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ORIGINAL PAPER

Ectomycorrhizal fungus communities of *Quercus liaotungensis* Koidz of different ages in a northern China temperate forest

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Abstract Ectomycorrhizal (ECM) fungal communities of *Quercus liaotungensis* of different ages (seedlings, young trees and mature trees) in the growing seasons (June and September) between 2007 and 2009 were studied in a temperate forest of northern China. A total of 66 ECM fungal taxa were identified based on ECM morphotyping, PCR-RFLP, and DNA sequence data. Of these fungal taxa, 51 were Basidiomycetes (77.3%) and 15 were Ascomycetes (22.7%). *Cenococcum geophilum* was the dominant species. Thelephoraceae (16 taxa), Sebacinaceae (12 taxa) and Russulaceae (seven taxa) were the most species-rich and abundant ECM fungi, accounting for 19.5%, 17.6% and 8.3% of the total ECM root tips, respectively. Results of multiple response permutation procedure (MRPP) analysis indicated that there were marginally significant effects of tree ages (A=0.01801, P=0.054) and growing seasons (A=0.01908, P=0.064) on the ECM fungal species composition of *Q. liaotungensis* in a temperate forest.

Keywords Ectomycorrhizal fungi · ITS · *Quercus liaotungensis* · Community pattern

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Introduction

Ectomycorrhizas are symbiotic associations formed between soil fungi and plant roots. Ectomycorrhizal (ECM) fungi exchange soil-derived nutrients for carbohydrates from host plants and are beneficial to host species in resistance to abiotic or biotic stresses (Smith and Read 2008). ECM fungi therefore play an important role in nutrient transportation, interspecific interactions, and maintenance of biodiversity in ecosystems (Simard et al. 1997). Knowledge about the successional dynamics of ECM fungal community is key to understand fungal diversity and ecosystem functioning.

Investigations of ECM fungal communities only based on above-ground fruit bodies cannot provide a complete picture of ECM communities in natural ecosystems (Gardes and Bruns 1996; Jonsson et al. 2000) because some ECM fungi do not form easily visible fruit bodies or do not fruit every year (Horton and Bruns 2001). Recently, molecular techniques, such as PCR-RFLP combined with DNA sequencing, have provided rapid and accurate identification of fungal taxa from ECM roots and have greatly increased our understanding of the diversity and composition of below-ground ECM fungal communities in natural ecosystems (Horton and Bruns 2001; Smith et al. 2007, 2011; Azul et al. 2010; Jumpponen et al. 2010; Wang and Guo 2010; Wang et al. 2011).

ECM fungal community composition has been widely studied in temperate (Gebhardt et al. 2007; Smith et al. 2007; Jumpponen et al. 2010; Wang and Guo 2010), as well as subtropical (Liang et al. 2007; Wang et al. 2011) and tropical forests (Tedersoo et al. 2007; Peay et al. 2010; Smith et al. 2011). A few studies have also considered seasonal shifts (van der Heijden and Vosatka 1999; Buée et al. 2005; Koide et al. 2007; Smith et al. 2007; Jumpponen et al. 2010) and successional dynamics of ECM fungal

communities (Smith et al. 2002; Koide et al. 2007; Gebhardt et al. 2007; Twieg et al. 2007), but results are sometimes contradictory. For example, on seasonal shifts, Buée et al. (2005) found that there was a seasonal shift over 1 year in the ECM community of *Fagus silvatica* in France. Yet, Smith et al. (2007) investigated ECM community in a *Quercus douglasii* woodland in winter and spring over 2 years and found that 92% of the frequent ECM fungi were detected on roots in both seasons and years. On successional dynamics, Gebhardt et al. (2007) and Twieg et al. (2007) found that ECM fungal diversity was much lower in young stands than in older stands of temperate forests. Whereas, Smith et al. (2002) found no strong difference in cumulative species richness of ECM fungal sporocarps among three age classes (young, rotation-age, and old-growth) of *Pseudotsuga menziesii* in Oregon, USA. Therefore, the successional and seasonal dynamics of ECM communities need further study and our understanding of the successional dynamics of ECM communities remains limited (Read 2002).

Quercus species are geographically widespread and ecologically diverse, and ECM communities of *Quercus* species have been widely investigated in North America (Smith et al. 2007; Hynes et al. 2010; Jumpponen et al. 2010) and Europe (Gebhardt et al. 2007; Azul et al. 2010; Leski et al. 2010). However, little information is available on ECM fungi associated with *Quercus liaotungensis*, a widely distributed woody tree species in northern Chinese forests.

By combining observation of ECM morphotypes with PCR-RFLP and internal transcribed spacer (ITS) sequence analyses, we investigated the variation of ECM fungal communities of *Q. liaotungensis* in different tree ages (seedlings, young, and mature) and growing seasons (June and September) between 2007 and 2009 in a northern Chinese forest. The objectives were (1) to determine the diversity of the below-ground ECM fungi and (2) to evaluate the effects of host age and growing season on the ECM fungal communities of *Q. liaotungensisi* in a temperate forest of northern China.

