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ORIGINAL PAPER

Ectomycorrhizal communities associated with silver fir seedlings (*Abies alba* Mill.) differ largely in mature silver fir stands and in Scots pine forecrops

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Abstract
• ***Context*** The requirement for rebuilding forecrop stands besides replacement of meadow vegetation with forest plants and formation of soil humus is the presence of a compatible ectomycorrhizal (ECM) fungal community.
• ***Aims*** This study aims to assess ectomycorrhizal fungi diversity associated with silver fir (*Abies alba* Mill.) seedlings regenerating in silver fir stands and Scots pine forecrops.
• ***Methods*** One-year-old seedlings were sampled in six study sites: three mature fir forests and three pine forests. ECM fungi were identified by polymerase chain reaction amplification and sequencing of the internal transcribed spacer of rDNA.
• ***Results*** The mean mycorrhizal colonization exceeded 90 %. Thirty-six ectomycorrhizal taxa were identified in fir stands and 23 in pine forecrops; ten out of these species were common to both stands. The fungal communities were different between study sites (R=0.1721, p=0.0001). *Tomentella stuposa* was the only species present at all sites.
• ***Conclusion*** Silver fir seedlings in Scots pine forecrops supported smaller ECM fungal communities than communities identified in mature silver fir stands. Nevertheless, fungal colonization of seedling roots was similar in both cases. This suggests that pine stands afforested on formerly arable land bear enough ECM species to allow survival and growth of silver fir seedlings.

Keywords Ectomycorrhiza (ECM) · Forecrop rebuilding · Molecular identification · Scots pine · Silver fir

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1 Introduction

Silver fir (*Abies alba* Mill.) is widely distributed across the European highlands and is one of the most important forest trees in the mountainous regions of Poland. Silver fir is predominant in the foothills and lower subalpine regions of the Carpathians and forms monocultured and mixed forests with *Picea abies* L. and *Fagus sylvatica* L. (Horvat et al. 1974). The understory light regime is an essential environmental condition for the regeneration and survival of the silver fir. Seedlings and saplings require only 15–25 % of full light (Jaworski 2011), which dictates the methods available for regeneration and cultivation during the young stage of the fir beneath the understory. Thus, during silviculture, silver fir should be preceded by forecrop stands that provide shading for the seedlings; it cannot be introduced as the first generation on formerly cultivated areas. The conditions required for the regeneration of silver fir seedlings are usually found in Scots pine (*Pinus sylvestris* L.) and European larch (*Larix decidua* Mill.) stands (Dobrowolska 2008).

Since the middle of the twentieth century, some of the former arable areas in the Polish Carpathians have been afforested with Scots pine and European larch as forecrop stands. These stands have reached the age of rebuilding, which occurs by natural seed regeneration of silver fir from adjacent stands or by planting seedlings under the canopy of

pine stands. Silver fir is ectomycorrhizal (ECM) (Trappe 1962). ECM fungi colonize new seedlings and protect them against root disease ensuring healthy seedling growth (Marx 1969) and are involved in the process of regeneration of the plants and sustainability of ecosystems; this increases seedling survival of the silver fir (Perry et al. 1989). The current knowledge of silver fir symbionts is predominantly based on morphological and anatomical descriptions of ectomycorrhizas (Agerer 1987–2007; Berndt et al. 1990; Comandini et al. 1998, 2001; De Román et al. 2005; Dominik 1961; Farfał 2008; Kowalski 1982, 2008; Kowalski et al. 1996; Pachlewski 1955; Stępniewska and Rębisz 2004) and fungal fruiting bodies (Laganà et al. 1999, 2002). Few molecular investigations of the ECM symbionts that associate with silver fir seedlings appear in the literature (Cremer et al. 2009; Eberhardt et al. 2000; Smutek et al. 2010; Ważny 2011).

The requirements for rebuilding forecrop stands are (a) replacement of meadow vegetation with forest plants, (b) formation of soil humus because soil microorganisms hasten the decay of plant tissues, and (c) the formation of ectomycorrhizas on tree roots (Jaworski 2000). At one site in the Carpathians, 3 cm of soil humus had formed 30 years after meadow afforestation with Scots pine (Maciaszek and Zwydak 1996). Farfał (2008) and Kowalski et al. (1996) described the morphological and anatomical features of ectomycorrhizas of the silver fir seedlings regenerating in pine stands, although ECM fungal symbionts have not been identified.

