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The Major Factors Affecting Ectomycorrhizal Fungi Diversity in the Forest Ecosystem

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Abstract: Ectomycorrhizal fungi are important for plants growth which can enhance their resistance to drought, diseases, infertility and so on in the forest ecosystem, so researches on Ectomycorrhizal fungi are of necessity and great significance. As the basis for Ectomycorrhizal fungi research, the Ectomycorrhizal fungi diversity is attributed to many factors which can be divided to two parts in this review: biotic factors and abiotic factors. Biotic factors include chronosequences, bacteria living with ECM, habitat area, host genotype and abiotic factors include forest fire, toxic metal contamination, nitrogen content, survey method and microenvironment. The effects by these factors are deeply analyzed and discussed based on the plenty of literatures, our previous work and current research. The review not only introduces the current research result, but also arouses new ideas on Ectomycorrhizal fungi diversity research.

Keywords: Abiotic factor, biotic factor, diversity, ectomycorrhizal, review

INTRODUCTION

Ectomycorrhiza (ECM) which is formed by ectomycorrhizal fungi and the dominant tree roots in the forest ecosystems is widely observed. The ectomycorrhiza improves host performance by enhancing nutrient and water uptake from the soil and protecting host roots from being damaged by salinity, pathogens and toxic compounds (Smith and Read, 1997). Ectomycorrhizal fungi plays an important role in keeping the forest community dynamic in forest ecosystems, so doing research on Ectomycorrhizal fungi is necessary and significative.

The Ectomycorrhizal fungi diversity is the basis for Ectomycorrhizal fungi research, so many studies pay attention to the diversity of Ectomycorrhizal fungi (Dahlberg *et al*., 2001; Poole *et al.*, 2001; Lilleskov *et al*., 2001; Koide *et al*., 2011). Their diversities are complexity, for example, different researches show different diversity though in the same forest composition (Palmer *et al*, 2008; Ma *et al*.,2010; Buscardo *et al*., 2011) or in the same experiment place (Twieg *et al*., 2009; Richard *et al*., 2004, 2005), that is owing to too many factors which have an effect on Ectomycorrhizal fungi diversity.

These factors include biotic factors and biotic factors, while most people only focus on a few of them and they do not have a system summary. Every factor may cause the change of Ectomycorrhizal fungi diversity in nature or in the experiment, so reviewing the major factors affecting on Ectomycorrhizal fungi diversity is necessary and significant. This review has not only introduced the current research result, but also supplied the new ideas on Ectomycorrhizal fungi diversity research.

MATERIALS AND METHODS

The method for the aboveground survey of fruiting bodies and the belowground survey of root tipssurveying is vital for identification of ECM fungi, moreover sample time and sample volumes are also important factors. These effects were found in our research, during research on ECM diversity of *Castanea mollissima* in north China, we only found three ECM of C. *mollissima* by the morphological identification in 2010 (Chai *et al.*, 2010), however, by using the molecular identification method and increasing the sample time and sample volumes of fruitbodies collecting, a great more number of ECM species were found living with C. *mollissima* until 2012, so we conclude that survey method is a major factor which affects the ECM diversity of C. *mollissima*.

The aboveground survey of fruiting bodies, morphological classification is vital for surveying aboveground fruiting bodies. If it is used alone, however, it is less effective in identifying community diversity. A combination of morphological classification and molecular analysis is preferable for studying ECM diversity (Dahlberg *et al*., 2001; Taylor, 2002) and also with continued sequencing of fruiting bodies from aboveground surveys, there should eventually be a reduction in the number of unidentified

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root tip sequences in ECM diversity studies (Horton and Bruns, 2001; Dahlberg *et al*., 2001), assuming the fruiting body sequences are made available in DNA databases.

