Forest litter as the mulch improving growth and ectomycorrhizal diversity of bare-root Scots pine (*Pinus sylvestris*) seedlings

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In this paper, we report the influence of pine, oak and spruce forest litter on the growth and ectomycorrhizal (ECM) formation of Scots pine seedlings after the first growing season in a bare-root forest nursery. The mixture of collected forest litters and humus were used to obtain a 20-cm mulching layer on the prepared seedbeds. The concentrations of all nutrients and the C/N ratio of growth media were significantly higher in forest litter treatments than in negative control represented by mineral soil without litter. Addition of each forest litter type significantly enhanced pine seedling height and root-collar diameter compared to negative control. A significant positive influence on dry mass of stem, needles, roots and total dry mass of the seedling has been found only for pine litter. Based on molecular identification, seven ECM fungal taxa (*Wilcoxina mikolae*, *Suillus luteus*, *Cenococcum geophilum*, *Meliniomyces bicolor*, *Laccaria laccata*, unidentified *Atheliaceae*, unidentified *Ascomycetes*) were distinguished in the observed mycorrhizal communities. Each forest litter type significantly increased the total number of mycorrhizal tips and ECM fungal diversity compared to the control soil. However, results showed a lack of significant differences in species composition and relative abundance of ECM fungi between different litter types. Such result suggests that forest litter has not been a key source of inoculum for tested fungal species, as root systems of all pine seedlings from different litter types were dominated by a few nursery-adapted ECM fungi, probably originating from natural air-borne inoculum. Our data rather indicate that forest litter considerably improves environmental conditions for development of ECM fungi previously present in the nursery soil. Therefore, any of the forest litter types used in our studies may be able to promote planting stock quality on a small scale in the nursery phase.

Keywords: ***Pinus sylvestris*, Seedlings, Forest Nursery, Ectomycorrhiza**

Introduction

Scots pine (*Pinus sylvestris* L.) is a broadly distributed forest tree species relevant in terms of planted areas and harvest yields (Richardson 1998). Scots pine grows only naturally in Lithuania (Danusevičius 2000) and represents approximately 37% of the forest area (Lithuanian State Forest Survey Service 2003). Lithuania produces close to 80 million tree seedlings per year for reforestation and afforestation of abandoned agricultural lands, and approximately 20% of these are Scots pine (Lithuanian State Forest Management Institute 2001). The entire seedling stock in Lithuania is produced in bare-root forest nurseries. The mass production of tree seedlings in bare-root nurseries drastically reduces the soil organic matter content without the possibility of its natural regeneration (Gorzelak 1998). The decline of organic matter content in nursery soil often leads to decreased microbiological activity and negatively influences the diversity of ectomycorrhizal (ECM) fungi (Kropp & Langlois 1990). ECM fungi are a component of all forest trees and play a significant role in the uptake of soil nutrients and water, and inhibit the negative influence of soil borne pathogens and abiotic stresses (Smith & Read 2008). Deficiency of mycorrhizae or low species richness of ECM fungi on the root systems of tree seedlings in forest nurseries is one of the most common causes of poor seedling establishment and growth after transplanting (Amaranthus & Perry 1987, Pera et al. 1999, Dunabeitia et al. 2004). In contrast, high diversity of ECM fungi in bare-root seedlings production improves their growth and survival, and as a consequence the use of mineral fertilizers may be significantly reduced (Khasa et al. 2001).

Forest litter is known as a rich source of soil organic matter and nutrients (Jurgensen et al. 1997, Giardina et al. 2001, Franklin et al. 2003), and may significantly improve the physical characteristics of the soil (Balisky et al. 1995, Hallsby 1995). The use of forest litter in nursery management practices may also revitalize the soil by the input of thousands of individual living organisms which decompose organic matter and facilitate seedling competition for nutrients (Rose et al. 1995, Lukac & Godbold 2011). The investigations performed by the Dunemann scheme indicated a better germination, growth and survival of conifer seedlings in spruce litter than in mineral soil (Hutt 1956). However, data regarding the effect of litter addition on ECM colonization and community structure are inconsistent. Both positive and negative effects of forest litter amendment have been observed in different experiments (Brearley et al. 2003, Cullings et al. 2003, Aučina et al. 2007).

