

PAPER • OPEN ACCESS

Ecological Aspects of *Pinus sibirica* Du Tour Mycotrophy in Forest Ecosystems of West Siberia

To cite this article: O Vaishlya *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **224** 012049

View the [article online](#) for updates and enhancements.

Ecological Aspects of *Pinus sibirica* Du Tour Mycotrophy in Forest Ecosystems of West Siberia

O Vaishlya¹, K Karbysheva¹, D Sarsekova², G Aidarkhanova²

¹ National Research Tomsk State University, Tomsk, Lenin Ave., 36, 634050, Russia

² S.Seifullin Kazakh AgroTechnical University, Astana, Pobeda ave., 62, Kazakhstan

E-mail: plantaplus@list.ru

Abstract. Samples of *Pinus sibirica* Du Tour ectomycorrhizas (ecm), the correlations of ecm-tips morph-anatomical indicators and host tree growth parameters, biotic and abiotic factors were analysed. The correlation between the numbers of ectomycorrhizal morphotypes per sample and the vegetation period was shown. High occurrence of contact exploration type in the most disturbed biotope was indicated. A positive relationship between the radius of the tree root and the thickness of the fungal mantle was recorded.

1. Introduction

Pinus sibirica Du Tour is one of the most important forest-forming species of dark-coniferous taiga from 48° up to 66° north latitude and grows over a large territory of Russia from the Timan Ridge to Kazakhstan, Mongolia and China. Pure cedar forests in West Siberia mostly exist due to plant community dynamics of during the Holocene and the anthropogenic impact: first Russian settlers used other tree species for firing and building but favored *P. sibirica* for its edible seeds. Thus unique forest ecosystems have been preserved on soils near many Siberian villages. This study was carried out in Western Siberia, where most valuable and large *P. sibirica* stands are located. Despite the fact that *P. sibirica* is an obligate mycotrophic plant, which does not grow nor develop without ectomycorrhizae, the mycosymbionts for this tree have not been fully identified. It is well known, that the richness and structure of ectomycorrhizal associations are influenced by a number of biotic and abiotic factors, including the host tree genotype, mycosymbiont interaction strategy, soil characteristics, availability of nutrients, and activity of pathogens, quality and quantity of soil organic matter. Detailed studies of *Pinus* genus mutualistic associations have not yet been conducted in Western Siberia [1 - 3]. The aim of the study was to investigate *Pinus sibirica* du Tour mycotrophy in three different biotopes of Tomsk Region. It was planned to describe the ectomycorrhizae biodiversity, ecm-tips anatomical and morphological structures, fungal symbiont DNA identification, analysis of exploration types distribution in different biotopes of morph types on the basis of in various biotopes.

2. Methods

The sampling took place in three remote biotopes. Loskutovo (L): Mixed-herb *Pinus sibirica*-*Picea abies* mixed stand. Tree canopy density ranges from 0.2 to 0.4. Moss cover is about 10%. Debris and trampling of ground vegetation are observed. The soil is characterized as gray forest soil. Timiryazovo (T): *Pinus sylvestris* green-moss forest. Tree canopy density is 0.7. Moss cover is 85-100%. The sight is disturbed with past cuttings and fires. The ground vegetation is trampled near roads and paths. Soil type is alfegumus podzol. Zorkaltsevo (Z): Mixed-herb *Pinus sibirica* forest. Tree canopy density is



0.8–0.9. The soil type is dark gray. A combination of negative impacts is observed: mechanical damage of *Pinus sibirica* tree trunks after harvesting the seeds, trampling of ground vegetation, soil horizons compaction and destruction of the soil body structure; *Phaeolus schweinitzi*, *Phellinus pini*, dot infections; strong wind damage.

