FOREST LITTER AS THE MULCH IMPROVING GROWTH AND ECTOMYCORRHIZAL DIVERSITY OF BARE-ROOT SCOTS PINE (*PINUS SYLVESTRIS*) SEEDLINGS

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Introduction

Most forest trees live in symbiosis with ectomycorrhizal (ECM) fungi which facilitate both nutrient and water uptake, increase resistance to certain root diseases and enhance stress tolerance of the plant. The entire seedling stock in Lithuania is produced in bare-root forest nurseries where the mass production of tree seedlings drastically reduces the soil organic matter content without the possibility of its natural regeneration. The decline of organic matter content in nursery soil often leads to decreased microbiological activity and negatively influences the diversity of ECM fungi.

The aim of the present investigation was to evaluate how different forest litter types (pine, oak and spruce) affect growth and ECM formation of Scots pine seedlings after the first growing season in a bare-root forest nursery.

Materials and methods

The study was performed in a bare-root forest nursery of Vilnius University Botanical Garden (54°43′N, 25°24′E) in Lithuania. Each forest litter was collected in healthy, natural pine, oak or spruce stand of similar age, where natural seedling regeneration was abundant. The mineral nursery soil was used as a control. The pine seeds originated from the local provenance of Labanoras (55°16′N, 25°50′E). Seedlings were manually maintained and were not fertilized. After the first year of growth under nursery conditions, 50 seedlings per growth medium were randomly selected, and their stem height and root-collar diameter were measured. Ectomycorrhizal fungal community developing in treatments with different forest litter addition was characterized by combination techniques of traditional morphotyping and molecular methods (PCR and sequencing).

Results

The properties of growth media from all litter treatments are summarized in Table 1. Among seven detected ECM fungal taxa (*Wilcoxina mikolae, Suillus luteus, Cenococcum geophilum*, *Meliniomyces bicolor*, *Laccaria laccata*, unidentified *Atheliaceae*, unidentified *Ascomycetes*) two were found in control soil, seven in pine and oak litter treatment and six in soil with spruce litter amendment (Fig. 1 and Table 2). *W. mikolae* was the most common in all litter treatments. Significant differences in seedling height and root-collar diameter between forest litter treatments and control soil were found (Fig. 2).

Table 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).

Treatment	N_{total} (%)	mg×kg^{-1}						C (%)	C/N	pH_{KCl}
		NH_4^+	NO_3^-	P	K^+	Ca^{2+}	Mg^{2+}			
None (control)	0,06±0,01 **d**	0,12±0,01 **d**	0,24±0,02 **d**	2,08±0,04 **b**	9,88±0,07 **d**	6,62±0,08 **d**	2,73±0,03 **d**	0,7±0,02 **d**	12,4±1,41 **c**	5,66±0,01 **a**
Pine litter	0,12±0,01 **c**	0,28±0,01 **c**	0,47±0,01 **c**	1,28±0,01 **d**	15,7±0,08 **c**	10,7±0,05 **c**	4,31±0,05 **c**	1,88±0,01 **c**	16,0±0,91 **bc**	4,81±0,04 **b**
Oak litter	0,22±0,01 **b**	0,86±0,01 **b**	1,57±0,01 **b**	1,79±0,02 **c**	20,0±0,13 **b**	14,7±0,08 **b**	5,69±0,05 **b**	3,77±0,03 **b**	17,5±0,57 **b**	4,65±0,04 **c**
Spruce litter	0,31±0,02 **a**	1,77±0,01 **a**	4,12±0,03 **a**	10,3±0,04 **a**	62,4±0,37 **a**	39,3±0,17 **a**	15,9±0,08 **a**	6,64±0,06 **a**	22,3±1,23 **a**	3,93±0,03 **d**
P>F	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001

Within a column, values with different letters are significantly different (P < 0.05, Tukey's test).

Fig.1. Plan views of mycorrhizas observed on *Pinus sylvestris* seedlings from tested bare-root nursery – *Wilcoxina mikolae* (a); *Meliniomyces bicolor* (b); *Cenococcum geophilum* (c); unidentified *Ascomycetes* (d); *Amphinema* sp. (e); *Suillus luteus* (f); *Laccaria laccata* (g).

Table 2. 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).

