Studies of Ectomycorrhizal Fungi Above- and Belowground in the 50-year-old *Pinus sylvestris* L. Forest

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Abstract

The assemblage structure of ectomycorrhizal fungi above- and belowground in the 50-year-old pine (*Pinus sylvestris* L.) forest was determined in this study. The community was composed of 53 species, the majority of which belonged to the genera *Cortinarius, Russula, Amanita* and *Tricholoma*. *Cantharellus cibarius* and *Paxillus involutus* were the dominant species making up the largest part of the sporocarp biomass and determining harvest in the studied forest type. Twenty ectomycorrhizal morphotypes were determined belowground. Morphanatomical analysis and PCR–RFLP were used to determine morphotypes. The maximum species richness above ground was characteristic of September while variation in the diversity of ectomycorrhizal morphotypes per vegetation season was very marginal and not significant.

Key words: ectomycorrhizal fungi, sporocarps, ectomycorrhizae, pine forest

Introduction

Symbiotic associations of organisms are very important to the viability and productivity of ecosystems. Macrofungi - especially ectomycorrhizal - are organisms vitally important to the forest ecosystem. Ectomycorrhizae (ECM) play a key role in nutrient cycling and energy flow of temperate and boreal forests, also protecting plants against pathogens and pollutants (Yamaji *et al*. 2005, Smith and Read 1997). It has been proposed that high ECM diversity increases plant productivity and the loss of ECM due to the impact of various negative factors could have important functional consequences, not just for the host plants but for the ecosystem as a whole, due to an altered ability of nutrient release by ECM (Baar 1997, Read and Perez-Moreno 2003). The best known mycobionts of ECM belong to the basidiomycota. Host specificity plays an important role for the distribution of ectomycorrhizal fungi. Under natural conditions, a wide range of ectomycorrhizal fungi develop ectomycorrhizal symbiosis with *Pinus sylvestris* L. *P. sylvestris* is one of the dominant components of coniferous forests in Lithuania. Conifers make up 58.8% of Lithuanian woodland territory; 36.4 % of the territory is occupied by *P. sylvestris* and 22.4 % by *Picea abies* (L.) Karst. (Navasaitis *et al*. 2003). Variations in richness, distribution, and sporocarp abundance of fungal species among different forest sites have been observed and may be attributed to microclimatic and macroclimatic factors, soil properties, vegetation parameters etc. Forest age has been observed to be an important factor determining the composition of ectomycorrhizal fungi (Dighton and Mason 1985, Molina *et al*. 1992, Ohenoja 1993, Dahlberg *et al*. 1997). We investigated the assemblage structure of ectomycorrhizal fungi above- and belowground associated with the 50-year-old *P. sylvestris*. The objectives of the present study were: 1) to perform an inventory of ectomycorrhizal fungus diversity above- and below the ground, 2) to examine sporocarp and ECM root abundance with the aim of determining the dominant species in the investigated territory, 3) to perform phenological observations of symbionts of *P. sylvestris* above- and below the ground per vegetation season.

Materials and methods

Study site

The investigations were conducted on a permanent study site, situated in Lazdijai district, southern Lithuania (53°58'06"NL, 23°30'20"EL) at 125 – 135 m above sea level. The mean annual temperature and precipitation are 6.4° C and 550 mm, respectively. This territory occupies about 20 ha and is located in a Lithuanian – Poland transborder region where public access is prohibited. This factor was important for obtaining objective investigational data because most

of the ectomycorrhizal fungi are edible and intensively collected. Nine study plots (1 – 9) were set in the 50 year-old pine forest of the *Cladonio-Pinetum sylvestris* (Juraszek 1927) association. The area of each study plot was 900 m^2 (30x30 m). The dominant tree species was *Pinus sylvestris* L. In some locations *Betula pendula* Roth., *Quercus robur* L. were intermixed. The shrub layer was dominated by *Juniperus communi* L., with occasional *Q. robur*, *Frangula alnus* Mill., *Sorbus aucuparia* L. About 80 % of the ground area (evaluation according to the Braun-Blanquet method (Natkevičaitė–Ivanauskienė 1983) was occupied by moss and lichen (Table 1). The dominant species of moss were *Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., lichen – *Cladonia rangiferina* (L.) Weber ex F. H. Wigg., *Cladonia arbuscula* (Wallr.) Flot.