The aboveground survey of fruiting bodies is vital for identification of ECM fungi, when combining with the belowground survey; it is a good indicator of community diversity (Gardes and Bruns, 1996; Dahlberg *et al*., 2001; Horton and Bruns, 2001; Taylor, 2002). The belowground survey of root tips, morphological classification is an effective identification method for belowground surveys (Wurzburger *et al*., 2001; Sakakibara *et al*., 2002). However, the results obtained with this method can be affected by poor conservation, phenotypic expression, tip age, environmental conditions, different experimental times and the operators' procedures (Wurzburger *et al*., 2001; Horton and Bruns, 2001; Sakakibara *et al*., 2002; Burke *et al*., 2005). For these reasons, molecular analysis is an ideal identification method for belowground surveys.

In many studies, the aboveground results are not match well with the belowground result. Discrepancies have been explained by the fact that some species rarely or never produce fruiting bodies (Horton and Bruns, 2001), some species form inconspicuous fruiting bodies and therefore are under sampled (Gardes and Bruns, 1996; Tedersoo *et al*., 2003) and some species (such as the Cantharellales) do not sequence well (Feibelman *et al*., 1994).

Sample time and sample volumes are also important influences for identification diversity of ECM fungi. In certain ECM diversity studies, a large number of belowground root tip samples were collected only at a single time point. This approach could not reveal the composition of the entire community (Horton and Bruns, 2001; Taylor, 2002). A methodology based on sampling at multiple time points throughout the growing season should be used in the study. As molecular analysis is affected by DNA degradation in storage, processing samples promptly is very important. Small sample volumes should be collected in the study to ensure that the samples could be processed within a week. Palmer used a similar method to identify ECM forming during one growing season (Palmer *et al*., 2008).

RESULTS AND DISCUSSION

A**biotic factors:** ECM root species richness and community structure can be strongly influenced by abiotic factors, including nitrogencontent, forest fire, toxic metal contamination, survey method and microenvironment.

Nitrogen content: Nitrogen (N) content of soil is the main limiting factor for plants and it can also influence the diversity of EMF communities (Agerer and Göttlein, 2003), particularly under a deficiency or oversupply of nitrogen (N) (Alexander and Fairley, 1983; Nilsson, 2004; Parrent *et al*., 2006; Parrent and Vilgalys, 2007). Peter reported that after 2 years of further N supply significant changes were observed. The abundance of species that form large sporocarps (cap diameter about 5-10 cm) decreased on the fine roots in the underground community while the abundance of species with no or resupinate sporocarps increased (Peter *et al*., 2001a). Similarly, through an increasing anthropogenic N deposition gradient in long-term experiment, species richness of both sporocarp surveys and sampling of the fine roots were decreased (Lilleskov *et al*., 2001).

However, responses of the relatively rapid decline of sporocarp abundance and diversity to surplus N are different; the belowground community is faster (Lilleskov *et al*., 2001, 2002a). The diversity of EMF communities in Minnesota (US) was measured by Avis in a 16 year N-addition (fertilization) field experiment (Avis *et al*., 2003). The evenness and diversity of sporocarp species decreased in the fertilization treatments and total sporocarp species richness was reduced by more than 50%. Carfrae also demonstrated that after a 3-year nitrogen, sulphur and acidity treatment, N deposition suppressed the appearance of EM fungi, producing larger sporocarps in young plantations (Carfrae *et al*., 2006). Fewer sporocarps and lower EMF sporocarp diversity were generally found under the N treatment plots, while the number of mycorhizal root tips was greatest. In contrast, Kårén and Wiklund found the opposite; they reported a 50% reduction in sporocarp number and species richness of mycorrhizal species during the N fertilization experiment, while the EMF species richness and diversity on the roots were not changed (Kårén and Nylund, 1997; Wiklund *et al*., 1995).

