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ORIGINAL PAPER

Ectomycorrhiza communities of red oak (*Quercus rubra* L.) of different age in the Lusatian lignite mining district, East Germany

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Abstract Ectomycorrhizal (ECM) communities were assessed on a 720 m^2 plot along a chronosequence of red oak (*Quercus rubra*) stands on a forest reclamation site with disturbed soil in the lignite mining area of Lower Lusatia (Brandenburg, Germany). Adjacent to the mining area, a red oak reference stand with undisturbed soil was investigated reflecting mycorrhiza diversity of the intact landscape. Aboveground, sporocarp surveys were carried out during the fruiting season in a 2-week interval in the years 2002 and 2003. Belowground, ECM morphotypes were identified by comparing sequences of the internal transcribed spacer regions from nuclear rDNA with sequences from the GenBank database. Fifteen ECM fungal species were identified as sporocarps and 61 belowground as determined by morphological/anatomical and molecular analysis of their ectomycorrhizas. The number of ECM morphotypes increased with stand age along the chronosequence. However, the number of morphotypes was lower in stands with disturbed soil than with undisturbed soil. All stands showed site-specific ECM communities with low similarity between the chronosequence stands. The dominant ECM species in nearly all stands was *Cenococcum geophilum*, which reached an abundance approaching 80% in the 21-year-old chronosequence stand. Colonization rate of red oak was high (>95%) at all stands besides the youngest chronosequence stand where colonization rate was only 15%. This supports our idea that artificial inoculation with site-adapted mycorrhizal fungi would enhance colonization rate of red oak and thus plant growth and survival in the first years after outplanting.

Keywords Chronosequence · Diversity · Ectomycorrhiza · *Quercus rubra* · Reclamation sites

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Introduction

In temperate forest ecosystems, mycorrhizal communities with remarkable richness levels have evolved over long periods. This richness is coupled with diverse ecological functions such as ecophysiological characteristics across the taxa from nutrient acquisition to pH-tolerance (Mc Afee and Fortin 1986). Trees establishing during primary succession depend on fungal inoculum from neighboring forests (Jumpponen et al. 2002; Ashkannejhad and Horton 2006). Ectomycorrhizal (ECM) fungi have been found to be critical for tree establishment during primary succession of a subalpine glacier forefront (Cázares et al. 2005), coastal sand dunes (Ashkannejhad and Horton 2006), and a volcanic desert on Mount Fuji (Nara 2006).

Studies on primary succession of ECM fungi in post mining landscapes are rare (Schramm 1966; Marx 1975; Münzenberger et al. 2004). Forest reclamation sites, i.e., heavily disturbed sites, differ crucially in their abiotic soil

conditions from temperate forests established on undisturbed sites. The lack of a humus layer, extreme low pH values, nutrient deficiency, high amounts of heavy metals, and presence of coal particles in the soil substrate causing extreme soil temperatures make it difficult to reestablish forest ecosystems on reclamation sites (Tate and Klein 1985). ECM propagules or hyphal networks are few or absent in non-vegetated areas of disturbed sites (Jumpponen et al. 2002; Kottke 2002). With regard to the reafforestation of these landscapes and the functional importance of ECM for tree growth, it is essential to know more about the development of ECM communities under such conditions. The question arises whether the diversity of the mycorrhizal fungal species changes over time leading to a more diverse mycorrhizal community. As mycological studies are rare in post mining landscapes, it is necessary to improve our knowledge about the succession of ECM fungi on reclamation sites. A better understanding of the development and the function of these fungal species would help to use them to improve survival and growth of forest trees in such landscapes.

Afforestation of landscapes in the Lower Lusatia mining district was mostly carried out with indigenous tree species that are typical for this region, but the results were often unsatisfactory (Preussner 1998). Allochthonic red oak (*Quercus rubra* L.) was chosen as a more adequate tree species because this tree grows comparatively fast and has a larger amplitude of site requirements such as frost hardiness, light demand, and requirements for nutrients and water supply compared to indigenous oak (*Quercus petraea* Liebl.; Preussner 1998; Kutschera and Lichtenegger 2002). The main objectives of this study were to characterize the diversity and succession of ECM sporocarps and morphotypes of red oak growing on forest reclamation sites of the Lusatian lignite-mining district, East Germany. For assessment of the disturbance level of the ECM communities from the chronosequence sites, a red oak reference site on undisturbed soil was investigated in addition.

