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Ectomycorrhizal Fungi in Jiangsu Province, China*[1]

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ABSTRACT

A survey was conducted for about 3 years to study the abundance and diversity of ectomycorrhizal fungi (EMF) in Jiangsu Province, China. The identification of the fungal species was based on the microscopic and macroscopic characteristics of their fruiting bodies. About 126 species of EMF were found in Jiangsu Province. These fungi were largely categorized into three orders (of 121 species), four families (of 96 species), and six genera (of about 86 species).

Key Words: biodiversity, ectomycorrhizal fungi, geographical distribution, Jiangsu Province

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As one of the major terrestrial ecosystems, forests play an important role in a region's ecology, helping regulate climate, wind flow, sand and dust movement, soil quality, air quality, and so on. In temperate and boreal ecosystems, ectomycorrhizal fungi (EMF) colonize more than 90% of the fine roots of forest trees (Markkola *et al.*, 1996). The stability of forest ecosystems depends on the EMF colonization rate and species composition (Haselwandter and Bowen, 1996), especially on disturbed soils (Beckjord and McIntosh, 1984; Berry, 1982; Grossnickle and Reid, 1983; Mullins *et al.*, 1989). As a result, closing hills for afforestation is crucial to improve the local ecological system.

Ectomycorrhizae are formed as a result of the association between fungi and roots of some plant species. This is a symbiotic relationship in which the plant and fungal components benefit from each other. EMF enhance uptake and utilization of nutrients by plant roots, strengthen self-defense ability of plants, facilitate development of plants, and improve soil quality (Donnelly *et al.*, 2004; El Karkouri *et al.*, 2005).

In addition, the fungal symbionts of ectomycorrhizal plants excrete major quantities of enzymes, such as chitinases, phosphatases, and proteases. These enzymes might allow the organic residue to be tapped directly for nutrients such as N and P (Tibbett and Sanders, 2002). Therefore, these symbiotic fungi are important in the afforestation and rehabilitation of vegetative ecosystems, the remediation of soil and water loss, and the diversification of biological species. Many of EMF are also high-quality food with high economic value. This has generated considerable interest in the taxonomy and classification of EMF as a biospecies.

The presence of EMF is often indicative of a healthy ecosystem. When ecosystems deteriorate, EMF sometimes disappear, which interrupts or even terminates the interrelation between these fungi and plants (Wiemken and Boller, 2002).

EMF belong to the divisions Basidiomycota, Ascomycota, and also Zygomycota, and it is estimated that more than 5 000 species exist in EMF (Molina *et al.*, 1992). Common EMF genera include: *Suillus*,

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Boletus, *Gomphidius*, *Russula*, *Lactarius*, *Tricholoma*, *Hebeloma*, *Laccaria*, and *Amanita* belonging to the order Agaricales; *Thelephora*, *Pisolithus*, *Scleroderma*, and *Rhizopogon* belonging to the order Thelephorales; *Cenococcum* and *Tuber* belonging to the division Ascomycotina, and some species belonging to the family Amanitaceae. Species belonging to Thelephoraceae and Russulaceae are among the most frequent and abundant EMF in Europe and North America (Horton and Bruns, 2001). The results of a survey on fungal resources in various areas indicate that China is rich in EMF (He *et al.*, 1994; Mao, 1998; Xing and Li, 1999; Yu and Liu, 2002), and thus there is great potential for the development and utilization of EMF in ecosystem restoration.

In Jiangsu Province, China, the limited mountainous and forested areas, as well as poor biodiversity, contribute to the vulnerability of the ecosystem, with human activities easily influencing the ecological stability. However, the roots of a majority of the trees in the province can form mycorrhizae with EMF. As a part of the provincial afforestation program, the protection of local ectomycorrhizal species and the introduction of appropriate EMF from outside are equally important and practical. Rebuilding plant-EMF relationships is probably a fundamental step in improving the vegetation. Therefore, it is imperative to survey and analyze the now limited EMF in this area. The objective of the study was to determine the abundance and diversity of EMF present in Jiangsu Province, with a view toward future afforestation and ecosystem restoration.

MATERIALS AND METHODS

Site description

Jiangsu Province, China, lies between latitude N 30° 45′–35° 7′ and longitude E 116° 22′–121° 5′ and is in the transition zone between a subtropical monsoon climate and a temperate monsoon climate. The boundary of the two climatic zones falls approximately along the Huaihe River and the Subei General Irrigation Trench. Annual mean temperature is 13–16 °C and increases from the north to the south. Because of the monsoon climate, rainfall is abundant with a large variation in precipitation among localities. Generally, the eastern and southern parts receive more precipitation than the western and northern parts.