Two hypotheses were developed: (a) the diversity of ECM communities of silver fir seedlings is higher in mature silver fir seedling forecrops than in Scots pine forecrops and (b) ECM colonization of roots in forecrop stands is elevated ensuring seedlings survival and successful rebuilding of pine forecrops. Molecular methods were used in this study to identify the fungal species associated with silver fir seedlings regenerating in fir stands and pine forecrops, which are potentially relevant to the subsequent steps in ecological succession towards a mature silver fir stand.

2 Materials and methods

2.1 Study sites

Three silver fir (*A. alba*) stands (F1, F2, and F3) and three Scots pine (*P. sylvestris*) stands (P1, P2, and P3) with natural regeneration of silver fir seedlings were selected for investigation. Each pine stand was adjacent to at least one fir stand and was established on an abandoned area after cultivation. Several woody and herbaceous plants, such as *P. abies* (L.) H. Karst, *F. sylvatica* L., *Acer pseudoplatanus* L., *Corylus avellana* L., *Dentaria glandulosa* Waldst. and Kit., *Galium odoratum* (L.)

Table 1 Forest characteristics and soil properties in the study sites

	Silver fir stands			Scots pine forecrops		
	F1	F2	F3	P1	P2	P3
Tree species composition	Silver fir (90 %), Scots pine (10 %)	Silver fir (100 %)	Silver fir (100 %)	Scots pine (100 %)	Scots pine (100 %)	Scots pine (100 %)
Tree age (years)	45–100	55–115	75–95	60	55	50
Soil type	Proper brown (cambic)	Acid brown (cambic)	Acid brown (cambic)	Acid brown (cambic)	Acid brown (cambic)	Acid brown (cambic)
Stand area (ha)	16	24	6	17	9	6
pH in H_2O	5.0	5.0	4.3	5.7	5.0	4.5
pH in KCl	3.7	3.7	3.3	4.8	3.9	3.6
C (%)	3.56	3.03	4.38	4.98	4.20	2.74
N (%)	0.25	0.22	0.28	0.32	0.32	0.19
C/N (%)	14.1	13.6	15.5	15.4	13.2	14.3
Ca (mg/kg)	1,652.9	1,226.7	441.3	3,196.5	2,019.8	301.8
K (mg/kg)	117.1	81.2	80.6	139.1	111.0	79.9
Mg (mg/kg)	134.8	102.2	59.2	259.8	193.7	42.0
Na (mg/kg)	7.2	7.5	6.8	10.5	8.5	6.2
P (mg/kg)	0.6	0.5	1.2	0.8	0.7	8.2

Scop., *Paris quadrifolia* L., and *Maianthemum bifolium* (L.) F. W. Schmidt, were sparsely distributed in the understory and fir seedlings of between 1 and 20 years old had regenerated in each stand. The stands were located in the Experimental Forestry Unit in Krynica (southern Poland: 49° 21′ N, 20° 58′ E). Table 1 presents their characteristics. For ECM analysis, 30 1-year-old seedlings were sampled from each site in autumn 2010 along transects spaced 10–20 m apart with minimum intervals of 10 m between adjacent seedlings. Seedlings were sampled in an area of 6 ha in each of the stands. The seedlings were collected together with adjacent soil, placed in plastics bags, and stored at –20 °C until analysis. In addition, in each stand, one bulk soil sample (each being composed of 10 subsamples) was collected for chemical analysis.

2.2 Seedling parameters

The following data were collected: number of mycorrhizal seedlings, number of live and dead ectomycorrhizas and non-mycorrhizal roots, height and diameter of shoot, and dry weight of shoot and root.

2.3 ECM assessments

The root system was gently washed in tap water to remove organic and mineral soil. Different morphotypes of ectomycorrhizas were selected according to characteristics as mentioned by Agerer (1987–2007). Mycorrhizal colonization was confirmed by microscopic examination (Reichert, Austria). For the same morphotypes from each site, two to six root tips were placed in cetyl trimethyl ammonium bromide. DNA extraction was performed according to Lanfranco et al. (1998) with a minor modification (chloroform was not used and DNA was diluted in water). Amplification of the internal transcribed spacer (ITS) rDNA region was carried out with ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) primers. The reagents of the polymerase chain reaction (PCR) and their final concentrations were: 1× Pol Buffer, 0.2 mM dNTPs (each), 50 pmol of each primer, and 1 unit Taq DNA polymerase (Eurx).