In our previous experiment, natural forest litter cover on the surface of the nursery bed soil considerably improved two years old Scots pine seedling survival and positively affected their ECM communities (Aučina et al. 2007). However, in present forestry practice the use of two years old seedlings tends to be rare and the use of one-year-old seedlings, especially on dry sites, is becoming increasingly frequent (Barzdajn 2009). Nonetheless, information about the effectiveness of different forest litter types on one year old planting stock quality is limited to only laboratory studies with tree species other than Scots pine (López-Barrera & González-Espinosa 2001).

The aim of the present investigation was to evaluate how different forest litter types (pine, oak and spruce) affect growth and

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ECM formation of Scots pine seedlings after the first growing season in a bare-root forest nursery. We hypothesize that each of the forest litter type will differently affect: (i) the tested growth parameters of the seedlings; and (ii) quantitative and qualitative structure of ECM fungal community compared with nursery soil without litter amendments.

Materials and methods

Litter collection and seedling material

In March 2010, forest litter was collected in healthy, natural oak, pine and spruce stands of similar age, where natural seedling regeneration was abundant. The 90-years-old *Quercus robur*-dominated stand was classified as an aegopodiosa type with soil characterized as Eutric Planosols (FAO 1998). To get a growth medium, litter (2 cm deep) on the top layer of the soil and humus horizon (12 cm deep), both taken from the same spot, was thoroughly mixed. The 80-years-old Scots pine-dominated stand was classified as a nemoral type with *Vaccinium myrtillus* in the understory and soil characterized as Albic Arenosols (FAO 1998). The humus horizon was thin and spread to a uniform depth of 4 cm. The 70-years-old Norway spruce-dominated stand was classified as an oxalidosa type with soil characterized as Haplic Arenosols (FAO 1998). Litter (3 cm deep) on the soil top layer was mixed with a 10-cm-deep humus horizon. The mixture of litter and humus has been stored in a forest as a heap and protected from direct sunlight with twigs of trees until further processing (ca. 3 weeks). From each growth medium, fine and coarse roots have been carefully picked up.

In April 2010, the mixture of collected forest litters and humus were transferred to the experiment site located in the bare-root nursery of the Vilnius University Botanical Garden (54° 43′ N, 25° 24′ E) and used as mulching layer (20 cm deep, approx. 40 kg m^{-2}), allowing the largest part of the root system to grow up in the litter. A mixture of vermiculite and perlite (as chemically neutral substances) at a volume of 15% and 10%, respectively, was used to maintain the appropriate microclimate (moisture content, temperature and porosity) for plant growing in each growth medium (with and without litter amendments). The experimental design was divided into five complete blocks with four plots per oak litter, pine litter, spruce litter and control (mineral nursery soil), randomly allocated in each block. Each treatment variant was spaced approximately 1 m. The pine seeds for the trial originated from the local provenance of Labanoras (55° 16′ N, 25° 50′ E). The seeds of Scots pine required no stratification and a 3x3 cm sowing stencil was used. Two seeds were sown directly by hand into a single hole at the end of April 2010. Germinants were then thinned to one per hole. During the first growing season seedlings were manually weeded and irrigated, but not fertilized.

After the first year of growth under nursery conditions, 40 randomly selected seedlings were harvested from each block (50 seedlings per growth medium, 200 seedlings in total). The excavated seedlings with their surrounding soil were placed into labelled plastic bags and transported to the laboratory for subsequent mycorrhizal assessment and shoot height, root collar diameters and biomass measurement. Seedling biomass was determined after drying the plant material at 70 °C for 48 h.

