Diversity of ectomycorrhizal structures of *Larix sukaczewii* and *Pinus sylvestris*

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Abstract. The ecological value of ectomycorrhizal associations is not restricted to the organism level. They play an essential role in plant communities as key to the diversity and sustainability of species. The sustainability of forest stands in urbanised areas depends on preserving mycorrhizal communities' structural and functional organisation. Reducing species diversity of ectomycorrhizal associations does not lessen the resilience of forest ecosystems in industrial centres but is balanced by morphological diversity.

1. Introduction

Symbiont relations with mycorrhizal fungi are important biological and ecological properties of light conifers, forming unique ectomycorrhizal structures. The formation of mycorrhizae may vary depending on the growing conditions of arboreal plants. Mycorrhization is an essential process for the sustainable functioning of the ecosystem and for adapting tree plants to adverse factors. Ectomycorrhiza acts as a root, forming a dense fungal cover around the root, from which the hyphae forming the Hartig net enters the primary cortex. Significant and often irreversible physiological and anatomical changes occur in the organs and tissues of woody plants following toxic emissions from industrial plants and transport. Therefore, studies aimed at assessing mycorrhizal growth's anatomical diversity in extreme plant conditions are particularly relevant. [1].

The work aimed to study the structural diversity of the ectomycorrhizal root tips of *Larix sukaczewii* and *Pinus sylvestris*.

2. Materials and methods

The *Larix sukaczewii* and *Pinus sylvestris* stands of artificial origin, which form part of the sanitary protection zones of the urbanized areas of PreUrals, were chosen as the object of study. *Larix* species (*Larix* Mill.) are frequently used in urban sanitation, as they grow well and develop well beyond their natural range. This is an ecological plastic species with a wide range of adaptive capacities, with deciduous foliage, which contributes to resilience to a wide range of environmental factors and can also be assumed to be a feature of the structure of the absorbing roots [2]. Therefore, numerous scientists are actively studying many aspects of larch growth and development, particularly in Russia. However, it should be noted that the formation of mycorrhizae in *Larix* species is less studied than in *Pinus* and *Picea* species [1] and thus requires further and more detailed examination. This study is of
particular importance regarding the establishment of protective forest plantations in major industrial centres to improve the environmental situation.

The samples were selected within the two largest industrial centres of the Republic of Bashkortostan located in the forest-steppe zone of PreUrals: the city of Ufa (54°47´ N, 56°02´ E.) and the city of Sterlitamak (53°37´ N, 55°57´ E). The Ufa Industrial Centre (UIC) is characterised by a predominantly petrochemical type of pollution, while a predominantly polymetallic type of pollution characterises the Sterlitamak Industrial Centre (SIC). Samples were taken in 2009 and 2016 using standard methods [3]. The root tips (absorbing part of roots up to 3 mm) of pine and larch were selected at the end of the growing season (September). The test areas were laid in the northern part of cities in the industrial zone of pollution and the southern part – "in conditional control". In order to determine the structural diversity of ectomycorrhiza, cross-sections of 10-15 μm thickness prepared on a sledge microtome by freezing were analysed. At least 100 slices were viewed for each test area, with a total of about 4,800 cross-sectional sections studied using the AxioImager A2 microscope (Carl Zeiss, Germany). The Veselkin procedure [4] was used to evaluate and interpret the material received.

3. Results and Discussions

*Pinus sylvestris* is an obligatory mycorrhizal tree, forming a typical eumycete chalmophagus ectomycorrhiza. Based on our observations, mycorrhizae are dichotomically branched and have a predominantly furlcral and double furlcral form, as noted by other researchers [5-6]. In *Larix sukaczewii*, the small absorbing part of the root system also forms mycorrhizal associations. Simple clubbed forms represent most mycorrhizae, a monopodial branch with a pronounced central axis, occasionally found with a cystic shape in structure. The life expectancy of mycorrhizal roots is estimated in various ways in the literature, most commonly in annual form, performing their functions during a single growing season [1;6]. The intensity of larch mycorrhizal formation in Ufa varies between 81-92%, while in the city of Sterlitamak, it ranges between 77-92%. The intensity of pine mycorrhizal formation is lower than that of larch (which is not entirely typical of pine as an obligatory mycorrhizal tree), and in the city of Ufa is 63-82%, and in Sterlitamak varies from 61-87%. Detailed studies on the variability of general dimensional and qualitative parameters of the ectomycorrhizal roots of pine and larch have already been conducted [7-8].

The impact of pollution on the variety of fungal covers, expressed as a change in the abundance of mycorrhizae with sheaths of different addition types, has been identified. The more physiologically active are slightly coloured, well-structured pseudo parenchymal and double fungal covers [9]. According to the literature, the variety of fungal cover subtypes depends primarily on mycorrhizal fungal species' diversity because different fungal species form morphologically different fungal covers [3]. Phytobiont in ectomycorrhizal symbiosis is regarded as having a low level of specificity, as a single plant species may be associated with thousands of fungal species that may provide plants with access to a broader range of nutrients in the soil [10]. In turn, the species composition of the biocoenosis is affected by environmental factors. Despite many fungal sheath subtypes, isolating four types of ectomycorrhizal covers is more practical: plectenchymatic, pseudoparenchymal and binary coverage types (often analysed together) non-structural (SR), which are more informative when comparing samples against each other [1;3].