PCR conditions were as follows: initial denaturation 10 min at 93 °C followed by 35 cycles consisting of 1-min denaturation at 95 °C, 1-min annealing at 60 °C, 2-min extension at 72 °C, and 10-min final extension at 72 °C. The presence of amplified products was visualized in 1.5 % agarose gel stained with ethidium bromide. Sequencing was carried out at the Laboratory of Molecular Biology of Adam Mickiewicz University in Poznan, Poland. The ITS4 primer was used for reading sequences. The sequences were edited using BioEdit (Hall 1999) and Chromas (www.technelysium.com.au) software and then compared with published sequences in UNITE (Abarenkov et al. 2010) and NCBI (www.ncbi.nlm.nih.gov) databases. A positive identification of a mycorrhizal species was confirmed if they shared ≥98 % ITS region sequence identity with the most similar (reference) sequence from the UNITE or NCBI databases. The obtained sequences within 2 % nucleotide difference were categorized as a single operational unit and assigned an identical name.

2.4 Soil assessment

The pH was determined in H_2O and KCl, the C content was analyzed by the Tiurin method, the N content by Kjeldahl method, and the macronutrient (Ca, K, Mg, Na) content in the soil was determined in 1 M CH_3COONH_4 with the spectrophotometer ICP-OES Thermo iCAP 6500 DUO (Ostrowska et al. 1991).

2.5 Data analysis

Statistical analysis was performed with the parametric *t* test and non-parametric Mann–Whitney *U* test using Statistica 10.0 (StatSoft 2011) at the level of significance α=0.05. The non-parametric test was used because normality through the Shapiro–Wilk test and homogeneity of variance by the Levene's test were not always found. Communities of ECM fungi were described by species richness (number of identified

Table 2 Parameters of shoots and roots of 1-year-old *Abies alba* seedlings regenerating in fir stands (F) and pine forecrops (P)

Parameter	F1	F2	F3	P1	P2	P3	F	P
Height (cm)	4.64	4.96	5.06	4.76	4.43	4.82	4.89a[a]	4.67a
Diameter (mm)	0.82	0.98	0.98	0.88	0.98	0.89	0.93a[a]	0.92a
Dry weight (g)	0.039	0.061	0.050	0.040	0.050	0.040	0.050a[a]	0.042b
Mycorrhizal seedlings (%)	90.0	96.7	100.0	96.7	93.3	83.3	95.6	91.1
Live mycorrhizas (%)	79.4	99.7	100.0	81.8	97.8	88.6	93.0	89.4
Non-mycorrhizal roots (%)	18.9	0.3	0.0	18.2	2.2	0.6	6.4	7.0
Dead mycorrhizas (%)	1.7	0.0	0.0	0.0	0.0	10.8	0.6	3.6

[a] Numbers with the same letters indicate lack of statistical differences between F and P sites

Table 3 Diversity of ectomycorrhizal fungal communities colonizing 1-year-old *Abies alba* seedlings in fir stands (F) and pine forecrops (P)

Diversity parameter	F1	F2	F3	P1	P2	P3	F	P
Richness per site	13	14	22	9	7	12	36	23
Richness per seedlings	1.7	2.3	3.3	1.5	1.4	1.5	2.4	1.5
Shannon–Wiener (*H'*)	2.32	2.29	2.44	1.67	1.78	2.10	2.94	2.71
Simpson (1-*D*)	0.88	0.87	0.85	0.74	0.81	0.83	0.91	0.90
Evenness (*e^H/S*)	0.78	0.71	0.52	0.59	0.84	0.68	0.52	0.65

mycorrhizal taxa), relative abundance (number of mycorrhizas of a given mycorrhizal species per total number of mycorrhizas in each site), and frequency (ratio of number of seedlings with given ECM taxa to total number of seedlings in each stand). To assess whether a sufficient number of samples were collected, the observed species accumulation curve and jackknife first-degree estimator curve with 100 randomization with sample replacement (allowing comparison of Sobs F with Sobs P *t* test) were plotted in the Estimates 8.2.0 program (Colwell 2006). To assess the ECM species diversity, Shannon–Wiener's (*H'*), Simpson's (1-*D*), and evenness (*e^H/S*) indicators were performed. The above coefficients, the analysis of similarities (ANOSIM), and the non-metric multidimensional scaling (NMDS) analysis were calculated in PAST 1.89 (Hammer et al. 2001). ANOSIM was used as an analysis of similarity to determine if the fungal symbionts communities differed between study sites. The variability of ECM composition among seedlings of different forest stands was visually modeled using NMDS. For ANOSIM and NMDS analyses standardized relative abundance data of each taxa after square root transformation was used. The above mentioned analyses were based on the Bray–Curtis coefficient (Bray and Curtis 1957).

