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Abundance, diversity, and vitality of mycorrhizae of Scots pine (*Pinus sylvestris* L.) in lignite recultivation sites

Abstract Scots pine (*Pinus sylvestris* L.) stands cover large areas in the Lusatian and the Middle German lignite mining districts. Due to adverse chemical substrate conditions, the root systems of the trees are restricted to the ameliorated top-spoil and the organic forest floor layers. To investigate functioning of fine root systems under the prevailing site factors, we studied mycorrhizal colonization rate and frequency as well as mycorrhizal diversity, vitality and growth phases in Scots pine ecosystems along a chronosequence in both mining districts. Mycorrhizal rate was close to 100% in both districts. Mycorrhizal abundance was higher in the organic forest floor layer than the mineral soil layer. In total, 25 morphotypes were recorded. Diversity differed between the districts. The mycorrhizae of *Amphinema byssoides*, *Tuber puberulum*, Pinirhiza discolor, Pinirhiza cf. bicolorata and E-type were present in both mining areas. These morphotypes are typical of nutrient-rich soils with high pH values. Compared with the undisturbed sites, vitality of mycorrhizae was very high at the test sites on spoil substrate, correlating with the high growth dynamics of mycorrhizae at recultivation sites. A relatively high carbon flow to the mycorrhizal root systems at these sites seems likely. Thus, mycorrhizal root systems are able to cope with the ameliorated top-spoil and the organic layer. The main reason for the adaptation is the large number of ectomycorrhizal fungal species available in this area where *Pinus sylvestris* is indigenous.

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Introduction

There are two large lignite mining areas in eastern Germany, the Lusatian and the Middle German districts. In the Lusatian area near Cottbus, Brandenburg, 80,000 ha of land have been disturbed by open-cast mining (Weber et al. 1999). This district is the largest recultivation area of Europe (Fig. 1). In the Middle German district near Halle, Saxony-Anhalt and Leipzig, Saxony, 60,000 ha of land have been utilized for brown coal production (Haubold-Rosar et al. 1991). Up to the 1990s, only about 50% of either area was recultivated. The prevailing open-cast mining technology in these districts deposits spoil substrates with adverse chemical properties. These substrates are predominantly sandy with high amounts of pyrite and often large amounts of lignite particles of varying size. Oxidation of pyrite results in high concentrations of Fe^{2+}/Fe^{3+} and sulphuric acid in the soil. Resulting low pH values (as low as pH 2) cause intensive mineral weathering and produce phytotoxic substrates (Weber et al. 1999; Knoche et al. 2000; Hüttl and Weber 2001). These substrates are low in pedogenic organic matter and in moisture-holding capacity and have an extremely low nutrient content, excessive matter leaching and a high risk of wind and water erosion.

The low productivity of these substrates results primarily in forest ecosystems on the post-mining landscapes. The substrates are ameliorated with alkaline materials such as limestone or fly ashes from brown coal combustion, which compensate for the high soil acidity (Hüttl et al. 2000; Schaaf et al. 2001). Soil solutions of these substrates are marked by high Fe, Al, Ca, Mg and SO_4 concentrations. However, low N and P availability is growth limiting for forests on juvenile acid mine soils and fertilizer applications are necessary for successful afforestation (Heinsdorf 1996).
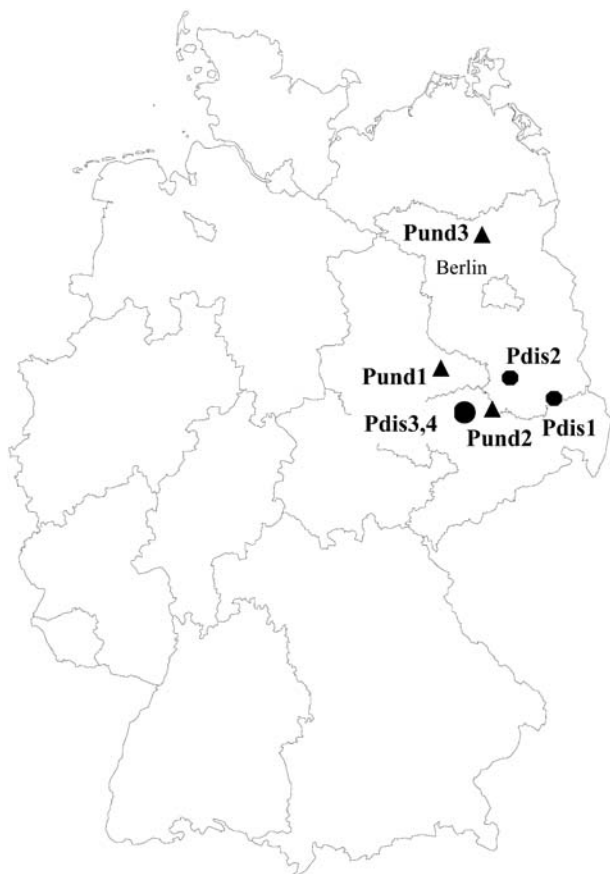
Fig. 1 Experimental sites. Pdis1 and 2 are disturbed sites in the Lusatian lignite mining district, Pdis3 and 4 are disturbed sites in the Middle German lignite mining district. Pund1–3 are sites with undisturbed soil

soils has been used successfully for artificial inoculation (Schramm 1966; Marx 1975; Berry and Marx 1978; Walker et al. 1985; Castellano 1996).

