

論文の内容の要旨

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Ectomycorrhizal fungal communities in mine tailings and heavy-metal contaminated soils in central south China

(中国南中部の重金属の鉱山尾鉱廃地と汚染土壌における外生菌根菌群集構造の解析)

Mining activities destroy vegetation and remove surface soil to reach buried ore deposits. Mineral extraction processes are often accompanied by pulverization of rocks and chemical use. The residue of the extracted substrates is stored in tailings, producing large areas of bare ground and piles of tailings. Ideally, these mine areas should be restored to the original vegetation. However, the toxicity of heavy metals (HMs), lack of macronutrients, and abnormal soil structures in mining areas make restoration difficult without careful planning (e.g., storage of surface soil to cover the bare ground after mining activities). In China, many mining areas have been abandoned, causing severe ecological and environmental problems. Soils in these abandoned mine areas are highly contaminated by HMs, which are dispersed by wind or dissolved in water, causing human health disorders in the surrounding regions. Many of the abandoned mine areas in China were originally covered by forests dominated by Pinaceae and Fagaceae. These tree species depend on ectomycorrhizal fungi (EMF) for nutrient absorption and cannot grow in their absence.

Some reports have demonstrated that EMF helps trees survive in soils contaminated with HMs by alleviating the toxicity of the metals. Thus, application of EMF in abandoned mine areas may help with forest restoration. Unfortunately, our knowledge about the function of EMF in heavy-metal tolerance is largely from in vitro experiments using a few easily culturable strains. To develop an effective reforestation strategy in the mining areas, we need better understanding of EMF on major forest tree species in mining areas.

In this dissertation, ectomycorrhizal fungal communities associated with two dominant forest trees, Masson pine (*Pinus massoniana*) or oak (*Quercus* spp.), were investigated in four mine sites (three types of HM, including lead-zinc (Chapter II), manganese (Chapter III) and copper (Chapter IV)) in the central south of China. In addition, seedling bioassay experiment was applied to screen EMF propagule communities in the mine tailing soil and guest soils covering tailings (Chapter V). The goals of this dissertation were to characterize EMF communities on Masson pine or oak growing in these different mining areas and to evaluate the effects of HMs and other soil factors.

Root systems were collected from selected trees in each mine sites and the corresponding rhizospheric soil samples and tree leaves were also collected for chemical analysis. Soil pH and electrical conductivity were determined by mixing the soil in water. For the element determination (K, Pb, Cd, Cu, Zn, and Mn) in soil and leaf samples, soils and leaves were digested by nitric acid and perchloric mixture and then determined using the atomic absorption spectrometer. For the

determination of total N and P in soils, soils were digested by sulfuric acid and perchloric acid, and then N and P were determined by indophenol-blue method and molybdenum blue method, respectively.

Ectomycorrhizas were identified by combining morphotyping and molecular analyses. For molecular analysis, fungal internal transcribed sequence region of nuclear rDNA were amplified by polymerase chain reaction and then subjected to sequencing analysis.

As a summary, in total, 107 species-level operational taxonomic units (OTUs) based on 97% ITS sequence similarity were identified from the Masson pine (103 OTUs) and oak (20 OTUs) ectomycorrhizal root tips from all of study sites. Of these OTUs, 84 belong to basidiomycete, 22 belong to Ascomycete. The richest family is Thelephoraceae, including 24 OTUs, followed by Russulaceae (22 OTUs) and *Cenococcum* (7 OTUs). These OTUs formed a phylogenetically diverse community associated with Masson pine. However, different structures of ectomycorrhizal fungal community were found among the different mine sites investigated.