3 Results

3.1 Seedling parameters

Mean heights of seedlings were 4.89 cm in the fir stands and 4.67 cm in the pine stands and were not significantly different. Mean diameter of seedlings did not differ between seedlings regenerating in the fir and pine stands. The dry weight of fir seedlings in the fir stands was statistically higher than that of seedlings in the pine stands (Table 2).

3.2 ECM colonization

A high mean percentage of mycorrhizas were observed in both types of seedlings (95.6 % in fir stands and 91.1 % in pine stands) and live ectomycorrhizas (93.0 and 89.4 %, respectively) in both groups of sites. The mean percentage of dead mycorrhizas was 0.6 % in the fir stands and 3.6 % in the pine forecrops (Table 2).

Sequencing analysis revealed a total of 49 mycorrhizal taxa on *A. alba* seedlings (Supplementary Table 1); 36 taxa were present on fir seedlings regenerating in the fir stands, and 23 taxa were present on fir seedlings regenerating in the pine forecrops (Table 3). The jackknife species richness values were estimated as 42 and 25, respectively (Fig. 1). Hence, the observed number of taxa was 86 % of the estimated richness in fir stands and 92 % of the estimated number of ECM species in pine forecrops. The observed number of ECM taxa in the fir stands was significantly higher than that in the pine forecrops (p=0.0001). Ten out of 49 taxa (20 %) were common to both stands. Thirty-one ECM taxa were identified to the species level (Supplementary Table 1, Fig. 2). Three morphotypes were not matched to any taxa; one of which was a brown ectomycorrhiza with a Hartig net and without a mantle (Supplementary Table 2). Molecular analysis revealed that this morphotype was formed by various taxa, which included *Russula integra*, *Thelephora terrestris*, *Tylospora* sp., and *Xerocomus badius* (data not published).

Species richness per site was higher in particular fir stands (13–22) than in pine (7–12) stands, showing similarity to the mean species richness per one seedling (Table 3). The Shannon–Wiener and Simpson's indices were higher for fir

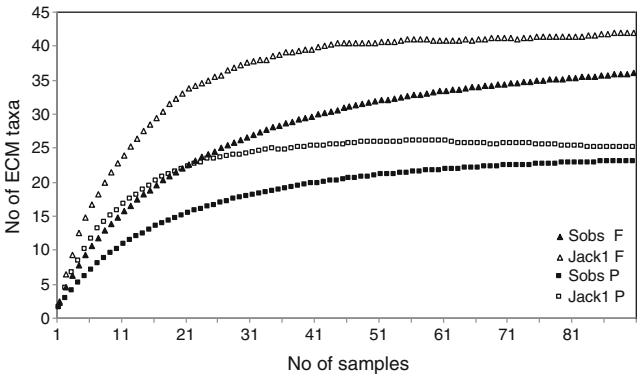

Fig. 1 Ectomycorrhizal species richness estimation curves for 1-year-old *Abies alba* seedlings regenerating in fir (*F*) and pine (*P*) stands. Sobs-species observed, Jack1-first order jackknife estimator (100 randomized runs with sample replacement were used)

stands (2.29–2.44 and 0.85–0.88) than for pine forecrops (1.67–2.10 and 0.74–0.83), respectively (Table 3).

Tomentella stuposa was the only species present at all sites, followed by *Cenococcum geophilum* noted in four stands (Table 4). *R. integra*, *Clavulina cristata*, *Tylospora asterophora*, *Tuber puberulum*, and *Clavulina* sp.1 were also commonly occurring species and noted at half of the investigated sites (Table 4). The most abundant species on seedlings

Fig. 2 Morphological features of selected ectomycorrhizas observed on 1-year-old *Abies alba* seedlings (magnification 20–40×)

Table 4 Relative abundance (RA) and frequency (FR) of ectomycorrhizal taxa on the roots of 1-year-old *Abies alba* seedlings regenerating in fir stands (F1, F2, F3) and pine forecrops (P1, P2, P3)