Only relatively few ECM morphotypes were identified on such sites. Schramm (1966) described the following morphotypes from anthracite mines in Pennsylvania: *Pisolithus tinctorius, Scleroderma citrinum, Thelephora terrestris, Amanita rubescens, Astreus hygrometricus, Cenococcum geophilum, Inocybe* sp. and *Boletus* sp. Zak et al. (1982) identified E-type mycorrhizae from mine spoils in Alberta. Danielson (1991) reported about 10 mycorrhizal morphotypes on coal spoils (e.g. *Cenococcum geophilum, Amphinema byssoides, Thelephora terrestris, Suillus* sp., *Tuber* sp., *Tomentella* sp. and E-type). Studies on mycorrhization of forest trees on recultivated coal mining areas are lacking in Germany.

The ECM root system of forest trees undergoes continuous ageing and regeneration. Investigations on vitality of mycorrhizae measured at the tissue level are rare. Qian et al. (1998a) stated that the efficiency of the system in terms of nutrient uptake versus carbohydrate consumption is a function of the physiological activity of the mycorrhizae and their turnover rate. Life span and vitality of individual mycorrhizae are related to soil conditions such as humidity, texture, chemistry and nutrient availability, as well as the fungal species involved in the symbiosis (Ritter 1990; Kottke et al. 1993; Qian et al. 1998a).

Vitality of mycorrhizal tissues can be studied by staining with fluorescein diacetate (Söderström 1977; Ritter et al. 1986; Ritter 1990; Taylor and Peterson 1998). Fluorescein stains hyphae with a permeable cell wall, an intact outer membrane, active esterases in the cytoplasm and a non-acidic cytoplasmic pH (Taylor and Peterson 1998). This method can be used to measure the proportion of hyphal mass with the potential to absorb ions (Taylor and Peterson 1998) and thus allow conclusions about mycorrhizal function. This is particularly true for comparative studies of ECM vitality of a tree species in forest stands at comparable sites. There is no information about mycorrhiza vitality of Scots pine at recultivation sites.

So far it is unclear, how ECM tree root systems function under such extreme site conditions. The aim of the present investigation was to compare the abundance, diversity and vitality of mycorrhizae of Scots pine along a chronosequence after recultivation in the Lusatian and Middle German lignite mining districts. Our data provide ecologically important information about the stability of forest ecosystems on disturbed sites in post-mining areas compared with those on undisturbed soils of the general region.

Scots pine (*Pinus sylvestris* L.) covers large areas of both districts, forming about 60% of the Lusatian area (Preußner 1998). An important feature of afforestation stands on these substrates is that tree root systems are restricted to the ameliorated top layer of the substrate and the developing organic forest floor layer. This specific ecological situation probably has consequences for nutrient and water uptake.

The root systems of most forest trees, including pines, form ectomycorrhizae (ECM) with various fungi and the stability of forest ecosystems depends on ECM colonization rate and species composition (Haselwandter and Bowen 1996), especially on disturbed soils (Berry 1982; Grossnickle and Reid 1983; Beckjord and McIntosh 1984; Mullins et al. 1989). Most information on the mycorrhization of trees on disturbed soils is available from North American recultivation sites of hard coal regions. There it was found that seedlings of pine and oak inoculated with mycorrhizal fungi that were adapted to adverse soil conditions had a lower rate of mortality and/or a better growth rate (Berry 1982; Grossnickle and Reid 1983; Beckjord and McIntosh 1984; Mullins et al. 1989). *Pisolithus tinctorius*, a fungus very well adapted to the high soil temperatures and low pH of such recultivation

Materials and methods

Site description

Two disturbed sites, Pdis1 (Pinus disturbed 1) and Pdis2, were chosen in the Lusatian and two, Pdis3 and Pdis4, in the Middle

Table 1 Stand characteristics of disturbed sites (Pdis1–Pdis4) in two lignite mining districts. Data for stand basal area and number of stems are from Grote (1999) and Bräunig (personal communication) (*n.s.* no specification)

Parameter	Pdis1	Pdis2	Pdis3	Pdis4
Annual precipitation (mm)	541	594	600	600
Tree age (years)	18	32	27	41
Soil type	Regosole	Regosole	Regosole	Regosole
Thickness of organic layer (cm)	3.0	5.5	4.5	7.5
pH (H_2O) organic layer	4.7–5.5	4.0–5.4	4.4–6.2	5.3–5.9
pH (H_2O) mineral soil	4.4–7.3	3.1–6.5	4.7–6.2	4.8–6.8
Stand basal area ($m^2 \cdot ha^{-1}$)	24.7	43.0	35.3	28.9
Number of stems·(ha^{-1})	8944	3409	n.s.	n.s.

