoculation of *Suillus* on masson's pine seedlings can change the habitat environment, which indirectly changed the community structure of dominant fungi in rhizosphere soil, and thus allows for such a metal-tolerant *Suillus-Pinus* symbiont to growth in large scaled metal-contaminated soils.

Materials And Methods

Site description

The study area located in Mount Manao, Suxian, Hunan, China (113°08'09", 25°42'05"), which was near to the upper reaches of Xiangjiang River. The mean annual precipitation is 1500-2000mm, the rainfall concentrates in March, June and August. Within the sampling areas, a former mining area had been previously exploited and contained Pb (131.25 mg/kg) and Zn (262.67 mg/kg) pollutants of concentrations exceeding the safety limits. *Miscanthus* sp, *Equisetum ramosissimum*, *Esholtzia cypriani* are the dominate herbaceous plants species growing in Mount Manao that are seriously polluted by heavy metals, worse still, during the rainy season, heavy metals in soil are easily washed by rain and converge into the Xiangjiang River, resulting in a large area of water pollution.

Experimental design and soil sampling

Suillus luteus was screened from masson's pine forest soils and provided by Hunan academy of forestry. The seedlings inoculated with EMC were cultivated according by our previous research [15]. Briefly, *S. luteus* was cultivated on sterilized Potato Dextrose Agar solid medium for about one week, and then, these mycelium cultivated in modified Kottke in aseptic room for another week (25 °C, 120 r/min) was used as inoculum. Masson's pine seedlings were surface sterilized and cultured in incubator (37 °C, 24h) in sterilized petri dishes, then transferred to plastic pot in growth chamber for 4 weeks. Four-week-old seedlings were washed three times with aseptic water to clean the roots, and roots of the seedlings were immersed in mycelium suspensions were as the inoculated plants (WE), and roots of another seedlings immersed in autoclaved (121 °C, 120 min) mycelium suspension were as the non-inoculated plants (NE).

The ECM fungi-inoculate seedlings were cultivated in greenhouse with light intensity of 8,000 lx, relative humidity of 55%, photoperiod of 13 h, 25 °C at light and 18 °C in the dark for 2 months. And then, the roots with and without mycorrhizal were transplanted in the field in November 2016 (Fig. 1a, b). We established nine 4×5 m planting plots with three groups: masson pine seedling inoculated with *S. luteus* (WE), masson's pine seedling inoculated without *S. luteus* (NE), and bulk soil without plants (BS) as control in the field, each treatment had three replications. For each plant in the group of WE and NE, the size of planting hole was 30cm×30cm×40cm and then covered with soil for 30cm after placing the masson's pine seedling in the hole. In October 2017, we surveyed the experiment area, and the sporocarp of *Suillus luteus* was found in plots inoculated with ECM fungi (Fig. 1d).

We followed the method of Vergani et al. [53] to collect the rhizospheric soils. Briefly, three root samplings of each plant inoculated with (WE)/ without (NE) *S. luteus* were carefully excavated and shaken for 5 times, and the soil that still attached to the root surface was the rhizospheric soil, we also collected three soil samples in the plots without plants (BS) as control, and the depth of sampling was 10 to 20cm. Totally, we collected 9 samples, including three WE, three NE, and three BS. The soil was divided into two parts, one part was kept in liquid nitrogen canister and later transfer to the laboratory for fungal community analysis, the other part was kept on dry ice box for determining soil physicochemical properties analysis.

Soil physicochemical characterization

The soil samples were air-dried at room temperature for soil physical and chemical analysis. For the soil pH, 5 g of fresh soil collected from tailing area was suspended in 25 ml distilled water in a ratio 1:5(w:v) and measured using a pH meter (Sartorius PB-10, Sartorius Scientific Instruments, Beijing, China). The soil total carbon (TC) and total nitrogen (TN) were analyzed on an Elemental analyzer (VarioEL III, Elementar, Germany) using combustion method. Available phosphorus (AP) was determined by means of Ammonium Molybdate-Tartaric Emetic-Ascorbic Acid Colorimetry (0.5mol/L NaHCO₃), Available potassium (AK) was measured by means of flame photometer (1mol/L NH₄OAc), and Available nitrogen (AN) was measured by means of alkaline hydrolysis diffusion method [54].