The total area of the province is 102 600 km^2. The majority of land comprises a plain covering 70 600 km^2, including 68.3% arable land (47% of the whole province). The major plain areas, from north to south, are the Huanghuai Plain, Jianghuai Plain, Binhai Plain, and the Yangtze River Delta. Jiangsu Province has the lowest average elevation in China, with most of its area less than 50 m above sea level. Massif clusters, grouped in the northern and southwestern parts, cover about 14.3% of the province. They include Laoshan, Ningzhen, Maoshan, Yili, and Yuntai Mountains. Yunvfeng (625 m above sea level and the highest point in Jiangsu Province) is located in a suburb of Lianyungang City. In this area having both subtropical and temperate zones, because of the transitional climate as well as ample sunlight, heat, and water resources, trees grow very well. The major topographic forms are plains (most of which are in agricultural fields) and water bodies, with limited mountainous areas. The forest cover is low, at 3.4% of the whole area, compared with the national average of 12.98%.

In this province, the main foresting tree species are *Populus tomentosa* Carrière, *Salix matsudana* Koidzumi, *Albizia julibrissin* Durazz., *Sophora japonica* L., *Pinus thunberqii* Parl., *P. densiflora* Siebold & Zucc, *P. canadensis* Bong, *Cedrus deodara* Loud., *Sabina chinensis* Antoine, *Cryptomeria fortunei* Otto & Dietr., *Metasequoia glyptostroboides* Hu & Cheng, *Taxodium ascendens* Brongn., *T. distichum* H.B. & K., *Quercus variabilis* Blume, *Q. acutissima* Carruth., *Castanea mollissima* Blume, *C. seguinii* Dode, *Fraxinus chinensis* Roxb., *etc.* (Yan, 1998; Mao *et al.*, 1996).

Procedures

From April to October of 2003, 2004, and 2005, Jiangsu Province was surveyed for the presence of EMF. This investigation covered the forests in hilly areas of Nanjing, Zhengjiang, and Yangzhou.

These were mainly afforested areas, where the tree species were *Pinus thunbergii* Parl., *P. densiflora* Siebold & Zucc, *P. canadensis* Bong, *Cedrus deodara* Loud., *Quercus variabilis* Blume, *Q. acutissima* Carruth, *Castanea mollissima* Blume, *C. seguinii* Dode, *Populus tomentosa* Carrière, *Fraxinus chinensis* Roxb., *Platanus sp.* L., *Salix matsudana* Koidzumi, *Albizia julibrissin* Durazz., and *Sophora japonica* L. The species of fungi were identified on the basis of the microscopic and macroscopic characteristics of the fruiting bodies, and a previously published guide (Wei, 1979) was also used to make species identifications.

RESULTS

The survey showed about 126 species of EMF, identified either by investigation on the species type or using data available in previously published reports, were found in Jiangsu Province (Table I). They belonged to 5 orders, 15 families, and 27 genera and most of them were concentrated in 3 orders, 4 families, and 6 genera (Fig. 1).