Table 1. Vegetation cover (%) of study plots (evaluation according to Braun-Blanquet method)

Vegetation groups	Plots								
	1	2	3	4	5	6	7	8	9
Trees	50	60	60	60	50	50	60	70	60
Shrubs	60	50	10	10	10	10	15	15	20
Herbaceous plants	50	30	10	10	10	10	20	20	40
Mosses, lichens	80	70	80	70	70	70	70	80	80

Aboveground studies

Species composition of fungi was inventoried at each selected forest plot every second or third week during vegetation periods in 2003 – 2005. The investigation started at the beginning of June and lasted until the first snowfall. Identification of specimens was carried out according to Moser (1983), Hansen and Knudsen (1992), Urbonas (2005) using a Jenaval Carlzeiss Jena microscope. Voucher specimens of infrequent species found as part of this study have been deposited in the fungal collections of the herbarium at the Institute of Botany (BILAS). The number of sporocarps collected in each investigation plot was counted, they were weighed and the biomass of fresh sporocarps (kg/ha) was calculated.

Belowground studies

Samples for ectomycorrhizal investigations were taken in 2004 from the 4th study plot which was distinguished by the highest species richness. Sporocarp abundance was also high in this plot. To investigate the dynamics of ectomycorrhizae per vegetation season, samples were taken every month from June until October (21 June, 20 July, 23 August, 15 September, 12 October). Twenty soil cores of 4.5 cm in diameter and 6 cm deep (it is known (Gardes and Bruns 1996; Stankevičienė 2003) that the most of the ectomycorrhizae is located in the upper soil layers) were randomly taken from the study plot each sampling time. These original samples were mixed together in the laboratory to prepare the representative samples for each research plot. For investigation of ECM from the representative sample, 100 g of soil were taken. Soil samples were stored in plastic bags in the dark at 4^{o}C until processing. Ectomycorrhizal roots were processed within three weeks after the collection of soil cores. Cleaning was performed by soaking the samples in tap water overnight and then roots were gently cleaned under running water using a 0.5 mm sieve. Living ectomycorrhizal roots of each sample were isolated and analysed under a Nikon C-PS stereoscopic microscope and sorted into morphotypes using the criteria of Agerer (1991) (size of tips, colour, branching patterns, presence or absence of hyphae and rhizomorph). The number of tips which belonged to separate morphological types was counted. Mantel, hyphae and rhizomorph preparations were used for the purpose of identifying the ECM to the genus or, if possible, to the species level using a Janaval Carlzeiss Jena light microscope (Agerer 1987–2004, Agerer *et al.*1996–2004).

ECM morphotypes isolated from soil samples collected in September were used for DNA analyses. Genomic rDNA was isolated from individual root tips as described by Gardes and Bruns (1993). The identification of fungal symbionts was based on PCR amplification of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA using the primer pair ITS1 and ITS4. Some morphotypes did not yield PCR product. The ITS region was characterized by RFLP analysis. Three restriction enzymes (*Tag*I, *Hinf*1 and *Mbo*I) in single enzyme digests were used to characterize and match fungal ITS-RFLP patterns. Restriction fragments were subsequently separated on agarose gels (3% agarose) and made visible using 0.5% ethidium bromide under ultraviolet light. Agarose gel was photographed digitally and the gel photographs were imported into the program Taxotron® 2000 (Grimont 2000). The patterns were identified by matching the sample and reference specimens (obtained from a regional collection of sporocarps or pure culture). Different restriction fragment length polymorphism (RFLP) patterns were denoted as separate species if the fragments varied above 4%.

Soil analyses

Chemical composition of the soil directly influences functioning of fungi in the ecosystem. Therefore, the concentration of the main nutrient elements – N, P, K, humus and soil pH –was determined. Soil samples for

chemical analyses were taken with a soil corer of 4.5 cm in diameter and 6 cm deep in August of each investigation year. The representative sample for each research plot was prepared from 18-20 randomly taken sub-samples from each plot. These soil samples were dried and sieved before the following analyses. The concentration of nitrogen and phosphorus was determined photometrically using a SPEKOL11 photometer; of potassium – applying a FLAPHO41 flame photometer; and the content of humus was ascertained colorimetrically (Mineev 1989). Soil pH_{KCL} was measured potentiometrically with a glass electrode in a 1.0 M KCl suspension.

Data analyses

The diversity of the ectomycorrhizal fungi above- and below the ground was expressed as the number of identified species (species richness) in the study territory. Simpson's index of diversity (1 – D) was calculated using the equation: $D = (n/N)^2$, where D – Simpson's diversity index, n – the total number of sporocarps/root tips of the particular species (genus/morphotype), N – the total number of organisms of all species (Krebs 1989). The species sporocarp abundance was calculated as the number of sporocarps of a given ECM species, the abundance of ectomycorrhizae was expressed as the number of living ectomycorrhizal root tips per 100 cm^3 soil volume or mass of living ECM in mg per 1 g dry soil. The relative species/genus/morphotype abundance in a soil was calculated as a percent of each species/genus/morphotype per total number of determined living mycorrhizal root tips in 100 cm^3 of the soil. Correlations between some climatic (meteorological data were obtained from the State Meteorological Service) factors and abundance of ECM fungi were calculated. Data analysis was carried out employing the methods of statistics using the software *Statistica* 5.5.