Table 2 Mean anion and cation concentrations of the soil solution ($mg \cdot l^{-1}$) at a depth of 20 cm (Knoche et al. 1999) at two disturbed soil sites

Site	Ca^{2+}	Mg^{2+}	K^+	Al^{n+}	Fe^{n+}	NH_4^+	NO_3^-	SO_4^{2-}
Pdis1	200.4	4.8	3.3	4.8	0.3	<0.1	4.0	527.4
Pdis2	61.1	3.1	3.3	0.8	0.3	0.1	34.9	124.7

Table 3 Stand characteristics of undisturbed sites

Parameter	Pund1	Pund2	Pund3
Annual precipitation (mm)	566	565	595
Tree age (years)	60	40	60
Soil type	Typic Dystrochrepts	Typic Dystrochrepts	Typic Dystrochrepts
Thickness of organic layer (cm)	9.7	6.5	6.1
pH (KCl) organic layer	4.1	3.3	2.8

German lignite mining districts (Fig. 1). The conditions at these sites are summarized in Table 1. Pdis1 and Pdis2 are characterized by high interception loss (about 50%) reflecting extremely dry site conditions (Knoche et al. 1999). These sites were ameliorated with power plant ashes down to a depth of 30–60 cm (Knoche et al. 1999). At site Pdis3, limestone was used for amelioration and the amelioration material at site Pdis4 is not known. At Pdis1 and Pdis2, the parent material is tertiary carboniferous loamy sand and at Pdis3 and Pdis4 tertiary loamy sand mixed with quaternary material. Because of the substrate heterogeneity, the pH varied considerably. Chemical soil solution data are only available for the Lusatian sites, where there are high concentrations of SO_4, Al and Ca, especially at the younger site (Table 2).

Undisturbed, i.e. semi-natural sites of the general region, were chosen as controls to study mycorrhiza vitality. The undisturbed sites Pund1 (Pinus undisturbed 1), Pund2, and Pund3 are also stocked with Scots pine. The site conditions for these undisturbed sites are summarized in Table 3. Until the early 1990s, Pund1 near Bitterfeld/Halle was heavily impacted by atmogenic deposition of N, S, and alkaline fly ash, i.e. high input rates of Ca and Mg oxides. Pund2 near Leipzig received lower deposition loads, especially of fly ash. Pund3 near Berlin received only background deposition loads and is, therefore, a reference site for Pund1 and Pund2.

As mycorrhiza vitality is strongly correlated with soil humidity, humidity of the organic forest floor layer was determined gravimetrically at each mycorrhizal sample date (Table 4).

Mycorrhizal parameters

At the disturbed sites, mycorrhization of Scots pine was investigated at 2-month intervals from April until November in 1996 and 1997 in the Lusatian district and from May until November 1996 and from March until October 1997 in the Middle German district. Samples were taken randomly with a soil corer (6.5 cm in diameter) with (8–)10 replicates and the cores divided into organic and mineral soil layers (0–20 cm). Samples were stored in plastic bags at 4°C. Root samples were taken from fresh soil and adhering soil particles carefully removed with fine forceps and preparation needles. For quantitative analysis, mycorrhizal rate (percent mycorrhizae per total amount of root tips) and mycorrhizal abundance (mycorrhizae per 100 cm^3 soil volume) were deter-

Table 4 Soil humidity (% dry wt.) at disturbed and undisturbed sites

Date	Pdis1	Pdis2	Pund1	Pund2	Pund3
1996					
April	64±30	80±28	-	-	-
June	127±31	145±34	-	-	-
July	85±31	74±22	-	-	-
September	65±29	127±20	-	-	-
November	159±42	158±50	-	-	-
1994					
April	-	-	157±16	214±37	221±14
1993					
July	-	-	150±63	173±52	127±51
August	-	-	146±23	71±15	76±14
September	-	-	112±25	97±36	91±52
October	-	-	100±24	74±19	95±47
November	-	-	119±19	86±10	154±24

mined. Mycorrhizal morphotypes were classified under a dissecting microscope according to morphological features. Freshly prepared hyphal mantle tissue was used for identification. The different cell layers of the hyphal mantle were investigated with the help of Normarski differential interference contrast microscopy (Olympus BX 50). Known morphotypes were identified according to Agerer (1987–2002). Eight unknown morphotypes were described (Golldack et al. 1996a, b, c, d; 1997a, b; 1998a, b, c, d, 1999a, b).

To study vitality of mycorrhizae, 10 soil cores were taken randomly using a soil corer (8 cm in diameter) at Pdis1 and Pdis2 in April, June, July, September, and November 1996. Only mycorrhizae of the organic layer were investigated. A further 10 samples were taken at the same locations to determine gravimetric water content (Table 4). At Pund1, Pund2 and Pund3, sampling was carried out as above in July, August, September, October, and November 1993 and in April 1994. Thus, the vitality of mycorrhizas from disturbed and undisturbed sites was investigated in different years and this has to be taken into account when interpreting the data. However, all sites are located in an area