Chemical analysis

For each plot, five soil cores were randomly sampled from the upper 15 cm of growth medium, where bare-root seedlings grew. Soil samples from growth medium were mixed, dried at 40 °C, and soil carbon content and the nutrient composition (N, P, K, Mg and Ca) were determined in four repetitions. A complete description of the growth medium analysis is presented in our earlier paper (Aučina et al. 2007).

Mycorrhizal evaluation

One-year-old seedlings with surrounding soil were stored at -20 °C until processing. Frozen seedlings were processed in sets of ten to minimize the impact of thawing on fine root observations. The root systems of seedlings and single roots were extracted from the soil using a 0.5 mm mesh-sieve under tap water. The clean roots were cut into approximately 2-cm long fragments and placed in a Petri dish filled with water. Only roots with a diameter ≤ 2 mm were observed. The assessment with the aid of a stereomicroscope Zeiss Stemi 2000-C (Carl Zeiss, Germany; 10-60× magnification) was performed by counting all ECM root tips (85-249 root tips on average were sampled from each growth medium). Live roots (identified as swollen, without root hair or covered by fungal mantles) were considered ECM-colonized roots. Mycorrhizas were classified into morphotypes based on morphological characteristics (ramification system; colour, shape, texture and thickness of the mantle; presence and organization of the emanating hyphae; rhizomorphs; and other elements) according to Agerer (1987) and database from our Laboratory of Mycorrhizal Research at the Institute of Dendrology. In doubtful cases (*e.g.*, thin or non-evident mantle) we made a cross-section of potential mycorrhizas to determine the presence of a Hartig net and a mantle under the compound microscope Axio Imager A1 (Carl Zeiss, Germany) at 400-1000× magnification. The number of live mycorrhizas of each morphotype was recorded separately for each seedling. The relative abundance of each morphotype (number of root tips of each morphotype/total number of mycorrhizas) was calculated for each seedling.

The representative samples of three to four root tips for each morphotype from each growth medium were stored in a 2% cetyltrimethylammonium bromide buffer for molecular identification. No attempt was made to relate morphotypes between growth media until molecular analysis was complete; thus, each morphotype was treated separately in the molecular identification and pooled for abundance calculation only after the molecular analysis indicated that morphotypes were identical and belonging to the same taxa. The full methods used for molecular identification of mycorrhizas are reported in Pietras et al. (2012). Fungal symbionts were identified using polymerase chain reaction (PCR) amplification of the internal transcribed spacer of rDNA (ITS rDNA) using the ITS-1F and ITS-4 primers (White et al. 1990). The selected samples with ECM root tips were then sequenced, and in total 98 sequences were obtained. Sequencing of the PCR products was performed with a CEQ 20000XL automatic sequencer using the same set of primers. Consensus sequences were constructed, with manual editing of ambiguous readings, and were compared to sequences published in the GenBank and UNITE databases (Kõljalg et al. 2005) using the BLAST tool. Species-level identification of mycorrhizae was defined as sharing ≥ 98% of the ITS region sequence identity with the reference sequence. The best representative of each unique ITS sequence type was deposited in NCBI GenBank with the accession numbers KJ596425-KJ596431.

Statistical analysis

The chemical composition of soils, growth and biomass parameters, the amount of mycorrhizal tips and the mean fungal species richness per seedling were analysed by two-way analysis of variance, with the growth medium as a fixed factor and the block as the random factor. Normal distribution of data and homogeneity of variance were tested through the Shapiro-Wilk's and Levene's test, respectively. *Post-hoc* comparisons of means among the examined growth media were made using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). As for relative abundances, no homogeneity of variance was found; therefore, differences in the relative abundance of morphotypes between growth media were tested using the Kruskal-Wallis and Mann-Whitney U tests. Prior to analysis, the relative abundance of mycorrhizal morphotypes was arcsine square root transformed. Computations were performed using the statistical software package Statistica 5.5 (StatSoft Inc. 2000). Estimates of the true species richness (bootstrap and

Chao 2) were calculated using the EstimateS program version 8.2.0 (Colwell 2006). Shannon's diversity indices for the ECM assemblages of Scots pine seedlings were carried out using the PAST1.89 software (Hammer et al. 2001) and were based on square root transformed data.