Sampling was carried out from May to September in 2016 - 2018 in *Pinus sibirica* stands of Toms region. Sampling points were associated with cedar seedlings of 1–8 years old. The seedlings were extracted with the soil cores of 10×10×20 cm and root system. In habitats with no *P. sibirica* undergrowth, soil cores of 10×10×20 cm were extracted within the projection of mature trees canopy. Before sampling, the non-decomposed litter layer was removed. The samples were wrapped in aluminum foil and stored at +4°C. The roots were washed with running water, ectomycorrhizal tips were separated with forceps and scissors. Morphotyping was performed with a binocular with due consideration of ramification type, tip color, mantle surface features, mycelium or rhizomorphs characteristics according to the established methods [1]. Selected ectomycorrhizas were photographed and fixed with 70% ethanol for DNA-identification.

A microscopic study was conducted to explore the fungal mantle and Hartig net characteristics, as well as to estimate the proportion of mycelium in the sampled ectomycorrhizas [4]. The transverse sections of the fixed tips were performed with the freezing microtome “MZ-2”, measured in glycerol without staining with a Zeiss Axiostar plus microscope “LCL - 217 HS Digital” video camera and “Siams Mesoplant” soft (USA). The four directions of the following anatomical characteristics were measured: the mantle thickness, the cortex thickness, the stele diameter, and the diameter of the tip. The ratio of the cortex area (S_c) and the mantle area (S_m) was calculated by the formula: $S_m \times 100 / S_c$.

The age of seedlings was determined with LinTab-6 Tree-ring Station. Data analysis was carried out with one- and two-factor ANOVA Statistic 10, MS Excel, R free software environment for statistical computing and graphics.

Ectomycorrhizal tips DNA extraction was performed with the standard AxyPrep Multisource Genomic DNA Miniprep Kit according to the standard protocol. ITS1F and ITS4B primers were applied for nuclear DNA ITS amplification and sequencing according to standard protocols. Fermentas Genomic DNA Purification Kit was used for the PCR products purification. The data processing was performed with the BioEdit Sequence Alignment Editor application. Identification of ectomycorrhizal fungi was implemented via GenBank database BlastN algorithm. The 97–98% threshold established for ITS fungi satisfying sequences were selected for further analysis [5].

3. Results

In total 75 *Pinus sibirica* roots samples were selected in each of three biotopes, 15 soil cores were sampled in mature stands in spots where seedlings were not registered, and 60 were seedlings with soil cores, 250 ectomycorrhizal tips were selected and morphologically described. For each sample, the mycorrhization index was calculated: the number of ectomycorrhizal tips found was divided by the total length of the roots in the sample (cm), thus the number of ectomycorrhizas per 1 cm of root was found. The correlations between the mycorrhization index and biotopes, the host tree age were analyzed. The Kruskal-Wales criterion did not show a significant difference in mycorrhization between 3 biotopes. When combining two closely located biotopes and applying the Mann-Whitney criterion, significant differences were found ($p < 0.05$), which indicated that the mycorrhization index in Loskutovo is 2 times higher than in Timiryazev and Zorkaltsevo (Fig. 1).