Fungal species	F1		F2		F3		P1		P2		P3	
	RA	FR	RA	FR	RA	FR	RA	FR	RA	FR	RA	FR
Amanita muscaria (L.) Lam.					1.6	13.3						
Amanita spissa (Fr.) P. Kumm.									7.1	16.7		
Amphinema byssoides (Pers.) J. Erikss.			0.5	3.3								
Boletus edulis Bull.	1.8	6.7			0.7	6.7						
Boletus pruinatus Fr. and Hök			0.3	3.3	3.9	13.3						
Byssocorticium sp.							0.4	3.3				
Cenococcum geophilum Fr.	25.2	46.7	7.4	13.3	2.7	16.7	12.7	30.0				
Clavulina cristata (Holmsk.) J. Schröt	4.3	10.0					45.8	50.0	31.0	33.3		
Clavulina rugosa Bull. J. Schröt											12.8	26.7
Clavulina sp. 1					1.4	10.0	4.4	3.3			1.0	3.3
Clavulina sp. 2									20.1	33.3		
Cortinarius sp. 1					0.5	10.0						
Cortinarius sp. 2			2.0	3.3	1.4	10.0						
Elaphomyces muricatus Fr.					2.1	6.7						
Hydnotrya sp.					2.8	6.7						
Hydnum repandum L.			12.2	10.0								
Hydnum rufescens Schaeff.					2.3	3.3						
Inocybe terrigena (Fr.) Kühner	5.3	6.7										
Laccaria amethystina (Huds.) Cooke	3.9	10										
Laccaria maritima (Theodor.) Singer ex Huhtinen			4.8	16.7								
Lactarius aurantiacus (Pers.) Gray					8.2	30.0						
Lactarius salmonicolor R. Heim and Leclair	3.9	6.7	5.7	20.0								
Leotiomycetes					6.2	13.3						
Paxillus involutus (Batsch) Fr.					1.1	3.3						
Piloderma byssinum (P. Karst.) Jülich	5.3	10.0			0.9	3.3						
Piloderma fallax (Lib.) Stalpers					2.5	13.3						
Pseudotomentella sp.							2.8	6.7				
Russula amethystina Quél.			11.8	16.7	4.8	20.0						
Russula integra (L.) Fr.	5.7	16.7	2.6	6.7			10.4	26.7				
Russula nigricans Fr.			11.6	23.3								
Russula olivacea (Schaeff.) Fr.					5.7	23.3						
Russula puellaris Fr.											5.7	3.3
Russula xerampelina (Schaeff.) Fr.							12.4	10.0				
Sebacina sp. 1											6.1	13.3
Sebacina sp. 2					0.7	3.3						
Sebacina sp. 3	8.2	13.3							11.4	6.7		
Thelephora terrestris Ehrh.											11.8	20.0
Thelephoraceae											4.1	6.7
Tomentella ellisii (Sacc.) Jülich and Stalpers									10.2	6.7		
Tomentella sp.	13.5	13.3									33.4	30.0
Tomentella stuposa (Link) Stalpers	7.4	16.7	5.9	30.0	7.5	30.0	2.0	13.3	3.8	20.0	0.7	3.3
Tomentellopsis sp.											4.1	13.3
Tuber puberulum Berk. and Broome			3.3	6.7					16.5	23.3	6.4	13.3
Tuber sp.							9.2	10.0				
Tylospora asterophora (Bonord.) Donk			7.0	23.3	6.8	16.7					5.1	10.0
Unidentified 1			24.8	56.7	35.1	70.0						
Unidentified 2	12.1	13.3										
Unidentified 3	3.5	3.3										
Xerocomus badius (Fr.) Kühner					1.1	3.3					8.8	10.0

from the fir stands were unidentified fungus 1 (24.0 % in relative abundance), followed by *C. geophilum* (9.0 %), *Russula amethystina* (6.9 %), *T. stuposa* (6.8 %), and *T. asterophora* (5.6 %). Fir seedlings from the pine forecrops formed mycorrhizas mostly with *C. cristata* (25.2 %), *Tomentella* sp. (10.5 %), *T. puberulum* (8.9 %), and *Clavulina* sp. 1 (5.1 %) (Fig. 3).

According to the ANOSIM similarity analysis, the ECM fungal communities differed (R=0.1721; p=0.0001) between seedlings regenerated in the fir stands and pine forecrops; this was confirmed visually by NMDS analysis (Fig. 4).

3.3 Soil parameters

Table 1 provides soil parameters showing the pH value was similar among study sites, in the range of 4.3–5.7 in H_2O and 3.3–4.8 in KCl; the C/N ratio had a range of 13.2–15.5 between study sites. A high content of Ca and Mg was noted in the pine forecrops P1 and P2. The content of potassium had a range of 0.5–1.2 in the F sites to 0.7–8.2 mg/kg in the P sites.