Multiple regressions were applied to assess the relationships between nutrient composition and the pH of growth media and the total number of mycorrhizal tips, species richness of ECM fungi, and growth parameters of the seedlings. Stepwise multiple regression with backward elimination was used after screening the potential independent variables (total number of mycorrhizal tips, species richness of ECM fungi) for significant autocorrelation. Dependent variables were log-transformed to improve their linearity. The total number of mycorrhizal tips and species richness of ECM fungi were log(n+1) transformed.

Results

The nutrient content and pH of growth media after one year of seedling growth are summarized in Tab. 1. Significant differences between forest litter types and control soil were found for all nutrient concentrations, C/N ratio and pH_{KCl} of growth media. Concentrations of nutrients and the C/N ratio were highest in spruce litter. Mean values of soil total N, NH_4^+, NO_3^-, K, Ca, Mg, C and the C/N ratio were intermediate in oak and pine litters, and lowest in the control soil. The concentration of soil P was intermediate in the control soil and oak litter, and lowest in pine litter. The pH_{KCl} was highest for the control soil, intermediate for pine and oak litters, and lowest for the spruce litter. The block effect was not significant (two-way ANOVA, $P = 0.859$).

The statistical analysis of growth parameters revealed an overall significant differen-

Tab. 1 - Nutrient composition and pH of growth media at harvest of *Pinus sylvestris* L. seedlings after one year of growth in a bare-root nursery with mineral soil and growth media with pine litter, oak litter and spruce litter (values are mean ± SE, n=16). Within a row, values with different letters are significantly different after Tukey's test ($P < 0.05$).

Parameter	Growth medium				P>F
	Pine litter	Oak litter	Spruce litter	Mineral nursery soil (control)	
N_{total} (%)	0.12 ± 0.01 ^{c}	0.22 ± 0.01 ^{b}	0.31 ± 0.02 ^{a}	0.06 ± 0.01 ^{d}	0.0001
C (%)	1.88 ± 0.01 ^{c}	3.77 ± 0.03 ^{b}	6.64 ± 0.06 ^{a}	0.70 ± 0.02 ^{d}	0.0001
C/N	16.00 ± 0.91 ^{bc}	17.50 ± 0.57 ^{b}	22.30 ± 1.23 ^{a}	12.40 ± 1.41 ^{c}	0.0001
NH_4-N (mg kg^{-1})	0.28 ± 0.01 ^{c}	0.86 ± 0.01 ^{b}	1.77 ± 0.01 ^{a}	0.12 ± 0.01 ^{d}	0.0001
N-NO_3(mg kg^{-1})	0.47 ± 0.01 ^{c}	1.57 ± 0.01 ^{b}	4.12 ± 0.03 ^{a}	0.24 ± 0.02 ^{d}	0.0001
P (mg kg^{-1})	1.28 ± 0.01 ^{d}	1.79 ± 0.02 ^{c}	10.30 ± 0.04 ^{a}	2.08 ± 0.04 ^{b}	0.0001
K (mg kg^{-1})	15.70 ± 0.08 ^{c}	20.00 ± 0.13 ^{b}	62.40 ± 0.37 ^{a}	9.88 ± 0.07 ^{d}	0.0001
Ca (mg kg^{-1})	10.70 ± 0.05 ^{c}	14.70 ± 0.08 ^{b}	39.30 ± 0.17 ^{a}	6.62 ± 0.08 ^{d}	0.0001
Mg (mg kg^{-1})	4.31 ± 0.05 ^{c}	5.69 ± 0.05 ^{b}	15.90 ± 0.08 ^{a}	2.73 ± 0.03 ^{d}	0.0001
pH_{KCl}	4.81 ± 0.04 ^{b}	4.65 ± 0.04 ^{c}	3.93 ± 0.03 ^{d}	5.66 ± 0.01 ^{a}	0.0001

Tab. 2 - Growth parameters of *Pinus sylvestris* L. seedlings after the first growing season in a nursery with mineral soil and growth media with pine litter, oak litter and spruce litter (values are mean ± SE, n=50). Within a row, values with different letters are significantly different after Tukey's test ($P < 0.05$).