Materials and methods

Site description

The study was conducted in the lignite open-cast mining district of Lusatia in Northeastern Germany 70 m above mean sea level. This mining area is still active, and produced brown coal is fired in power plants of the region to supply the European Power Net and Berlin with energy. This region is the largest recultivation area of Europe, as almost 1,000 km^2 of land is devasted as a result of mining activity (Hüttl and Weber 2001). Due to the special mining procedure (conveyor bridge technology), a mixture of quaternary and tertiary sandy sediments form the new substrates of the post mining landscapes (Knoche et al. 2002). These substrates lack organic matter, are extremely poor in nutrients, and are strongly acidified by spoil dumps with high saline concentrations (Schaaf et al. 1999; Hüttl and Weber 2001). The acid soil reaction is a result from the oxidation of pyrite, a characteristic mineral of tertiary sediments. To compensate the acidic potential of this mineral, extensive amounts of lime or alkaline ashes from lignite-fired power plants have been applied generally to the top 30 cm of the soil before afforestation (Gast et al. 2001). To improve the poor nutrient amounts of these substrates, mineral fertilizers have usually been used (Katzur and Haubold-Rosar 1996).

Extended drought periods during the growing season with mean annual precipitation of 560 mm and an average annual temperature of 8.5°C are typical for the regional continental climate (Groer 1998). The drought combined with the low field capacity of the mining substrates constitutes a further problem for achieving successful afforestation (Häge 1996; Hangen et al. 2004). Red oak has been favored for afforestation of reclamation sites in the last decades, as it is more suitable to compensate phytotoxic conditions such as low pH values than indigenous oak species (Stratmann 1985). To date, about 15% of the Lusatian mining district has been afforested with this allochthonic tree species (Preussner 1998).

In this study, a chronosequence consisting of four red oak stands on reclamation sites was investigated. Tree ages of the four red oak stands were 5 years (51°46′N, 13°44′E), 21 years (51°49′N, 13°51′E), 33 years (51°32′, 13°45′E), and 43 years (51°48′N, 13°32′E). In the oldest chronosequence stand, some individuals of *Tilia cordata* Mill. were intermingled. A 46-year-old red oak stand (51°48′N, 13°31′E) established in the intact landscape adjacent to the mining area was chosen as a naturally grown reference forest site. Site characteristics such as number of trunks, soil type, humus type, and soil chemistry are summarized in Table 1.

Experimental design

Sampling of morphotypes and mycorrhiza parameters

With the exception of the youngest plantation, an area of 720 m^2 was selected on each oak stand and sub-divided into eight plots. Each plot was 6×15 m in size. Samples were taken at a distance of 3-m width and 6-m length between the sampling points. Sampling was carried out on each plot within a 3-month interval in June 2002, October 2002, January 2003, and May 2003. At each sampling date, eight soil cores were collected with a soil corer (5 cm in diameter) from each oak stand. Each soil core was 15 cm in depth. Provided that the organic layer was present, the soil cores were divided into humus horizon and mineral soil

Table 1 Site characteristics (mean values with standard deviation)

	Chronosequence stands				Reference stand
Stand age (year)	5	21	33	43	46
Tree species	*Q. rubra*	*Q. rubra*	*Q. rubra*	*Q. rubra*	*Q. rubra*
Number of trunks ha^{-1}	4,838	5,000	3,663	1,724	2,000
Substrate	Tertiary carboniferous, pyritic loamy sand	Quaternary carboniferous, pyritic pure sand	Quaternary carboniferous, pyritic loamy sand	Quaternary carboniferous, pyritic loamy sand	Pure sand
Soil type	Dystric regosol	Dystric regosol	Dystric regosol	Dystric regosol	Podsolic cambisol
Humus type	–	–	Moder	Moder	Typical moder
Thickness of the organic horizon (cm)	0.0±0.0	2.0±1.2	3.0±0.7	4.7±1.7	8.6±1.7
pH (H_2O)					
Organic horizon	–	3.7	3.6	5.2	3.1
Mineral soil layer	6.5	3.5	3.1	5.2	3.2
Electric conductivity ($\mu S \cdot cm^{-2}$)					
Organic horizon	–	69.7±40.0	71.9±31.2	82.1±44.9	75.1±25.9
Mineral soil layer	105.1±79.1	56.6±15.7	64.9±15.5	144.8±83.3	66.5±13.3
Phosphate ($mg\ kg^{-1}$)					
Organic horizon	–	13.0±10.2	10.9±6.1	12.7±5.6	17.9±9.1
Mineral soil layer	10.7±16.6	0.8±1.8	05.2±6.8	06.0±4.0	02.0±2.0
Nitrate ($mg\ kg^{-1}$)					
Organic horizon	–	0.65±0.58	0.61±0.55	7.07±4.68	0.71±0.70
Mineral soil layer	0.83±1.81	0.21±0.26	0.25±0.24	1.10±0.91	0.28±0.34
Ammonium ($mg \cdot kg^{-1}$)					
Organic horizon	–	9.23±5.32	13.82±7.56	17.57±5.83	21.38±12.07
Mineral soil layer	0.99±0.37	1.71±0.68	02.72±2.14	02.02±0.70	2.65±1.06
N_t (%)					
Organic horizon	–	0.18±0.10	0.44±0.23	0.94±0.29	0.88±0.29
Mineral soil layer	0.02±0.01	0.02±0.02	0.08±0.05	0.16±0.04	0.10±0.07
C_t (%)					
Organic horizon	–	4.4±2.7	11.0±5.0	20.5±5.6	21.4±7.5
Mineral soil layer	4.4±0.4	0.7±0.4	03.6±2.6	06.2±2.2	03.0±2.4
S_t (%)					
Organic horizon	–	0.04±0.03	0.14±0.05	00.2±0.07	0.16±0.07
Mineral soil layer	0.04±0.02	0.02±0.01	0.09±0.06	0.11±0.04	0.02±0.02