4 Discussion

Mycorrhizal colonization of fir seedlings was very similar to that of other coniferous trees (Aučina et al. 2011; Leski and Rudawska 2012; Teste et al. 2009). Sequencing analysis identified 49 ECM taxa associated with 1-year-old *A. alba*. In agreement with the present work, Comandini et al. (2001) classified a similar number of ECM morphotypes (48) on mature trees of *A. alba* in the Apennines. This stands in contrast to only 25 morphotypes found on seedlings at the same site (Comandini et al. 1998). Cremer et al. (2009) identified 33 taxa of *A. alba* symbionts in German forests by molecular means. Based on anatomical and morphological descriptions, Kowalski (2008) found 35 ECM types on silver

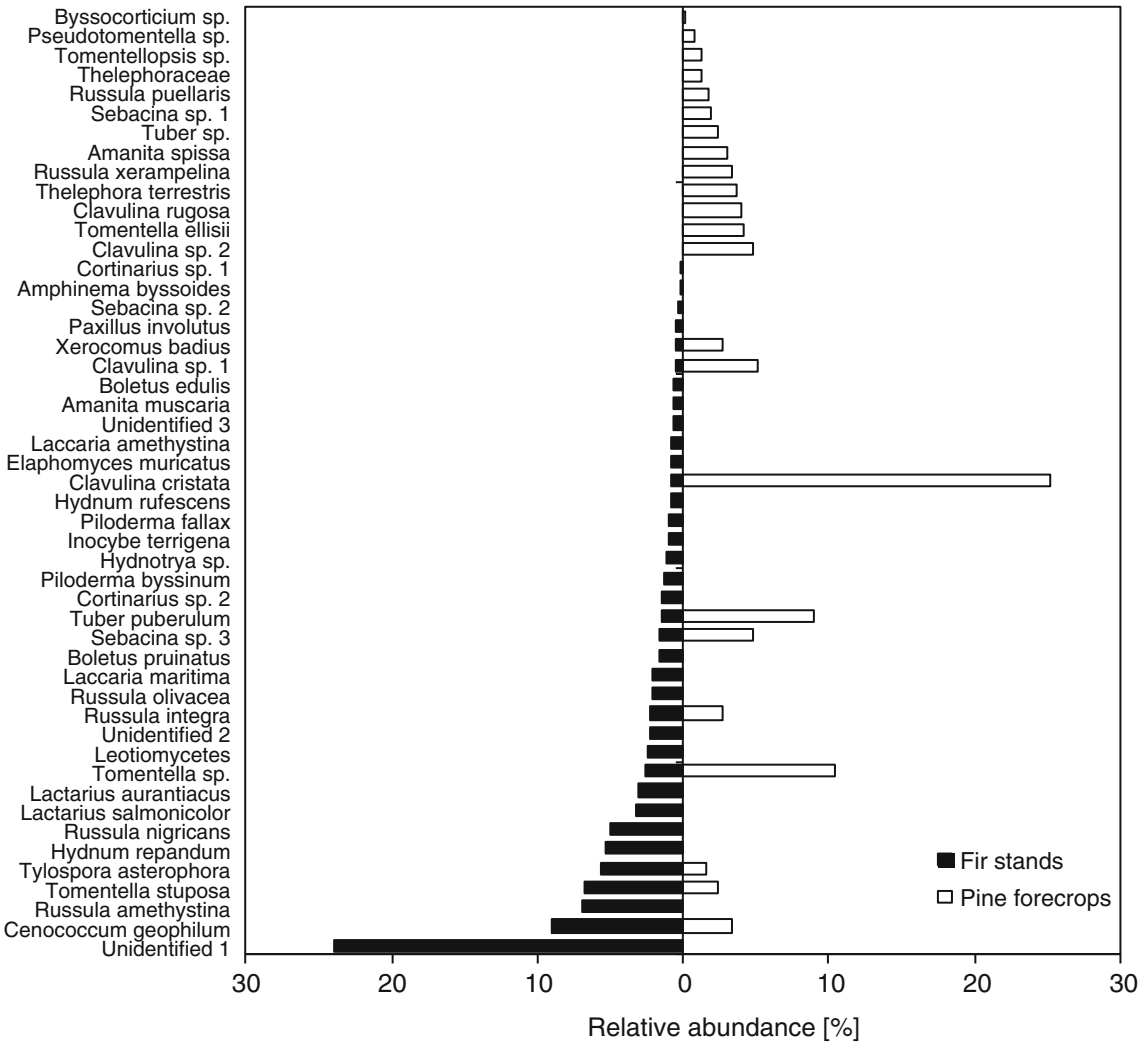

Fig. 3 Mean relative abundance of ectomycorrhizal fungi on 1-year-old *Abies alba* seedlings regenerating in fir stands and pine forecrops