Results

Chemical characteristics of soil

Some differences were observed between study plots when obtained soil chemical composition data were analysed. The highest concentration of N, K and humus was determined in the 9th investigation plot (Table 2). These concentrations were several times higher as compared to the other study plots. The lowest content of the aforementioned elements and humus was characteristic of the 7th plot while this plot was also distinguished by the highest pH value. The 8th and 9th plots were distinguished by the lowest concentration of P. Other values were more or less similar among the study plots. Soil chemical composition is especially important for ectomycorrhizal fungi because the concentration of nutrients alters ectomycorrhizal formation and community structure (Avis *et al*. 2003, Stankevičienė 2003, Tarvainen *et al*. 2003, Edwards *et al*. 2004, Harrington and Mitchell 2005).

Table 2. Chemical composition (macroelement, humus concentration, pH) of soil (dry weight) from the study plots (means from 4 composite samples for 1, 2, 7 and 9 plots or from 5 composite samples for 3 – 6 and 8 plots; SE in brackets)

Plots	N(%)	P(%)	P_2O_5 mg/kg)	K (mg/kg)	Humus (%)	pH (KCl)
1	0.084(0.011)	0.027(0.006)	159.9(43.1)	50.35(12.05)	3.75(0.74)	3.67(0.135)
2	0.043(0.026)	0.035(.008)	182.1(31.9)	37.05(0.95)	3.79(0.86)	3.65(0.11)
3	0.054(0.025)	0.042(0.015)	101.6(27.96)	24.17(3.51)	2.89(0.06)	3.67(0.13)
4	0.062(0.03)	0.041(0.008)	136.4(14.7)	28.95(10.53)	2.96(0.63	3.53(0.15)
5	0.052(0.019)	0.038(0.007)	126.5(17.1)	25.3(3.93)	2.43(0.46	3.68(0.04)
6	0.054(0.016)	0.051(0.019)	131.5(29.76)	24.33(5.62)	2.47(0.44)	3.67(0.09)
7	0.026(0.007)	0.037(0.008)	126.35(6.35)	16.5(2.4)	2.05(0.52)	3.9(0.08)
8	0.064(0.006)	0.027(0.012)	81(33.56)	30.53(4.78	2.56(0.42)	3.43(0.11)
9	0.189(0.008)	0.028(0.005)	87.2(17.4)	74.05(6.95)	8.02(1.28)	3.32(0.15)

Aboveground diversity and sporocarp abundance

The diversity of ectomycorrhizal fungi recorded above the ground at nine permanent study plots over a three year period consisted of 53 taxa (Table 3). Most species belonged to the genus *Cortinarius* (23 % of the total number of species) followed by *Russula* (19 %) and the rest of the species were *Amanita* (9 %), *Tricholoma* (9 %), *Lactarius* (≈8 %), and *Hebeloma* (≈6 %). Other genera were represented by 1 – 2 species. *Cantharellus cibarius, Lactarius necator, L. rufus*, *Paxillus involutus, Russula aeruginea* and *Tricholoma saponaceum* were the most frequent and were found in each study plot. The rarest species (only 1 – 2 sporocarps/investigation time were found in 1 or 2 plots) were *Boletus pinophilus, Cortinarius causticus, C. bovinus, C. delibutus, C. evernius, Hebeloma. pussilum, Laccaria bicolor, Lactarius vietus, Russula claroflava, R. decolorans* and *Tylopilus felleus*.

The lowest species richness (15 species) was found in plot 1 and a twice bigger number of species was found in the 4th plot which was characterized by the highest species richness (32 species) among the study plots.