characterized by a continental climate with moderate atmospheric fluctuations. As mycorrhiza vitality can be influenced by the fungal partner (Kottke et al. 1993; Qian et al. 1998a), the different morphotypes were sorted. Only mycorrhizae with a well-developed hyphal mantle were chosen. Mycorrhizae (100–250 per sampling date) were sectioned longitudinally with a razor blade and the sections stained immediately with fluorescein diacetate (Ritter et al. 1986). The presence of unspecific esterases and proteinases in the cytoplasm of living fungal cells, cleaving fluorescein diacetate into fluorescein and acetic acid (Rotman and Papermaster 1966), was examined under a fluorescence microscope (Axioscop, filter BP 365/12, lamp HBO 50, camera MC 80, Carl Zeiss). Vitality was scored as: ++++ (fluorescence of stele, meristem, hyphal mantle, Hartig net), +++ (fluorescence of stele, meristem, Hartig net), ++ (fluorescence of stele, meristem), + (fluorescence only of the stele) and − (no fluorescence). Metacutin layers visible in longitudinal sections because of the blue autofluorescence of suberin and lignin indicate the number of growth phases of a mycorrhiza (Ritter et al. 1989; Kottke et al. 1993). Therefore, metacutin layers were also recorded using the same sections to obtain information on growth dynamics of the mycorrhizae in relation to the age of the afforested recultivation sites.

Statistics

Data for mycorrhizal abundance were log transformed before statistical analysis. A two-way variance analysis was performed separately for the organic and mineral soil layers ($P<0.05$). Thereby, data for two collections were summarized for spring 1996, autumn 1996, spring 1997 and autumn 1997. The association of morphotypes with different soil layers was examined using chi-square. The probability with which a specific morphotype appears in a given soil layer depends on the number of root tips inside this layer. Accordingly, expected frequencies were calculated assuming that species with no preference for a particular soil fraction colonize an equal proportion of the tips in each fraction. For each species, observed frequencies were determined from the number of mycorrhizae formed in each soil fraction. The expected number of mycorrhizae was then estimated by multiplying the total number of mycorrhizae for a species in each fraction by the proportion of total root tips that occurred in that soil fraction. Only data from cores in which the species actually occurred were used to calculate the proportion of total root tips in each soil fraction.
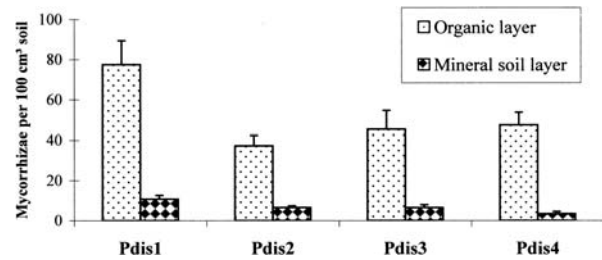

Fig. 2 Mycorrhizal abundance (mycorrhizae per 100 cm3 soil volume) at the disturbed sites

Results

Mycorrhizal rate was normally high in both soil layers at all four recultivation sites (Table 5), in many samples 100%. Only minor seasonal fluctuations occurred, for example, somewhat lower rates were found in spring 1997.

Mycorrhizal abundance was always higher in the organic than in the mineral soil layer (Fig. 2) and was highest in the organic layer of Pdis1. At this site, thickness of the organic layer was low and tree density high (Table 1).

Twenty-five morphotypes were determined at the four sites (Table 6), 11 of which were identified from the literature. Eight morphotypes were described for the first time: *Pinirhiza cyaneoviridis*, *Pinirhiza dimorpha*, *Pinirhiza discolor*, *Pinirhiza flexipila*, *Pinirhiza granulosa*, *Pinirhiza lactogelatinosa* and *Pinirhiza lactariosimilis* (Golldack et al. 1996a, b, c, d; 1997a, b; 1998a, b, c, d, 1999a, b). Five morphotypes were common to all sites: *Amphinema byssoides*, *Tuber puberulum*, E-type, *Pinirhiza* cf. *bicolorata* and *Pinirhiza discolor*. Twelve morphotypes were exclusively found at sites in the Lusatian district and five in the Middle German district, i.e. species composition was site specific in the two mining districts. At all disturbed sites, *Amphinema*

Table 5 Mycorrhizal colonization rate (%) of Scots pine in the organic layer (*OL*) and mineral soil layer (*MS*) of disturbed sites

| Date | Lusatian | | | | Middle German | | | | Date |
| | Pdis1 | | Pdis2 | | Pdis3 | | Pdis4 | | |
	OL	MS	OL	MS	OL	MS	OL	MS	
1996									
April	98	100	100	100	-	-	-	-	April
May	-	-	-	-	97	100	99	100	May
June	99	100	98	98	98	99	98	98	June
July	98	96	99	97	-	-	-	-	July
September	93	99	100	100	100	100	99	88	September
November	97	97	99	100	99	100	100	100	November
1997									
March	-	-	-	-	92	91	97	100	March
April	93	94	90	95	95	84	95	96	April
May	90	99	94	94	-	-	-	-	May
September	-	-	-	-	100	100	100	100	September
October	98	100	99	100	99	95	99	94	October
November	100	100	99	100	-	-	-	-	November
Mean	97	99	98	98	98	96	99	98	Mean

Table 6 Distribution of morphotypes found (as percent of all samples) (*OL* organic layer, *MS* mineral soil layer)