layer, and the soil volume was determined. On the youngest plantation, eight seedlings were chosen randomly, and soil cores (10 cm in diameter) were taken in the same interval as for the other oak stands. Preliminary sampling has shown that the harvest of eight soil cores is the maximum number of samples that can be handled on these reclamation sites. This is due to the extensive root system of deciduous trees of temperate forests that is comprised of numerous, very fine root tips (Rumberger et al. 2004).

Before removing the roots, each soil core was soaked in tap water for 1 h. Subsequently, the roots were gently cleaned under running tap water. Root tips were counted under a dissection microscope (Olympus SZH 10), and fine root density (fine roots, 0–1 mm per 100 cm^3 soil) was determined. Mycorrhizal colonization rate (number of mycorrhiza per total number of root tips) was ascertained. Abundance of a morphotype was defined as the number of mycorrhizal tips per total number of root tips present in all samples of each oak stand. Additionally, frequency of a morphotype, defined as presence per number of soil cores (n=32) of each oak stand, was calculated. Already described morphotypes were identified according to the keys of Agerer (1987–2006) and the online key DEEMY (http://www.deemy.de). Morphology of morphotypes such as color, luster, branching, and texture as well as the presence and color of emanating hyphae and rhizomorphs were investigated. Anatomy of morphotypes was studied on freshly prepared hand sections of hyphal mantle tissue using a Normarski differential interference contrast microscope (Olympus BX 50). For molecular identification, analyses of the fragments of the internal transcribed spacer (ITS) regions from nuclear rDNA were performed using approximately ten mycorrhizal tips per morphotype for DNA extraction. This relatively high number of root tips was necessary, as oak roots contain high amounts of polyphenols that interfere with the extraction. Type richness (number of morphotypes), diversity, and evenness were calculated.

Sampling of sporocarps

Sporocarp surveys were conducted for two consecutive fruiting seasons of the years 2002/2003 in the eight subsampling plots of each oak stand. The surveys were carried out in a 2-week interval (in the youngest stand in a 4-week interval) from September until November until the first frost period. Sporocarps that could not be identified in the field were collected for morphological and anatomical identification.

Molecular analysis

For molecular analysis, ten mycorrhizal root tips of the same morphotype were stored in 2 ml tubes containing 1.5 ml of CTAB (hexadecyltrimethylammoniumbromide)-buffer. One hundred milliliters of the CTAB-buffer consisted of the following chemicals: 3.00 g, 3 *w*/*v* CTAB; 8.18 g, 1.4 M NaCl; 1.21 g, 100 mM Tris (trishydroxymethylammoniumethan); 4.00 ml, 20 mM EDTA; and 1 g, 1 *w*/*v* PVP (polyvinylpyrrolidone). Consecutively, the tubes were shaken over night. After shaking, the root tips were collected from the tubes and placed in a new tube containing a tungsten carbide ball, 100 µl of the AP1 buffer of Qiagen DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) and 10 mg PVP. The mycorrhizal root tips were shredded, and the solution was transferred to a new tube where the DNA was extracted following the Qiagen DNeasy instruction manual.

PCR amplification was performed using the Qiagen PCR Kit (Qiagen GmbH) and the primer pair ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) synthesized by TIB MOLBIOL (Berlin, Germany). The amplification program was: initial denaturation at 94°C for 3 min followed by 35 cycles of 30 s at 94°C, 45 s at 53.8°C and 60 s at 72°C, and a final hold of 5 min at 72°C. The quantity and quality of PCR products were checked by gel electrophoresis. For purification, PCR products were run on 1.8% agarose gel for 60 min, gel bands were cut out and purified with the QIAquick gel extraction kit (Qiagen GmbH). Each DNA fragment was sequenced using the primer pair ITS1F and ITS4. The sequencing program started with denaturation at 96°C for 6 min followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The sequence products were purified by ethanol precipitation. Sequencing was performed with an ABI Prism 310 genetic analyzer (Applied Biosystems, Darmstadt, Germany).

DNA sequences were aligned pairwise using the Seqman module of the program DNASTAR (DNASTAR, Madison, USA). Consensus sequences were compared with sequences from the GenBank database (National Center for Biotechnology Informations) using the blast algorithm (Altschul et al. 1997). Following the recommendations of Tedersoo et al. (2003) and Ashkannejhad and Horton (2006), ITS sequences sharing more than 97% identity to a sequence from a reference species were considered as belonging to this latter species. A morphotype whose ITS sequence did not share any significant homology with a known sequence was named by its sample number (e.g., type 01). When possible, morphological identification was performed to confirm some of the results obtained using molecular analyses.