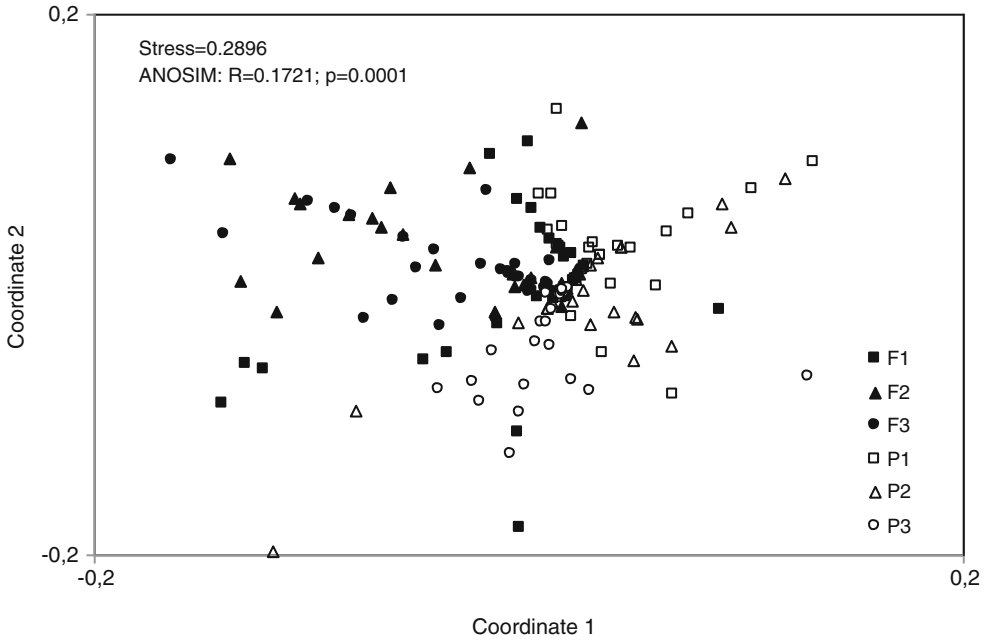

Fig. 4 Non-metric multidimensional scaling ordination of ectomycorrhizal fungal communities on 1-year-old *Abies alba* seedlings regenerating in fir stands (F1, F2, F3) and pine forecrops (P1, P2, P3)

fir seedlings in the Karkonosze National Park (Poland). In a forest nursery, Stępniewska and Rębisz (2004) found only seven different types of ECM on fir seedlings.

Some of the ECM taxa described here have been recognized as symbionts of silver fir in previous studies, including: *Amphinema byssoides*, *C. cristata* (Cremer et al. 2009), *Lactarius aurantiacus*, *Piloderma fallax*, *T. puberulum*, *T. asterophora* (Smutek et al. 2010), *Boletus pruinatus*, *C. geophilum*, *Laccaria amethystina*, *T. stuposa* (Cremer et al. 2009; Smutek et al. 2010), and *Lactarius salmonicolor* (Agerer 1987–2007; Eberhardt et al. 2000). Some of the identified symbionts of silver fir have also been found as symbionts of other fir species. Kranabetter et al. (2009) identified *C. geophilum*, *Amphinema byssoides*, and *T.* cf. *stuposa* among the ECM symbionts of *Abies lasiocarpa*, whereas Matsuda and Hijii (1999, 2004) found *C. geophilum*, *Russula* spp., *Lactarius* spp., *Tuber* sp., and the thelephoroid fungus on *Abies firma*. ECM symbionts of *Abies homolepis* belonged mainly to the following genera: *Amanita*, *Boletus*, *Cenococcum*, *Cortinarius*, *Inocybe*, *Laccaria*, *Lactarius*, *Russula*, *Sebacina*, *Tomentella*, and *Tuber* (Ishida et al. 2007).

There were identified *Clavulina rugosa* and *Inocybe terrigena* as novel ECMs that were associated with *A. alba*. In addition, there were identified several ECM fungal species known to associate with other coniferous tree species from the genus *Picea*, *Pinus*, and *Pseudotsuga* and with deciduous species *F. sylvatica* as symbionts of silver fir for the first time on record. These ECM species were *Amanita spissa* (De Román et al. 2005), *Boletus edulis* (Korkama et al. 2006), *Elaphomyces muricatus* (Menkis et al. 2010), *Hydnum rufescens* (Agerer 1987–2007), *Laccaria maritima* (Lang et al. 2011), *Russula nigricans*, *Russula xerampelina* (Agerer 1987–2007; Teste et al. 2009), and *Tomentella ellisii* (Obase et al. 2009).