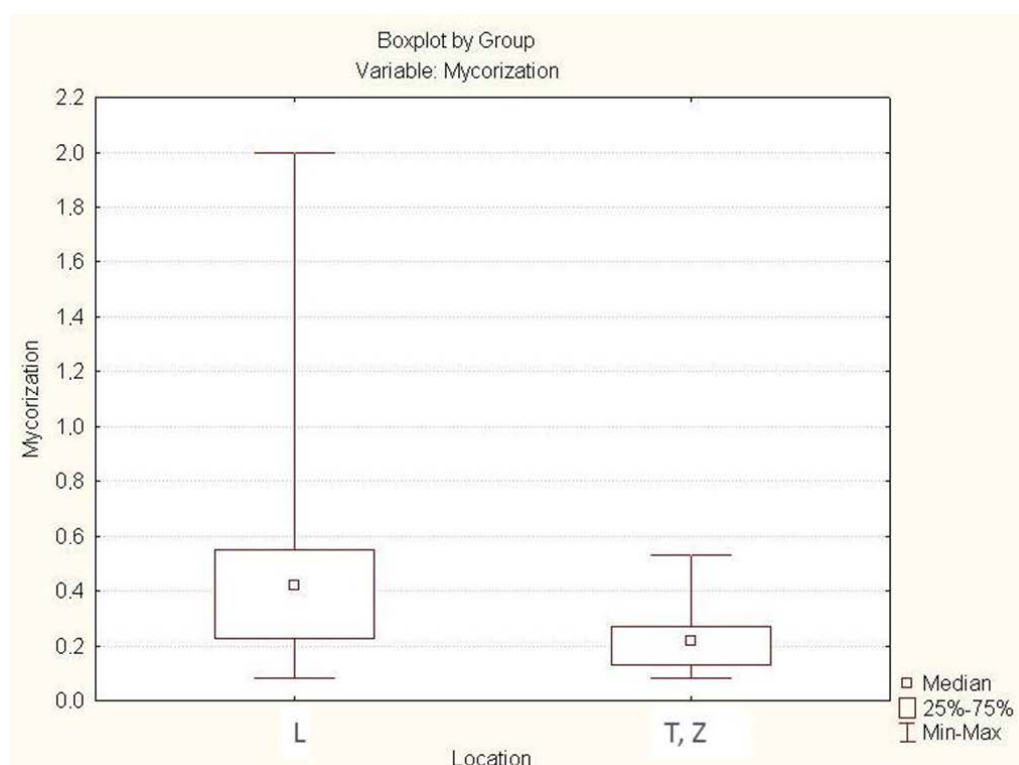


Figure 1. Reliability of differences in the mycorrhization index in Loskutovo (L), Zorkaltsevo(Z) and Timiryazev (T).

Samples were divided into two age groups. Group 1: 0–10 years old for the seedlings and Group 2: trees over 100 years old for the ectomycorrhized roots from soil samples. It worth noting that the average index of mycorrhization of mature trees roots (0.29 for Loskutovo, 0.21 for Zorkaltsevo) which is 1.8 lower in than seedlings (0.52 for Loskutovo, 0.46 for Zorkaltsevo). But the statistical analysis showed that these differences are not reliable enough: $F=(1.73)=3.4046$, $p=0.06907$.

The most frequent or rare ectomycorrhizas were described: A Dichotomously branched light-brown cottony ECM with proximal hydrophilic emanating hyphae and soil particles, short-distance exploration type; B Unramified ECM with many white hydrophobic filamentous rhizomorphs, medium-distance fringe/mat exploration type; C Irregularly pinnate light-brown ECM with whitish mantle dots, contact exploration type; D Dichotomously branched stringy ECM, contact exploration type; E Coralloid light-brown smooth ECM with whitish tips, contact exploration type; F Dichotomously branched light-brown ECM with whitish tips, contact exploration type; G Irregularly dichotomous smooth ECM associated with soil particles, contact exploration type; H Coralloid ECM with semi-transparent dark-brownish mantle, contact exploration type; I Monopodial pinnate light-brown smooth ECM with distinguished new parts, contact exploration type; J Unramified ECM with hydrophobic slivery rhizomorphs, medium-distance fringe/mat exploration type; K Irregularly dichotomous black smooth ECM with distinguished new parts, contact exploration type; L Tuberculate ECM covered with hydrophobic silvery hyphae, long distance exploration type. The fungal symbiont species were identified for some morphotypes by comparing the valid 18S – ITS1F–5.8S – ITS4B – 28S ribosomal DNA gene sequences with the NCBI references: B - *Cortinarius* sp.; D - *Tylospora fibrillose*; E - *Suillus subluteus*; H - *Suillus pictus*; K - *Tomentellopsis submollis* (Fig. 2).



Figure 2. Photos of various morphotypes of *Pinus sibirica* ectomycorrhizas.
Zeiss Stemi 2000C microscope, AxioCam ERc 5s camera.

Based on the anatomical and morphological description of the selected ectomycorrhizas, the number of morphotypes per sample was calculated: the correlations between the number of morphotypes per sample (seedling or soil core) and biotopes, host-tree age were not indicated. However, the average number of morphotypes per sample was higher in Loskutovo than in Timiryazev and Zorkaltsevo. The analysis also showed the correlation between the number of morphotypes per sample and the sampling period. The highest number of 5.8 morphotypes per sample, was indicated for the period 3 (August-September) with 4.3 for the period 2 (July-August) and 4.6 for the period 1 (June- July), $F(2,70)=5.1815$, $p=0.00797$ (Fig. 3).

