FREQUENCY OF MICROSCOPIC FUNGI IN THE UPPER LAYER SOIL OF CONIFEROUS TREE STANDS

Nijolė Maršalkienė[1], Maris Senkovs[2]

[1]*Vytautas Magnus University, Lithuania*
[2]*University of Latvia, Latvia*

Abstract. The present study was designed to examine the frequency and prevalence of microscopic fungi in the top layer of mineral soil of native *Pinus sylvestris* L., *Picea abies* (L.) H. Karst. and alien *Thuja occidentalis* L. and *Larix sibirica* Lebed tree stands. Studies on fungi frequency were conducted in January, March, May, July, August and November of 2020, the prevalence of fungi genera – in March of 2021. Biochemical composition (N, C_{org}, Ca, lignin) of investigated tree litter was also rated. By the carbon and nitrogen (C:N) and lignin and nitrogen (Lig:N) ratios, the slowest decompositions were of the T. *occidentalis* litter and the fastest one – of *P. abies* litter. Most abundant microscopic fungi were found in early spring (March) and late autumn (November) and the least one – at the end of summer (August). The biggest average frequency of microscopic fungi was in the soil of *T. occidentalis* and the least one – in *P. sylvestris* stands. The higher lignin content and Lig:N ratio in litter positively influenced the number of microscopic fungi in upper layer of mineral soil. The largest part (80 – 39%) of fungi in all investigated stands soil consisted of *Penicillium* Link, *Geomyces* Traaen and *Mucor* P. Micheli ex L. genera. The composition of fungi genus differed most from other stands in *T. occidentalis soil.* The greatest diversity of fungal genera was found in *P. sylvestris* stand soil.

Keywords: litter, lignin, soil, microscopic fungi, genera.

Introduction

Tree litter and their biochemical composition are an important factors influencing forest floor mass and degradation (Vaičys et al. 1996; McClaugherty, 2014). The soil quality and composition, abundance and activity of the biota depend on the structure of tree litter (Hagen - Thorn et al., 2004; Trocha et al., 2012). Soil biota is an integral indicator of soil functions, indicating its physical and chemical properties (Eitminavičiūtė, 2015). Tree litter strongly influences the abundance of microscopic fungi and their diversity in the forest floor and mineral soil (Kubartova et al, 2009). Microscopic fungi are one of the most abundant groups of eukaryotic microorganisms in the soil, capable of degrading difficult-to-degrade and poorly tolerable bedding materials to other microorganisms, such as lignin (Лугаускас, 1988; Baldrian, 2017). Currently, the interaction of mycorrhizal and pathogenic fungi in native and alien tree stands has been much studied, but too little attention has been paid to saprotrophic fungi which are the most abundant in the floor and soil and are major degraders of organic matter (Urcelay, 2019, VLK et al, 2020). According to the data of our previous research (Maršalkienė, Nikolajeva, 2020), the greatest diversity of microscopic fungal genera was found in the upper layer of mineral soil.

The aim of the study was to evaluate the frequency and predominant genera of microscopic fungi in the top layer of mineral soil of the most common native coniferous – Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) H. Karst) and alien – northern white cedar (*Thuja occidentalis* L .) and *Siberian larch* (*Larix sibirica* Lebed) tree stands.

Metodics

The investigation was performed in the stands of coniferous native (*Picea abies* (L.) H. Karst., *Pinus sylvestris* L.) and alien (*Larix eurolepis* Henry., *Thuja occidentalis* L.) trees at the stand park of the of Vytautas Magnus University, Agriculture Academy, Kaunas distr., Lithuania. Monoculture tree stands were planted 60 years ago at the same soil and climatic conditions of the temperate mixed forests biome. There Endocalcari-Epihypogleic Cambisols with anthropogenic influence dominate. The average annual temperature was 6,0-6,5 ^{o}C, and rainfall was 600-650 mm (Juodis et al., 2013) (Table 1).

Table 1. Species and area of investigated tree stands of Vytautas Magnus University, Agriculture Academy

Tree species	*Area of stands, ha*	Family
Paprastoji pušis (*Pinus sylvestris* L.)	0,27	Pušiniai (*Pinaceae* Lindl.)
Paprastoji eglė (*Picea abies* (L.) H. Karst.)	0,27	Pušiniai (*Pinaceae* Lindl.)
Vakarinė tuja (*Thuja occidentalis* L.)	0,14	Kiparisiniai (*Cupressaceae* Ritch. Ex Bartl.)
Sibirinis maumedis (*Larix sibirica* Lebed.)	0,17	Pušiniai (*Pinaceae* Lindl.)