Especially, nitrogen should be available and well balanced (Walker *et al*., 1995; Rygiewicz *et al*., 1997; Treseder, 2004), but species seem to react differently to supply (Alberton and Kuyper, 2009). Avis found that different species responded differently to the treatment and so the composition of the above-ground ECM community differed across the fertilization treatments. Moreover, they found that the treatment to different species responded differently and the composition of the aboveground EMF community differed. The same response was found in the below-ground result; however, it was smaller than the aboveground result (Avis *et al*., 2003). N fertilization with ammonium nitrate promoted the growth of *Tomentellopsis submollis*, while inhibited the growth of *Piloderma croceum* mycelium (Arnebrant and Söderström, 1992), this species-specific N uptake capacity under different N availability shows two fungal species have different optimum range of N. A shift in ectomycorrhizal

community structure with reference to N availability has been observed (Fransson *et al*., 2000, Parrent *et al*., 2006). The same result that ECM taxa can differ in response to N deposition was found by Lilleskov (Lilleskov *et al*.,2002b).The reaction to nitrogen may be the different sensitivity of both species to the availability of this nutrient (Alberton and Kuyper, 2009).

To summarise the results of the above fertilisation studies, we can conclude that N surplus is a factor which have an effect on the diversities of ECM community and its effect is sooner in the aboveground response than belowground changes, different EMF taxa can differ in response to N deposition. Therefore, a refined approach with nitrogen amendment adjusted to the special demands of either species would be necessary.

Forest fire: Forest fire is the major forest disturbance pattern and hazard (Byrot, 2009) which can affect ECM fungal communities (Grogan *et al*., 2000; Chen and Cairney, 2002; Anderson *et al*., 2007) by the cessation of carbohydrate flow to the established, native ECM fungal population. However, it is difficult to predict the extent to the forest fire affects the population of ECM fungi as there are multiple factors (Koide *et al*., 2011). For example, after fire changes in the chemical or biological properties of the soil (Amaranthus *et al*., 1993; Colinas *et al*., 1994) and the presence of plant species which can resprout or quickly establish (Borchers and Perry, 1990)? However, some environmental factors are not affected by the fire, belowground soil temperature during the fire probably did not rise beyond 200°C. Consequently, concentration of phosphorous which would be affected by even higher temperatures than nitrogen remained almost unchanged (Knoepp *et al*., 2005). Soil pH was slightly more alkaline at post-fire sites, a common effect in burnt soils and in this case probably mainly due to the increased concentration of soluble calcium which is a product of ash deposition (Knoepp *et al*., 2005).

Though the fire influence is complex, we still found some regularity that the frequency of the fire is a major factor for ECM diversity changing.

Many studies on fire influence show that low-frequency fire events do usually not cause substantial change but the relative abundance of ECM. The result of the ECM community of the *Quercus ilex* forest at Nazar between 1998 and 2001 demonstrated that there are differences in the relative abundance of certain ECM morphotypes when comparing the stand affected by fire and the undisturbed plot (Román and Miguel, 2005). Anderson study changes in vertical stratification of soil basidiomycetes by molecular tools, result showed no substantial change in species richness (Anderson *et al*., 2007). A similar trend was observed by Tuininga on ectomycorrhizal roots of adult pine trees previously exposed to low-frequency, they found no consistent stand-specific changes in fungal diversity but clear shifts in morphotype richness between soil horizons compared to unburned plots (Tuininga and Dighton, 2004). Compared ectomycorrhizal morphotype richness on seedlings from the burnt sites with the control seedlings, only a slight small richness effect by low-frequency fire (Palfner *et al*., 2008). Fungal species diversity is often not substantially changed by low-frequency fire, which has been reported generally (Torres and Honrubia, 1997; Anderson *et al*., 2007; Tuininga and Dighton, 2004). Similar results have been reported in the ECM community of a *Pinus sylvestris* forest in Sweden (Jonsson *et al*., 1999) and a *Picea* forest in Canada (Mah *et al*., 2001). A decrease in the number of mycorrhizal tips after low-frequency fire has also been reported by several studies (Dahlberg *et al*., 2001; Stendell *et al*., 1999; Torres and Honrubia, 1997). It was concluded that low-frequency fire induced a shift in the relative abundance of each species rather than a change in the species composition.

In contrast, several published studies report that the intensity fire results differ in several aspects from the above results. For example, after the intensity fire in a *Pinus muricata* forest in California, a shift in the species composition of the ECM community were found by Baar and Dahlberg found both a decrease and a change in the species composition in the ECM diversity of several conifer forests affected by fire in Scandinavia (Baar *et al*., 1999; Dahlberg *et al*., 2001). The intensity of fire is an important factor which would influence the impact of fire on the ECM community (Dahlberg *et al*., 2001; Dahlberg, 2002; Grogan *et al*., 2000).