Statistical analysis

Evenness and the Shannon–Weaver index of diversity were calculated for each ECM community in the different oak stands (Zar 1996). Differences in mycorrhiza frequency

across the different treatments were tested using the Mann–Whitney *U* test (Zöfel 1998). For testing similarity of the investigated oak stands, a hierarchical cluster-analysis was performed according to Ward's method (Ward 1983).

Results

Fine root density and mycorrhizal colonization rate

With the exception of the youngest stand, where the organic horizon was missing, all stands exhibited a higher fine root density in the organic horizon compared to the mineral soil layer (Table 2). Fine root density varied significantly between the five oak stands. The youngest stand was characterized by the lowest fine root density, whereas fine root density was higher in the 21- and 33-year-old chronosequence stand and lower in the 43-year-old stand. Fine root density of the 21- and the 33-year-old stands was nearly twice as high compared to the reference stand, whereas fine root density of the 43-year-old chronosequence stand was well below the one of the reference stand. With the exception of the youngest chronosequence stand, all stands showed high mycorrhizal colonization rates (>95%). In the youngest stand, only 15% of all counted root tips were mycorrhizal.

Sporocarp surveys

Sporocarp surveys were conducted only qualitatively at the investigated stands. Due to the drought in summer and autumn 2003, few sporocarps were observed. In total, ten ECM species were recorded on the oak stands. The highest number of species was found in the 46-year-old *Q. rubra* reference stand. In the chronosequence stands, no sporocarps were present in the youngest stand, and the highest number of species was found in the 21- and 33-year-old stands. In the 43-year-old chronosequence stand, only one species *Scleroderma citrinum* was observed. *Amanita citrina*, *A. muscaria*, *Paxillus involutus*, *Boletus edulis*, and *S. citrinum* were only found as sporocarps (Table 3). In contrast, *Laccaria amethystina* and *Tricholoma sulphureum* were also found belowground as mycorrhizal morphotype (Tables 3 and 4).

Mycorrhizal surveys

Sixty-one ECM types could be distinguished by morphotyping. From these, 12 ECM types occurred only sporadically and were therefore not further determined. They were combined in the group 'combined minor types' (Table 4). Seventy-eight percent of the remaining 49 ECM types were successfully sequenced. Only 12 ECM types could not be amplified. The alignment of 25 of the 49 ECM types with GenBank sequences showed an identification >90%. From these, 14 types could be identified at species level and 5 to genus level (Table 4). Six morphotypes could not be identified at genus or species level.

The most dominant ECM species was *Cenococcum geophilum* (Fig. 1). This fungus was found with both high frequency and high abundance in all oak stands (Table 4). Regarding the chronosequence, the abundance of *C. geophilum* increased from the 5-year-old to the 21-year-old stand (about 80%) and decreased then to the 33- and 43-year-old stands. However, in comparison to the two

Table 2 Fine root density (fine roots per 100 cm^3 soil), diversity index (Shannon–Weaver), and evenness of ECM morphotypes in oak stands of different age

	Fine root density		Diversity index			Evenness		
	Organic horizon	Mineral soil layer	Organic horizon	Mineral soil layer	Total	Organic horizon	Mineral soil layer	Total
Chronosequence								
Q. rubra (5 years)	–	11±8[d]	–	1.366	1.366	–	0.762	0.762
Q. rubra (21 years)	930±883[a,b]	107±95[a]	1.769	1.756	1.833	0.712	0.762	0.695
Q. rubra (33 years)	799±361[b]	53±55[b]	2.746	2.536	2.464	0.990	0.937	0.853
Q. rubra (43 years)	196±147[d]	24±32[c]	2.234	2.243	2.747	0.746	0.762	0.889
Reference								
Q. rubra (46 years)	462±255[c]	114±91[a]	2.853	2.441	2.826	0.876	0.815	0.839

Significant differences in fine root density between stands are indicated by different letters ($p<0.05$, *U* test) and standard deviations

Table 3 Occurrence of sporocarps of ECM species

Species	Chronosequence stands				Reference stand
	5 years	21 years	33 years	43 years	46 years
Amanita muscaria (L.) Pers.		X	X		
Amanita citrina (Schaeff.) Pers.					X
Boletus edulis Bull.: Fr.		X			X
Boletus aestivalis Fr.					X
Laccaria amethystina Cke.					X
Paxillus involutus (Batsch: Fr.) Fr.					X
Russula vesca Fr.			X		
Russula spec. 02					X
Scleroderma citrinum Pers.		X	X	X	X
Tricholoma sulphureum (Bull: Fr.) Krumm.					X

younger stands of the chronosequence, abundance of *C. geophilum* was lower at the red oak reference stand.