According to the abundance of formed sporocarps, there was strong variation among the species. Seven species were the most abundant and formed more than 100 sporocarps/species/investigation time: *C. cibarius, P. involutus, Rozites caperata, L. rufus, Hygrophoropsis aurantica, Cortinarius mucosus* and *R. aeruginea.* They were the dominant fungi in the 50-year-old pine forest. All other species formed not more than 100 sporocarps/investigation time in the study plots. A total of 10,358 sporocarps of ectomycorrhizal

Table 3. Species of ectomycorrhizal basidiomycete fruited in study area in 2003-2005

Species	Sporocarps[1]	Biomass[2]	Fruiting time[3]
Amanita citrina (Schaeff.) Pers.	51	1329	S; O
A. fulva (Schaeff.) Fr.	26	371	A; S
A. muscaria (L.) Lam.	49	1042	S; O
A. porphyria Fr.	28	238	A; S; O
A. rubescens Pers.	42	574	Jl; A; S; O
Boletus edulis Bull.	18	1056	A; S; O
B. pinophilus Pilát et Dermek	2	227	Jl
Cantharellus cibarius Fr.	5527	13989	Jn; Jl; A;S;O
Cortinarius alboviolaceus (Pers.) Fr.	4	25	O
C. armilatus (Alb. et Schwein.) Fr.	11	280	S
C. causticus Fr.	1	25	S
C.bolaris (Pers.)Fr.	3	76	S
C. bovinus Fr.	1	5	O
C. cinnamomeus (L.) Fr.	5	19	O
C. delibutus Fr.	2	12	O
C. evernius (Fr.) Fr.	2	11	O
C. mucosus (Bull.) Cooke	146	1399	S; O
C. salor Fr.	3	76	S
C. semisanguineus (Fr.) Gillet	5	31	O
C. traganus (Fr.) Fr.	44	1020	A; S; O
Hebeloma crustuliniforme (Bull.) Quél.	44	185	O
H. longicaudum (Pers.) P. Kumm.	3	11	S
H. pussilum J. E. Lange	1	1	O
Hygrophoropsis aurantiaca (Wulfen) Maire	173	298	A; S; O
Laccaria bicolor (Maire) P. D. Orton	2	27	S
Lactarius necator (Bull.) Pers.	96	2775	A; S; O
L. rufus (Scop.) Fr.	392	3800	A; S; O
L. torminosus (Schaeff.) Gray	28	400	S; O
L. vietus (Fr.)	1	6	A
Leccinum scabrum (Bull.) Gray	19	737	Jl; S; O
	2475	40030	Jn; Jl; A; S; O
Paxillus involutus (Batsch.) Fr.			
Rozites caperata (Pers.) P. Karst.	405	5824	S; O
Russula adusta (Pers.) Fr.	24	808	A; S; O
R. aeruginea Fr.	99	1821	Jl; A; S; O
R. claroflava Grove	1	18	S
R. decolorans (Fr.) Fr.	2	37	S; O
R. emetica (Schaeff.) Pers.	79	848	Jl; A; S; O
R. nigricans (Bull.) Fr.	3	44	A; S
R. rhodopoda Zvára	14	286	A; O
R. sanguinea (Bull.) Fr.	3	53	S
R. vesca Fr.	32	683	Jl; A; S; O
R. xerampelina(schaff. ex Secr.) Fr.	4	198	A
Sarcodon imbricatus (L.) P. Karst.	92	1706	S; O
Suillus bovinus (Pers.) Kuntze	27	782	S; O
S. variegates(Sw.) Kuntze	24	464	Jl; S
Tylopilus felleus (Bull.) P. Karst.	2	43	Jl; A
Tricholoma equestre (L.) P. Kumm.	90	2209	O
T. pesundatum (Fr.) Quél.	4	238	O
T. portentosum (Fr.) Quél.	40	1094	O
T. saponaceum (Fr.) P. Kumm.	81	688	O
T. sejunctum (Sowerby) Quél.	9	225	O
Xerocomus badius (Fr.) Kühner	85	2840	Jl; A; S; O
X. subtomentosus (Fr.) Fr.	34	895	Jl; S; O
Total	10358	91879	

[1] – number of sporocarps collected in study area/whole investigation time; [2] – biomass of sporocarps collected in study area/whole investigation time; [3] – fruiting time of separate species: Jn – June, Jl – July, A – August, S – September, O – October

basidiomycetes during the 3 year study period were collected. This made up 918.8 kg (fresh weight)/8,100 m^2 (study area) or 1,134 kg/ha per investigation time or 378.1 kg/ha per one vegetation season on average.

Total sporocarp abundance, like species richness, differed among the study plots and ranged from 312 to 1,637 (Figure 1). Most of the plots yielded 1,000 – 1,600 sporocarps per investigation time. The lowest amount of sporocarps (3-5 times lower than in the other study plots) was found in plot 7.

