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SHIFT IN FUNGAL COMMUNITIES ASSOCIATED WITH *PINUS SYLVESTRIS* STANDS AFFECTED BY ROOT ROT

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Root rot caused by the wood-decay fungus *Heterobasidion annosum* sensu lato damages both below- and above-ground parts of Scots pine trees (*Pinus sylvestris* L.). The disease progress is likely to be affected by reshaping occurred in a forest such as soil properties, vegetation composition, and tree age. These changes are apparently followed up by paralleled shifts in fungal community composition in forest soil with potential feedback on ecosystem functioning. In the present study, we tried to evaluate fungal communities across diseased *P. sylvestris* stands and investigated correlations between taxonomic composition and forest health. Not surprisingly, root rot infestation had a significant effect on root-associated fungal abundance and diversity. During the development of the disease, the root-associated fungal community shifted in composition from dominance by saprotrophic fungi to ectomycorrhizal and pathogenic fungal species. Our results suggested that the maintenance of functional diversity in the root-associated fungal community may sustain long-term forest health or even root rot resistance to some extent by retaining a capacity for symbiosis-driven recycling of organic nutrients. However, it is necessary to thoroughly examine this hypothesis.
Key words: Scots pine, *Heterobasidion annosum* s.l.

Introduction. Scots pine (*Pinus sylvestris* L.) covers large areas in European regions with significant economic importance to the Ukrainian forest industry. It is the most important and main forest-forming tree species in Ukraine growing on poor sandy soil and degraded habitats (Ustskyi et al. 2010, Ustskyi 2011). This species is tolerant to many abiotic factors, such as poor soil, drought, wind, and frost (Durant et al. 2016), and hundreds of thousands of hectares have been planted with this species, e.g., in Ukraine. Most of this pine wood decay is caused by the root and butt rot pathogen *Heterobasidion annosum* sensu lato (Ustskyi et al. 2010).

For many years, a gradual decline of Scots pine forests has been observed in Ukrainian regions due to various factors (Ustskyi et al. 2010, Ustskyi 2011, Meshkova 2022). Nevertheless, the main cause of pine decline and dieback is root rot infection. *P. sylvestris* is susceptible to root rot caused by fungi from the genus *Heterobasidion* (Pitkänen et al. 2021) – the most economically important pathogens of conifers in the Northern Hemisphere (Piri et al. 2021). There are three *Heterobasidion* species presented in Europe that have different tree host preferences: *Heterobasidion parviporum* Niemelä and Korhonen, *H. abietinum* Niemelä and Korhonen and *H. annosum* s.s. (Fr.) Bref. (Piri et al., 2021). *H. annosum* s.s. is mostly associated with *Pinus* species, especially Scots pine, but it can attack several other conifers as well as some broadleaved tree species; *H. parviporum* demonstrates a narrow specialization for Norway spruce (*Picea abies* Karst.), while *H. abietinum* prefers European silver fir (*Abies alba* Mill.) and other species of the genus *Abies* (Dalya et al. 2021).

Extensive logging of pine and spruce forests has changed the environment into favoring this pathogen in stands where it originally was rare. The proportion of infested forest stands and associated production losses are expected to enlarge in the future due to the increase in sanitation logging.

Primary infections of the pathogens are established by airborne basidiospores, landing on surfaces of freshly cut conifer stumps (Garbelotto & Gonthier 2013) and growing over the stump surface into the inner part of the stump, finally colonizing roots. Secondary infection can spread via the growing of lateral roots connection of adjacent trees or stumps (Swedjemark & Stenlid 2001). Moreover, *Heterobasidion* spp. could effectively infest the roots of trees that were planted on infested land after sanitation clear-cuts or final harvesting, creating secondary infections (Vasiliauskas & Stenlid 1998, Lygis et al. 2004). Also clear-cutting exposes pine stands to primary spore infection, while in Scots pine stands wounds might not be a significant infection pathway for *H. annosum* s.s. via root contact and infect nearby growing trees (Garbelotto & Gonthier 2013).

The old-growing coniferous forests are composed of trees of different species and ages, but nowadays most forest areas in Ukraine were managed into even-aged stands, where sanitation clear-cuts in pine stands are very common due to the *H.annosum* s.s. infection. This has major effects on forest biodiversity, carbon storage, and forest resilience (Kyaschenko et al. 2017, Hagenbo et al. 2018), as well as nutrient and water availability, soil microclimate, and litter quantity and quality (Jurgensen et al. 1997). Moreover, fungi are able to form symbiotic, pathogenic, or neutral associations with plant roots and can be the triggers of shifts in associated micobiome (Hagenbo et al. 2018). To date, there is very little research in Ukraine on the fungal community associated with a root system.

The root rot infection is currently controlled by means of chemicals, biocontrol agents, and silvicultural measures (sanitation logging). For instance, the saprotrophic fungus *Phlebiopsis gigantea* has for several years been used as a biocontrol agent against *H. annosum* s.l. in spruce and pine stumps in the EU (Lygis et al. 2004, Garbelotto & Gonthier 2013) but it does not apply in Ukraine. A major problem is that, although the effectiveness of *P. gigantea* as a biocontrol agent has empirically been shown in many countries, the long-term biological effect of this fungus on other decomposing wood microbiota including different pathogens has not been proven. Moreover, there is little concern over the study of the complex of different pine pathogens dominated by *H.annosum s.l.* which caused root rot in pine stands.