Other ECM morphotypes often found were *Boletus aestivalis*, *Tricholoma muricatum* and *Tuber* spec. 01. These morphotypes were frequent in nearly all stands and occurred with the highest abundance in the 21-year-old chronosequence stand. However, mycorrhiza of the morphotypes *Tuber* spec. 01 and Ascomycota-01 showed highest abundance in the youngest chronosequence stand. Other common morphotypes in the older chronosequence stands and the reference stand were *Laccaria amethystina*, *Lactarius chrysorrheus*, and Thelephoraceae-01. Whereas mycorrhiza of *Laccaria amethystina* occurred with the highest abundance in the two oldest chronosequence stands, *Lactarius chrysorrheus* and Thelephoraceae-01 reached only a low abundance in all stands where these fungi appeared. Some of the dominant ECM morphotypes were either only restricted to one or a few stands or appeared only on disturbed or undisturbed soil. For example, morphotypes of *Piloderma croceum* (one of the dominant species), *Cortinarius* spec. 01, *Cortinarius* cf. *paleaceus*, type-14, *Lactarius quietus*, and *Tomentella* spec. 01 were only found at the red oak reference stand with undisturbed soil.

In contrast, mycorrhiza of *Cortinarius* cf. *alboviolaceus* were restricted to the 21-year-old chronosequence stand, the morphotype of *Russula fragilis* occurred with high abundance only in the 33-year-old chronosequence stand, and the morphotypes type-04, type-32, type-09, and Pezizales-02 appeared only in the 43-year-old chronosequence stand. The morphotypes type-27 and type-28 were restricted to the youngest chronosequence stand.

All stands exhibited stand-specific ECM fungal communities with low similarity (Fig. 2, Table 4). The red oak reference stand showed the highest dissimilarity to the chronosequence stands. Along the chronosequence, the similarity of the ECM communities decreased with age of the trees (Fig. 2).

Type richness, diversity, and evenness

The richness of ECM types increased with tree age along the chronosequence. However, in comparison to the red oak stand on undisturbed soil, type richness in the 43-year-old stand of the chronosequence was lower (Fig. 3). In the chronosequence stands, ECM richness differed only marginally between organic horizon and mineral soil layer. Nearly all morphotypes of the organic horizon were also found in the mineral soil layer. In contrast, at the red oak reference stand on undisturbed soil, the number of morphotypes differed significantly between these two horizons. A greater number of ECM types occurred in the organic horizon (Table 4). Similar to type richness, Shannon diversity and evenness increased with tree age along the chronosequence. The ECM richness of red oak at the oldest chronosequence stand showed similarly high Shannon diversity and evenness values as naturally grown red oak from the reference stand (Table 2).

Discussion

It was striking that the chronosequence stands (21 and 33 years) exhibited a relatively high fine root density in comparison to the reference stand. High densities of root tips might be related to reduced thickness of the organic horizon at these reclamation sites. Together with lower nutrient availability, water retention is reduced obviously leading to a stimulation of root extension (Chiatante et al. 1999; Münzenberger et al. 2004). In contrast, fine root density of the oldest chronosequence stand was relatively low. Apparently, the low number of trees and the competition with individuals of basswood (*Tilia cordata*) might have caused this root development at this stand. Naturally grown red oak reached a similarly high fine root density at the reference stand. This supports our assumption

Table 4 Substrate preferences (sub. pref.), abundance, and frequency of the ECM types from the different oak stands