Tree litter were collected after the massive November fall, on October 30th. Mineral soil samples (0–4 cm) were taken in 2020 (January, March, June, August, November 1-5st. Biochemical studies of tree litter were performed at the Institute of Agriculture of the Lithuanian research centre for agriculture and forestry according to standard methodologies: total N - Kjeldahl method (ISO 11261: 1995), Corg. - dry burning method (DIN / ISO 13878), lignin - according to P. J. Van Soest's fiber fractionation method (Faithfull, 2002), soil $pH_{KC}l$ in 1 mol / l KCl suspension – ISO 10390: 2005; Ca is determined in the BaCl2 extract – ISO 11260: 1994.

Studies on the frequency of microscopic fungi were performed at the Research Laboratory of Climate Change Impact on Forest Ecosystems at Vytautas Magnus University, Agriculture Academy. Dilution and direct seeding methods were used to isolate fungi from the soil (Bilaj, 1982). Mix 10 g of soil with 100 ml of distilled water and was shaked for 10 minutes. The resulting suspension is diluted to 1:1000. The prepared suspensions are added to 1 ml of sterile Petri dishes and filled with PDA (Potato Dextrose Agar) medium. Chloramphenicol (0.5 g / l) was added to the medium to inhibit bacterial growth. The inoculated plates are incubated in a thermostat at 25˚C for 4-6 days. The assay was performed in triplicate in four plates.

The microscopic fungi detection frequency in 1 g of soil was determined according to the formula:

$$(a \times b \times c) / d$$

where a is the volume of suspension made (ml); b – the number of detected colonies; c - dilution, d – the weight of soil used for research (g).

The predominant genera in the soils were identified in the laboratory of the University of Latvia, Latvian Collection of Microorganism Cultures, according to the macro- and micro-morphological features, using light microscopy methods and microscopic fungal descriptors.

The population density of fungal genera was calculated according to the formula:

$$p / q \cdot 100\%$$

where p is the number of colonies per fungal genus; q - total number of fungal colonies.

Statistical calculations were performed using Excel (90.6926SP-3) program. Standard deviation was used to show the distribution of the mean sample.

Rezults and discusion

Biochemical studies showed that the litter of northern white cedar was slightly acidic (according to Staugaitis, Vaišvila, 2019), and the remaining stands were acidic (Table 2). According to the literature, the increase in pH of both organic and mineral soil layers is associated with an increase in Ca concentration in the litter (Reich et al., 2005). In our case, this trend was not confirmed in the thuja stand, which had the highest Ca concentration in the litter but the lowest pH among the studied stands (Table 2).

Table 2. Biochemical composition of litter of investigated tree stands (in dry matter)

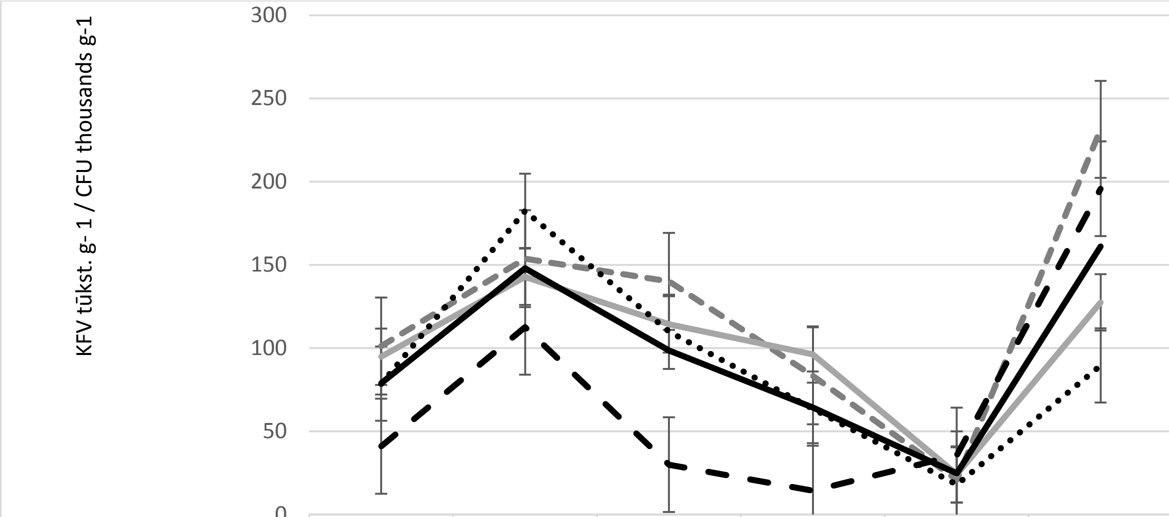
Tree stand	pH_{KCl}	N %	Ca %	$C_{org.}$ %	Lignin%	C:N	Lig:N
Pinus sylvestris	5,9±0,23	0,78±0,075	1,19±0,102	37,9±0,91	27,5±1,565	48,3±0,420	35,1±0,614
Picea abies	5,6±0,420	0,79±0,081	0,72±0,042	33,4±1,60	23,0±1,204	42,3±1,066	29,2±0,369
Thuja occidentalis	5,4±0,342	0,96±0,310	2,11±0,124	52,7±1,31	32,7±0,342	81,6±1,86	50,8±0,342
Larix sibirica	5,7±0,126	0,64±0,042	1,10±0,093	47,8±0,87	33,8±0,342	49,6±0,682	35,1±0,196