Determination of enzyme activity in rhizospheric soil

Urease (URE), saccharase (SAC), and alkaline phosphatase (AKP) activity analysis were measured referred as Guan et al. [55] and modified slightly. Briefly, 5 g fresh soil sample was homogenized, and cultured in incubator (37 °C) for 24 hours, then centrifuged in 4000r/min for 5 min, the absorbance of the reaction mixture was determined by using UV/visible spectrophotometer (UV-5100B, METASH, China) at a relative wavelength of 578 nm, 508nm and 660nm, respectively; and for catalase (CAT) activity, the soil reaction mixture was cultured in incubator (37 °C) for 20min, and recorded at a wavelength of 240 nm according to Guan et al. [55].

DNA extraction and High-Throughput sequencing

Microbial DNA was extracted from 0.5g soil of each sample plot (n = 3) using the PowerSoil DNA Isolation Kit (MoBio PowerSoil® DNA Isolation Kit, Norcross, GA, USA). Then, the extracted genomic DNA was subsequently analyzed by agarose gel electrophoresis system (0.8% w/v) (BIO-RAD, Hercules, CA, USA). Real-time PCR amplification was carried out on a GeneAmp 2720 PCR system (GeneAmp 2720, ABI, Foster City, CA, USA), and the primers 817F-1196R was used for 18S rRNA genes according to [27]. The reactions of PCR system was conducted following by the program of [20, 56]. Each sample was amplified three times, and the finally amplified products were mixed and tested by 2% agarose gel electrophoresis. Sequencing was done on Illumina MiSeq platform (San Diego, CA, USA) by Wuhan FraserGen Bio-pharm Technology (Wuhan, Hubei, China). The sequences date with low-quality sequences including overlapped sequences, polyclonal sequences or the sequences shorter than 200 bp had to be truncated. The SILVA ribosomal RNA gene sequences database was used to classify the sequences. After quality control, the obtained sequences were merged and classified into OTUs according to 97% of the sequence similarity by using the QIIME software and UCLUST sequence alignment tool [57].

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted with confidence level of 95% by using SPSS (version 19.0; SPSS, Chicago, IL, USA). A non-metric multidimensional scaling (NMDS) ordination diagrams was performed to visualize the effects of ECM fungi inoculation on the structure of soil fungal community. T-test of the intra-group and inter-group distance mean of Weighted's UniFrac distance matrix was calculated by using QIIME software, and the statistical significance was judged by 1000 Monte Carlo permutation test. Redundancy Analysis (RDA) was used to assess relationships among soil fungal community and environmental factors, soil physicochemical factors were randomly replaced by forward selection of Monte-Carlo permutation test for 999 times to test the similarity of fungal communities, and visualized by Cano Draw [58]. Effect size measurements (LEfSe) based on linear discriminant analysis (LDA) was used to screen the keystone species among groups via the Galaxy online analytics platform (<http://huttenhower.sph.harvard.edu/galaxy/>). The top 50 dominant fungi were clustered and demonstrated in the form of heatmap by using R software.

Abbreviations

AK

Available potassium

AKP

Alkaline phosphatase

AN

Available nitrogen

AP

Available phosphorus

BD

Bulk density

CAT

Catalase

ECMF

Ectomycorrhizal fungi

LEfSe

Linear discriminant analysis Effect Size

LDA

Linear Discriminant Analysis

NMDS

Non-Metric Multidimensional Scaling

OTUs

Operational Taxonomic Unit

RDA

Redundant analysis

SAC

Saccharase

SWC

Soil water content

URE

Urease

Declarations

Acknowledgments

We would like to thank Chen Ning for the linguistic editing and improving this manuscript, and the following undergraduates for their assistance in laboratory chemical analysis: Simeng Li, Xiao Zhou and Xuechun Feng.

Funding

Key Research and Development Project of Hunan Province (2019SK2191); Key Research and Development Project of Changsha local government (kq1801080); Scientific Innovation Fund for Post-graduates of Central South University of Forestry and Technology (20181011).