ECM type	Best match (NBCI)		Sub. Pref.	Abundance (%)					Frequency (%)				
	Accession no.	%	O/M[a] (%)	Chronosequence				Reference	Chronosequence				Reference
				5 years	21 years	33 years	43 years	46 years	5 years	21 years	33 years	43 years	46 years
Cenococcum geophilum			86:14	23.7	81.1	41.0	10.3	57.0	12.5	100.0	93.8	73.4	100.0
Boletus aestivalis	AY130295	100	67:33	–	5.3	2.1	0.1	0.3	–	23.4	25.0	1.6	6.3
Ascomycota-01	AJ534696	98	86:14	11.8	–	<0.1	–	3.1	3.1	–	1.6	–	40.6
Lactarius chrysorrheus	AF096983	97	86:14	–	0.4	2.0	–	0.4	–	1.6	10.9	–	3.1
Thelephoraceae-01	AF184742	92	99:1	–	0.1	0.4	0.3	<0.1	–	1.6	4.7	4.7	1.6
Tricholoma muricatum	AF458440	100	87:13	–	6.3	1.9	1.9	1.1	–	23.4	17.2	4.7	1.6
Laccaria amethystina	AY299225	98	98:2	–	–	3.4	7.2	0.9	–	–	17.2	25.0	10.9
Tuber spec. 01	AF458440	99	95:5	–	–	5.6	2.4	0.1	–	–	26.6	17.2	6.3
Type-02			76:24	–	1.2	0.6	3.8	–	–	17.2	20.3	18.8	–
Tricholoma sulphureum	AF377244	99	80:20	–	0.2	–	–	5.2	–	3.1	–	–	10.9
Cortinarius spec. 02	AJ534712	94	100:0	–	0.3	–	–	1.2	–	1.6	–	–	10.9
Type-07			40:60	–	1.1	–	3.2	0.9	–	12.5	–	23.4	10.9
Type-01			98:2	–	–	3.7	–	1.5	–	–	7.8	–	29.7
Russula vesca	AF418610	99	73:27	–	–	1.6	–	0.3	–	–	6.3	–	4.7
Thelephoraceae-02	AF184747	94	77:23	–	<0.1	–	6.5	–	–	1.6	–	6.3	–
Hebeloma velutipes	AF430254	99	57:43	–	1.8	–	–	0.2	–	18.8	–	–	4.7
Type-08			84:16	–	<0.1	–	–	–	–	1.6	–	–	–
Type-03			100:0	–	–	2.6	–	<0.1	–	–	4.7	–	3.1
Cortinarius spec. 03	AJ549968	99	99:1	–	–	20.0	6.7	–	–	–	29.7	28.1	–
Cortinarius spec. 01	AJ534713	92	99:1	–	–	–	–	2.3	–	–	–	–	12.5
Cortinarius cf. *paleaceus*	AJ236078	96	80:20	–	–	–	–	5.2	–	–	–	–	1.6
Lactarius quietus	AJ272247	100	77:23	–	–	–	–	0.9	–	–	–	–	17.2
Cortinarius bolaris	AF389169	97	88:12	–	–	–	–	0.3	–	–	–	–	6.3
Pezizales-01	AJ534699	94	70:30	–	–	–	–	0.8	–	–	–	–	12.5
Piloderma croceum			91:9	–	–	–	–	10.7	–	–	–	–	53.1
Russula spec. 01	AJ534905	99	91:9	–	–	–	–	1.0	–	–	–	–	25.0
Tomentella spec. 01	AJ534912	96	100:0	–	–	–	–	1.1	–	–	–	–	10.9
Tomentella terrestris	AF272901	100	74:26	–	–	–	–	0.5	–	–	–	–	17.2
Type-14			93:7	–	–	–	–	3.6	–	–	–	–	34.4
Type-10			100:0	–	–	–	–	0.3	–	–	–	–	3.1

Table 4 (continued)

ECM type	Best match (NBCI)		Sub. Pref.	Abundance (%)					Frequency (%)				
	Accession no.	%	O/M[a] (%)	Chronosequence				Reference	Chronosequence				Reference
				5 years	21 years	33 years	43 years	46 years	5 years	21 years	33 years	43 years	46 years
Type-13			98:2	–	–	–	–	0.9	–	–	–	–	7.8
Pezizales-02	AJ534701	96	97:3	–	–	–	8.6	–	–	–	–	26.6	–
Russula parazurea	AF418611	98	100:0	–	–	–	2.7	–	–	–	–	18.8	–
Thelephoraceae-04	AF430259	94	95:5	–	–	–	6.0	–	–	–	–	21.9	–
Thelephoraceae-05	U83467	97	98:2	–	–	–	6.8	–	–	–	–	7.8	–
Type-04			93:7	–	–	–	7.6	–	–	–	–	26.6	–
Type-09			96:4	–	–	–	9.4	–	–	–	–	25.0	–
Type-32			92:8	–	–	–	10.8	–	–	–	–	31.3	–
Type-33			91:9	–	–	–	4.1	–	–	–	–	14.1	–
Type-34			88:12	–	–	–	0.9	–	–	–	–	3.1	–
Russula fragilis	AF230897	99	97:3	–	–	9.2	–	–	–	–	51.6	–	–
Thelephoraceae-03	AF272922	100	100:0	–	–	1.0	–	–	–	–	4.7	–	–
Tomentella atramentaria	AF272904	98	99:1	–	–	0.5	–	–	–	–	6.3	–	–
Type-30			86:14	–	–	2.7	–	–	–	–	18.8	–	–
Type-31	AF630020	98	0:100	–	–	1.6	–	–	–	–	10.9	–	–
Cortinarius cf. *alboviolaceus*	AY083178	96	98:2	–	2.1	–	–	–	–	7.8	–	–	–
Type-27			0:100	10.6	–	–	–	–	21.9	–	–	–	–
Type-28			0:100	6.7	–	–	–	–	3.1	–	–	–	–
Type-29			0:100	47.2	–	–	–	–	43.8	–	–	–	–
Combined minor types			–	–	0.1	–	0.7	0.4	–	3.1	–	4.8	12.4