The most predominant fungal taxon was *T. stuposa*, which was found in all sites. This was followed in frequency by *C. geophilum*, *C. cristata*, *Clavulina* sp.1, *R. integra*, *T. asterophora*, and *T. puberulum*, which were present at a minimum of three sites. These results agree with previous reports that identified *C. geophilum* and *T. stuposa* as frequent and abundant ECM symbionts of fir (Cremer et al. 2009). *C. geophilum* is ubiquitous and a dominating ECM species (Aučina et al. 2011; Teste et al. 2009). The genus *Tomentella* forms mycorrhizas with different tree species and develops morphotypes as has been frequently described in the literature (Jakucs and Erős-Honti 2008). In addition, the genus *Tylospora*, represented in this investigation by *T. asterophora*, is one of the most abundant genera-forming ectomycorrhizas on other coniferous tree species (i.e., *P. abies*) (Eberhardt et al. 1999).

A surprisingly high relative mean abundance (25 %) of ectomycorrhizas formed by *C. cristata* was detected on fir seedlings regenerating in pine forecrops. This finding was hypothesized to be a result of the high concentrations of Ca and Mg in the soil of P1 and P2 forecrops. Rineau and Garbaye (2009) showed that liming (Ca–Mg soil amendment) positively affects the development of *C. cristata* ectomycorrhizas in spruce (from 0 to 53 %) and beech (from 8 to 19 %) stands. The abundant brown mycorrhizas with the Hartig net and without a mantle were among the unidentified ECM morphotypes in fir stands. Molecular analysis revealed that this morphotype was made of various taxa; this suggests that the initial stadia of mycorrhiza of these taxa are similar and differ later on. For instance, this morphotype was not as frequent on 2-year-old *A. alba* seedlings (data not published). Nylund and Unestam (1982) reported that

the Hartig net can appear before the fungal mantle. *L. salmonicolor*, one of the symbionts known to be specific to *A. alba*, was noted only in two sites and was not as frequent as in studies carried out in central Italy (Comandini et al. 2001).

ECM richness was significantly higher in particular fir stands (13–22) than in pine stands (7–12); this was also confirmed by Shannon–Wiener's and Simpson's diversity indices. According to the analysis of similarity, the ECM fungal communities on *A. alba* seedlings differed between fir and pine stands. Because common mycorrhizal networks (CMN) are present in forest soils (Kennedy et al. 2003; Teste et al. 2009), fir seedlings regenerating under a canopy of pine trees that are in close proximity to fir can be colonized by ECM fungi from mature silver fir trees in adjacent stands. However, in this case, because the pine and fir stands support different species of fungi, the seedlings in pine stands may be affected by pre-existing ECM communities of mature pine caused by the presence of CMN and spore banks.

Only 20 % of symbionts of silver fir seedlings were common to fir and pine stands. This suggests that succession of ECM fungi specific to fir regenerating on formerly arable land had been afforested with pine about 50 years ago is ongoing and continues in the present day. Because silver fir invades under the canopy of pine forests, it is positioned to become the dominant plant species after the pines are lost in the future to competition. Natural regenerating and outplanted seedlings belong to the first age class; this may also indicate that these seedlings initially shared ECM with coexisting pine trees and became colonized by specific ECM many years after their establishment. Ishida et al. (2007) showed that the ECM community of *Abies homolepis* in the secondary forest site, which had been clear-cut over 70 years ago, more closely resembled the EMF communities of broadleaf trees from the same forest than that of *A. homolepis* in the primary old-growth forest.

It was found that ECM species richness was significantly lower in pine forecrops than in fir stands, but fungal colonization of seedling roots in pine forecrops was as high as in mature silver fir forest. The both hypotheses were confirmed. This suggests that the development of ECM of fir seedlings regenerating in pine stands afforested on formerly arable land allows these seedlings to survive and develop, enabling the potential process of secondary succession (Jaworski 2000).

This is the first report concerning ECM of *A. alba* seedlings regenerating in pine forecrops using molecular approaches. These results significantly increase our knowledge of the ECM fungal species associated with *A. alba*. Future work aimed at understanding why ECM fungal communities were different in seedlings of fir stands than those of pine forecrops will complement these findings. In addition, studying how differences in the pre-existing ECM fungal communities between fir stands and pine forecrops affect the mycorrhizal status and establishment of fir seedlings of different age classes in pine forecrops would be worthwhile. Such a study would provide more information on the diversity of ECM fungal partners of silver fir seedlings regenerating in pine stands on formerly arable areas and the possibilities of their successful regeneration.

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