Author information

College of Life Science and Technology, Central South University of Forestry and

Technology, 498 South Shaoshan Road, Changsha, Hunan, 410004, China

Peiyi Yu, Wenbin Zeng & Lijuan Jiang

National Engineering Laboratory for Applied Technology of Forestry & Ecology in South China, Central South University of Forestry and Technology, 498 South Shaoshan Road, Changsha, Hunan, 410004, China

Chen Ning & Fan Zhu

State Key Laboratory of Utilization of Woody Oil Resource, Hunan Academy of Forestry, 658 South Shaoshan Road, Changsha, Hunan, 410004, China

Jingzhen Chen & Airong Shen

Corresponding author

Wenbin Zeng; Lijuan Jiang

References

1. Gall JE, Boyd RS, Rajakaruna N. Transfer of heavy metals through terrestrial food webs: a review. *Environ Monit Assess*. 2015;187:201.
2. doi:10.1007/s10661-015-4436-3.
3. Ayangbenro AS, Babalola OO. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int J Environ Res Public Health*. 2017;14:94.
4. doi:10.3390/ijerph14010094.
5. Chen L, Swenson NG, Ji N, Mi X, Ren H, Guo L, et al. Differential soil fungus accumulation and density dependence of trees in a subtropical forest. *Science*. 2019;366:124–8.
6. doi:10.1126/science.aau1361.
7. Pulford ID, Watson C. Phytoremediation of heavy metal contaminated land by trees: a review. *Environ Int*. 2003;29:529–40.
8. doi:10.1016/S0160-4120(02)00152-6.
9. Dixit R, Wasiullah, Malaviya D, Pandiyan K, Singh UB, Sahu A, et al. Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*. 2015;7:2189–212.
10. doi:10.3390/su7022189.
11. Nara K, Hogetsu T. Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. *Ecology*. 2004;85:1700–7.
12. doi:10.1890/03-0373.
13. Smith SE, Read DJ. *Mycorrhizal Symbiosis*. Cambridge: Academic; 2008.
14. Huang J, Nara K, Lian C, Zong K, Peng K, Xue S, et al. Ectomycorrhizal fungal communities associated with Masson pine (*Pinus massoniana* Lamb.) in Pb-Zn mine sites of central south China. *Mycorrhiza*. 2012;22:589–602.

15. doi:10.1007/s00572-012-0436-0.
16. Li N, Guo B, Li H, Fu Q, Feng R, Ding Y. Effects of double harvesting on heavy metal uptake by six forage species and the potential for phytoextraction in field. *Pedosphere*. 2016;26:717–24.
17. doi:10.1016/S1002-0160(15)60082-0.
18. Shi L, Deng XP, Yang Y, Jia QY, Wang CC, Shen ZG, et al. A Cr (VI)-tolerant strain, *Pisolithus* sp1, with a high accumulation capacity of Cr in mycelium and highly efficient assisting *Pinus thunbergii* for phytoremediation. *Chemosphere*. 2019;224:862–72.
19. doi:10.1016/j.chemosphere.2019.03.015.
20. Policelli N, Bruns TD, Vilgalys R, Nuñez MA. Suiloid fungi as global drivers of pine invasions. *New Phytol*. 2019;222:714–25.
21. doi:10.1111/nph.15660.
22. Krznaric E, Verbruggen N, Wevers JHL, Carleer R, Vangronsveld J, Colpaert JV. Cd-tolerant *Suillus luteus*: a fungal insurance for pines exposed to Cd. *Environmental pollution*. 2009;157:1581–8.
23. doi:10.1016/j.envpol.2008.12.030.
24. Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K. How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. *Ann For Sci*. 2011;68:17–24.
25. doi:10.1007/s13595-010-0003-9.
26. Op De Beeck M, Lievens B, Busschaert P, Rineau F, Smits M, Vangronsveld J, et al. Impact of metal pollution on fungal diversity and community structures. *Environ Microbiol*. 2014;17:2035–47.
27. doi:10.1111/1462-2920.12547.
28. Yu PY, Sun YP, Huang ZL, Zhu F, Sun YJ, Jiang LJ. The effects of ectomycorrhizal fungi on heavy metals' transport in *Pinus massoniana* and bacteria community in rhizosphere soil in mine tailing area. *J Hazard Mater*. 2020;381:121203.
29. doi:10.1016/j.jhazmat.2019.121203.
30. Mueller RC, Paula FS, Mirza BS, Rodrigues JL, Nüsslein K, Bohannan BJ. Links between plant and fungal communities across a deforestation chronosequence in the Amazon rainforest. *ISME J*. 2014;8:1548–50.
31. doi:10.1038/ismej.2013.253.
32. Brundrett MC, Tedersoo L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol*. 2018;220:1108–15.
33. doi:10.1111/nph.14976.
34. Crowther TW, van den Hoogen J, Wan J, Mayes MA, Keiser AD, Mo L, et al. The global soil community and its influence on biogeochemistry. *Science*. 2019;365:eaav0550.
35. doi:10.1126/science.aav0550.
36. Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, et al. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their