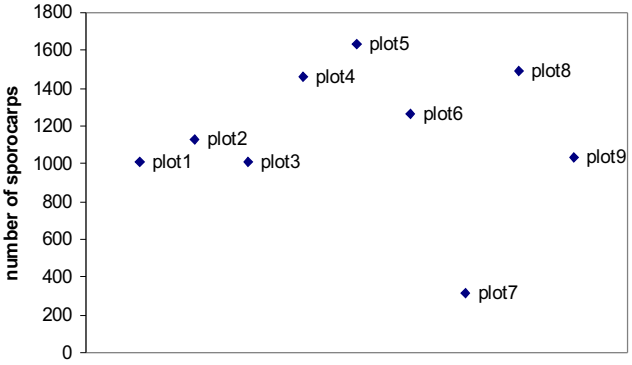

Figure 1. Distribution of study plots according to sporocarp abundance (total number of sporocarps found in study plot 1 - 9/whole investigation time)

To analyse the dynamics of fruiting per vegetation season, sporocarps were monitored every second or third week between June and October. Simpson's index of diversity (1-D) demonstrated differences among the separate months (Figure 2).

The start of fruiting varied strongly among the species. The longest period of fruiting was characteristic of *C. cibarius* and *P. involutus*. Sporocarps of these species started to grow in June and fruited until the end of the vegetation season. A long fruiting period was also characteristic of *Laccinum scabrum, Boletus edulis*, and of some species from genus *Amanita*, *Lactarius*, *Russula,* and *Xerocomus*. The fruit bodies of the aforementioned fungi started growing in July or August. Species from genera *Cortinarius, Tricholoma, Hebeloma* as well as *Sarcodon imbricatus* were found from September and their fruiting period was relatively short.

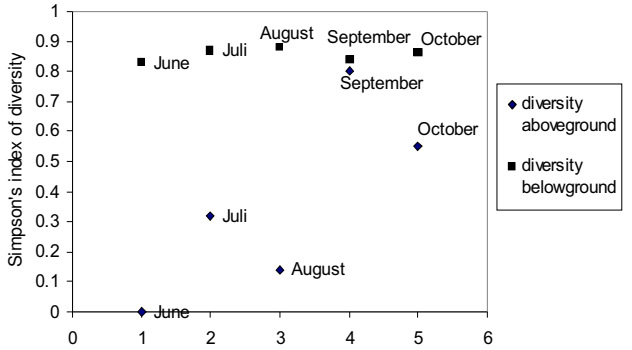

Figure 2. Simpson's index of diversity (1-D) above- and belowground per vegetation season

Belowground diversity and ECM root abundance

A total of 4,809 ectomycorrhizal tips, assigned to 20 different ECM morphotypes, were observed. The mean number of mycorrhizal tips found per 100 cm^3 soil

sample was 962 ranging from 642 to 1,148. The mean biomass of dried ECM roots made up 0.65 mg per 1 g of dry soil ranging from 0.35 to 0.95 mg.

Variation in diversity of ectomycorrhizal morphotypes per vegetation season was very marginal and no significant differences between the samples collected in different months (June – October) were found. Simpson's index of diversity (1-D) did not demonstrate any marked difference among the separate samples (Figure 2).

Identification of ECM morphotypes was based on morphanatomical and molecular (PCR; RFLP) analyses. Morphotypes formed by fungi of species/genera *Cenococcum geophylum, Paxillus involutus, Russula, Tomentella* were identified when morphological and anatomical features were analysed. PCR-amplification and RFLP-analysis were successful with 14 morphotypes (Table 4), six of which were reliably identified to the species level. They were: 4/8LP - *Suillus luteus*, 24/8LP – *S. variegatus*, 5/8LP – *Tricharina ochroleuca*, 7/8LP – *Amanita fulva*, 16/8LP – *Russula aeruginea,* 17/8LP – *Tricholoma equestre*. Fungi of samples 1/8LP, 12/8LP, were close to *Amanita,* 3/8LP - to *Rozites*, 22/8LP - to *Tricholoma*, and 19/8LP - to *Tricharina* species and it is possible to predict that these types are formed by species from the aforementioned genera. Basing on RFLP-analyses it was impossible to identify the samples 14/8LP, 18/8LP and 23/8LP. However, 14/8LP morphotype had morphanatomical features typical of *Cortinarius* (Agerer 1986-2004) and was likely formed by fungi of this genus. PCR amplification of type 26/8LP and 28/8LP was not successful and it was not possible to ascribe them to any taxonomical unit according to morphanatomical features also.

ECM types were classified into exploration types according to Agerer (2001). Five morphotypes were attributed to the contact and short-distance types, by four – to the long- and medium-distance smooth and only two morphotypes were attributed to the medium-distance fringe type. The most abundant were the contact and medium-distance fringe types and the lowest relative abundance was determined for the medium-distance smooth type (Fig. 3).