Many trees survive and the organic matter remains intact after a low-intensity fire and this situation causes small affect of the fire on the ECM community. Loss of fungal species richness under the ground but may alter community structure. However, high burning frequency may lead to a loss of ectomycorrhizal fungal diversity as reported by Bastias (Bastias *et al*., 2006).

After fire influence, the diversities of ectomycorrhizal fungi are finding in the forest and most of them are belonged to Ascomycotaand Basidiomycetes.

Most studies are about Ascomycota fungi. Post-fire fungi which is a series of typically fungi appeared only after a fire, largely belonging to the order Pezizales and these fungi produced fruiting nearly 6 weeks after a fire (Petersen, 1970). Post-fire Pezizales are often referred to as facultatively mycorrhizal. For example, *Biotrophism* which belongs to Pezizalean is supported by direct observation of the fungi colonizing root tips in vitro (Warcup, 1990; Dahlstrom *et al*., 2000) as well as in vivo (Vrålstad *et al*., 1998). Some species of post-fire Pezizales are reported to form ectendomycorrhizal

associations with members of the *Pinaceae* (Danielson, 1984; Egger and Paden, 1986; Dahlstrom *et al*., 2000). *Wilcoxina* sp. is common found on post-fire pine (*Pinus muricata*) seedlings (Baar *et al*., 1999). *Geopora cooperi* is widely distributed in undisturbed forest conditions (Maia *et al*., 1996; States and Gaud, 1997) as well as in nutrient-poor, volcanic cinder soils with species of the Pinaceae (Gehring *et al*. 1998).*Wilcoxina rehmii* and *Geopora cooperi*belongs to the Pezizalean species, the probability of recovery of them have been increased because the fire removed most of the speciose mycorrhizal fungal community that typically occurs in undisturbed habitats (Horton *et al*., 1998; Baar *et al*., 1999; Stendell *et al*., 1999; Fujimura *et al*., 2005). They found very low richness of mycorrhizae, mainly represented by a hymenoscyphus-like morphotype and *Cenococcum geophilum*, both of them were more abundant on seedlings from unburnt forest than on those from the burnt areas (Palfner *et al*., 2008). Ascomycota mycorrhizal may be less abundant on post-fire seedlings tha n on trees not affected by fire (Izzo *et al*., 2006; Fujimura *et al*., 2005). The study on the ecosystem function of *Morchella* showed that some species fruiting may be mycorrhizal without a fire influence (Dahlstrom *et al*., 2000; Hobbie *et al*., 2001), however, they may not form mycorrhizas in burned sites, if they do, they may revert to being saprobic (Buscot, 1994; Dahlstrom *et al*., 2000). Isotopic experiment on *Morchella* species from burned and nonburned sites may help distinguish between mycorrhizal and saprobic functions of post-fire *Morchella* species (Hobbie *et al*., 2001, 2002).Every year, the wildfires of Mediterranean cause the loss of forested lands where soil and climatic characteristics are suitable for *truffle* cultivation. After the forest fires the competitiveness of *Tuber melanosporum* was highlighted within the ECM community in these soils (Aragón *et al*., 2012).