Morphotype	Pdis1		Pdis2		Pdis 3		Pdis4	
	OL	MS	OL	MS	OL	MS	OL	MS
Amphinema byssoides	59	42	40	32	69	58	85	54
Cenococcum geophilum	1	-	12	4	-	-	27	4
E-type	40	30	38	43	25	38	40	19
Inocybe sp.	-	-	-	-	42	31	23	12
Lactarius deliciosus	9	8	-	-	-	-	8	6
Pisolithus tinctorius	-	1	-	-	-	-	-	-
Rhizopogon roseolus	12	9	-	-	2	2	6	2
Tuber puberulum	13	12	6	12	33	23	60	27
Pinirhiza cf. bicolorata	52	34	2	1	4	2	4	4
Pinirhiza cyaeneoviridis	6	-	-	-	12	4	-	-
Pinirhiza dimorpha	38	44	53	68				
Pinirhiza discolor	32	14	10	1	12	6	19	4
Pinirhiza flexipila	1	-			4	2	19	-
Pinirhiza granulosa	-	-	16	10	-	-	-	-
Pinirhiza lactariosimilis	3	5	2	1	-	-	-	-
Pinirhiza lactogelatinosa	3	5	-	-	-	-	-	-
Pinirhiza stellannulata	-	-	39	10	-	-	-	-
Unidentified 1	2	-	3	3	-	-	-	-
Unidentified 2	19	30	-	-	-	-	-	-
Unidentified 3	-	-	24	15	-	-	-	-
Unidentified 4	-	-	2	1	4	4	12	6
Unidentified 5	-	-	-	-	10	14	2	0
Unidentified 6	-	-	-	-	4	8	6	2
Unidentified 7	-	-	-	-	2	2	10	-
Unidentified 8	-	-	-	-	-	-	17	10

Table 7 Substrate preference of morphotypes (*OL* organic layer, *MS* mineral soil layer, + weak association: $0.01<P<0.05$, ++ strong association: $0.001<P0.01$, +++ very strong association: $P<0.001$)

Morphotype	Pdis1		Pdis2		Pdis3		Pdis4	
	OL	MS	OL	MS	OL	MS	OL	MS
Amphinema byssoides	+++	-	+++	-	+	-	+++	-
Cenococcum geophilum	-	-	+++	-	-	-	+	-
E-type	-	+++	+++	-	+++	-	-	+++
Inocybe sp.	-	-	-	-	-	+++	-	+++
Pinirhiza cyaneoviridis	+++	-	-	-	++	-	-	-
Pinirhiza dimorpha	-	+++	-	+++	-	-	-	-
Pinirhiza discolor	+	-	+++	-	+++	-	-	-
Pinirhiza flexipila	+++	-	-	-	+	-	+++	-
Pinirhiza granulosa	-	-	+++	-	-	-	-	-
Pinirhiza lactariosimilis	+++	-	+++	-	-	-	-	-
Pinirhiza lactogelatinosa	-	+++	-	-	-	-	-	-
Unidentified 2	-	+++	-	-	-	-	-	-
Unidentified 3	-	-	+++	-	-	-	-	-
Unidentified 5	-	-	-	-	-	+++	-	-
Unidentified 8	-	-	-	-	-	-	-	+++

byssoides and E-type mycorrhizae were the dominating morphotypes, i.e. they constituted more than 5% of the total number of collected mycorrhizae (Erland et al. 1998). *Amphinema byssoides* was present at 11–17% in the Lusatian and 40% in the Middle German area. In the latter area, *Tuber puberulum* and *Inocybe* sp. were also dominating with 7–18% and 24%, respectively. All three *Lactarius* species, namely *Lactarius deliciosus*, *Pinirhiza lactariosimilis* and *Pinirhiza lactogelatinosa*, were only found at Pdis1,but these morphotypes were not dominating.

Substrate preference of morphotypes is summarized in Table 7. Fifteen morphotypes showed a significant relationship to one of the soil horizons. Eight morphotypes, e.g. *Amphinema byssoides* and *Cenococcum geophilum*, were positively correlated to the organic layer. Seven morphotypes, e.g. Pinirhiza dimorpha, E-type and *Inocybe* sp., were positively correlated to the mineral soil layer.

The morphotypes collected for the study of mycorrhiza vitality at Pdis1 and Pdis2 are shown in Figure 3. Only three morphotypes, namely the grainy brown type, the brown type with cystidia and Pinirhiza discolor, were found at both disturbed sites; the other morphotypes were site specific. In general, the number of morphotypes present varied with the season. Only morphotypes with a well-developed hyphal mantle were investigated.