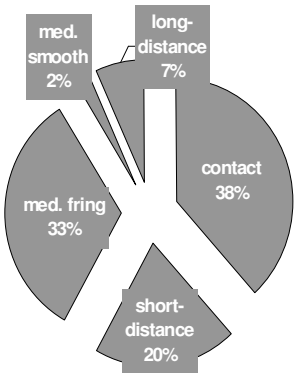

Figure 3. Exploration types (contact, short-distance, med. fringe and med. smooth - fringe and smooth subtypes of medium-distance type and long-distance) of ectomycorrhizae izolated from study plots

Discussions and conclusions

The diversity (species richness) of ectomycorrhizal fungi determined above ground in a 50-year-old *Pinus sylvestris* stand in Lithuania was very close to the diversity in a 50-year-old *P. sylvestris* forest in central Sweden (56 species) (Taylor 2002). Also a similar

Table 4. Genetic diversity among ITS regions of ectomycorhizal morphotypes determined in examined 50 year old pine forest as approximate restriction fragment sizes (bp)

Morphotype	*Hinf*1	*Mbo*1	*Taq*1	Species/Genus
1/8LP	352	419, 158, 112	342, 325	unknown
3/8LP	416, 297	261, 143	326	*Tricharina*
4/8LP	219, 147	240, 139	188, 95	*Suillus luteus*
5/8LP	319	312, 224	246	*Tricharina ochroleuca*
7/8LP	291	328, 233	284, 250	*Amanita fulva*
12/8LP	413, 292	384, 199	307, 259	*Amanita*
14/8LP	311, 245	391, 153	557, 310, 192	unknown
16/8LP	343, 281	272, 212, 184	291, 198, 104	*Russula sanguinea*
17/8LP	390, 244, 127	445, 282	381, 325	*Tricholoma equestre*
18/8LP	329, 291	427, 201	331, 248	*Amanita*
19/8LP	293	415, 310, 201	228, 106, 61	*Rozites*
22/8LP	274, 246, 200	294, 152, 106	400, 274, 151	unknown
23/8LP	319, 232	396, 307, 229	355	unknown
24/8LP	220, 189 ,143	239, 152	88, 80	*Suillus variegatus*

aboveground diversity of these fungi was recorded in a temperate oak savannah – 59 species (Avis 2003).

Species richness differed among study plots. Plots with lower species richness were characterized by higher coverage of herbaceous plants and shrubs, while plots with the most species (4th plot – 32 species; 9th – 30; 3rd – 28) were characterized by a lower coverage of shrubs and grasses (Fig. 4). The chemical features of the soil are an important factor controlling species composition and fruiting intensity also, because varying demands of different species for soil conditions are expressed. It was determined that different soil ion concentrations (N, P, S, K, Na, pH etc.) influence the above- and belowground community structure of ectomycorrhizal fungi (Tyler 1985, Agerer 1990, Erland and Söderström 1990, Fransson *et al*. 2000, Lilleskov *et al.* 2002, Agerer and Gotlein 2003, Tarvainen *et al*. 2003, Iwanski *et al*. 2006, Stankevičienė and Urbonas 2006). In the present studies, the plot with the minimum amount of sporocarps (plot 7) was distinguished by the lowest concentration of N, K, humus and the highest value of pH (Table 2). However, this could be interpreted more as a tendency than a strong correlation because statistical reliable negative correlation was found only between sporocarp abundance and pH value (r = -0.52). Thus, many factors would influence the species composition and fruiting intensity in an ecosystem including vegetation type and soil chemical properties.

Present investigations determined that sporocarp abundance of ectomycorrhizal fungi in half-life pine forest varied from 2,487 to 5,025 sporocarps per vegetation season over the study period. Studies of fungal sporocarps in a mixed forest in Switzerland showed that sporocarp abundance varied over the years of the investigation and ranged from 58 to 5,559 per vegetation season (Straatsma *et al*. 2001). Our studies showed a considerably tighter range. The reason for this seems to be climatic conditions which highly influence sporocarp production (Senikova 1984; Vogt 1992; Kasparavičius and Stankevičienė 2004).