Parameter	Growth medium				P>F
	Pine litter	Oak litter	Spruce litter	Mineral nursery soil (control)	
Seedling height (cm)	9.00 ± 0.39 ^{a}	7.90 ± 0.46 ^{a}	8.50 ± 0.36 ^{a}	5.10 ± 0.30 ^{b}	0.0001
Root collar diameter (mm)	1.70 ± 0.04 ^{a}	1.60 ± 0.07 ^{a}	1.60 ± 0.05 ^{a}	1.40 ± 0.06 ^{b}	0.0001
Stem dry weight (g)	0.09 ± 0.01 ^{a}	0.07 ± 0.01 ^{ab}	0.08 ± 0.01 ^{ab}	0.04 ± 0.00 ^{b}	0.001
Needles dry weight (g)	0.29 ± 0.04 ^{a}	0.17 ± 0.03 ^{b}	0.16 ± 0.02 ^{b}	0.09 ± 0.01 ^{b}	0.0001
Roots dry weight (g)	0.17 ± 0.03 ^{a}	0.10 ± 0.02 ^{b}	0.10 ± 0.01 ^{b}	0.06 ± 0.00 ^{b}	0.0001
Total seedling dry weight (g)	0.55 ± 0.07 ^{a}	0.33 ± 0.05 ^{b}	0.32 ± 0.03 ^{b}	0.18 ± 0.02 ^{b}	0.0001
Ratio of above-/ belowground weight	2.30 ± 0.09	2.40 ± 0.16	2.30 ± 0.15	2.20 ± 0.10	0.723

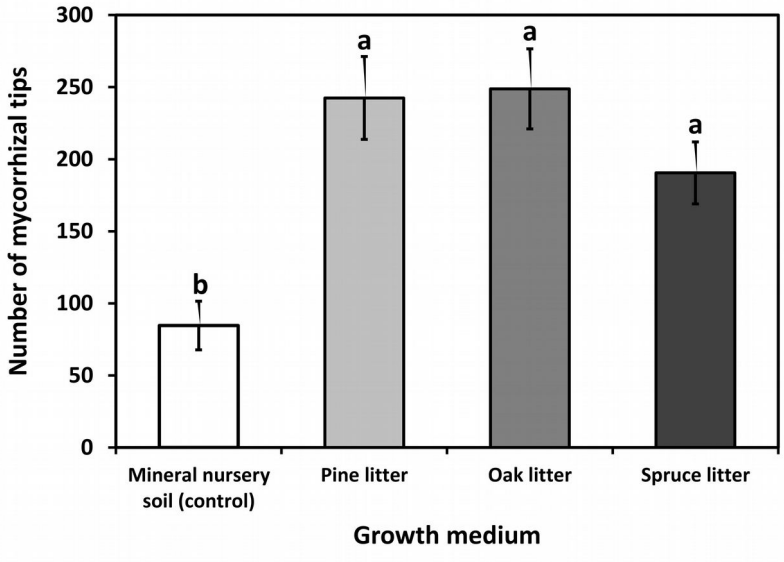

Fig. 1 - Total number of mycorrhizal tips on Scots pine seedlings after the first growing season in a bare-root nursery with mineral soil and growth media with pine litter, oak litter and spruce litter. Each bar shows the mean for 50 replicates ± standard error (SE). Different letters indicate significant differences between growth media ($P<0.05$, Tukey's test).