Basidiomycetes were the most abundant fungi on both plant groups (post-fire and control), with a clear dominance of a single mycorrhizal morphotype, especially on the post-fire sites with almost double as many (42.7%) colonized root tips as on control seedlings (22.1%) (Palfner *et al*., 2008).The studies by Palfner and Godoy and Palfner already yielded some evidence that mycorrhizae of *Descolea flavoannulata Horak* are especially abundant on seedlings or young trees at disturbed or ruderal sites: they were found in such distinct habitats as on seedlings of *Nothofagus pumilio*, establishing on thick ash layers in Patagonia in the aftermath of a volcanic eruption, on 2-year old nursery seedlings of *Nothofagus alpina* and on planted trees of the same species in park-like environments (Palfner,1997; Palfner,2001;Godoy and Palfner,1997). The less frequent fungal species such as *Cortinarius magellanicus* whose mycorrhiza is characterized by conspicuous pseudosclerotia, russuloid morphotypes and tomentelloid morphotypes (Palfner, 2001; Palfner *et al*.,2002), the first morphotypes were found only on seedlings from the undisturbed control area and the other two morphotypes were appeared almost exclusively on roots from the burnt sites. It should be mentioned that Bastias also report absence of Cortinariaceae in the aboveground horizon of a frequently burnt *Eucalyptus pilularis* forest (Bastias *et al*., 2006). Increased richness of a single fungal species or species complex on mycorrhizal roots after the fire influence has already been studied: 6 years after the fire event, *Suillus brevipes* were found the most abundant species associated with *Pinus banksiana* (Visser,1995), early stage after forest fire, *Rhizopogon* spp. was dominant colonizers of bioassay seedlings of *Pinus muricata* and *Pinus jeffreyi*, respectively fruiting patterns of macromycetes often revealed a boost of ascomycetous taxa (Baar *et al*., 1999; Izzo *et al*., 2006).

Heavy metal contamination: It was shown that the metal-contaminated soils had a negative impact on the abundance and variability of ectomycorrhizas in the root systems.

Different kinds of Heavy Metal (HM) environment showed different diversities of ECM, these effects have been found in the birch root systems (Bojarczuk and Barbara, 2010). Under natural conditions, *Betula pendula* develops Ectomycorrhizal (ECM) symbiosis (Smith and Read, 1997). *Betula pendula* often has been found on soils contaminated with metals, such as Zn (Denny and Wilkins, 1987); Pb (Eltrop *et al*., 1991); Zn and Pb (Kopponen *et al*., 2001); Cd, Cd and Zn (Regvar *et al*., 2006); and Cu and Pb (Klink *et al*., 2006), however, the diversity of Ectomycorrhizal fungi are not same in each place. The reason is that ECM diversity has different response to the different HM type. However, the same ectomycorrhizal fungi when hosted different trees has also different response to the same HM type. For example, the phytoextraction capacity of willow or poplar associated with mycorrhiza has been studied by Lingua (Lingua *et al*., 2008). Lingua reported that *Populus alba* (clone Villafranca) inoculated with *Glomus mosseae* or *Glomus intraradices* accumulated lower Zn concentrations; *Populus nigra* (clone Jean Pourtet) also accumulated lower Zn concentrations when inoculated with *G. mosseae* whereas its Zn concentrations remained unchanged when inoculated with *G. intraradices* (Lingua *et al*., 2008). On the other hand, Sell reported that the Cd concentration were enhanced in *Populus canadensis* when associated with an ectomycorrhizal fungal species (*Paxillus involutu*s), however, when associated with this ectomycorrhizal fungi, the Cd concentration remained unchanged in *Salix viminalis* (clone 78198) (Sell *et al*., 2005). By contrast, Baum reported higher Cd, Zn and Cu concentrations in the

stems of *Salix×dasyclados* (clone SDHM) associated with the same ectomycorrhizal fungi (Baum *et al*., 2006).

Living at high metal concentrations causes a high variability in tolerance to ECM fungal species. (Fomina *et al*., 2005; Leski *et al*., 1995). The effects of heavy metals are inconsistent to different ECM as a result of this fungal variability (Godbold *et al*., 1998; Meharg and Cairney, 2000). Metal enriched soils can lead to the selection pressure of metal toxicity ECM genotypes (Hartley *et al*., 1997; Leyval *et al*., 1997; Markkola *et al*., 2002; Colpaert *et al*., 2004; Adriaensen *et al*., 2005). ECM has the ability to alleviate the effects of heavy metal toxicity for themselves and their host trees (Adriaensen *et al*., 2005, 2006; Krznaric *et al*., 2009), by providing a more balanced access to mineral nutrition (Marschner and Dell, 1994).