- biogeochemical functions. ISME J. 2012;6:1749–62.
37. doi:10.1038/ismej.2012.11.
38. Banos S, Lentendu G, Kopf A, Wubet T, Glöckner FO, Reich M. A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. BMC Microbiology. 2018;18.
39. doi:10.1186/s12866-018-1331-4.
40. Yuan X, Niu D, Wang Y, Boydston A, Guo D, Li X, et al. Litter decomposition in fenced and grazed grasslands: A test of the home-field advantage hypothesis. Geoderma. 2019;354:113876.
41. doi:10.1016/j.geoderma.2019.07.034.
42. Dean SL, Farrer EC, Taylor DL, Porras-Alfaro A, Suding KN, Sinsabaugh RL. Nitrogen deposition alters plant-fungal relationships: linking belowground dynamics to aboveground vegetation change. Molecular ecology. 2014;23:1364–78.
43. doi:10.1111/mec.12541.
44. Compant S, Van Der Heijden MGA, Sessitsch A. Climate change effects on beneficial plant-microorganism interactions. FEMS Microbiol Ecol. 2010;73:197–214.
45. doi:10.1111/j.1574-6941.2010.00900.x.
46. Liu SH, Zeng GM, Niu QY, Liu Y, Zhou L, Jiang LH, et al. Bioremediation mechanisms of combined pollution of PAHs and heavy metals by bacteria and fungi: A mini review. Bioresour Technol. 2017;224:25–33.
47. doi:10.1016/j.biortech.2016.11.095.
48. Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, et al. Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere. 2017;171:710–21.
49. doi:10.1016/j.chemosphere.2016.12.116.
50. Thion C, Cébron A, Beguiristain T, Leyval C. Long-term *in situ* dynamics of the fungal communities in a multi-contaminated soil are mainly driven by plants. FEMS Microbiol Ecol. 2012;82:169–81.
51. doi:10.1111/j.1574-6941.2012.01414.x.
52. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, et al. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 2010;4:1340.
53. doi:10.1038/ismej.2010.58.
54. Peay KG. The mutualistic niche: mycorrhizal symbiosis and community dynamics. Annu Rev Ecol Evol Syst. 2016;47:143–64.
55. doi:10.1146/annurev-ecolsys-121415-032100.
56. Liu J, Ngoc Ha V, Shen Z, Dang P, Zhu H, Zhao F, et al. Response of the rhizosphere microbial community to fine root and soil parameters following *Robinia pseudoacacia* L. afforestation. Applied soil ecology. 2018;132:11–9.

57. doi:10.1016/j.apsoil.2018.08.004.
58. Fernandez CW, Kennedy PG. Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? *New phytologist*. 2016;209:1382–94.
59. doi:10.1111/nph.13648.
60. Demenois J, Carriconde F, Bonaventure P, Maeght JL, Stokes A, Rey F. Impact of plant root functional traits and associated mycorrhizas on the aggregate stability of a tropical Ferralsol. *Geoderma*. 2018;312:6–16.
61. doi:10.1016/j.geoderma.2017.09.033.
62. Thion C, Cébron A, Beguiristain T, Leyval C. Inoculation of PAH-degrading strains of *Fusarium solani* and *Arthrobacter oxydans* in rhizospheric sand and soil microcosms: microbial interactions and PAH dissipation. *Biodegradation*. 2013;24:569–81.
63. doi:10.1007/s10532-013-9628-3.
64. Sun YJ, Yu PY, Chen JZ, Li SM, Jiang LJ. Effects of Slippery Jack (*Suillus luteus*) on the heavy metal accumulation and Soil Properties of Masson's Pine (*Pinus massoniana* Lamb) in a Mining Area of China. *Applied ecology environmental research*. 2020;18:3741–55.
65. doi:10.15666/aeer/1802_37413755.
66. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol*. 2013;11:789–99.
67. doi:10.1038/nrmicro3109.
68. Hannula SE, de Boer W, van Veen JA. In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. *Soil Biol Biochem*. 2010;42:2211–23.
69. doi:10.1016/j.soilbio.2010.08.020.
70. Phillips RP, Fahey TJ. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology*. 2006;87:1302–13.
71. doi:10.1890/0012-9658(2006)87[1302:TSAMAI]2.0.CO;2.
72. De Miguel AM, Águeda B, Sánchez S, Parladé J. Ectomycorrhizal fungus diversity and community structure with natural and cultivated truffle hosts: applying lessons learned to future truffle culture. *Mycorrhiza*. 2014;24:5–18.
73. doi:10.1007/s00572-013-0554-3.
74. Li Q, Zhao J, Xiong C, Li X, Chen Z, Li P, et al. *Tuber indicum* shapes the microbial communities of ectomycorrhizosphere soil and ectomycorrhizae of an indigenous tree (*Pinus armandii*). *PLOS ONE*. 2017;12:e0175720.
75. doi:10.1371/journal.pone.0175720.
76. Liu P, Wang XH, Li JG, Qin W, Xiao CZ, Zhao X, et al. Pyrosequencing Reveals Fungal Communities in the Rhizosphere of Xinjiang Jujube. *Biomed Res Int*. 2015;2015:1–8.
77. doi:10.1155/2015/972481.