By analysing the mycorrhizal formation of pine and larch during different years of research (2009 and 2016 - own research, 2003 results are provided by literary data [1] from the same test areas), it has been found that the proportion of non-structural fungal cover types in the pollution area is growing steadily and unambiguously (figures 1 and 2). The proportion of pine mycorrhizae with non-structural covers increases from 21-34% to 47% in pollution conditions at Sterlitamak, and in Ufa increases from 19-39% to 33-45%. This type of addition of the fungal cover is characteristic of the final phase of morphogenesis, and physiologically they are not as active. In larch, the ratio of non-structural cover types is independent of pollution level and ranges from 40%.
Figure 1. The abundance of mycorrhizal cover subtypes of Pinus sylvestris (A) and Larix sukaczewii (B) under various growing conditions, 2009.
In contrast to larch, in 2009, plectenchymatic fungal cover of pine was abundant in control (34-60%), double the pollution (27-36%) (figure 1A). However, in 2016, the share of pine plectenchymatic fungal covers in pollution conditions in Ufa is twice as high as in control and varies between 20% and 52%, which may be due to the stimulating influence of hydrocarbon pollution (figure 2A). In larch, the formation of plectenchymatic covers depends on the growing conditions (contamination level). At the Sterlitamak Industrial Centre, under pollution conditions, the abundance of plectenchymatic fungal covers (5-25%) was fewer than in control (21-35%) (figure 1B). At the Ufa Industrial Centre, the proportion of plectenchymatic fungal covers in larch trees increases when pollution occurs.

Figure 2. The abundance of mycorrhizal cover subtypes of *Pinus sylvestris* (A) and *Larix sukaczewii* (B) under various growing conditions, 2016.
Pseudoparenchymal and double addition covers are the most varied in structure. In larch and pine trees, pseudoparenchymal and double covers are the most represented in control compared to contaminated areas. This group comprises eight to nine fungal cover subtypes (F, G, H, K, L, N, O, P, Q), whereas, in plectenchymatic types, these are mainly 2-3 subtypes of fungal covers (A, B, E). It is not easy to associate a subtype of the structure of a fungal cover with a particular mycobiont, as a phytobiont may form a mycorrhiza simultaneously as dozens of symbionts. However, some studies have shown that the G fungal cover subtype is formed with *Cenococcum geophilum* (*Ascomycota*). This species of fungus is the most common ectomycorrhizal symbiont globally and often dominates the tree root system in the arctic, temperate and subtropical areas, especially in severe environmental conditions [11]. It forms a typical melanized mantle with dark pigmented fibrous hyphae around the root tips. Hyphae growing from a fungal cover are rigid, bristly, blunt-pointed, black in colour, fungal covers are yellow-brown to dark brown [12].

While studying the diversity of *Larix sukaczewii* ectomycorrhiza, we have identified another subtype of HN that was not found in the study of *Pinus sylvestris* mycorrhiza. The distinguishing feature of this subtype is the lack of an external fungal cover, with the preservation of a well-developed Hartig net between the root cortex cells. In 2009, the share of this subtype in mycorrhizal covers within Sterlitamak industrial centre was 3-22 times higher than in Ufa industrial centre, but in 2016 the situation was reversed.

4. Conclusion

The structural diversity of fungal covers varies across all test areas. At the same time, the variety of fungal covers decreases in the same manner in these tree species when contamination occurs, but the ratio of mycorrhizal abundance with different cover types is not uniform. Pine ectomycorrhiza reacts more strongly to industrial pollution, resulting in the reduced intensity of mycorrhizal cover formation and decreased diversity of fungal cover subtypes. The response of larch ectomycorrhiza to contamination increases the intensity of mycorrhizae formation.

Obligatory mutualism allows the phytobiont and mycobiont of pine and larch trees to respond effectively to anthropogenic pressures, increases the adaptive potential of these tree species and enables them to grow under severe environmental conditions. Mycorrhiza is associated with growing conditions but also with tree species.

References

[1] Veselkin D V 2013 *Morphological variability and adaptive value of coniferous ectomycorrhizae: Pinaceae Lindl.* (Ekaterinburg: Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences)

[2] Simonenko A P, Klostnikov M V and Paramonov E G 2008 Larch in protective forest stands of steppe zone *Bulletin of Altai State Agrarian University* 7(45) 23-28

[3] Selivanov I A 1981 *Mycosymbiotrophism as a form of consortium ties in the vegetation cover of the Soviet Union* (Moscow: Nauka)

[4] Veselkin D V 2003 Variability of anatomical parameters of ectomycorrhizal endings of different structure *Mycology and phytopathology* 37(1) 22-29

[5] Shemakhanova N M 1962 *Mycosimbiosis of tree species* (Moscow: Publishing House of the USSR Academy of Sciences)

[6] Lobanov N B 1971 *Tree plant mycotrophy* (Moscow: Forest industry)

[7] Mukhametova G M and Zaitsev G A 2017 Morphological and anatomical variability of *Larix sukaczewii* mycorrhiza formation in urbanized territories of the Cis-Urals. *Izvestia of the Ufa Scientific Center of the Russian Academy of Sciences* 3 5-172

[8] Zaitsev G A, Mukhametova G M and Safiullina L M 2021 Mycorrhizal formation of light-coniferous species of urbanized ecosystems. *IOP Conf. Ser.: Earth Environ. Sci.* 677 052080

[9] Tvorozhnikova T A 2008 *Structural-functional organization of mycorrhizal root tips of Picea obovata Ledeb* (Syktyvkar: Institute of Biology, Komi Science Centre, Ural Branch of the
Russian Academy of Sciences)

[10] Sizonenko T A 2018 Modern ideas about the structure of ectomycorrhizal associations. Bulletin of the Institute of Biology of Komi Scientific Centre UB RAS 1 2-14

[11] LoBuglio K F, Chambers S M and Cairney J W G 1999 Cenococcum Ectomycorrhizal Fungi Key Genera in Profile (Berlin: Springer) 287-309

[12] Peter M, Kohler A and Ohm R 2016 Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus. Cenococcum geophilum Nat Commun 7 12662