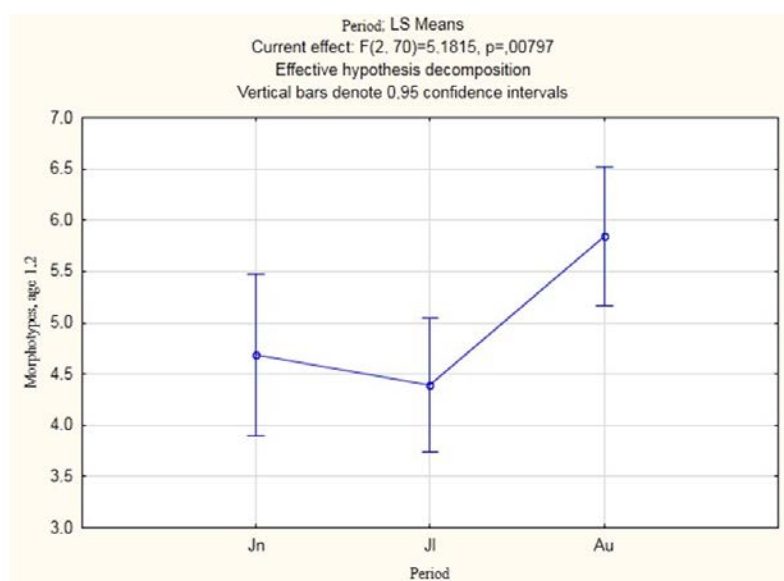


Figure 3. Dynamics of the number of morphotypes per sample during the 2018 growing season

The analysis showed that long distance exploration type was the rarest in all studied biotopes (2-3%). But occurrence of other exploration types had some peculiarities. Pearson's Chi-squared test for R ($X^2 = 22.587$, $df = 6$, $p\text{-value} = 0.00094720$) showed that contact exploration type in Zorkaltsevo is 1.5 and 2 times higher than in Loskutovo and Timiryazevo and makes 67% of the registered morphotypes, with 44% in Loskutovo and 32% in Timiryazevo. Medium distance fringe/mat was also rare in Zorkaltsevo with 5% of the morphotypes. In Loskutovo medium distance smooth was rare with 6% of the registered morphotypes. In Timiryazevo, medium distance smooth and medium distance fringe/mat were 2 times less common than contact and short distance exploration types.

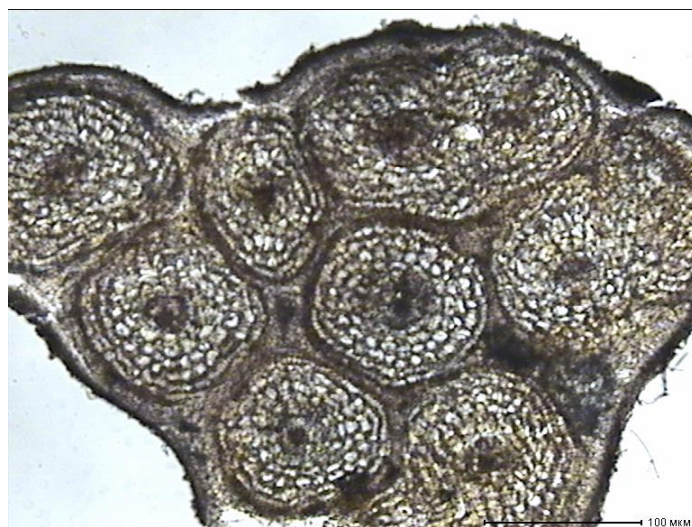


Figure 4. Transverse section of tuberculate *P. sibirica* – *Suillus sibiricus* ectomycorrhiza