Nitrogen (N) concentration in the litter of the studied stands ranged from 0,64 to 0,96 (Table 2). The highest number of N was found in the litter of northern white cedar, the lowest – in Siberian larch. The highest concentration of organic carbon (C_{org}) was also found in the sediments of northern white cedar, the lowest - in the sediments of Norway spruce. The C_{org} cycle is closely related to the N cycle (Christopher and Lal, 2007; Mohanthy et al, 2011), and the rate of mineralization is determined by the ratio of organic carbon and nitrogen concentrations (C: N) (Vaičys and Kubertavičienė, 1998). The most favorable ratio of organic carbon and nitrogen for mineralization was of Norway spruce (42,3), the least favorable – of northern white cedar (81,6) (Table 2).

One of the most common polymers in nature is lignin, which is characteristic of woody plant cells. Lignin is one of the slowest degrading components of dead vegetation (Blanchette, 2000; Boerjane et al., 2003). The highest lignin concentration was found in the sediments of the Siberian larch stand (33,8) and northern white cedar. (Table 2). The lowest lignin concentration was found in the litter of Scots pine. According to the research data, the highest lignin and nitrogen ratio (Lig: N) – 50,8 and the slowest decomposition were northern white cedar litter, the lowest L: N ratio (29,2) and the fastest decomposing – of Norway spruce litter (Table 2).

The frequency of microscopic fungi detection in the upper mineral soil layer was highest in early spring (March) and late autumn (October), after leaf fall (Fig. 3). The detection rate of microscopic fungi in the soil of all studied stands tended to decrease in May and June and was the lowest in August.

According to the literature, moisture is one of the main factors limiting the activity of soil fungi (Kimmins, 1987; Лугаускас, 1988), such a decrease in the number of microscopic fungi (compared to the spring and early summer months) may have been determined by dry weather in August.



	Sausis/ January	Kovas/ March	Gegužė/May	Liepa/July	Rugpjūtis/ August	Lapkritis/ November
Thuja occidentalis	101.2	153.8	140.1	83.4	20.8	231.4
Picea abies	94.8	142.8	114.2	96.2	24.0	127.4
Larix sibirica	78.6	182.4	109.8	63.6	18.0	89.5
Pinus sylvestris	41.0	112.5	29.9	14.3	35.7	195.7
Vidurkis/Average	78.9	147.9	98.5	64.3	24.6	161.0

Fig. 1. The abundance of microscopic fungi colonies in uper mineral soil layer of investigated tree stands (CFU thousands g^{-1})

The graph of microscopic fungi frequency dynamics (Fig. 3) shows, that the most abundant microscopic fungi in early spring were found in the soil of the Siberian larch stand, but in November it was the lovest. Siberian larch was the only one of the studied conifers to drop all the needles in autumn, but the fresh, lignin–rich needle cover did not facilitate the development of fungi in the soil. The frequency of microscopic fungi of Scot pine in January – July was the lowest among investigated tree stands, but one of the highest in November (Fig. 3).