The kinds of ECM which are always found in HM polluted soils are mainly Ascomycete. For example, on the roots of *Salix caprea* studied at the polluted site 15 ECM taxa were found by combining the ECM phenotyping and sporocarp identification, the members of Sordariaceae were the most frequent, followed by The lephoraceae (Regvar, 2010). A study from a primary succession site at the forefront of a receding glacier also reported Sordariaceae as dominant ECM fungi of willows (Trowbridge and Jumpponen, 2004). *Glomusmosseae* which has frequently been identified in HM soil (Hassan *et al*., 2009; Vallino *et al*., 2006), these ascomycetes could be intercellular endophytes or rhizoplane colonisers and their role for hosts deserve further attention. Ascomycete ECM fungal taxa seem to be most numerous at the plots with high metal and low levels of organic matter and more sparse vegetation cover.

Survey method: This part was in material sand methods.

Microenvironment: ECM living environment which has great effect on the diversity of ECM are complex and hard to control, the research on microenvironment factors can help us clearly know the ECM living habit. Vitro environment is the best way to realize microenvironment, our laboratory is focus on the vitro of C. *mollissima*, we have already built up the regeneration system of C. *mollissima* (Hou *et al*., 2010), three new ECM species were separating from the C. *mollissima* ectomycorrhiza and two of them were successful infecting with the roots of C. *mollissima* in vitro environment. During the whole process of growth, we found the vitro environment factors have different influence on different kind of C. *mollissima* ECM which include carbon dioxide concentration, relative humidity, PH value, growth hormone levels and so on, these factors which affect the ECM diversity of C. *mollissima* are our new current research.

Biotic factors: ECM root species richness and community structure are also influenced by a range of biotic factors, including chronosequences; bacteria lived with ECM, host genotype, habitat area and so on.

Chrono sequences: The age of plants living with ECM is an important factor to show their nutritional status and the structure, which are correlated with the ECM diversity. So the chronosequence affects the ECM species compositions. For instance, Peter showed different EMF species compositions were found in different stand ages (35, 100–200 years old) (Peter *et al*., 2001b). They explained that the sites with various ages may lead to their results. The same study was carried out by Visser, in which he indicated that sequence of mycorrhizal fungi related to stand age has undergone regeneration following wildfire disturbance in both fruiting body and root tip survey (Visser, 1995). Visser found different diversities in different stand ages, so he divided mycorrhizal fungi into early-stage, middle-stage and late-stage fungi (Visser, 1995).

Different experiment showed different results, most people believe that as the ages increase ECM diversities increase too, however some people showed that as the ages increase the diversities of EMF decrease, there are still some researchers found that ECM diversity are different though in the same ages.

As the ages increase ECM diversities increase. Our laboratory is major research on the ECM diversity of chestnut (*Castanea mollissima*) in north China, Chronosequence factor which affects the ECM diversity of C. *mollissima* are our current research. During the long term research, our sample plot included 15 years old C. *mollissima* stand and over 80 years C. *mollissima* stand, both aboveground and belowground diversities were much more found in the over 80 years stand, so we believe that as the C. *mollissima* forest ages increase their ECM diversities increase too. A progressive increase in species richness of mycobionts along chronosequences of Sitka spruce forests (6, 12, 30, 40 years) in the result of both sporocarp survey and root tip sampling has been demonstrated by Palfner (Palfner *et al*., 2005).Gebhardt showed that the diversity of ECM sporocarps and root tip morphotypes of red oak growing on forest reclamation sites(Gebhardt *et al*., 2007). Each stand (5, 21, 33, 43 and 46 years old) results were demonstrated stand specific EMF communities with low similarity to other stands by both methods. The total number of EM species was the largest in the 46 year-old nature standin both sporocarp survey and the root sampling. ECM fungal community structure was surveyed in four combinations of age class (5-, 26-, 65- and 100-year-old), each representing critical seral stages of stand development in Interior Cedar-Hemlock (ICH) forests of southern British Columbia. ECM fungal community structure was more strongly influenced by stand age and in particular, no

pattern that paralleled the strong increase in ECM fungal diversity seen from 5-year-old to 26-year-old age classes (Twieg *et al*., 2009). Tabea Kipfer investigated the diversity of ECM fungal communities on a chronosequence of 12 *Pinus sylvestris* stands in Valais (Switzerland) and Val d'Aosta (Italy) between 1990 and 2006, the number of ECM species was significantly lower in samples from recently (2-5 years)(Kipfer *et al*., 2011).