Materials and methods

Study site

The study site is located in a mosaic stand of *Q. liaotungensis* with different tree ages in Dongling mountain, the Beijing Forest Ecosystem Research Station of the Chinese Academy of Sciences, 117 km west of Beijing (39°58′N, 115°26′E, 1,240 m above sea level). The mean annual temperature is 2–7°C, and the mean annual precipitation is ca. 500 mm. Soil is mountainous brown earth, with 2.64% organic matter, pH 7.20, 0.21% total nitrogen, 0.008% total phosphorus, and 0.625% K_2O (Sun 1997). Three age classes of *Q. liaotungensis* were selected as seedlings (<5 years old), young trees (~15 years old), and mature trees (~50 years old). The mature tree plots are naturally regenerated, while the young tree plots were planted after forest clear-cutting 15 years ago. Tree ages were estimated by counting knots and tree rings observed under a microscopy.

A sampling site (1,200×2,400 m) was established in the *Q. liaotungensis* stand (Fig. 1). Three plots (20×60 m for each plot, >120 m apart) as replicates were selected for young trees and mature trees, respectively. The plots were selected using the following criteria: The individuals of *Q. liaotungensis* were more than 90% of the total individuals of all trees and no other ECM plant species exists in the plots. Each plot had the same age trees only and was divided into 24 subplots (5×5 m for each subplot). There was at least 5 m buffer zone around the plots.

Sampling and ECM morphotyping

Soil samples were collected within growing seasons on 22 September 2007, 9 June 2008, 24 September 2008, and 8 June 2009. At each sampling date, ten soil samples (20×10×20 cm, length × width × depth) were randomly collected from ten subplots in each plot by a shovel. To minimize the spatial community heterogeneity and improve the precision

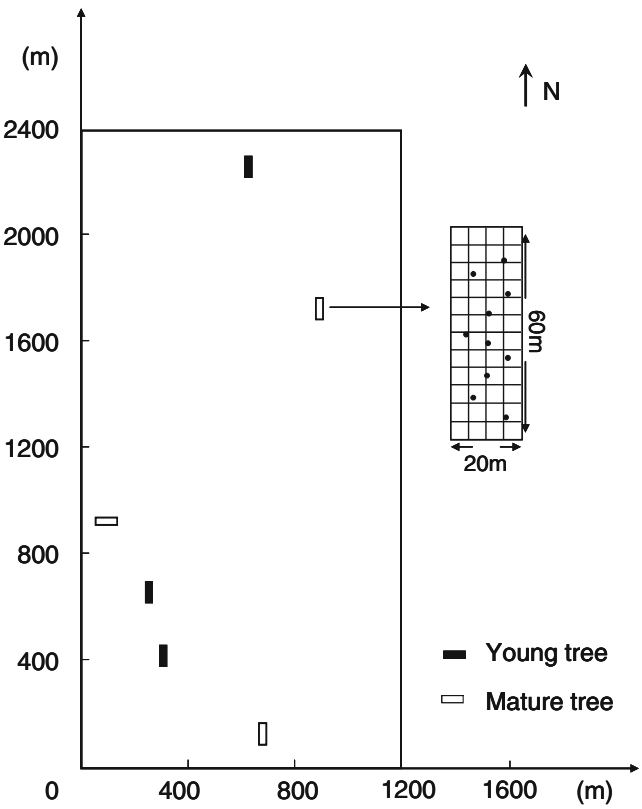

Fig. 1 Distribution of sampling plots in the study site. Roots of seedlings were collected from the whole site due to no enough seedlings in the plots

of temporal community dynamics, soil samples were collected from the same subplots in the four sampling dates. Due to the lack of available seedlings in each plot, only a total of 18 root samples of *Q. liaotungensis* seedlings were collected by tracing roots from seedling stems in the whole study site on each sampling date. Samples were placed in plastic bags and stored at 4°C. A total of 312 (78 samples × 4 sampling dates) samples were collected in this study.

Root samples were washed free of soils over a 380-μm sieve in running tap water. Fine roots (<2 mm diam.) of *Q. liaotungensis* were manually selected and trimmed into ca. 1-cm-long fragments. Fifty fine root fragments from each sample were randomly selected for ECM examination under a SMZ-B2 stereomicroscope (Chongqing Optec Optical Instrument Co., Ltd., Chongqing, China). Live ECM root tips were divided into different morphotypes based on general appearance, such as color, luster, size, ramification type and texture, as well as the presence and color of emanating hyphae and rhizomorphs. For each morphotype, three root tips as one ECM sample were placed in a 1.5-ml microcentrifuge tube and stored in −20°C for DNA extraction. Three ECM samples of each morphotype were selected for DNA extraction, PCR, and RFLP analysis.