The microscopic study showed that the radius of *Pinus sibirica* ectomycorrhizal tips varied from 104.5 to 389.9 μm , the mantle volume fraction comprised 3 to 43% and averaged 22%. The average radius of the root (from the center of the stele to the mantle) was 184.0 μm and varied from 96.1 to 364.2 μm . The average mantle thickness was 23.8 μm and varied from 4.9 to 74.2 μm . Statistical analysis showed that the data had normal distribution. No correlation was found between the ectomycorrhizae anatomical structure indices and biotope, host-tree age nor morphotype. However, a positive correlation between the radius of the tree root and the mantle thickness and, accordingly, between the areas of the root and the mantle was revealed, i. e. the thickness of the fungal mantle increased as the root area increased, which corresponds the publication data [2]. It is estimated that the difference between the maximum and minimum radius of the roots reached 3 - 4 times, and the maximum and minimum values of the mantle thickness had 16 - 17 times variation.

4. Discussion

The distribution of mycelium in the soil is crucial for the detection and effective consumption of nutrients. It is considered that ectomycorrhizae exploration type depends on the fungal species [6]. Short Distance exploration type ectomycorrhiza mycelium on the average occupies 89 mm^2 , Medium Distance fringe ectomycorrhiza mycelium occupies 165 mm^2 , Long Distance ectomycorrhiza mycelium occupies 1.336 mm^2 [7]. Contact exploration type forms a smooth mantle with a limited number of single and rare hyphae. This type is predominantly hydrophilic, provides direct contact with organic and inorganic substrate. Short distance exploration type forms numerous hyphae, mostly hydrophilic, which extend for a limited distance in the substrate. Medium Distance exploration type fungal species forms either hyphae and rhizomorphs, which can extend over considerable distances in the soil. The most frequent subgroup of this type is the Medium Distance fringe/mat exploration type

with numerous rhizomorphs interconnected with each other. This subtype is hydrophobic. Medium Distance smooth exploration type forms smooth rhizomorphs, mostly hydrophilic. Long Distance exploration type is characterized by very long, highly differentiated rhizomorphs with vascular-like hyphae. These rhizomorphs can be up to several decimeters in length and, as a rule, are hydrophobic. Rhizomorphs of this type are suitable for transporting solutes. Most Long-Distance exploration type fungi specialize in N absorption from organic sources [7].

The computer modelling of ectomycorrhiza position in the soil revealed zones where different fungi species mycelium exists within the same microenvironment. It may seem therefore that one host tree mycosymbionts compete each other for the resources. It is noted however, that hydrophobic mycelium is usually hydrophilic and able to absorb the nutrients at the periphery. Therefore, Contact exploration type and Short Distance exploration type fungal symbionts are supposed to use the nutrients which were not consumed by a Medium and Long-Distance exploration types without competition [8, 9, 10].

It is noted that factors such as litter quality, soil pH, availability of nutrients affect the strategy, but root density is supposed to determine the exploration type. When the roots are located at a considerable distance, it is more difficult for species with a shorter exploration types to colonize new roots via the mycelium, while the Medium and Long-Distance types effectively distribute and colonize new roots. While the roots density increase, Contact and Short Distance become more advantageous in terms of carbon supply. The results of this study are consistent with this assumption, since the highest occurrence of Contact exploration type among the studied biotopes was observed in the most densely populated biotope, Zorkaltsevo, and in Loskutovo, with the highest occurrence of Medium Distance, a low tree canopy density index was described. As Long-Distance exploration type probably requires significant amounts of host-tree carbon input to form rhizomorphs a negative anthropogenic impact in Zorkaltsevo where soil structure is disturbed also leads to the Long-Distance exploration type loss of competitive advantages, since this strategy needs more resources and time to regenerate than Contact and Short Distance exploration type which do not have extensive mycelia [11, 12].

Conifer root cortex is shown to immobilize carbohydrates, phosphorus. It is shown that exploration type not the host tree nor ecm-fungi parameters influence the potential ecm-tips enzymatic activity. Thus, the activity of β -glucosidase, cellobiohydrolase, acid phosphatase, β -glucuronidase and leucine aminopeptidase, as a rule, is higher in Long Distance than in Contact, Short Distance and Medium distance exploration types. On the contrary, laccase activity is significantly higher in Contact exploration type ectomycorrhiza [12].