1. Ectomycorrhizal fungal communities associated with Masson pine in Pb–Zn mine sites

The diversity and composition of EMF associated with Masson pine and soil chemistry were investigated in Taolin Pb–Zn mine tailings (TLT), two fragmented forest patches in Huayuan Pb–Zn mineland (HY1 and HY2), and a non-polluted forest in Taolin (TLC). The two study sites in the Huayuan mineland (HY1 and HY2) were significantly different in soil Pb, Zn, and Cd concentrations, but no significant difference was observed in ectomycorrhizal colonization, EMF richness, diversity, or rank–abundance. In addition, the similarity of communities between HY1 and HY2 was quite high (Sørensen similarity index = 0.47). Thus, the concentration of HMs may not be determining factors in the structure of these communities. In TLT, however, significantly lower ectomycorrhizal colonization and EMF richness were observed. The amounts of Pb and Zn in the tailing sand were higher than those in the non-polluted forest but far lower than in HY1. Thus, these HMs did not account for the reduced colonization and EMF richness in TLT. The community in TLT was dominated by four pioneer species (*Rhizopogon buenoi*, *Tomentella ellisii*, *Inocybe curvipes*, and *Suillus granulatus*), which collectively accounted for 93.2% of root tip colonization. The immature soil conditions in tailing (low N and P, sand texture and lack of organic matter) may only allow certain pioneer EMF species to colonize the site. When soil samples from four sites combined, I found the occurrences of major EMF taxa were not clearly related to the concentrations of Pb, Zn, and Cd. In conclusion, my results suggest that ectomycorrhizal fungal communities in these Pb-Zn mining areas are not necessarily affected by HMs themselves but could be largely determined by soil maturity.

2. Ectomycorrhizal fungal communities associated with Masson pine and oak growing in a manganese mine site

The ectomycorrhizal fungal communities associated with Masson pine and oak were investigated

in Xiangtan Mn mining area. The sampled trees were divided into three groups based on soil color: pine trees growing in yellow soils (normal color in local site), pine trees in black soils (polluted with Mn tailing wastes) and oak trees in gray-black soils. The results showed that average Mn concentrations in black soils ($4034.5 \pm 4692.5 \text{ mg kg}^{-1}$) and gray-black soils ($4246.8 \pm 3983.0 \text{ mg kg}^{-1}$) were significantly higher than those in yellow soils ($2019.8 \pm 2205.2 \text{ mg kg}^{-1}$). On the contrary, ectomycorrhizal colonization rates of Masson pine growing in black soils ($39.1 \pm 34.4\%$) and oak trees ($38.9 \pm 15.2\%$), being not significantly different from each other, were lower than that of pine in yellow soils ($54.6 \pm 18.7\%$). In total, 53 OTUs were identified at 97% similarity threshold, of which 48 were found in pine trees and 20 in oak trees. Compared to pine trees in yellow soils, a lower EMF richness of pine in the black soils and oak was observed. Correlation analysis indicated a significantly negative dose effect of Mn on EMF richness. The three ectomycorrhizal fungal communities associated with the three groups of trees showed different structure. The pine growing in yellow soils were dominated by Atheliaceae with relative abundance of 38.2%, followed by Russulaceae (34.9%) and Thelephoraceae (8.7%). The pine growing in black soils were dominated by Atheliaceae with relative abundance of 37.8%, followed by Thelephoraceae (34.3%) and *Cenococcum* (17.0%). On the other hand, the most dominant species in oak was members of Thelephoraceae, followed by *Cenococcum* with relative abundance of 61.9% and 19.4% respectively. Atheliaceae only accounted for 3.6% of ectomycorrhizal tips of oak. Russulaceae being dominant in pine from yellow soils became rare in pine in black soils (2.8%) and absent in oak. Russulaceae preferred to soils with lower Mn concentrations ($163.0\sim 3132.8 \text{ mg kg}^{-1}$) compared to Atheliaceae, Thelephoraceae, and *Cenococcum* (one-way ANOVA, $P < 0.01$). I concluded that the overburden of Mn tailing wastes may inhibit the ectomycorrhizal development and eliminate some Mn-sensitive ectomycorrhizal taxa, such as Russulaceae, resulting in the decrease of the EMF richness.