DNA extraction, PCR and ITS-RFLP analysis

The DNA was extracted according to protocol of Gardes and Bruns (1993). The ITS region (ITS1, 5.8S, ITS2) of rDNA from each ECM morphotype was amplified by PCR using the primer pairs ITS1-F/ITS-4, ITS1-F/ITS-4B or ITS1-F/ITS-4A, depending on their preceding success of the samples (White et al. 1990; Gardes and Bruns 1993) in a PTC 100TM Programable Thermal Controller (MJ Research, USA). The final 50 μl reaction mixture contained 1 μl template DNA, 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 15 pmol of each primer, and 2.5 U *Taq* polymerase (TransGen Biotech, Beijing, China). The amplification was programmed for a denaturation at 95°C for 5 min, followed by 35 cycles of denaturation for 40 s at 94°C, annealing for 50 s at 50°C, extension for 1 min at 72°C, and a final 10 min extension at 72°C. A negative control using sterile Milli-Q water instead of template DNA was included in the amplification process.

Ten microlitres of each PCR product was combined with 7.5 μl sterile Milli-Q water, 0.5 μl restriction endonuclease (either *Hin*fI, *Bsu*RI, *Hha*I, or *Alu*I; MBI Fermentas, Lithuania), 2 μl buffer and overnight at 37°C. ITS-RFLP products were size-fractionated on 2% agarose gels. The gels were stained with ethidium bromide and photographed by AlphaImager™ 2200 (Alpha Innotech Corporation, USA) under UV light. ITS-RFLP band sizes were estimated by comparison to a standard 100-bp molecular weight ladder. The fragment length error was ±3% as suggested by Glen et al. (2001). Molecular identifications were repeated at least once for each morphotype sample. Morphotypes with the same ITS-RFLP patterns were considered to be formed by the same fungus species.

DNA sequencing and identification

The PCR products were purified using the UNIQ-10 PCR production purification kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, Shanghai, China) according to the manufacturer's instruction. One representative PCR product of each ITS-RFLP pattern was sequenced using an ABI Prism 3700 Genetic Analyzer (Applied Biosystems, USA). In order to check the most likely chimera breakpoints, all ITS sequences were analyzed by the Chimera Check program of the RDP version 2.7 (Maidak et al. 1999).

A value of 97% ITS region identity was used as a DNA barcoding threshold for ECM fungal taxa (Tedersoo et al. 2008). This cut-off level is based on error rates generated by PCR, sequencing and inter-specific variability within ITS regions as employed in previous studies using ITS sequences for ECM fungal identification from roots and soils.

The ITS sequences generated in this study were used as query sequences to search for similar sequences in NCBI and UNITE (Kõljalg et al. 2005) to provide at least tentative identification for the ECM fungi. To assign names to operational taxonomic unit (OTU) we used a combination of the BLAST and phylogenetic analysis. ITS sequences of OTUs with high similarity (>96%) to vouchered specimens were assigned to a genus. If the ITS sequences of OTUs showed poor similarity (<96%) with reference sequences in NCBI and UNITE, a genus or family name was assigned to the OTU based on a combination of similarity and phylogeny (Southworth et al. 2009).

Data analysis

Frequency was the number of samples in which a species occurs divided by the total number of samples. Relative frequency was the frequency of a species divided by the sum of frequencies of all species (Gardes and Bruns 1996). The ECM fungi were considered as frequent species when they were detected in more than 5% soil cores. Colonization rate was the number of root tips colonized by ECM fungi divided by the total number of root tips detected in a sample.

Species rarefaction curves were constructed by pooling the soil samples from each age class in a single sampling time and were then drawn by plotting the mean of the accumulated number of expected species in pooled samples after 1,000 randomizations without replacement, using EstimateS version 8.0 (Colwell 2006). Estimated measurements of ECM fungal species richness in each sampling time were

Table 1 Identified ECM species on root tips of *Quercus liaotungensis* based on BlastN against the NCBI and UNITE