A larger cortex can provide a larger pool for minerals, proteins or lipids. The number of rows in cortex is stable for conifers (4-6), so changes in the cortex thickness are associated mainly with changes in cell size [5]. There is evidence of a positive correlation between soil characteristics and the diameter of the root, which indicates a positive effect of soil fertility on the ectomycorrhiza root tissues size. A high variability of the mantle thickness is traditionally considered as a result of the adaptation of coniferous roots to the conditions of soil as a heterogeneous environment. Thus, the adaptation of tree absorbing organs is possible via the fungal partner tissues. Coniferous trees are able to form symbiotic relationships with 200-300 species of ectomycorrhizal fungi. There are near 50 ectomycorrhizal fungi species which are capable of forming ectomycorrhizas with *Pinus sibirica* [13]. These species differ in anatomical and morphological features and are capable of performing different environmental functions. According to the molecular identification methods different ecm-morphotypes can perform various ecological functions. Studies of such associations showed the specialization of ectomycorrhiza for certain functions: water absorption, mobilization and assimilation of nutrients, growth regulation and protection against pathogens or toxins [14 - 19]. The availability of nitrogen is the main factor determining the type and function of ectomycorrhiza. If nitrogen is low and the soil is acidic, the trees increase the photo assimilates flow underground. In telephoroid and atelioid fungi with rezupinant fruit bodies, preference is given to a decomposing substrate [20-23]. Just in Zorkaltsevo biotope with huge anthropogenic impact we could see very often *Piloderma* sp. and *Tomentella* sp. morphotypes from «the contact exploration type» with high laccase activity.

5. Conclusions

Thus, it is not the ectomycorrhizal tips morphotypes nor their microstructural parameters that promote the sustainability of *Pinus sibirica* forests, but the optimum ratio of exploration types in the rhizosphere. The whole pool of environmental factors influences the host-tree to promote the ecm-fungal partner with the most effective, for this particular stage of the host-tree ontogenesis, foraging strategy. The Contact exploration type which is optimal for the rapid carbon transfer over short distances under conditions of high roots density dominates in depressed Zorkaltsevo stand. In *P. sibirica* seedlings sampled under scots pine mature trees in Timiryazev Short Distance exploration type dominates. This exploration type does not obtain an extensive hyphal system and therefore it easily recovers but does not distribute for vast distances which is very important in the absence of wide variety of traditional *P. sibirica* fungal partners. The optimal parameters of Loskutovo forest probably explains a greater biodiversity of ecm-macromycetes observed on this site and the observed combination of exploration types. Apparently, a higher variability in hydrophilic and hydrophobic hyphae functioning, variable mycelium enzymatic activity and different foraging strategies are necessary for the host tree to adequately and rapidly respond to the changes of environment; which we assume to be the fundamental significance of the obligatory mycotrophy of *Pinus* genus representatives.

The study was supported by the RFBR grant № 15-29-02588 and the Target Program of the Ministry of Agriculture of the Republic of Kazakhstan № IRN-BR06249252.

References

- [1] R. Agerer (ed.) 1996–2008 Colour Atlas of Ectomycorrhizae. Einhorn-Verlag, Schwabisch Gmund.
- [2] L. Tedersoo, M. E. Smith 2013 Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground *Fung. Biol. Rvs.* **3-4**, №27 83.
- [3] R. Agerer 2001 Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance *Mycorrhiza* **11** 7
- [4] I. Ostonen, K. Lohmus, R. Lasn 1999 The role of soil conditions in fine root ecomorphology in Norway spruce (*Picea abies* (L.) Karst.) *Plant and Soil* **208** № 283 <https://doi.org/10.1023/A:1004552907597>
- [5] B.E. Clasen et al. 2018 Characterization of Ectomycorrhizal species through molecular biology *Agric. Microbiol.* **75** № 3 246.
- [6] E.A. Lilleskov, E.A. Hobbie, T.R. Horton 2011 Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition *Fung. Ecol.* **4** 174
- [7] Weigt R.B, Raidl S., Verma R., Agerer R. 2012 Exploration type-specific standard values of extramatrical mycelium – a step towards quantifying ectomycorrhizal space occupation and biomass in natural soil *Myc. Progr.* **11** 287
- [8] Agerer R. 2006 Fungal relationships and structural identity of their ectomycorrhizae *Myc. Progr.* 5 67
- [9] Hobbie E.A., Agerer R. 2010 Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types *Plant and Soil.* **327** DOI 10.1007/s11104-009-0032-z
- [10] Chen W., Eissenstat D.M., Koide R.T. 2018 Root diameter predicts the extramatrical hyphal exploration distance of the ectomycorrhizal fungal community *Ecosph.* **9** № 4 <https://doi.org/10.1002/ecs2.2202>
- [11] Peay C.J., Cennedy P.G., Burns T.D. 2011 Rethinking ectomycorrhizal succession: are roots