Tab. 3 - Molecular identification, relative abundance, observed total and mean species richness (± SE), and estimated species richness of ECM fungi on the roots of *Pinus sylvestris* L. seedlings after one year of growth in a bare-root nursery with mineral soil and growth media with pine litter, oak litter and spruce litter (values are mean ± SE, n=50). Within a row, values with different letters are significantly different (P<0.05; Mann-Whitney U test for relative abundance and Tukey's test for mean species richness per seedling and Shannon diversity index).

Group	Identification	Accession	Closest match	Identity (%)	Relative abundance (%) - Growth medium			
					Pine litter	Oak litter	Spruce litter	Mineral nursery soil (control)
Asco-mycota	*Wilcoxina mikolae*	KJ596425	*Wilcoxina mikolae* (JQ310818)	99	84.8 ± 3.6	87.1 ± 3.2	89.1 ± 2.9	96.3 ± 3.7
	Meliniomyces bicolor	KJ596426	*Meliniomyces bicolor* (HM190124)	98	8.5 ± 2.4	8.8 ± 2.4	5.9 ± 2.6	-
	Cenococcum geophilum	KJ596427	*Cenococcum geophilum* (HM189727)	99	1.9 ± 0.6	0.9 ± 0.7	2.2 ± 0.8	-
	Ascomycetes	KJ596428	unidentified *Ascomycetes* (JN172989)	89	1.7 ± 1.2	0.5 ± 0.5	1.2 ± 1.0	-
Basidio-mycota	Atheliaceae	KJ596429	*Amphinema* sp. (JN943925)	91	0.5 ± 0.6	2.4 ± 1.8	-	3.7 ± 3.7
	Suillus luteus	KJ596430	*Suillus luteus* (UDB000930)	98	2.4 ± 1.7	0.3 ± 0.2	0.2 ± 0.2	-
	Laccaria laccata	KJ596431	*Laccaria laccata* (UDB000106)	98	0.1 ± 0.1	0.1 ± 0.1	1.5 ± 0.8	-
Statistics	Observed species richness			-	7	7	6	2
	Mean species richness per seedling			-	2.9 ± 0.4 [a]	2.8 ± 0.4 [a]	3.0 ± 0.3 [a]	1.1 ± 0.1 [b]
	Chao 2			-	8	7.67	6.5	2
	Bootstrap			-	7.91	8.02	6.46	2.35
	Shannon diversity index (H′)			-	0.47 ± 0.10 [a]	0.39 ± 0.09 [a]	0.37 ± 0.07 [a]	0.07 ± 0.07 [b]

ces among growth media (two-way ANOVA, F=6.477, *P*=0.0001), with the block having no significant influence (two-way ANOVA, F=0.388, *P*=0.819 - Tab. 2). The height and root-collar diameter of the seedlings from all litter types were significantly higher than seedlings from the control mineral soil. Only pine litter significantly increased standing biomass of seedlings (dry weight of total seedling, stem and needles; *post-hoc* Tukey's HSD test - Tab. 2).

In all growth media, the rate of mycorrhizal colonization of Scots pine seedlings was high and neared 100%. The total number of mycorrhizal tips was significantly higher (two-way ANOVA, *P*=0.0003) for the litter variants than for the control (Fig. 1). The block effect was not significant (two-way ANOVA, *P*=0.713). On seedlings from all growth media, seven ECM fungal taxa (*Wilcoxina mikolae, Suillus luteus, Cenococcum geophilum, Meliniomyces bicolor, Laccaria laccata*, unidentified *Atheliaceae*, unidentified *Ascomycetes*) have been found: two taxa in the control mineral soil, seven in the pine and oak litter types and six in spruce litter type (Tab. 3). The mean fungal species richness per seedling ranged from 1.1 to 3.0, with significant differences among the litter types and the control soil (two-way ANOVA, *P*=0.001). The block effect was not significant (two-way ANOVA, *P*=0.534). According to the bootstrap species richness estimates, seedlings from oak litter type were estimated to be the most rich in ECM fungi (8.02 taxa), with control seedlings showing the lowest species richness estimates (2.35 taxa - Tab. 3).