Morphotype	ECM fungus	GenBank accession No.	Closest blast match reference taxa (GenBank accession No.)	Query/reference ITS length (bp) (similarity %)
ECM1	*Boletus* sp.	HM105532	*Boletus erythropus* (AJ419188)	606/593 (93.6)
ECM2	*Cenococcum geophilum*	HM105500	*Cenococcum geophilum* (AM084698)	445/445 (99)
ECM3	*Clavulina* sp.	HM105511	*Clavulina* cf. *rugosa* (DQ974712)	610/615 (95)
ECM4	Clavulinaceae sp.	HM105503	Clavulinaceae sp. (GQ254856)	612/607 (93.1)
ECM5	*Cortinarius rubricosus*	HM105542	*Cortinarius rubricosus* (AY669673)	517/516 (99.2)
ECM6	*Cortinarius* sp. 1	HM105543	*Cortinarius cotoneus* (AY669597)	608/607 (97.7)
ECM7	*Cortinarius* sp. 2	HM105538	*Cortinarius* sp. (EU819467)	604/604 (96)
ECM8	*Cortinarius* sp. 3	HM105541	*Cortinarius* cf. *anomalus* (FJ039605)	613/601 (93.4)
ECM9	Helvellaceae sp.	HM105544	*Helvella dovrensis* (AF046223)	672/744 (73.8)
ECM10	*Humaria* sp. 1	HM105553	*Humaria* sp. (EU024878)	604/606 (97.7)
ECM11	*Humaria* sp. 2	HM105554	*Humaria* sp. (EU024873)	612/618 (91.4)
ECM12	*Hygrophorus* sp.	HM105505	*Hygrophorus cossus* (AY242852)	515/513 (85)
ECM13	*Hymenogaster* sp.	HM105539	*Hymenogaster arenarius* (DQ328124)	592/596(96.8)
ECM14	*Inocybe pseudoreducta*	HM105515	*Inocybe pseudoreducta* (EF644109)	635/634 (98.9)
ECM15	*Inocybe umbrinella*	HM105563	*Inocybe umbrinella* (FJ904165)	613/612 (99.7)
ECM16	*Inocybe* sp. 1	HM105504	*Inocybe* cf. *soriora* (EU819474)	618/619 (93.9)
ECM17	*Inocybe* sp. 2	HM105514	*Inocybe pseudoreducta* (EF644109)	638/634 (93.4)
ECM18	*Inocybe* sp. 3	HM105506	*Inocybe* sp. (FN669217)	552/530 (91.2)
ECM19	*Melanogaster* sp.	HM105535	*Melanogaster variegates* (AJ555534)	687/673 (92.8)
ECM20	*Pachyphloeus* sp.	HM105546	*Pachyphloeus thysellii* (EU543197)	576/576 (96.7)
ECM21	*Peziza* sp. 1	HM105501	*Peziza infossa* (DQ974817)	556/548 (96.7)
ECM22	*Peziza* sp. 2	HM105556	*Peziza ostracoderma* (EU819461)	551/555 (96.3)
ECM23	*Peziza* sp. 3	HM105547	*Peziza michelii* (DQ200839)	571/566 (90.6)
ECM24	Pyronemataceae sp.	HM105520	*Genabea cerebriformis* (AY920530)	547/555 (72.4)
ECM25	*Russula acrifolia*	HM105525	*Russula acrifolia* (DQ421998)	586/585 (99.1)
ECM26	*Russula* sp. 1	HM105560	*Russula risigallina* (DQ422022)	613/613 (96.5)
ECM27	*Russula* sp. 2	HM105526	*Russula pectinatoides* (EU819493)	609/609 (98)
ECM28	*Russula* sp. 3	HM105517	*Russula insignis* (AY061700)	602/602 (98.2)
ECM29	*Russula* sp. 4	HM105533	*Russula* sp. (AF495464)	631/628 (97.7)
ECM30	*Russula* sp. 5	HM105545	*Russula cascadensis* (EU526006)	575/576 (97.6)
ECM31	*Russula* sp. 6	HM105502	*Russula* sp. (GU134512)	593/599 (97.3)
ECM32	*Scleroderma* sp.	HM105510	*Scleroderma* sp. (EF644144)	650/650 (99.4)
ECM33	*Sebacina* sp. 1	HM105523	*Sebacina* sp. (AF440649)	529/529 (99.2)
ECM34	*Sebacina* sp. 2	HM105548	*Sebacina* sp. (EU668271)	540/538 (98.1)
ECM35	*Sebacina* sp. 3	HM105522	*Sebacina* sp. (GU134519)	536/537 (97.9)
ECM36	*Sebacina* sp. 4	HM105558	*Sebacina* aff. *epigaea* (EU819519)	534/535 (97.7)
ECM37	*Sebacina* sp. 5	HM105518	*Sebacina* sp. (EU668224)	539/539 (97.5)
ECM38	*Sebacina* sp. 6	HM105529	*Sebacina* sp. (EU668273)	529/532 (96.9)
ECM39	*Sebacina* sp. 7	HM105516	*Sebacina incrustans* (EU326155)	522/524 (96.8)
ECM40	*Sebacina* sp. 8	HM105512	*Sebacina* sp. (AF440664)	525/527 (95.2)
ECM41	*Sebacina* sp. 9	HM105524	*Sebacina* sp. (GU256183)	531/530 (94.4)
ECM42	*Sebacina* sp. 10	HM105507	*Sebacina* sp. (AF440644)	529/527 (94.2)
ECM43	*Sebacina* sp. 11	HM105521	*Sebacina* sp. (GQ219874)	531/528 (91.9)
ECM44	*Sebacina* sp. 12	HM105519	*Sebacina* sp. (FJ792845)	537/530 (92.7)
ECM45	*Tarzetta* sp. 1	HM105530	Uncultured *Tarzetta* (HM370482)	473/489 (86)
ECM46	*Tarzetta* sp. 2	HM105534	*Tarzetta catinus* (FM206478)	473/487 (82)
ECM47	*Tarzetta* sp. 3	HM105549	Uncultured *Tarzetta* (EU326167)	521/488 (85)
ECM48	*Tomentella* sp. 1	HM105559	*Tomentella* sp. (AY635173)	578/579 (98.9)

Table 1 (continued)