- density and hyphal exploration types drivers of spacial and temporal zonation? *Fung. Ecol.* **4** 233.
- [12] Tedersoo L., Naadel T., Bahram M., Pritsch K., Buegger F., Leal M., Kõljalg U., Põldmaa K. 2012 Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest *New Phytolog.* **195** 832.
- [13] Vaishlya O. B., Kudashova N. N., Gashkov S. I., Karbysheva K. S., Bakhtinskaya I. A. 2017 First list of macromycetes forming ectomycorrhizas in cedar and pine forests of Tomsk region of West Siberia *Int. J. of Env. Stud.* **74** №5 DOI: 10.1080/00207233.2017.1294422
- [14] Rosinger C., Sandén H., Matthews B., Mayer M., Godbold D. L. 2018 Patterns in Ectomycorrhizal Diversity, Community Composition, and Exploration Types in European Beech, Pine, and Spruce Forests *Forests* **9** №8 <https://doi.org/10.3390/f9080445>
- [15] Villarreal-Ruiz L., Neri-Luna C. 2018 Testing sampling effort and relative abundance descriptors of belowground ectomycorrhizal fungi in a UK planted scots pine woodland *Mycol.* **9** № 2 <https://doi.org/10.1080/21501203.2017.1394393>
- [16] Rudawska M., Wilgan R., Janowski D., Iwański M., Leski T. 2018 Shifts in taxonomical and functional structure of ectomycorrhizal fungal community of Scots pine (*Pinus sylvestris* L.) underpinned by partner tree ageing *Pedobiolog.* № 71 20.
- [17] Põlme S., Bahram M., Jacquemyn H., Kennedy P., Kohout P., Moora M., Oja J., Öpik M., Pecoraro L., Tedersoo L. 2018 Host preference and network properties in biotrophic plant-fungal associations *New Phytol.* **217** № 3 1230.
- [18] Ning C., Mueller G.M., Egerton-Warburton L. M., Wilson A. W., Yan W., Xiang W. Diversity and Enzyme Activity of Ectomycorrhizal Fungal Communities Following Nitrogen Fertilization in an Urban-Adjacent Pine Plantation *Forests* **9** № 3 <https://doi.org/10.3390/f9030099>
- [19] van der Linde S., Suz L.M., Bidartondo M. I. 2018 Environment and host as large-scale controls of ectomycorrhizal fungi *Nature* **558** 243.
- [20] Finlay R.D. 2004 Mycorrhizal fungi and their multifunctional roles *Mycologist* **18** № 2 91.
- [21] Finlay R.D. 2005 Mycorrhizal symbiosis: myths, misconceptions, new perspectives and future research priorities *Mycologist* **19** № 3 90
- [22] Ang L., Fahey T. J., Pawlowsky T. E., Fisk M. S., Burtis J. 2015 Fine root decomposition, nutrient mobilization and fungal communities in a pine forest ecosystem *Soil Biol. and Biochem.* № 83 76.
- [23] Courty P-E. Buée M., Gamby A., A.G. Diedhiou, Pascale F-K., Le Tacon F., Rineau F., Turpault M-P., Uroz S., Garbaye J. 2010 The role of ectomycorrhizal communities in forest ecosystem process: New perspectives and emerging concepts *Soil Biol. and Biochem.* **42** 679.