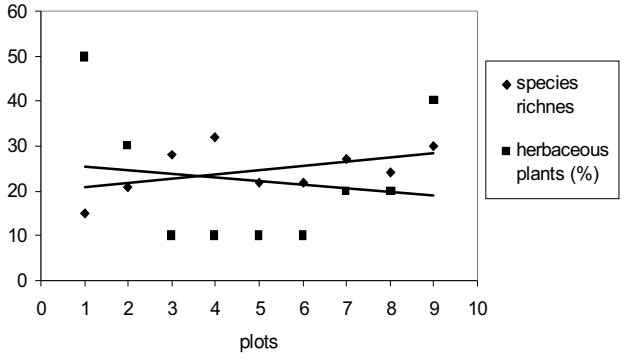

Figure 4. Comparison of herbaceous plant cover (%) of study plots and species richness of ectomycorrhizal basidiomycetes (above ground) in the 50-year-old pine forest

It is known that each forest type has its own dominant ectomycorrhizal fungi species and that dominants make up the main biomass of sporocarps and usually determine harvest in different forest types (Skryabina and Sennikova 1981). In the present studies, *C. cibarius* was the most abundant species, its sporocarps made up about a half of the total number of all collected sporocarps. *P. involutus* fruited quite abundantly also. The number of fruit bodies formed by this species made up about a quarter of the total amount of fruit bodies while their biomass made up about half.

In comparison to the above/belowground dynamics over the vegetation season (June – October), the greatest abundance of both sporocarps and ectomycorrhizal roots was determined in September (year 2004). The lowest amount of sporocarps was found in June. However, the abundance of ECM tips in June and July (1,016 and 1,046 tips/100 cm^3 soil, respectively) only slightly differed from the greatest abundance of tips in September (1,148). It was determined that seasonal physiological and phenological changes in the host plant affected both symbionts. The allocation of carbohydrates to the roots of host trees is a major factor affecting the density of fine roots and mycorrhizas (Jonson *et al*. 2000, Becerra *et al.* 2005). The increased root colonization by ectomycorrhizal fungi in spring and autumn could be associated with a period of intensive root growth and production of fruit bodies of ECM fungi. The difference between the maximum value of ECM in September and the minimum in August was approximately 44%. Meanwhile this difference in the case of sporocarps was twice bigger - approximately 86%. The reason for this variation may explain the individual fruiting entries of species. Only *C. cibarius* sporocarps were found in June while seventeen species fruited in September. The highest ECM colonization of roots of *Alnus acuminate* was determined in autumn also (Becerra *et al. 2005*). It is known that ectomycorrhizae and sporocarp formation mainly depends on the soil and climatic conditions (Senikova 1984, Vogt *et al*. 1992, Stankevičienė 2003, Kasparavičius and Stankevičienė 2004). Our data show a tendency towards reverse dependence (not statistically significant) of the temperature and abundance of both sporocarps and ectomycorrhizae. However, a reliable positive correlation was observed between the abundance of ectomycorrhizae and soil moisture (r = 0.60, number of tips/soil moisture; r = 0.82, biomass of ECM mgg^{-1} soil/soil moisture). A higher value of soil H_2O

holding capacity was obtained in September and ECM fungi respond with higher root colonization and sporocarp formation. Our data are in accord with other studies since drought has been shown to have a negative effect on mycorrhizal colonization (Lanzac *et al*. 1995, Nilsen *et al* 1998, Becerra *et al.* 2005).

The mean relative abundance of each ECM morphotype in each study site is shown in Fig. 5. Two ECM types showed markedly greater relative abundance than others: 14/8LP (probably *Cortinarius*) and 23/8LP. C. *geophilum* and *Tomentella* were frequent on mycorrhizal roots also. It was noted that in ECM community structures it is typical of a few ECM taxa to be widespread whereas the majority of species are only rarely encountered (Erland and Taylor 2002; Taylor 2002, Baier *et al*. 2006, Grogan *et al*. 2000). Moreover, it was shown that fungi not forming obvious fruiting structures (*e.g. C. geophilum, Tomentella* spp.) form the major mycorrhizal abundance. The ECM relative abundance curve of this study was dominated by *Cortinarius* (14/8LP), *C. geophyllum*, *Tomentell*a, and accompanied by a decreasing number of ECM of other taxa and was thus similar to those of other studies (Jonsson *et al*. 2000, Avis *et al.* 2003, Baier *et al*. 2006).

Some of the most common species fruiting aboveground were rarely detected on belowground roots. *P. involutus*, *S. variegatus, S. bovinus* were rare components belowground (Fig. 5) while their sporocarp abundance was considerable. Similar observations were reported for *S. pungens* in natural bishop pine stands, for *S. tomentosus* in a natural, mature jack pine stand, and for *Laccaria laccata* in a Douglas-fir nursery and radiate pine plantation (Danielson 1984, Chu-Chou and Grace 1988, Gardes and Bruns 1996). Although these species produced abundant sporocarps, their mycorrhizae were represented by low abundance or were not isolated from mycorrhizal samples. The reasons for this imbalance are not clear but the differences among the species in ecological requirements and also in terms of patterns of resource allocation could explain this discrepancy partly. Some species, such as *P. involutus*, may additionally access saprotrophic sources of carbohydrates (Wallander and Söderström 1999) and this possibility could influence abundant fruiting. Species from genera *Cortinarius* were frequent above and belowground in the studied half-life pine forest. Meanwhile, the ectomycorrhizae of *C. cibarius*, which was the dominant species aboveground, were not identified (probably were attached to the type/group of unidentified ECM which did not place between dominants) or even were not isolated. Thus, the correlation between fruiting abundance and mycorrhizal dominance belowground seems to be absent.