Morphotype	ECM fungus	GenBank accession No.	Closest blast match reference taxa (GenBank accession No.)	Query/reference ITS length (bp) (similarity %)
ECM49	*Tomentella* sp. 2	HM105555	*Tomentella* sp. (EU668200)	576/576 (98.7)
ECM50	*Tomentella* sp. 3	HM105551	*Tomentella* sp. (EU625876)	572/572 (98.6)
ECM51	*Tomentella* sp. 4	HM105528	*Tomentella* sp. (AY299218)	576/578 (98.2)
ECM52	*Tomentella* sp. 5	HM105536	*Tomentella* sp. (EU668206)	578/578 (98)
ECM53	*Tomentella* sp. 6	HM105565	*Tomentella* sp. (EU668203)	583/584 (97.4)
ECM54	*Tomentella* sp. 7	HM105561	*Tomentella* sp. (GQ469531)	581/581 (97.2)
ECM55	*Tomentella* sp. 8	HM105509	*Tomentella* sp. (GQ223454)	584/584 (96.9)
ECM56	*Tomentella* sp. 9	HM105564	*Tomentella* sp. (EU526855)	580/580 (96.8)
ECM57	*Tomentella* sp. 10	HM105552	*Tomentella* sp. (GQ240906)	578/576 (96.1)
ECM58	*Tomentella* sp. 11	HM105527	*Tomentella* sp. (DQ974780)	577/577 (96)
ECM59	*Tomentella* sp. 12	HM105550	*Tomentella* sp. (AJ534913)	567/568 (95.6)
ECM60	*Tomentella* sp. 13	HM105531	*Tomentella* sp. (EU668203)	580/584 (95)
ECM61	*Tomentella* sp. 14	HM105562	*Tomentella* sp. (FJ210775)	577/579 (94.3)
ECM62	*Tomentella* sp. 15	HM105557	*Tomentella* sp. (U92537)	584/581 (91.6)
ECM63	*Tomentella* sp. 16	HM105513	*Tomentella* sp. (U92537)	580/581 (90.4)
ECM64	*Tuber liaotongense*	HM105540	*Tuber liaotongense* (GU979037)	476/476 (99.8)
ECM65	*Tuber taiyuanense*	HM105508	*Tuber taiyuanense* (GU979033)	523/523 (99.8)
ECM66	*Tuber* sp.	HM105537	*Tuber* sp. (GU722194)	566/566 (99.1)

calculated by Chao2 and Jackknife2 (Jack2) estimators using the EstimateS version 8.0.

Multiple response permutation procedure (MRPP) is a nonparametric, multivariate procedure that tests the null hypothesis of no difference between groups. The MRPP was calculated based on the Sorensen coefficient using PC-ORD multivariate analysis of ecological data version 3.0 for windows (McCune and Medfford 1997). We used the MRPP to test whether there was significant difference in ECM fungal species composition between June and September and between young and mature trees. Because there were not enough seedlings in each plot, we collected seedling samples from the whole study site (within and out of plots). Therefore, the seedling samples were not from the true replicates and thus excluded in the MRPP analysis.

Results

ECM fungal colonization and diversity

Similar mean percentages of root ECM colonization were observed among the three age classes of *Q. liaotungensis* in the two growing seasons, i.e., 89.3% and 84% in seedlings, 91.3% and 90.2% in young trees, and 92.9% and 92.7% in mature trees in June and September, respectively.

A total of 178 ECM morphotypes were recovered from 97,265 ECM root tips in all 312 samples according to ECM morphological characteristics. Three root samples from each of the 178 ECM morphotypes displayed the same ITS-RFLP pattern. A total of 66 unique ITS-RFLP patterns were obtained from 534 samples (178 ECM morphotypes × 3 samples) based on the analyses of four restriction enzymes: *Hin*fI, *Bsu*RI, *Hha*I, and *Alu*I.

A total of 66 ECM fungal taxa, including 51 Basidiomycetes (77.3%) and 15 Ascomycetes (22.7%), were identified based on the similarity comparison of ITS sequences with references downloaded from GenBank and UNITE (Table 1). Among the ECM fungi, seven were identified to species level (*Cenococcum geophilum*, *Cortinarius rubricosus*, *Inocybe pseudoreducta*, *I. umbrinella*, *Russula acrifolia*, *Tuber liaotongense*, and *T. taiyuanense*), 56 to generic level, and three to family level.

One to 16 (mean 5.4) ECM fungal taxa were found per sample in the four sampling dates. The species rarefaction curves showed an overlap of the 95% confidence intervals among the mature trees, young trees and seedlings in these four sampling dates, except between the young and mature trees in June 2009. Although a high diversity of ECM fungal species were obtained from the roots of *Q. liaotungensis*, the species accumulative curves of ECM fungi observed not level off in the four sampling dates (Fig. 2).