As the ages increase, the diversities of EMF decrease. Species richness decreases as the age of host increases in the results of fruiting body surveys, however, the same result were not found in the results of belowground sampling (Richard *et al*., 2004, 2005).The high diversity of mycorrhizal fungal taxa existing in seedlings also confirms the applicability of using seedlings to effectively document mycobiont diversity in situ (Walker *et al*., 2008). Ectomycorrhizal (ECM) fungal communities of *Quercus liaotungensis* of different ages (seedlings, young trees and mature trees) in the growing seasons between 2007 and 2009 were studied by Wang in a temperate forest of northern China. He indicated that oak seedlings in natural forests are associated with a wide assemblage of ECM fungi as they are with the mature oak trees (Wang *et al*., 2012).

ECM diversities are different though in the same stand age. Differences in mycorrhizal fungus communities on roots of same-aged seedlings across a deglaciated chronosequence was evaluated by Helm, overall, the EM formation on transplants during the first 2 years was lower and less diverse than on naturally occurring plants in this same chronosequence (Helm and Allen,1999). Moreover, chronosequence affects are not limited by composition of forest, Twieg described the species composition of the ECM fungal community changed along a chronosequence of mixed forest stands that were similar in vegetation composition and site quality (Twieg *et al*., 2007).

Ectomycorrhiza Associated Bacteria (EMAB): The establishment of plant-fungi interactions may be influenced by bacteria that occur on the surface of mycorrhizal roots and at intercellular and intracellular locations within the ectomycorrhizal mantle and Hartig net (Nurmiaho-Lassila *et al*., 1997; Mogge *et al*., 2000). Parts of the bacteria which positively affect the formation of ectomycorrhizal associations were called EMAB (Garbaye, 1994) and these associated bacteria can enhance the symbiosis between host plants and mycorrhizal fungi and support mycorrhiza formation or function (Dunstan *et al*., 1998; Gryndler *et al*.,2000; Duponnois and Plenchette, 2003; Kataoka and Futai,2009). So far, positive effect of EMABon the mycorrhiza formation and indirect effect of mycorrhiza diversity have been described in many publications (Garbaye, 1994; Poole *et al*., 2001; Hrynkiewicz *et al*., 2009).

Different EMAB have different influence on stimulating mycorrhization and mycorrhiza diversity. For example, mycorrhization of *Amanita rubescens* or *Hebeloma sinapizans* by dual inoculating with EMAB*Pseudomonas putida* or *Bacillus cereus* on *Pinus sylvestris* seedling growth, the result showed that the increased growth of pine seedlings was especially seen for co-inoculation with P. *putida*, *Amanita rubescens* was more efficient in this stimulation than H. *sinapizans*, as H. *sinapizans*was hard to form ECM without the help of its associated EMAB and this may cause changes in mycorrhiza diversity (Kozdrój *et al*., 2007). The same report has been found that A. *rubescens* mycorrhization was hard to format in the presence of EMAB bacterial strains inoculation by Shishido (Shishido *et al*., 1996). The result indicates that bacteriaonly affect mycorrhization when they are associated with mycorrhiza, therefore mycorrhiza diversity is affected by their associated bacteria. Pine seedlings co-inoculation with some ECM was greatly stimulated by P. *putida*.of EMAB, many pseudomonads and bacilli promoting kinds of ECM formation have been identified as bacteria (Frey-Klett *et al*., 1997; Poole *et al*., 2001).