Identification	Accession	Closest match	Identity (%)	Relative abundance (%)			
				Growth medium			
				Pine litter	Oak litter	Spruce litter	Mineral nursery soil (control)
Ascomycota							
Wilcoxina mikolae	**KJ596425**	***Wilcoxina mikolae* (JQ310818)**	**99**	**84.8±3.60**	**87.1±3.19**	**89.1±2.86**	**96.3±3.67**
Meliniomyces bicolor	**KJ596426**	***Meliniomyces bicolor* (HM190124)**	**98**	**8.5±2.43**	**8.8±2.40**	**5.9±2.53**	**—**
Cenococcum geophilum	**KJ596427**	***Cenococcum geophilum* (HM189727)**	**99**	**1.9±0.59**	**0.9±0.65**	**2.2±0.76**	**—**
Ascomycetes	**KJ596428**	**unidentified *Ascomycetes* (JN172989)**	**89**	**1.7±1.22**	**0.5±0.49**	**1.2±0.96**	**—**
Basidiomycota							
Atheliaceae	**KJ596429**	***Amphinema* sp. (JN943925)**	**91**	**0.5±0.54**	**2.4±1.82**	**—**	**3.7±3.67**
Suillus luteus	**KJ596430**	***Suillus luteus* (UDB000930)**	**98**	**2.4±1.65**	**0.3±0.18**	**0.2±0.16**	**—**
Laccaria laccata	**KJ596431**	***Laccaria laccata* (UDB000106)**	**98**	**0.1±0.09**	**0.1±0.07**	**1.5±0.76**	**—**
Observed species richness				**7**	**7**	**6**	**2**
Mean species richness per seedling				**2.9±0.43a**	**2.8±0.36 a**	**3.0±0.30 a**	**1.1±0.10 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**

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)..

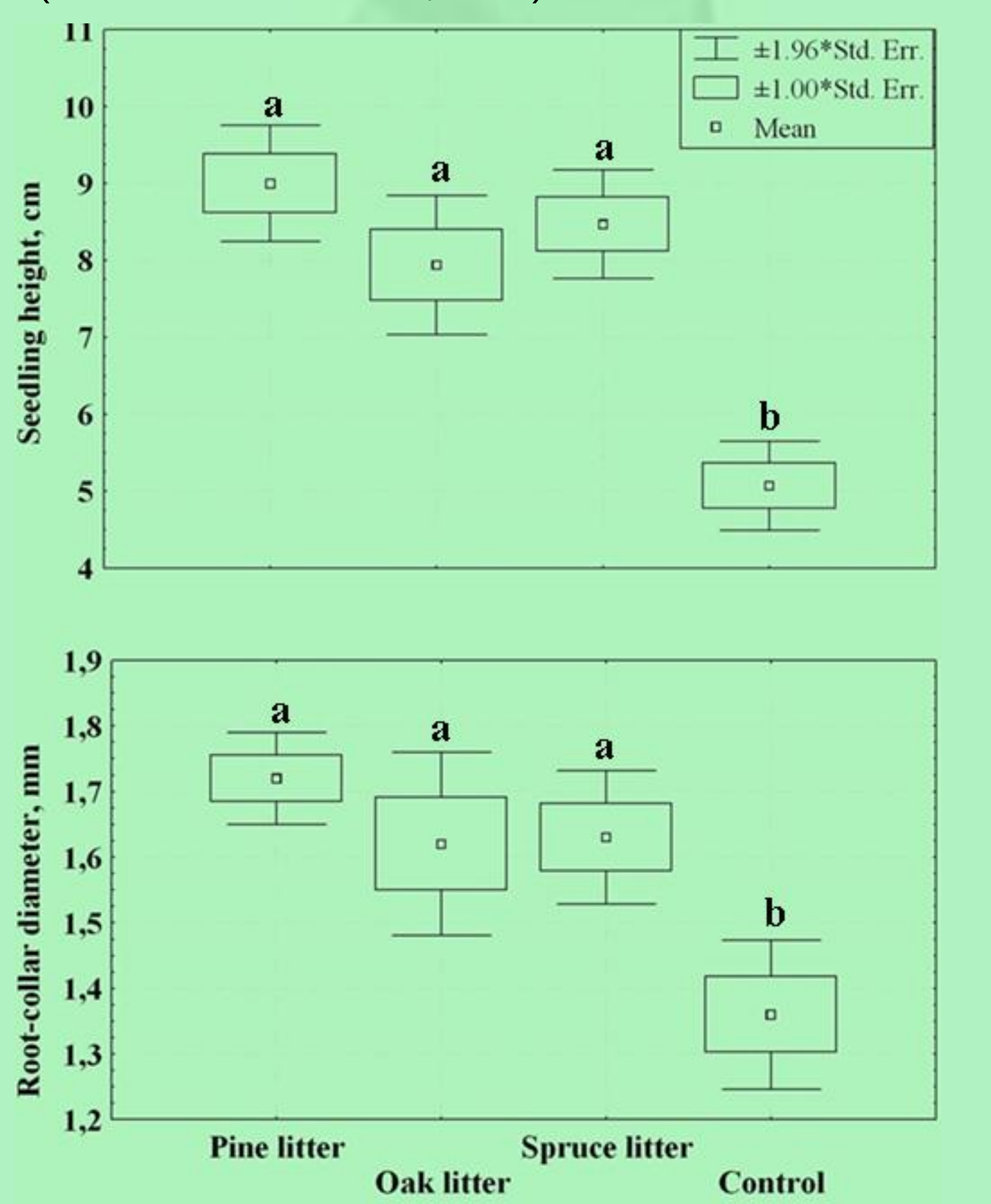

Fig.2. Height and root-collar diameter of *Pinus sylvestris* seedlings grown in bare-root nursery with different forest litter addition. Different letters indicate significant differences between treatments (P <0.05, Tukey's test).

Conclusions

Our 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.