ECM fungal community composition

In the ECM community of *Q. liaotungensis*, *C. geophilum* was the dominant species, occupying 80.4% of the total soil samples and 20.4% of the total ECM root tips. The

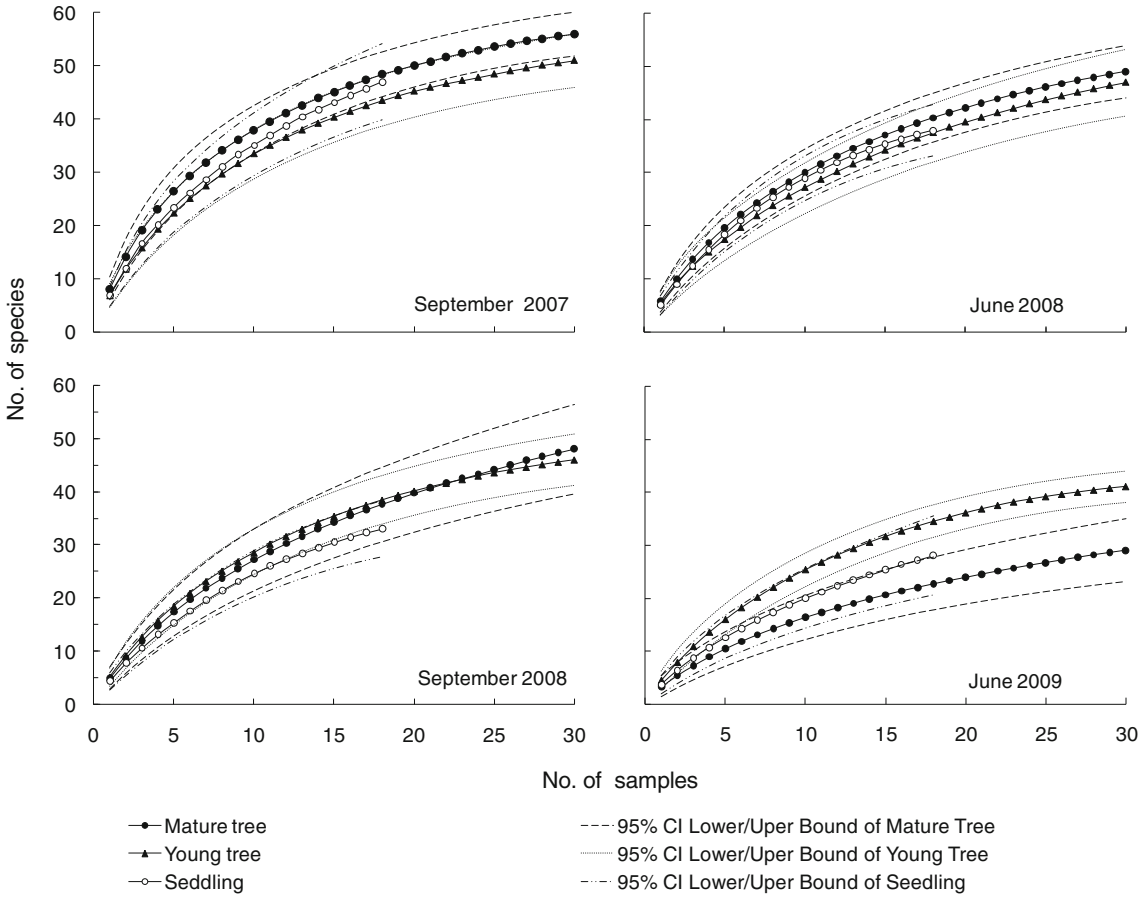

Fig. 2 Species rarefaction curves for ectomycorrhizal fungi on roots of *Quercus liaotungensis* of the seedlings, young trees and mature trees in the four sampling times

following five most frequent taxa, *Sebacina* sp. 3, *Humaria* sp. 1, *Russula* sp. 2, *Tuber taiyuanense* and *Tomentella* sp. 10, accounted for 32.1%, 28.8%, 25.6%, 23.4% and 23.1% of the total soil samples and 7.8%, 3.7%, 4.5%, 4% and 5.7% of the total ECM root tips, respectively. Another 33 taxa were rare (<5% of the total soil samples). Thelephoraceae (16 taxa), Sebacinaceae (12 taxa) and Russulaceae (seven taxa) were the most species-rich and abundant ECM fungi, accounting for 19.5%, 17.6% and 8.3% of the total ECM root tips, respectively.

Among the 66 ECM taxa, 59 were from seedlings, 64 from both young and mature trees, and 57 taxa (86.4%) concurrently appeared in the three age classes. Meanwhile, 63 out of the 66 taxa were from June, 66 from September, and 63 (95.5%) occurred in both growing seasons. Moreover, all 33 frequent ECM taxa were detected in both the three age classes and two growing seasons, except that *Russula* sp. 6 was not detected on the seedling roots (Fig. 3). Furthermore, almost all frequent ECM fungi had similar relative frequencies in both the three age classes and two growing seasons, although some fungal taxa showed a small variation in relative frequency.

Results of MRPP analysis indicated marginally significant effects of tree ages (A=0.01801, P=0.05) and growing seasons (A=0.01908, P=0.06) on the ECM fungal species composition.

Discussion

ECM fungal colonization, diversity and community structure

Our results indicated that the ECM colonization rates in *Q. liaotungensis* roots were similar (84–92.9%) among the three tree ages and sampling seasons of June and September. By contrast, ECM colonization was 15% in seedlings, while significantly higher (>95%) in older trees *Q. rubra* (Gebhardt et al. 2007).