[a] Organic horizon/mineral soil layer

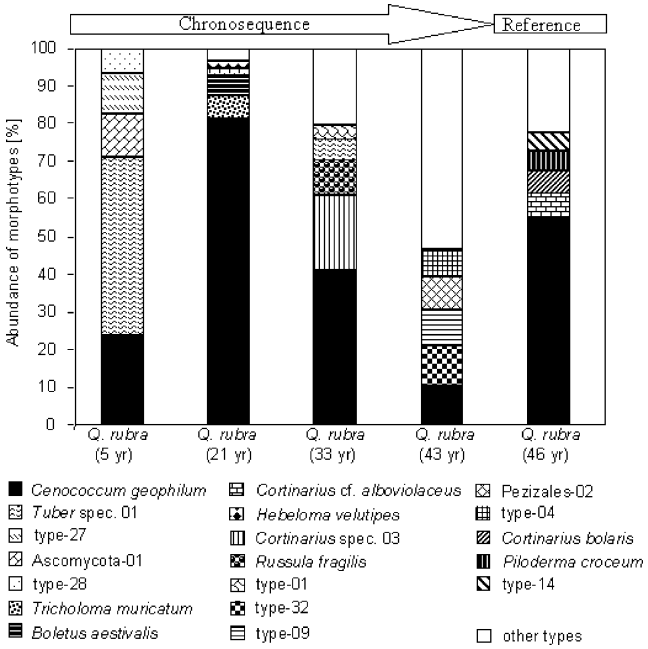

Fig. 1 Abundance of the five most frequent morphotypes from the different oak stands

that root growth of red oak is not hindered by the predominating soil conditions. Overall, fine root densities of the investigated stands are in the range of those found from other European oak stands, i.e., 500–1,500 fine root tips per 100 cm^3 soil (Egli 1980).

With the exception of the youngest chronosequence stand where the mycorrhizal colonization rate was only 15%, all stands exhibited a high colonization rate, which was comparable to those from naturally grown forest trees (Münzenberger et al. 2004; Palfner et al. 2005). The very low colonization rate at the youngest stand might be due to the lack of mycorrhizal propagules in the first years of reclamation (Fox 1986; Bâ et al. 1991). Mycorrhizal fungi that are brought in from seedlings grown in nurseries are mostly not adapted to the extreme soil conditions of the plant hostile reclamation stands (Herrmann et al. 1992). This leads to the assumption that artificial inoculation of forest trees with site-adapted mycorrhizal fungi could enhance the colonization rate and thus improves plant growth and survival in the first years after outplanting.

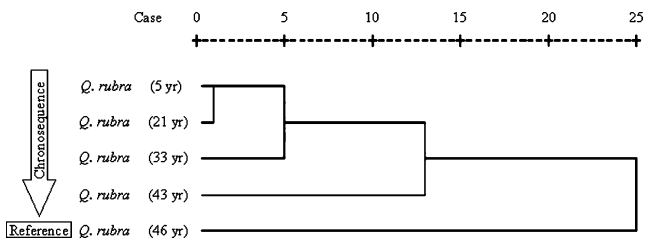

Fig. 2 Similarity between the ECM communities from the different oak stands

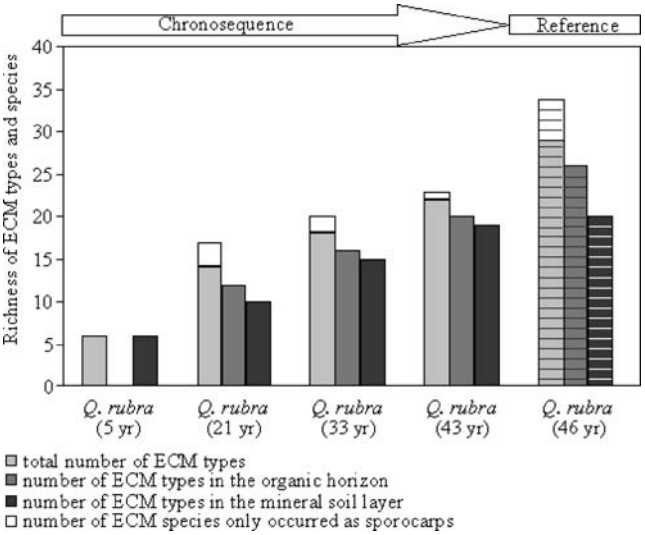

Fig. 3 Number of morphotypes and fungal species from the different oak stands. *Bars with horizontal stripes* show values of the reference stand

The diversity of ECM morphotypes increased with the age of the red oak reclamation stands. This is also well known from primary succession stands from both a forefront of a glacier (Helm et al. 1996; Jumpponen et al. 2002; Cázares et al. 2005) and a vulcano desert (Nara et al. 2003b; Nara 2006). In contrast to our results, Nara et al. (2003a) found an exceptionally large production of sporocarps and an increased number of fungal species present as sporocarps during succession on Mount Fuji. Increasing numbers of different morphotypes were also found during secondary succession, e.g., after wildfire and clear-cut logging (Visser 1995; Ingleby et al. 1998; Jones et al. 2003). An increase in morphotype diversity was also reported from a forest chronosequence (6-, 12-, 30-, and 40-year-old stands) from naturally grown Sitka spruce trees (Palfner et al. 2005) and from a 40- and 400-year-old mixed stand of Douglas fir and western hemlock (Horton et al. 2005). Linking all these results together, time seems to be the most important factor for mycorrhizal diversity.