W. mikolae was the most common taxon and dominated ECM communities on pine seedlings from all growth media. The mycorrhizas formed by *M. bicolor, C. geophilum*, unidentified *Ascomycetes, S. luteus* and *L. laccata* were present on pine seedlings grown in all forest litter types. Mycorrhizas formed by *Atheliaceae* sp. were detected on seedlings from the control soil and oak and pine litters. No significant differences in ECM abundance between the growth media were found (Tab. 3). The Shannon's diversity indices for the ECM assemblages of Scots pine seedlings ranged from 0.07 to 0.47 and revealed significant differences between litter types and the control soil.

With regard to seedling height, results from the stepwise multiple regression analysis indicated significant components of beta for the total number of mycorrhizal tips and species richness of ECM fungi. The rest of the tested growth parameters (root collar diameter and stem, needles and roots dry weight) showed significant positive interactions with total number of mycorrhizal tips, but not with species richness of ECM fungi. When carbon, mineral content and pH of growing medium were entered into the stepwise multiple regression model, total nitrogen content explained a significant amount of variation

Tab. 4 - Results from the stepwise multiple regression analysis between the total number of mycorrhizal tips, species richness of ECM fungi, nutrient composition and the pH of growth media and growth parameters of *Pinus sylvestris* L. seedlings.

Growth parameter, nutrient	Total number of mycorrhizal tips		Species richness of ECM fungi	
	β	P	β	P
Seedling height	0.43	0.005	0.33	0.025
Root collar diameter	0.42	0.016	0.11	0.508
Stem dry weight	0.46	0.009	0.01	0.969
Needles dry weight	0.46	0.009	0.01	0.943
Roots dry weight	0.47	0.008	-0.02	0.912
C	0.28	0.051	0.49	0.001
N_{total}	0.31	0.044	0.41	0.009
P	-0.14	0.469	0.26	0.172
K	0.12	0.480	0.45	0.010
Ca	0.14	0.399	0.46	0.006
Mg	0.12	0.448	0.46	0.006
pH_{KCl}	-0.23	0.125	-0.49	0.002

both in the total number of mycorrhizal root tips and the ECM species richness. Carbon, nitrogen, potassium, calcium and magnesium had a positive impact on species richness of ECM fungi. Moreover, pH of the growth medium showed significant negative interactions with the species richness of ECM fungi (Tab. 4).

Discussion and conclusions

Bare-root forest nurseries can be considered as a simplified ecosystem with a disturbed cycle of organic matter. Long-term use of nursery soil and organic matter reduction may have important implications for the physical, biological and chemical properties of the nursery soil (Gorzelak 1998) and thus negatively result in growth responses of the seedlings. Therefore, mulching the nursery seedbeds with forest litter may be an attractive way of supplementing nursery soil with organic matter. The results of the present investigation provide evidence that forest litter amendment can significantly influence some growth parameters and ECM formation of Scots pine seedlings even after a single growing season in a bare-root forest nursery. Improved heights and stem diameters of the seedlings from all litter types in respect to the control trees (Tab. 2) supported our first hypothesis and was consistent with data reported by Bakuzis (1952), suggesting that tree species seedlings rapidly develop a large root system and undergo a great height increment in undecomposed or partly decomposed forest litter. Forest floor as an appropriate rooting medium for planted tree seedlings has also been noted by other authors (Balisky et al. 1995, Hallsby 1995, McMinn 1982, Sutton 1993). Also in our previous study performed on two-years-old Scots pine seedlings, height and survival of seedlings were considerably improved by natural pine and oak litter cover on the surface of the nursery bed soil (Aučina et al. 2007). Although the foliar nutrient composition of the pine seedlings in our study was not examined, we presumed that the enhanced seedling growth and higher biomass parameters (in pine litter only) might be (at least partly) related to the nutritional effect of the tested forest litters. Forest litter with the organic horizon is an important reservoir of organic matter and mineral nutrients, especially nitrogen (Jurgensen et al. 1997). Different litter types and the environmental conditions in the litter layer affect the decomposition rate and the microbial activity of litter, and thus regulate the rate of nutrient release from the organic matter (Facelli & Pickett 1991, Scott & Binkley 1997, Conn & Dighton 2000). Consequently different litter types may selectively promote seedling growth responses (Brearley et al. 2003), as found in our experiment. Moreover, each forest litter type (pine, oak and spruce) increased the total number of mycorrhizal tips and ECM fungal diversity. The uptake of nutrients from decomposing litter by ECM fungi associated with seedling roots could further promotes seedling growth (Brearley et al. 2003). The presence of a high number of mycorrhizal tips and higher ECM fungal diversity in litter variants facilitate the access to an organic nutrient source and improve the seedling development, since mycorrhizal fungi often produce extracellular enzymes (Leake & Read 1997) capable of releasing nutrients from organic matter that are normally unavailable to plants. Baxter & Dighton (2005) found a positive influence of increased species richness of ECM fungi on the growth and nutrient uptake of *Pinus rigida* seedlings after one growing season in field soils.