ECM fungal diversity was similar among the three tree age classes of *Q. liaotungensis* in June and September in our study. Similarly, Smith et al. (2002) found no difference in cumulative species richness of ECM fungal sporocarps

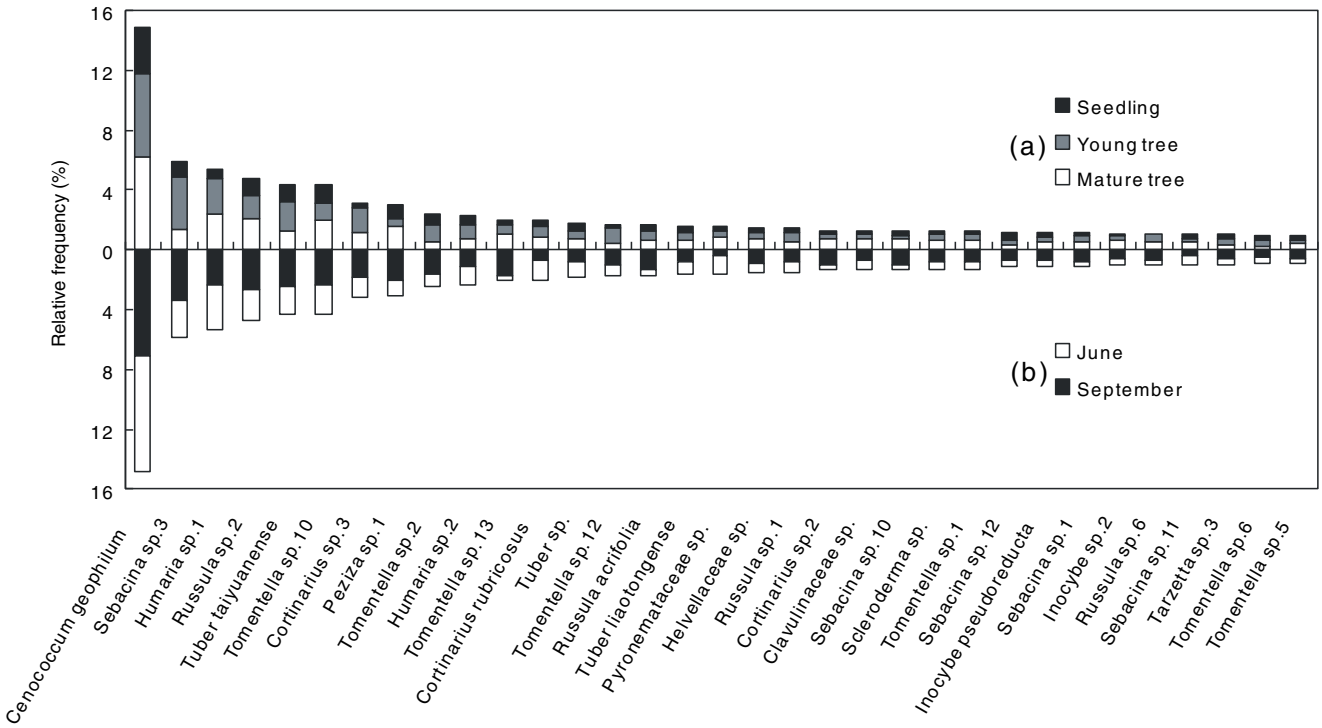

Fig. 3 Relative frequencies of the frequent ECM species on roots of *Q. liaotungensis* in the three age classes (**a**) and two growing seasons (**b**)

among the three age classes of *Pseudotsuga menziesii* in the Cascade Range of Oregon, USA. However, other studies show that the ECM fungal diversity was much lower in young than in old stands observed by the above-ground sporocarps (Nara et al. 2003) and below-ground roots in Douglas fir, oak, paper birch, spruce, and dwarf willow (Palfner et al. 2005; Nara 2006; Gebhardt et al. 2007; Twieg et al. 2007). Smith et al. (2007) found that the ECM fungal diversity was similar between winter and spring in a California *Q. douglasii* woodland, while Jumpponen et al. (2010) noted a decline in fungal diversity in the course of the growing season (May, June, and September) in a Kansas *Quercus* spp. woodland. Although our species rarefaction curves showed that the 95% confidence intervals did not overlap between the young and mature trees in June 2009, there was an overlap of 95% confidence intervals among the mature trees, young trees and seedlings in the other three sampling dates between 2007 and 2008. This difference may be caused by the inter-year change of environmental factors. Therefore, in order to completely understand the ECM community structure of *Q. liaotungensis*, the investigation should examine more years in future studies.