TABLE I

List of ectomycorrhizal fungi found in the Jiangsu Province

Order	Family	Species
Agaricales	Hygrophoraceae	*Hygrocybe cantharellus*
	Tricholomataceae	*Tricholoma flavovirens*; *T. terreum*; *T. equestre*; *Xeromphalina campanella*
	Hydnangiaceae	*Laccaria amethystea*; *L. laccata*
	Agaricaceae	*Macrolepiota rachode*
	Cortinariaceae	*Inocybe asterospora*; *I. decipientoides*; *I. fastigiata*; *I. rimosa*; *Rozites caperata*
	Lycoperdaceae	*Lycoperdon craniiformis*; *L. polymorphum*; *L. atropurpureum*; *L. asperum*; *L. pusillum*; *L. pyriform*; *L. perlatum*; *L. umbrinum*; *L. perlatum*; *L. wrightii*; *Calvatia lilacina*; *C. gigantean*; *C. cyathiformis*
	Aminataceae	*Amanita agglutinate*; *A. caesarea*; *A. ceciliae*; *A. citrine*; *A. excelsa*; *A. farinose*; *A. fulva*; *A. gemmata*; *A. manginiana*; *A. nivalis*; *A. phalloides*; *A. spissa*; *A. spissacea*; *A. spreta*; *A. solitaria*; *A. stribiliformis*; *A. vaginata*; *A. Verna*
Boletineae	Boletaceae	*Boletus edulis*; *B. erythropus*; *B. luridus*; *B. ornatipes*; *B. purpureus*; *B. queletii*; *B. radicans*; *B. retipes*; *B. speciosus*; *Strobilomyces retisporus*; *S. strobilaceus*; *Boletellus chrysenteroides*; *Porphyrellus pseudocaber*; *Phylloporus rhodoxanthus*; *Xerocomus badius*; *X. chrysenteron*; *X. subtomentosus*; *Pulveroboletus raverelii*; *Tylopilus ballovii*; *T. felleus*; *Leccinum crocipodium*; *L. nigrescens*; *L. scabrum*
	Gyroporaceae	*Gyroporus castaneus*
	Suillaceae	*Suillus granulatus*; *S. luteusl*; *S. grevillei*
	Sclerodermataceae	*Scleroderma areolatum*; *S. aurantium*; *S. bovista*; *S. cepa*; *S. polyrhizum*; *S. tenerum*; *S. verrucosum*
Russulales	Russulaceae	*Russula adusta*; *R. abbida*; *R. alutacea*; *R. crustosa*; *R. cyanoxanthaduo*; *R. decolorans*; *R. delica*; *R. densiforia*; *R. depalleus*; *R. emetica*; *R. fotens*; *R. fragilis*; *R. furcata*; *R. heterophylla*; *R. integra*; *R. lepida*; *R. lutea*; *R. mariae*; *R. mustelina*; *R. nigricans*; *R. ochroleuca*; *R. olivacea*; *R. pectinata*; *R. puellaris*; *R. pulchella*; *R. subdepallens*; *R. vesca*; *R. virescens*; *R. xerampelina*; *Lactarius camphoratés*; *L. deliciosus*; *L. insulsus*; *L. lignyotus*; *L. sanguifluus*; *L. scrobiculatus*; *L. subdulcis*; *L. subvellerreus*; *L. subzonarius*; *L. volemus*; *L. piperatus*; *L. liginosus*; *L. hygrophoroides*
Cantharellales	Cantharellaceae	*Cantharellus cibarus*; *C. cinnabarinus*; *Craterellus cornucopioides*
	Hydnaceae	*Hydnum repandum*
Thelephorales	Thelepheraceae	*Thelephora palmaata*; *T. terrestris*

Of the total number of EMF species, 121 species (96% of the total) belonged to the three orders (Fig. 1). Ninety-six species of EMF (76% of the total) belonged to 4 families of the total 15 families (Fig. 1). Among these families, almost one-third of the species belonged to Russulaceae. At the genus level, out of the total of 27 genera, 86 species (68% of the total number) belonged to the six genera shown in Fig. 1. The maximum number of species belonged to the genus *Russula*. Also, many species growing in Jiangsu Province were edible or had medicinal value. These fungi included *Boletus edulis*, *Cantharellus cibarus*, *Lactarius volemus*, *Russula lepida*, and *Scleroderma polyrhizum*.

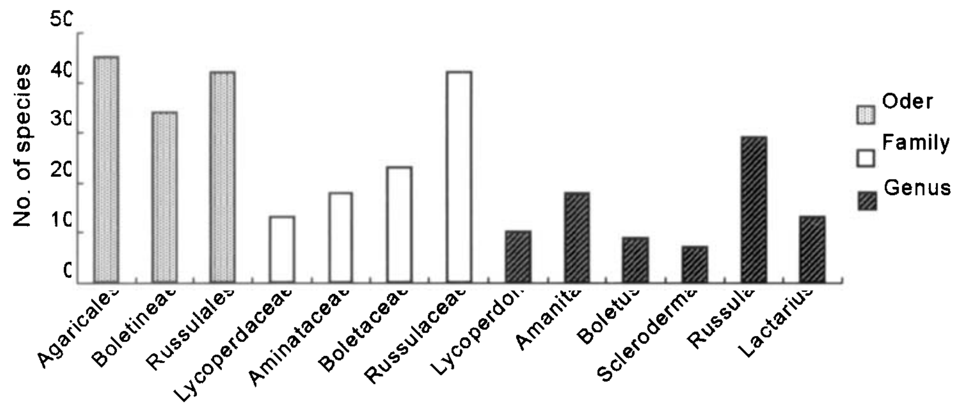

Fig. 1 Number of species of ectomycorrhizal fungi in the main taxonomic groups detected.

DISCUSSION

Despite the limited mountainous area in Jiangsu Province, the EMF species are important bioresources, contributing to the productivity and biodiversity of this area. Baxter and Dighton's report (2001) indicated that the diversity of ectomycorrhizal fungi was extremely beneficial to the ecosystem function. A majority of plants showed at least some kind of dependence on mycorrhizae. Van Scholl *et al.* (2006) believed that low molecular weight organic anions could enhance weathering of mineral grains. But ectomycorrhizal fungi did not increase the total concentration of low molecular weight organic anions compared with nonmycorrhizal seedlings, which depended on the fungal species; however, they affected the type of low molecular weight organic anions found. The association of ectomycorrhizal fungi with tree roots enhances the acquisition of phosphorus (P) from the soil. Compared with nonmycorrhizal seedlings, seedlings inoculated with *P. tinctorius* exhibit a greater capacity to survive under conditions of P limitation, as evidenced by the superior growth and the maintenance of normal foliar ion composition at low P concentrations (Cumming, 1993). In heavy-metal contaminated ecosystems, certain metal-adapted ectomycorrhizal combination may be suitable for large-scale land reclamation at phytotoxic metalliferous and industrial sites (Adriaensen *et al.*, 2005; Entry *et al.*, 1994; Kim *et al.*, 2004; Van Tichelen *et al.*, 1999).