Explanation of functional interactions between ectomycorrhizal symbionts and their EMAB is still a big challenge. Probanza reported that some strains of Bacillus, belonging to EMAB, directly affected the growth of mycorrhiza by gibberellin production (Probanza *et al*., 2002). de Boerfound a possible mechanism for selection of fungus associated bacterial strains by ectomycorrhizal fungi which could select antibiotic-resistant bacteria by exudation of soluble fungal storage sugars, polyols or organic acids which can increase the number of bacteria or exudation of inhibitory chemicals (wiestse *et al*., 2005).

Host genotype: ECM species richness and diversity are correlated with host plant diversity, the effect on vegetation composition forest ecosystem is not the same with that on mixed forest ecosystem.

In vegetation composition forest ecosystem, different hosts have different communities of ECM, taking broad leaved tree species and conifer for example, Palmer investigated ectomycorrhizal community of an American chestnut, finding that only ten putative ECM species were definitively associated with *Castanea dentate* (Palmer *et al*., 2008), however, Ma found that 22 ectomycorrhizal fungal species comprized the ECM community of *Pinus dens flora* (Ma *et al*., 2010). The differences were also found inside of broad leaved tree species and conifer, Palmer found that the ECM composition and diversity of *Quercus* spp. are different from that of *Castanea dentate* (Palmer *et al*., 2008).A total of 30 taxa colonized with *Pinus pinaster* were found by Buscardo, which is more than that with *Pinus densiflora* (Buscardo *et al*., 2011).

In mixed forest ecosystem, sporocarp inventory result showed that fruiting of some fungal species near either *Q. ilex* or *Arbutus unedo* and this phenomenon was also found in the belowground survey (Richard *et al*., 2004). EMF species richness and diversity is greatly different from the results in vegetation composition forest ecosystem, so in a mixed forest ecosystem, identification of host plants is imperative for studying EMF species richness and diversity (Richard *et al*. 2004).

Habitat area: Several studies have reported that habitat area affects the richness and diversity of ECM fungi, this affect is complex and interesting.

Some people believe that as the habitat area increase the richness and diversity of ECM fungi are also increase, for instance, Peay found that island size had a strong effect on diversity of ECM (Peay *et al*., 2007). Total species richness increased significantly with island area, independently of the approach used. *Pinus densiflora* Sieb. is one of the major ectomycorrhizal host trees in the eastern Asian biome. Twenty one species of ECM fungi fruit bodies were reported from a 700 m^2 study area (Fujita *et al*.,1989) while 63 species were found in a 3200 m^2 study area in P. *densiflora* forests in one year based on sporocarp production (Yamada and Katsuya, 1995). Four different study areas found different diversity within a 7,000 km^2 area New South Wales (Hitchcock *et al*., 2011).

On the opposite, some people found that as the habitat area increase the richness and diversity of ECM fungi are decrease. For example, Durall (Durall *et al*., 1999) studied the effects of small forest gaps and partial cutting on diversity of EM mushroom, results showed that sporocarp species richness were decreased as gap area increased along 100 m long transects. ECM richness on seedling root tips also decreased slightly with increasing distance from the edge of the intact forest. The maximum richness was found to be 7 m or less from the forest edge for both tree species investigated.

CONCLUSION

In this study, the abiotic and biotic factors effect on the ECM root species richness and community were discussed. Among the abiotic factors, the effect of N surplus is sooner in the aboveground response than belowground changes; low-frequency fire events do usually not cause substantial change but the relative abundance of ECM. Vitro environment factors, different carbon dioxide concentration, relative humidity, PH value and different growth hormone levels have different influence on the diversity of ECM.

The biotic factors effect on the ECM root species richness and community were also discussed. In many cases, ECM diversities usually increase with hosts aging. Effect of EMAB on the mycorrhiza formation and indirect effect of ECM diversity are positive; identification of host plants is imperative for studying EMF species richness, especially in mixed forests; the method for aboveground survey of fruiting bodies and the belowground survey of root tips is vital for identification of ECM fungi, moreover sample time and sample volumes are also important.

The knowledge of ECM diversity are more comprehensive by learning more affecting factors, so further studies on the diversity of ECM are still needed in the future.

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