3. Ectomycorrhizal fungal communities associated with Masson pine growing on guest soils on copper (Cu) mine tailing slope in Dexing Cu mine

Ectomycorrhizal fungal communities associated with Masson pine were investigated in the guest soils covering Dexing No.2 tailing dam slope (DXT) and one adjacent non-polluted forest (DXC) in Jiangxi Province. Soil chemical analysis showed significantly lower N concentration ($394 \pm 143.6 \text{ mg kg}^{-1}$) and relatively high Cu concentration ($100.7 \pm 71.2 \text{ mg kg}^{-1}$) in the DXT as compared to those in the DXC (N: $2492.9 \pm 703.5 \text{ mg kg}^{-1}$; Cu: $39.7 \pm 10.8 \text{ mg kg}^{-1}$), but the Cu concentrations in guest soils were significantly lower than that in tailings in DXT, suggesting that covering guest soils could significantly obstruct the movement of HMs from tailing substrates into surrounding environments. As compared to DXC, the ectomycorrhizal fungal communities in DXT showed a very uneven structure and low species richness. In addition, the similarity of communities between the two sites was low (Sørensen similarity index = 0.25). In DXT, ectomycorrhizal fungal community were overwhelmed by two OTUs of Atheliaceae (Atheliaceae sp.1 and *Tylospora* sp.1), which accounted for 78.2% ectomycorrhizal tips and collectively

colonized 29 of 30 trees. In DXC, the community was mainly comprised of *Cenococcum* (28.9%), Thelephoraceae (21.6%), Atheliaceae (16.9%) and Russulaceae (16.4%), collectively accounting for 83.8% relative abundance. The concentrations of Cu in the guest soils did not affect the ectomycorrhizal fungal community. The guest soils originating from the subsoils and plant community might be responsible for the low diversity and poor species richness of EMF in Dexing tailing slope.

4. EMF propagule communities in Taolin Pb-Zn tailings and guest soils on Dexing Cu tailing slope

The ectomycorrhizal propagule communities of in the tailing sites and HM contaminated soils have been given relatively little attention. A seedling bioassay method was used to investigate the resistant propagule communities of EMF in the Taolin Pb-Zn tailing (TLT) and the guest soils on Dexing tailing slope (DXT). Rhizosphere soils were collected for seedling growth from TLT, DXT and one non-disturbed mature forest (DXC). After eight months of sowing, the seedlings of Masson pine were harvested and analyzed. The growth of seedlings in the TLT and DXT soils was significantly inhibited with comparison to those growing in the soils of DXC. Correlation analysis showed that N concentrations in soils were the limiting factor for seedling growth ($R = 0.538$, $P < 0.01$). Poor ectomycorrhizal colonization were observed in the seedlings growing in TLT ($9.0 \pm 14.9\%$) and DXT ($22.4 \pm 17.7\%$) soils, in contrast, DXC seedlings were colonized well ($47.5 \pm 24.9\%$). Molecular analysis revealed that *Cenococcum*, Atheliaceae sp., *Rhizopogon*, *Inocybe* and *Suillus* were frequently encountered in seedling roots, but species assemblage was significantly different among the three sites. Comparing with the forest EMF communities of the same sites investigated, high similarity was observed in the TLT site (Sørensen similarity index = 0.71, 5 OTUs sharing). In DXC, little overlap was found (Sørensen similarity index = 0.12). Some early-stage fungi at TLT (*Inocybe curvipes*) and DXT (*S. granulatus* and *S. luteus*) were abundant in seedling roots, whereas DXC seedlings were dominated by the members of *Cenococcum* and *Rhizopogon*. In TLT, EMF species (5 OTUs shared between seedling and forest investigations) may be available candidates for culturing ectomycorrhizal seedlings for reforestation in the bare tailings.

In conclusion, Masson pine in central south China harbored phylogenetically diverse EMF, which is similar to that observed in many subtropical or boreal forest trees. In present study, the elevated concentrations of HMs (Pb, Zn, and Cd) may not be the determinants for ectomycorrhizal colonization and low diversity of EMF except Mn in the mine areas. Poor ectomycorrhizal colonization and low diversity in the Pb-Zn tailing site can be explained from the immaturity (low N and organic matter, poor texture) of tailing substrates and the stage of succession. The overburden of Mn tailing wastes may inhibit the ectomycorrhizal development and eliminate some Mn-sensitive ectomycorrhizal taxa, such as Russulaceae. Moreover, the occurrence of most of the EMF were not related to the concentrations of HMs in soils except Russulaceae. Russulaceae only occurred in the soils with low HMs (Mn, Cu) and rich N and may be sensitive to HMs stresses.