In the observed mycorrhizal communities, we distinguish seven ECM fungal taxa by morphotyping of 38 320 seedling root tips and sequencing of 98 mycorrhizas. Based on our sampling effort, the estimated numbers of species reach higher values by selected estimators for seedlings grown in litter variants than in the control soil. These values indicate that our estimated sampling completeness (percent of observed species) constitutes approximately 87% of estimated species richness. Similar sampling completeness is noted in many other studies as reported by Dickie (2007). All examined mycorrhizas are ubiquitous and the identity of EM fungi composing these morphotypes was established by molecular methods in bare-root forest nurseries in a previous study (Rudawska et al. 2001, Iwanski et al. 2006, Aučina et al. 2007, Leski et al. 2010). Our results indicate that the widespread *W. mikolae* mycorrhizae at the initial stages of seedling growth is better adapted to nursery conditions than other ECM fungal taxa, such as *M. bicolor, C. geophilum, Atheliaceae* sp., *S. luteus, L. laccata*, and unidentified *Ascomycetes*. This is consistent with numerous studies suggesting that E-strain fungi are the most common symbionts not only in conifer nurseries and degraded sites, but also in northern boreal forests (Danielson et al. 1983, Mikola 1988, Rudawska et al. 2006, Leski et al. 2010).

Contrary to second hypothesis, different litter types used in our experiment did not differentiated neither species composition nor relative abundance of ECM fungi. In previous experiments with seedlings from different pine species, an effect of different forest litter amendment was rather observed on the relative proportions of mycorrhizal symbionts than on their species composition (Conn & Dighton 2000, Jonsson et al. 2006, Aučina et al. 2007). Such results suggest that the forest litter has not been a source of inoculum for the identified fungal species, as root systems of pine seedlings from all litter types were colonized by a few nursery-adapted ECM fungi (Rudawska et al. 2001, Iwanski et al. 2006, Aučina et al. 2007, Leski et al. 2010), probably originating from a natural air-borne inoculum. Our data rather indicate that forest litter considerably improves environmental conditions for development of ECM fungi previously present in the nursery soil. We presume that forest litter amendment has constituted better microclimatic conditions for the maintenance of a high number of mycorrhizal tips and ECM fungal diversity in litter growth media than in the control without any supplement.

We conclude that the use of forest litter as a mulch improves the growth parameters and performance of ECM formation on Scots pine seedlings after the first growing season in a bare-root forest nursery. Therefore, any of the forest litter types used in our studies may be able to promote planting stock quality on a small scale in the nursery phase. It is likely that seedlings may benefit from litter growth media for their successful outplanting from nursery to dry field sites. Drought resistance of many ECM fungi is obviously of advantage for nursery seedlings following outplanting (Shi et al. 2002). Further experiments are in progress to examine growth performance and survival of such seedlings in the field.

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