To date, two species of the genus *Tricharina* have been registered in Lithuania. Sporocarps of *Tricharina gilva* (Boud) Eckblad and *T. cretea* (Cooke) K. S. Thind et Waraitch were found (Kutorga 2000). Belowground studies from the present investigation revealed the third species of this genus - *Tricharina ochroleuca* (Bres.) Eckblad; however, sporocarps of *T. ochroleuca* have not yet been found in Lithuania.

The contact exploration type of ECM was dominant in the studied pine forest. This type is represented by ectomycorrhizae with a smooth mantle - a few emanating hyphae and tips are often in close contact with the

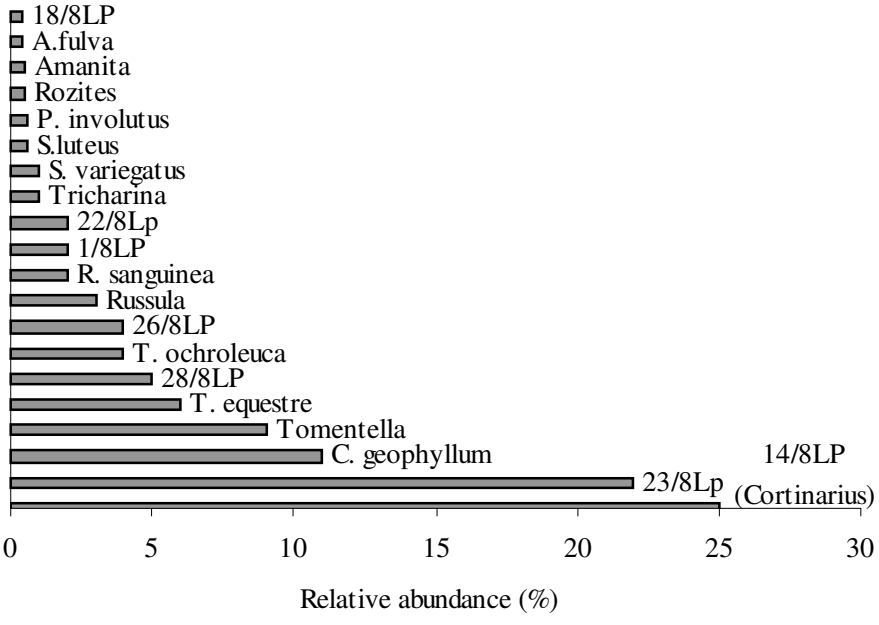

Figure 5. Relative abundance of ectomycorrhizal morphotypes in the soil of 50 year old pine forest

surrounding substrate. It was shown that the contact type ECM were associated with soil properties indicative of a mineral horizon (Agerer 2001, Baier *et al.* 2006). The ECM of this type are sandwiched between the surrounding substrate, and therefore, well equipped to explore soil horizons with narrow pores. Sandy soil with a very thin organic layer was characteristic of the studied half-life pine forest and this could probably explain the dominance of contact type ECM.

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ИЗУЧЕНИЕ НАЗЕМНОЙ И ПОДЗЕМНОЙ ГРУППИРОВОК ЭКТОМИКОРИЗНЫХ ГРИБОВ В 50-ЛЕТНЕМ СОСНОВОМ (*PINUS SYLVESTRIS* L.) ЛЕСУ

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Резюме

Исследована структура наземной и подземной группировок эктомикоризных грибов в 50-летнем сосновом бору. Наземная группировка состоит из 53 видов, основную массу которой составляют грибы родов *Cortinarius, Russula, Amanita* и *Tricholoma*. В исследованном типе леса доминировали *Cantharellus cibarius* и *Paxillus involutus*, которые и определяли биомассу плодовых тел грибов. Двадцать эктомикоризных морфотипов было выявлено в почве. Для определения морфотипов были использованы морфоанатомический и молекулярный (PCR–RFLP) методы. Наибольшее число видов в наземной группировке найдено в сентябре месяце, в то время как вариации эктомикоризных морфотипов во время вегетационного периода были незначительны.

Ключевые слова: эктомикоризные грибы, плодовые тела, эктомикориза, сосновый лес