78. Gosling P, Proctor M, Jones J, Bending GD. Distribution and diversity of *Paraglomus* spp. in tilled agricultural soils. *Mycorrhiza*. 2013;24:1–11.
79. doi:10.1007/s00572-013-0505-z.
80. Martins LF, Pinho RO, Siqueira JB, Guimarães SEF, Neto TM, Guimarães JD. Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* L. Batsch) seedlings. *J Anim Plant Sci*. 2011;21:746–50.
81. doi:10.4081/ijas.2011.e41.
82. Giordano L, Gonthier P, Lione G, Garbelotto M. The invasiveness of a non-native fungal forest pathogen is boosted by the presence of a congeneric native species. *Forestry: An International Journal of Forest Research*. 2018;92:157–66.
83. doi:10.1093/forestry/cpy042.
84. Lambers H, Mougél C, Jaillard B, Hinsinger P. Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant soil*. 2009;321:83–115.
85. doi:10.1007/s11104-009-0042-x.
86. Lastovetsky OA, Gaspar ML, Mondo SJ, LaButti KM, Sandor L, Grigoriev IV, et al. Lipid metabolic changes in an early divergent fungus govern the establishment of a mutualistic symbiosis with endobacteria. *Proceedings of the National Academy of Sciences*. 2016;13:15102–15107.
87. doi:10.1073/pnas.1615148113.
88. Kvaschenko J, Clemmensen KE, Hagenbo A, Karlton E, Lindahl BD. Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. *ISME J*. 2017;11:863.
89. doi:10.1038/ismej.2016.184.
90. Bödeker ITM, Lindahl BD, Olson Å, Clemmensen KE. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. *Funct Ecol*. 2016;30:1967–78.
91. doi:10.1111/1365-2435.12677.
92. Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, et al. Structure and function of the global topsoil microbiome. *Nature*. 2018;560:233.
93. doi:10.1038/s41586-018-0386-6.
94. Azarbad H, Niklińska M, van Gestel CAM, van Straalen NM, Röling WFM, Laskowski R. Microbial community structure and functioning along metal pollution gradients. *Environ Toxicol Chem*. 2013;32:1992–2002.
95. doi:10.1002/etc.2269.
96. Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecol*. 2015;13:10–22.
97. doi:10.1016/j.funeco.2014.08.002.

98. Burke DJ, Weintraub MN, Hewins CR, Kalisz S. Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. *Soil Biol Biochem.* 2011;43:795–803.
99. doi:10.1016/j.soilbio.2010.12.014.
100. Lindahl BD, Tunlid A. Ectomycorrhizal fungi-potential organic matter decomposers, yet not saprotrophs. *New Phytol.* 2015;205:1443–7.
101. doi:10.1111/nph.13201.
102. Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, et al. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat Genet.* 2015;47:410.
103. doi:10.1038/ng.3223.
104. Vergani L, Mapelli F, Marasco R, Crotti E, Fusi M, Guardo DA, et al. Bacteria associated to plants naturally selected in a historical PCB polluted soil show potential to sustain natural attenuation. *Front Microbiol.* 2017;8:1–13.
105. doi:10.3389/fmicb.2017.01385.
106. Bao SD. The soil agricultural chemistry analysis. Beijing: Chinese Agriculture Press; 2000..(in chinese).
107. Guan SY. Soil enzyme and its research methods. Agricultural, Beijing;1986.(in Chinese).
108. Hu H, Jiang C, Wu YP, Cheng YX. Bacterial and fungal communities and contribution of physicochemical factors during cattle farm waste composting. *Microbiol.* 2017;6:e00518.
109. doi:10.1002/mbo3.518.
110. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26:2460–1.
111. doi:10.1093/bioinformatics/btq461.
112. Ter Braak CJ, Smilauer P. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). [www. canoco. com](http://www.canoco.com); 2002.