Morphotype vitality (Fig. 4) showed seasonal variation. Over all, both sites were marked by a very high proportion of ++++ and +++ mycorrhizae and few + and −
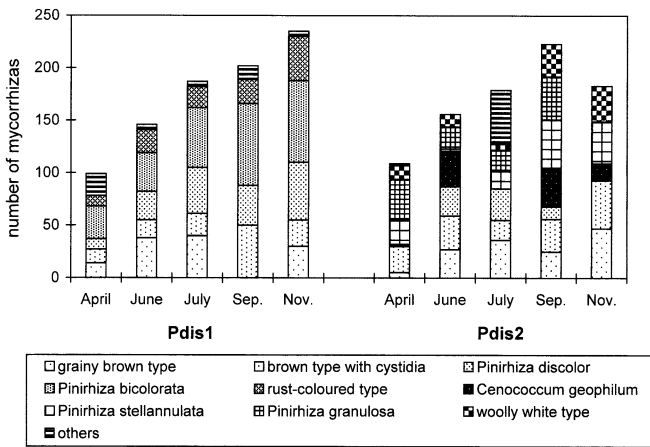
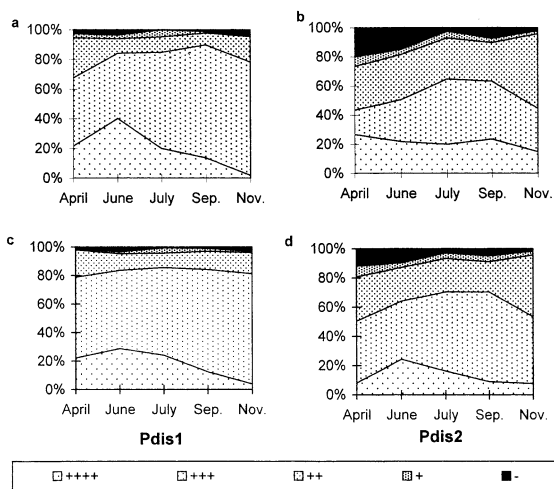**Fig. 3** Number and types of mycorrhizas investigated for mycorrhiza vitality

Fig. 4 Vitality patterns of mycorrhizae at the disturbed sites Pdis1 and Pdis2. **a, b** only the three types common on both sites were compared. **c, d** all investigated morphotypes were compared

Fig. 5 Comparison of the vitality patterns of mycorrhizae from the disturbed sites Pdis1 and Pdis2 with the undisturbed sites Pund1, Pund2 and Pund3

Fig. 6 Number of metacutin layers

mycorrhizae, but the vitality pattern was different at these two disturbed sites. At the younger site Pdis1, ++++ and +++ mycorrhizae were 21% higher than at the older site Pdis2. The influence of species on mycorrhiza vitality was negligible (Fig. 4), i.e. the vitality pattern was very similar for the morphotypes (grainy brown type, brown type with cystidia, Pinirhiza discolor) common to both disturbed sites (Fig. 3, Fig. 4a, b) and for morphotypes irregularly distributed at the two sites (Fig. 3, Fig. 4c, d).

In Figure 5, mycorrhiza vitality at the two disturbed sites is compared to that at the three sites with native soils (Pund1, Pund2, Pund3). At these latter sites, ++++ and +++ mycorrhizae were much less frequent, but − mycorrhizae were much more frequent than at the disturbed sites Pdis1 and Pdis2. At Pund1 and Pund2, relatively high percentages of the intermediate vitality classes were present. At the reference site Pund3, only low percentages of these vitality classes were observed, whilst values for −
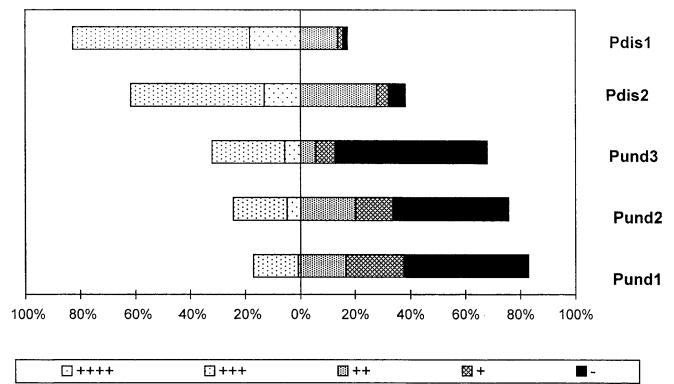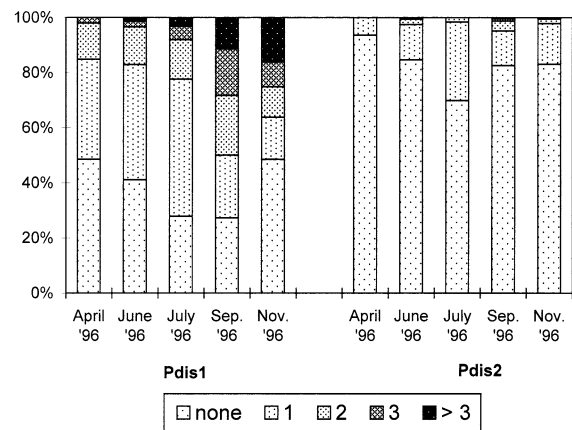
mycorrhizae were very high. Thus, the highest mycorrhizal vitality was clearly found at Pdis1 and Pdis2 (Fig. 5).

With regard to soil humidity (Table 4), no striking influence of this parameter on vitality was found in the organic layer at all sites. Soil humidity was slightly lower at Pdis1, the site with the highest mycorrhiza vitality.