Therefore, the application of EMF strains for environmental protection and afforestation can be very important. EMF is spatially associated with established ectomycorrhizal vegetation, but it is not well understood how distance from established vegetation affects the presence, abundance, diversity, and community composition of fungi. Dickie and Reich (2005) found that ectomycorrhizal infection of seedlings was spatially complex, with high infection and high fungal diversity near trees, high infection but lower diversity at intermediate distances from trees, and low infection and low fungal diversity at increased distance from trees. This spatial complexity should be considered as a potentially influencing factor in the establishment of ectomycorrhizal vegetation.

Through both field investigation and pure culture experiments, Wang *et al.* (2005) showed that the occurrence of mycorrhizal fungi was related to the tree species, the age of the tree, and the season. At least 20 EMF species were associated with more than two oak species. Buee *et al.* (2005) found that the species, structure of the ectomycorrhizal community, and metabolic activity of each morphotype changed depending on the season, temperature, and soil moisture; and a number of morphotypes were more abundant and active in winter than in summer. The most favorable month for occurrence of fruiting body was August, probably because the mycorrhizal fungus grew best at the temperature of 25–30 °C, and the optimum pH was 5–6.

EMF can boost absorption of nutrients from the soil, increase resistance to environmental challenges, and enhance growth and development of the plant. Aikio and Ruotsalainen (2002) found that due to the higher affinity of mycorrhizal roots at low nutrient levels, mycorrhizal plants appeared to be more sensitive to variation in nutrient concentration compared with nonmycorrhizal plants. Accordingly, EMF plays an important role in afforestation, revegetation, soil and water conservation, and biodiversity improvement. Furthermore, most ectomycorrhizal fruiting bodies have economic value as they are delectable food and sometimes have medical application.

Thus, the study of EMF taxonomy and evolution is both interesting and important. Identification of EMF is, however, very difficult in most situations, and new technology is often necessary to increase the potential of such studies. El Karkouri *et al.* (2005) examined the diversity of EMF naturally established on roots of containerized *Pinus* seedlings in a nursery using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and sequencing of the nuclear ribosomal internal transcribed spacer. Seventy-two samples, including ectomycorrhizae and fruiting bodies, were examined. Molecular typing assigned the fungal symbionts to four ectomycorrhizal species within Boletaceae: *Rhizopogon rubescens*, *Strobilomyces bovinus*, *Strobilomyces variegatus*, and *Rhizopogon luteolus*. *R. rubescens* was abundant (37.5%) and *Suillus* was moderately abundant (25%–26%), whereas *R. luteolus* was rare (2.8%). The combination of morphotyping and PCR-based analyses of parts of the rDNA is a well-accepted method to describe EMF and analyze the EMF community structure (Horton and Bruns, 2001; Kaldorf *et al.*, 2004).

The diversity and the plant growth-promoting ability of the naturally established symbionts investigated in this study could act as reference sources for studies monitoring the success of mycorrhizal application in both nursery and plantation conditions. The distributions of ectomycorrhizal fungi are influenced by the relative proportions of host tree species, which provide further support for the need to conserve stand diversity in Jiangsu Province. Cline *et al.* (2005) suggested that by enhancing the EMF diversity of seedlings, residual trees might maintain or accelerate the reestablishment of mycorrhizal communities associated with mature forests.

In this investigation, it was found that the EMF species occurring frequently were those that were edible or had medicinal value. These species included *Russula delica*, *Russula lepida*, and *Calvatia craniiformis*, which were often present on pine trees and Chinese fir trees. These fungi coexist with trees, which brought important economic value and environmental function. Therefore, if the mycelium of EMF is cultivated as a biofertilizer and the optimum conditions are provided for plants to develop relationships with these fungi, the process will benefit the growth of these trees. At the same time, when conditions are appropriate, the mycelium may develop fruiting bodies, and these can also be exploited. Therefore, afforestation and semicultivation of medicinal and edible fungi in nature are compatible and even mutually beneficial.

In summary, the evaluation of EMF resources is a very important and challenging aspect of sustainable development, and especially for Jiangsu Province. However, as current data on sources of EMF for the area are still sparse, the quantitative analysis and investigation in this study, based on the data currently available, are only preliminary. Further research will be necessary to better understand the overall picture of EMF in Jiangsu Province.

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