ECM fungal community consisted of a few most frequent ECM fungi (six taxa) and numerous rare taxa (33) in our study. This pattern is common for most ECM communities (Horton and Bruns 2001; Taylor 2002; Smith et al. 2002; Walker et al. 2008; Wang and Guo 2010; Wang et al. 2011). Thelephoraceae, Sebacinaceae and Russulaceae were the most species-rich and abundant in the ECM fungal community of *Q. liaotungensis*. Similar results have been reported in other oak forests (Smith et al. 2007; Courty et al. 2008; Walker et al. 2008; Jumpponen et al. 2010). For example, Morris et al. (2009) investigated the ECM community composition of *Q. crassifolia* Humb. & Bonpl and *Q. laurina* Bonpl. in a mixed forest of southern Mexico and found that Thelephoroid (38 taxa), Russuloid (21 taxa) and Sebacinoid (17 taxa) fungi were frequent, occupying 24.7%, 13.6% and 11.0% of the total number of ECM fungi, respectively. The other ECM fungi detected in this study have been reported to form typical ECM with *Q. crassifolia* in southern Mexico (Morris et al. 2009), *Q. petraea* in Poland (Kwasna et al. 2008), and *Q. agrifolia*, *Q. Douglasii*, *Q. Garryana*, *Q. prinus*, *Q. rubra* and *Quercus* spp. in the USA (Valentine et al. 2004; Walker et al. 2005, 2008; He et al. 2006; Smith et al. 2007; Moser et al. 2009; Southworth et al. 2009; Hynes et al. 2010; Jumpponen et al. 2010).

ECM community pattern in different host ages and growing seasons

Our results indicated that tree age and growing season had marginally significant effects on the ECM fungal community composition of *Q. liaotungensis*. Approximately 86.4% of the total fungal taxa were found in the seedlings, young trees, and mature trees. Particularly, 32 out of the 33 frequent ECM fungal taxa were detected among the three age

classes. Our findings indicated that oak seedlings in natural forests are associated with a wide assemblage of ECM fungi as they are with the mature oak trees. The high diversity of mycorrhizal fungal taxa existing in seedlings in this study also confirms the applicability of using seedlings to effectively document mycobiont diversity in situ (Walker et al. 2008). In addition, the similar ECM fungal composition between seedlings and mature trees has important implications for the potential of seedlings to acquire carbon and/or nitrogen from mature canopy trees through common mycelial networks (Simard et al. 1997; He et al. 2006). If seedling mycobiont diversity was low in a forest ecosystem, the potential for resource sharing would likely be reduced because fewer hyphal networks could be accessed (Walker et al. 2008).

Most ECM fungi (95.5% of total taxa) were found in June and September, and all frequent ECM fungi (33 taxa) were detected in the two growing seasons. Similarly, Smith et al. (2007) found that 92% of the frequent ECM fungal species were detected on ECM roots in a California *Q. douglasii* woodland in winter and spring over 2 years. Walker et al. (2008), however, found no statistical differences between ECM fungal assemblages of *Q. rubra* and *Q. prinus* seedlings by growing season based on the MRPP analysis.

C. geophilum was the dominant species in the three age classes of *Q. liaotungensis* (Fig. 3). Similarly, *C. geophilum* was the most frequent species in the 3-year-old *Q. agrifolia, Q. douglasii* and *Q. garryana* growing in Califonia (He et al. 2007) and in different ages (5, 21, 33, 43, and 46 years old) of *Q. rubra* in reclamation sites of the Lusatian lignite-mining district of East Germany (Gebhardt et al. 2007). *C. geophilum*, as a generalistic mycobiont, has commonly been found in variety of forest ecosystems (Jonsson et al. 1999; Izzo et al. 2005; Walker et al. 2005; Koide et al. 2007), particularly in drought habitats (Pigott 1982; Coleman et al. 1989; Jany et al. 2003). The genus *Quercus* is indeed characterized by high abundance of *C. geophilum* (Jumpponen et al. 2010), such as in *Q. garryana* (Valentine et al. 2004; Moser et al. 2009), *Q. ilex* (Richard et al. 2005), *Q. rubra* and *Q. prinus* (Walker et al. 2005; Gebhardt et al. 2007), and *Q. suber* (Azul et al. 2010).

In our study, oak seedlings are associated with ECM fungi typically considered as late-stage forest inhabitants, such as *Boletus* sp., *Cortinarius* spp., *Russula* spp., and *Tuber* spp. (Chu-Chou 1979; Danielson 1984; Dighton et al. 1986). Oak seedlings of *Q. rubra* and *Q. prinus* were also shown to be colonized by late-stage fungi *Albatrellus* spp., *Amanita* spp., Boletes, *Cortinarius* spp., *Gautieria* spp., *Hydnellum* spp., Russulales, *Tricholoma* spp., and Tuberales (Walker et al. 2005). Late-stage fungi also colonize birch seedlings planted under mature trees in a field (Fleming 1983) and cultivated in a glasshouse (Gibson and Deacon 1988).

Knowledge of ECM fungal communities improves our ability to maintain fungal biological diversity in seedlings, young tress, and mature trees. In summary, we found (1) a relatively high diversity (66 taxa) of ECM fungi associated with *Q. liaotungensis*, (2) a similar number of ECM fungal species among the three age classes and between June and September, and (3) a similar ECM fungal community composition between different host ages and growing season in a temperate forest. However, because we only selected three ECM samples from each morphotype for molecular analysis, some ECM fungal species might have been lost during the procedure of molecular identification. Therefore, the same morphotype in all soil samples should be analyzed by molecular methods in future studies. In addition, we only collected samples in June and September in this study, which represent the beginning and end of the host growth cycle. In order to completely understand the seasonal dynamics of ECM community structure of *Q. liaotungensis*, more sampling times, such as in winter or even between June and September, should be employed in future studies.

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