Growth dynamics, or the number of extended pauses followed by reinitiated root extension, is reflected by the number of metacutin layers visible in the mycorrhizal sections investigated for vitality (Fig. 6). This parameter was found to be site specific at each sampling date at the disturbed sites and varied with the season. At Pdis1, a maximum of eight metacutin layers was recorded, whilst Pdis2 exhibited only a maximum of four layers. At Pdis2, a striking number of mycorrhizal root tips without a metacutin layer was observed. There was a high proportion of mycorrhizae with numerous metacutin layers at Pdis1 in September and November 1996.

Discussion

Several authors have reported a reduction in mycorrhizal rate and/or reduced growth of forest trees growing on substrates of disturbed landscapes (Redell et al. 1999; Fay and Mitchell 1999). Results from the present study show that root systems can be highly colonized by ECM fungi after 20 years of forest recultivation (Scots pine stands). In the Lusatian and Middle German lignite mining districts, mycorrhizal rate was 96–99%. A lower mycorrhizal rate was observed at a Scots pine stand on a site with native soil in northeastern Germany (Münzenberger et al. 2000).

At Pdis1, mycorrhizal abundance was higher than at the other sites. As soil development is more advanced at Pdis2 than at Pdis1 (Heinkele et al. 1999), the latter site is characterized by more adverse soil chemical properties such as very low pH, very high electric conductivity, low humus content and low nutrient availability. Soil moisture was somewhat lower at Pdis1 than Pdis2 due to a higher interception and evapotranspiration rate as a consequence of the higher stem number (Knoche et al. 1999). In addition, the thin organic layer at Pdis1 leads to high fluctuation of soil moisture. At Pdis1, growth of mycorrhizae is obviously stimulated in the organic layer relative to the mineral soil layer and is higher than in both soil layers of Pdis2. A further reason for the more intensive growth of mycorrhizae at Pdis1 is seen in the adverse nutrient supply and presumed greater competition for nutrients with soil microorganisms. Obviously, root systems have adapted to these ameliorated, but physico-chemically still very heterogeneous soil conditions in the upper soil layer.

The number of ECM species at the investigated mining sites was similar to sites with native soil in the general region (Münzenberger et al. 2000). Despite the fact that a relatively high number of morphotypes was found, only five morphotypes dominated at each site. Erland et al. (1998) also observed only six morphotypes were dominating at sites of Norway spruce in southern Sweden. In a Norway spruce stand of the Höglwald in southern Germany, only 4–7 morphotypes were present in high numbers at all investigated sites (Brand et al. 1992).

Further, ECM species composition at our disturbed sites is similar to that of other mining regions. *Pisolithus tinctorius*, a fungus used successfully for inoculation of tree seedlings (Marx and Artman 1979; Grossnickle and Reid 1983; Beckjord and McIntosh 1984), was also found in anthracite and hard coal spoils (Schramm 1966; Medve and Gill 1982). Of the sites we investigated, this fungus was found at Pdis1 at a proportion of 2%. Mycorrhizae of *Amphinema byssoides* and of the genus *Tuber* were described for *Picea glauca* and *Pinus banksiana* growing on coal spoil and oil sands tailings in Alberta (Danielson and Visser 1989; Danielson 1991). On these coal spoils, species of *Tomentella* were among the dominating morphotypes (Danielson 1991). Mycorrhizae of Pinirhiza cyaneoviridis, which according to their morphological features belong to the genus *Tomentella*, were found at

the two younger sites Pdis1 and Pdis3. Schramm (1966) described *Inocybe* mycorrhizae with *Pinus rigida* and *Pinus virginiana* on anthracite spoils in Pennsylvania. *Inocybe* sp. was one of the dominating morphotypes in the Middle German lignite mining district.

The mycorrhizae of *Amphinema byssoides*, *Tuber puberulum* and E-strain are typical of nutrient-rich soils with high pH. Danielson (1991) observed *Amphinema byssoides* on seedlings of *Picea glauca* on a hard coal spoil 4 years after recultivation. By the seventh year, this fungus had reached a proportion of 90%, but was reduced to 19% by the tenth year. The pH of the substrate was approximately neutral. *Amphinema byssoides* and *Tuber* sp. were also found in nurseries with nutrient-rich soils (Weiss and Agerer 1988; Monaghan and Mitchell 1998). Fransson et al. (2000) found that both species were more abundant in fertilized plots than in control plots. *Amphinema byssoides* and *Tuber* mycorrhizae were dominating morphotypes at urban sites with pH values of 7–8 (Danielson and Pruden 1989). *Tuber puberulum* is typical of limed soils (Pegler et al. 1993; Qian et al. 1998b). These findings are in accordance with our results, where Ca concentrations (Table 2) and pH values were high at all four disturbed sites due to amelioration practices.