Tables

Table 1
Heavy metals and enzymatic activities of the rhizospheric soil under different treatments

	Concentration (mg/kg)		Enzymatic activities (mg/g)			
	Pb	Zn	CAT	AKP	URE	SAC
WE	64.27 ± 10.85b	27.47 ± 2.55c	2.81 ± 0.14a	0.28 ± 0.02a	0.29 ± 0.02a	23.19 ± 1.61a
NE	66.20 ± 0.81b	193.47 ± 9.51b	1.97 ± 0.25b	0.17 ± 0.03b	0.22 ± 0.02ab	16.37 ± 0.94b
BS	125.33 ± 4.49a	233.40 ± 23.81a	1.08 ± 0.70c	0.12 ± 0.01b	0.13 ± 0.03b	7.28 ± 1.03c
Different letters (a and b) in column refer to statistically significant differences (p<0.05) among 3 different sampling areas (n = 3), the same as follow.						

Table 2
Statistic of fungal diversity and richness from 9 soil samples.

	Simpson	Chao1	ACE	Shannon
Non-ECMF	0.95 ± 0.01a	1173.33 ± 75.12a	1174.63 ± 71.85a	6.56 ± 0.08ab
with-ECMF	0.96 ± 0.01a	1143.60 ± 49.48a	1049.36 ± 54.31a	6.87 ± 0.19a
bulk soil	0.73 ± 0.14a	791.87 ± 154.78b	792.81 ± 154.06b	4.60 ± 0.98b

Figures

Figure 1

Morphological structures of (a) non-ectomycorrhizal-fungi-inoculation root tips, and (b) ectomycorrhizal-fungi-inoculation root tips and mycelia of *Suillus luteus* in the lower right corner (Scale bar = 2mm), the roots without (c) and with mycorrhizal (d) in our plots.

0 5000 10000 15000 20000 25000 30000 35000

Figure 2

Rarefaction curves of fungal community based on the sequences per sample (label: 0.97). WE refers to the soil inoculated with ectomycorrhizal fungi, NE refers to the soil inoculated without ectomycorrhizal fungi and BS refers to the bulk soils.

Figure 3

Analysis of fungal community structure of soil samples (a) Non-metric multidimensional scaling (meta NMDS, Stress: 0.042) based on BrayCurtis dissimilarity among fungal communities of OTUs occurring on three sites, NE refers to the soil inoculated without ectomycorrhizal fungi, WE refers to the soil inoculated with ectomycorrhizal fungi, and BS refers to the bulk soil; (b) differences between inter-group and intra-group boxplot based on Weighted UniFrac distance matrix on the soil fungal community under different treatments.

Figure 4

Relative abundance of different fungal phylum in the rhizospheric soils under different treatments. WE refers to the soil inoculated with ectomycorrhizal fungi, NE refers to the soil inoculated without ectomycorrhizal fungi and BS refers to the bulk soils.

Figure 5

The LEfSe identified the most differentially abundant taxon among the soil with ectomycorrhizal fungi inoculation (WE), without ectomycorrhizal fungi inoculation (NE) and bulk soils (BS); the hierarchical relationship of all taxa from phylum to genus (from inner to outer circle) in the sample community was shown, the node size corresponds to the average relative abundance of the taxon, and the colored shadows refer to the trends of the significantly differed taxa.

Figure 6

Correlationship of the most fungal communities in the soil with ectomycorrhizal fungi inoculation (WE), without ectomycorrhizal fungi inoculation (NE), and bulk soils (BS) at genus level.

Figure 7

Redundancy analysis of the relationships between the soil physicochemical parameters and fungal community of dominant phylum inoculated with ectomycorrhizal fungi (WE), inoculated without ectomycorrhizal fungi (NE), and bulk soils (BS). The red arrows refer to soil physicochemical, available nitrogen (AN), available phosphorus (AP), available potassium (AK), total carbon / total nitrogen (C/N), bulk density (BD), soil water content (SWC), urease (URE), saccharase (SAC), catalase (CAT), alkaline phosphatase (AKP). The green arrows refer to Chytridiomycota (Chy), Ascomycota (Asc), Basidiomycota (Bas), and Cryptomycota (Cry) .