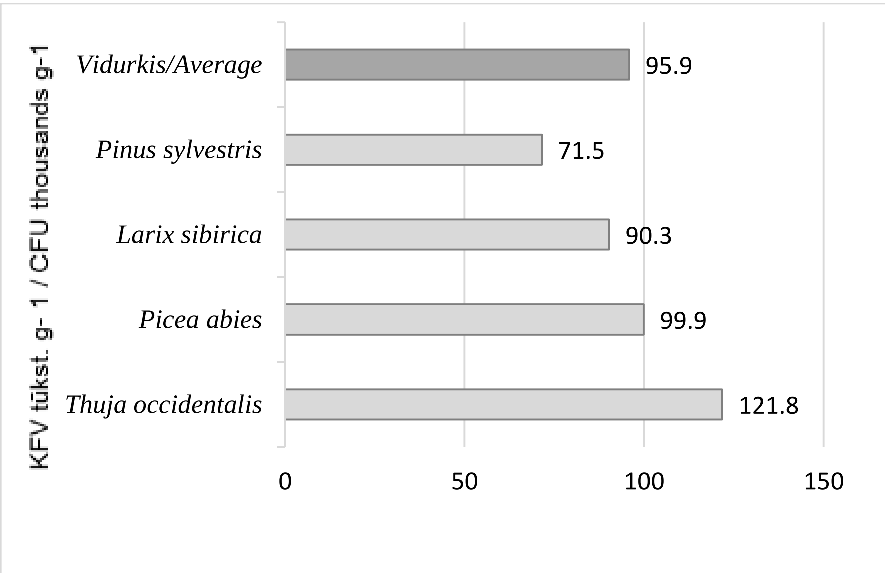


Fig. 2. The abundance of microscopic fungi colonies in uper mineral soil layer of investigated tree stands (CFU thousands g^{-1})

The highest average frequency of fungal detection (January – November), was in northern white cedar, the lowest – in pine stand soil (Fig. 4). According to literature, richer in heavy degrading components in the litter, influence the higher abundance of microscopic fungi (Lejon et al., 2005). Favorable conditions for the microscopic fungi in the northern white cedar soil could be determined by the high lignin content in litter and high L: N ratio (Table 2).

Table 3. The abundance of microscopic fungi genera (by percent) in the upper mineral soil layer of stands

Fungi genus / *Tree species*	*Penicillium*	*Geomyces*	*Trichoderma*	*Verticillium*	*Staphylotrichum*	*Aspergillus*	*Stenocephalopsis*	*Torulopsiella*	*Beauveria*	*Geotrichum*	*Stysanus*	*Paecilomyces*	*Mucor*	*Mortierella*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Thuja occidentalis	25,0	40,0			10,0		5,0						20,0	
Larix sibirica	21,4	14,3	10,7		7,1	14,3		3,6					3,6	25,0
Picea abies	38,1	2,4	7,1	14,3					21,4				11,9	4,8
Pinus sylvestris	33,3	7,0	6,4	6,9						13,3	6,0	7,4	7,0	6,4

The studied stands differed in the number and composition of the fungal genera (Fig. 3). 14 microscopic fungal genera were found in the soil of the studied stands, the majority of which (86%) belonged to the *Ascomycota* fungus division, the rest – to *Zygomycota* (Table 3). Fungi of *Penicillium* Link, *Geomyces* Traaen and *Mucor* P. Micheli ex L were detected in the soil of all studied stands and accounted for 80,0% (northern white cedar) to 39,3 percent. (Siberian larch) of all fungi found (Scot pine – 47,3%, Norway spruce – 52,4%). Fungi of the genus *Penicillium* were among the most commonly found in the soil of the studied stands. *Trichoderma* Pers and *Mortierella* Coem were common in all stands except northern white cedar stand soil. The northern white cedar soil fungi differed the most from the other stands in fungi composition and structure. The greatest diversity of fungal genera was found in the soil of the Scots pine stand.

Conclusions

1. The concentrations of calcium (Ca) and C_{org} differed the most in the litter of the studied coniferous stands. By the ratio of C : N tsnd Lig : N, the slowest decomposing and mineralization was of northern white cedar (*Thuja occidentalis*), the fastest – of Norway spruce (*Picea abies*).
2. The highest frequency of detection of microscopic fungi in the upper layer of mineral soil was in late autumn (November) and early spring (March), the lowest – in late summer (August).
3. The highest average frequency of fungal detection during the study period (January – November) was in the northern white cedar, the lowest – in the soil of the Scots pine (*Pinus sylvestris*) stand. The number of microscopic fungi was positively influenced by the higher lignin content and the L: N ratio in the litter.
4. *Penicillium*, *Geomyces* and *Mucor* fungi genera were detected in the soil of all studied stands and accounted from 80,0%. (northern white cedar) to 39.3 percent (Siberian larch (*Larix sibirica)*) of all fungi found. The fungi genera composition of the northern white cedar stand differed most from other stands. The greatest diversity of fungal genera was found in the soil of the Scots pine stand.

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About the authors

Nijolė Maršalkienė
Lecturer at the Faculty of Forest Sciences and Ecology,
Vytautas Magnus University Agriculture Academy, Lithuania
nijole.marsalkiene@vdu.lt

Maris Senkovs
PhD student at the Faculty of Biology, University of Latvia;
Researcher at the Microbial Strain Collection of Latvia
maris.senkovs@lu.lv