E-type and *Thelephora terrestris* are both symbionts adapted to high nutrient and water supply (Laiho 1965; Mikola 1965; Marx and Artman 1979; Danielson 1991). E-type mycorrhizae have a broad pH tolerance (Mikola 1965; Yu and Peterson 2001). Although *Thelephora terrestris* mycorrhizae have been found on several mine spoils in North America (Schramm 1966; Berry and Marx 1978; Danielson 1991), mycorrhizae of this species were not detected on our sites, but fruitbodies were collected at Pdis1. According to Thomas and Jackson (1979) and Mikola (1988), E-strain mycorrhizae do not persist longer than 1–2 years after outplanting of the seedlings as they are weak competitors. However, these mycorrhizae dominated with 25% in 10-year-old *Picea glauca* and *Pinus banksiana* stands on coal spoils and oil sands tailings in the Alberta-study (Danielson 1991). In our mining districts, E-type mycorrhizae belonged to the dominating morphotypes. Obviously, these morphotypes are adapted to the prevailing ecological site conditions; however, no information is available about their specific function in forest ecosystems. For example, hyphae of E-strain mycorrhizae grow very loosely around the root. Therefore, it is questionable whether the storage function of this mycorrhizal type is as efficient as that of morphotypes with a well-developed hyphal mantle.

At the recultivation sites Pdis1 and Pdis2, the proportions of the vitality classes ++++ and +++ were much higher than at the sandy Scots pine sites with undisturbed soils. High percentages of ++++ and +++ mycorrhizae are important, as such mycorrhizae indicate that both symbiosis partners are alive and may thus improve e.g. nutrient uptake. The high rates of these vitality classes reflect the high metabolic activity of most of the mycorrhizae at the recultivation sites but, given the high

rates of growth phases, also indicate that longevity of mycorrhizae at these sites was short.

In the comparison of forest ecosystems on disturbed post mining areas with comparable stands on undisturbed soils, relatively high percentages of ++ and + mycorrhizae were present at sites Pund1 and Pund2. The reference site Pund3 showed only low percentages of these vitality classes. Due to the high numbers of mycorrhizae of intermediate vitality classes, the life-span of mycorrhizae at Pund1 and Pund2 seems to be longer than at the reference site Pund3. Consequently, a higher mycorrhiza turnover is assumed at Pund3 (cf. Kottke et al. 1993; Münzenberger et al. 1995).

Vitality may be influenced by the fungal species involved in mycorrhiza formation (Ritter 1990). However, at the disturbed sites Pdis1 and Pdis2, the influence of fungal species on vitality was negligible. Accumulation of soil organic matter was different at Pdis1 and Pdis2. Hence, specific site parameters varied, for example soil humidity, which was low at Pdis1 where the organic forest floor layer was very thin. At this site, growth dynamics of mycorrhizae were related to corresponding fluctuations of soil humidity. This was documented by the high number of metacutin layers at Pdis1 (a maximum of eight). High numbers of metacutin layers were not detected at the other sites (Pdis2, Pund1, Pund2, Pund3).

A striking difference between disturbed and undisturbed sites is that mycorrhiza vitality was also high during the dry summer period. Whereas sites with undisturbed soil showed a drastic reduction of vitality with high quantities of − mycorrhizae (80–90%) between July and September (Münzenberger et al. 1995), vitality was extremely high at the recultivation sites during the whole vegetation period. This phenomenon is extraordinary, as mycorrhiza vitality is normally related to soil humidity (Kottke et al. 1993). However, soil humidity was low at Pdis1. This again underlines the importance of a high activity of mycorrhizal morphotypes at recultivation sites, especially at younger forest sites such as Pdis1 that are at the beginning of soil and ecosystem development.

The high amount of active mycorrhizae over the whole vegetation period and the high frequency of mycorrhizae growth phases indicate an increased carbon allocation to the mycorrhizal root system, with the consequence that less carbon is available for above-ground tree growth. This result is in agreement with forest growth studies by Böcker et al. (1998), who found three growth phases of *Pinus sylvestris* after afforestation of recultivation sites. In the first growth phase (1–8 years), the increment was drastically reduced compared with yield-tables of Scots pine from sites with undisturbed soil. In the second growth phase (9–25 years), yield was strongly increased, apparently because ecosystematic nutrient cycling was established. However, yield was still lower than at sites with undisturbed soil. At the age of 26–30 years, the pine trees reached increments typically found for pine trees from sites with undisturbed soil. After 30 years, growth

was as good or sometimes even higher than growth rates shown in yield-tables of stands on undisturbed sites.

Our investigations show that ECM frequency is relatively high on recultivated sites in the lignite mining districts of eastern Germany. The ECM rate and number of fungal species are similar to ecosystems on undisturbed soil. Species composition is similar to other recultivation sites but basically typical of Ca-rich soils of high pH.

The high metabolic activity of mycorrhizae indicates that the mycorrhizal root system is well adapted to the extreme soil conditions prevailing on recultivation sites and experiences few deleterious impacts of the extreme site conditions. We conclude, therefore, that the particular vitality pattern at the recultivation sites is the result of very adverse soil conditions, i.e. unbalanced nutrient availability due to high micro-scale substrate heterogeneity and large fluctuations in soil humidity, leading to higher carbon costs of the forest trees to the benefit of the mycorrhizal root system in the first 25 years of forestry recultivation. Our findings also suggest that the tree-fungus symbiosis is an essential strategy for the establishment of forest ecosystems on this type of "terra nova", whenever the appropriate fungi are present.

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