Similarity between above- and belowground ECM community composition is generally low (Dahlberg 1991; Mehrmann et al. 1995; Gardes and Bruns 1996). ECM species not producing conspicuous reproductive structures, such as *C. geophilum*, have usually been overlooked in sporocarp surveys, although this species may occur in high abundance (Dahlberg et al. 1997). In our survey, sporocarp yield was generally low due to a long drought period from summer until late autumn in 2003. Overall, the sporocarp production in red oak stands of the chronosequence was much lower than on the undisturbed reference stand.

Less than five ECM morphotypes made up more than 50% of the mycorrhizal community in all stands. The majority of ECM morphotypes was recorded from the samples of only a few tips. This high degree of rarity is a

common pattern described for almost all ecosystems and taxonomic groups (Gardes and Bruns 1996; Gehring et al. 1998; Grogan et al. 2000; Peter et al. 2001; Walker et al. 2005). The similarity of ECM community structure was low between the chronosequence stands. Likewise, the ECM community between the chronosequence stands and the reference stand differed considerably. We are aware that soil conditions vary considerably between the chronosequence stands and the reference stand caused by the mining procedure. However, to assess mycorrhizal diversity of red oak within the disturbed landscape, the comparison of red oak ECM diversity within the undisturbed landscape seems appropriate to us. Walker et al. (2005) found a total richness of 75 ECM fungal morphotypes on outplanted seedlings of *Q. robur* and *Q. prinus*, and Moser et al. (2005) detected 74 morphotypes on *Q. garryana*, values lying in the range of our results. Fungal species succession was observed along the studied chronosequence. *Russula* species, *Lactarius quietus*, *Lactarius chrysorrheus*, *Tricholoma sulphureum*, *A. citrina*, *B. edulis*, and *Cortinarius paleaceus* occurred only in the older oak stands (>20 years). These species are usually categorized as late-stage ECM fungi or belong to ECM fungal species associated with mid-aged or old trees (>25 years; Deacon and Fleming 1992; Keizer and Arnolds 1994; Visser 1995; Rao et al. 1997). However, in the younger stands, early-stage fungi were absent. *Hebeloma velutipes*, identified in the 21-year-old chronosequence stand, was the only early-stage fungus present (Deacon and Fleming 1992; Gryta et al. 1997). This is in agreement with Walker et al. (2005) who found no association of early and mixed-stage fungi with oak seedlings growing in mixed forests in the southern Appalachian Mountains.

Danielson (1991) pointed out that *Ascomycetes* such as E-strain fungi can dominate early stages of the mycorrhizal primary succession. In our case, *C. geophilum* was the most common species at nearly all investigated stands, and in the 21-year-old chronosequence stand, it reached an abundance about 80%. This very high abundance of *C. geophilum* was also observed by Walker et al. (2005) at sites where *Rhododendron maximum* thickets were present. The authors concluded that some component of the environment related to the shrub thickets was inhibitory to regular mycorrhizal colonization of rare mycorrhizal species thus favoring colonization by resistant *C. geophilum*. This is well founded by the fact that *C. geophilum* often dominates root systems in ecosystems with extreme environmental conditions such as drought (Mexal and Reid 1972; Read and Haselwandter 1981; Vogt et al. 1981; Pigott 1982). It might be that the genus *Quercus* is characterized by higher abundance of *C. geophilum* than other tree genera, as reported by Valentine et al. (2004) for *Q. garryana*, by Richard et al. (2005) for *Q. ilex*, and by Walker et al. (2005) for *Q.rubra*/*Q. prinus*.

The results of the present study suggest that most ECM morphotypes from the reclamation sites are not adapted to a well-developed organic horizon as they were found also in the mineral soil layer of the reference stand. In contrast, species collected from the organic horizon of the reference stand such as *P. croceum* or *Tomentella* spec. 01 were absent in the reclamation sites. The absence of *P. croceum*, for example, is not traced back to the fact that this fungal species might be a late-stage fungus as the oldest chronosequence stand and the reference stand exhibited the same age. In contrast, development of the organic horizon was not as far advanced at the chronosequence stands compared to the reference stand (e.g., thickness of organic horizon). Therefore, we suggest that development of the organic horizon might be an important selection factor for fungal presence. *Tuber* spec. and *Tomentella* species, which were frequent species in this study, are well known from other mining areas (Danielson and Visser 1989; Danielson 1991). However, some morphotypes such as *Inocybe* spec., *Thelephora terrestris*, *Hymenoscyphus* spec. and *Amphinema byssoides*, described from other stands with mining substrates (Schramm 1966; Münzenberger et al. 2004; Hohensee 2005), were not found on red oak at our reclamation stands.

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