The objective of our study was to screen fungal groups associated with the root system of *P. sylvestris* in stands affected by *H.annosum* s.s. to better understand the pathogenesis and development of root rot infection, as well as to recognize whether root size and disease severity affect diversity of fungi of the root system in the forest-steppe conditions of Ukraine. The additional object was to study other resident microflora of *P. sylvestris* root infested by *H.annosum s.s.* to find out whether the *H. annosum s.s.* impacts the overall diversity of other fungi.

Material and Methods. The field study was carried out in 2018–2020. The field study sites were pure pine forest stands located in Kharkiv region, Ukraine (compartment 126, subcompartment 7, tract Bugri, Kharkiv Forest Research Station). Wood core and root samples from *P. sylvestris* were collected in June 2018 in the forest sites previously identified as infested by *H.annosum s.l.* 7–8 years ago. Similarly, samples from non-infected, visually healthy trees within the same forest site were also collected. The site was a mature thinned *P. sylvestris* stand (age 60–70 years) after selective sanitation logging in 2014.

Wood cores and root samples were collected from the five infected (50–100 m apart from each other) and five non-infected trees (up to 500 m apart from the infested area and 50–100 meters apart from each other). One wood core from each tree (one core sample from the sapwood to the center (heartwood) of the stem) was sampled on ground level to check the presence/absence of *Heterobasidion annosum* s.l. Root samples (0.5–3.0 cm in thickness) were collected from the same infected and non-infected trees (0.3–1.0 m apart from the stem and 0.1–0.3 m in depth). All wood cores and root samples from each tree were pooled in a sterile falcon tube individually and stored at -20°C. Root samples from infected trees were visually divided by infected and non-infected zone (if applicable). In total, 10 wood samples (5 from infested trees and 5 from non-infested trees) and 40 root samples (4 from each tree) were collected, resulting in 50 samples.

Fungal culturing. Wood samples were surface sterilized according to Millberg et al (2006) and Lygis at al. (2004). Fungal isolation was carried out of the small sample fragments which were cut from each root/wood and placed in Petri dishes containing 3 % malt extract agar (MEA) supplemented with antibiotic chloramphenicol (0.5%) (to avoid the fast-growing fungi) and incubated at 23°C for 15 days in the dark. Pure fungal cultures were obtained from colonies morphologically classified and one representative isolate resembling the morphological group was selected. Fungal cultures were subjected to sequencing of the internal transcribed spacer of the fungal ribosomal RNA (ITS rRNA). Isolation of DNA, amplification and sequencing followed methods described by Menkis et al. (2006). Amplification by PCR was done using the two primers: ITS1F and ITS4 (Gardes & Bruns 1993).

Direct sequencing of fungi from the root samples. Frozen root samples were ground to a fine powder with liquid nitrogen. DNA extraction and purification was done by using Nucleo Spin® Plant II Midi kit (MACHEREY-NAGEL product). DNA quantification and quality control of the DNA samples were analyzed spectrophotometrically by way of NanoDrop ND-1000 (Wilmington, USA). Amplification by PCR was done using the ITS1F and ITS4 primers (Gardes & Bruns 1993). Each PCR reaction contained 200 µM deoxyribonucleotide triphosphates, 0.2 µM of each primer, 0.03 U/µl Thermo Green Taq polymerase with reaction buffer Green, and 2.75 mM final concentration of MgCl2. The thermal cycling was carried out using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Foster City, CA, USA). PCR products were size separated on 1% agarose gels and visualized under UV light. If only one DNA band was present on gel per sample following nested PCR, the PCR product was used for sequencing. Multiple-banded PCR products were separated on 2.0% agarose gels, individual bands were excised and re-amplified using universal primers ITS1 and ITS4 (Gardes & Bruns 1993). Resulting single-band products were sequenced in both directions using the same primers as for PCR amplification.

The PCR products were purified with Qiagen DNA extraction PCR M kit (Qiagen, Hilden, Germany). Sequencing was carried out by Macrogen Inc., Korea. The raw sequence data were analyzed using the SeqMan Pro version 10.0 software from DNASTAR package (DNASTAR, Madison, WI, USA). Databases at GenBank (Altschul et al. 1997) and at the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, were used to determine the identity of ITS rRNA sequences. The criteria used for the identification were sequence coverage > 80%, similarity to taxon level 98–100%, and similarity to genus level 94–97%.

Statistical analysis. All data were tested for adherence to the normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances using Bartlett's Test. To evaluate the influence of infection on the occurrence and frequency of fungi, we made a randomized block design, dividing the experiment on-site blocks, such that the variability within blocks is less than the variability between blocks. The richness of fungal taxa from the trees with different root rot statuses (infected vs non-infected) was compared using chi-squared tests. The relative abundance of fungal taxa was calculated from actual numbers of the observations (presence/absence data) as a proportion of observations (fungal cultures/ sequences) of a particular species relative to the total number of species of fungal community in the samples. Shannon diversity indices and quantitative Sorensen similarity indices were used to characterize the diversity and composition of fungal communities (Maggurran 1988). The Simpson diversity index (Mouillot & Lepretre 1999) was used to estimate the dominance in fungal diversity while taking into account both richness and evenness. Fungal dominance was determined by Camargo's index (Mouillot & Lepretre 1999). Comparisons between fungal communities were made using Sorensen similarity indices, Mandel statistics (Maggurran 1988), and principal component analysis (PCA). In PCA, data from the culturing and direct sequencing were analyzed jointly using Canoco 4.5. Statistical analyses were completed using STATISTICA® 7.0 (StatSoft, Inc., Tulsa, OK, USA) and PAST software (Hammer et al. 2001).

Results and Discussion. Fungal culturing from 10 surface-sterilized wood cores resulted in 21 fungal cultures, 2.1 per wood segment. Direct sequencing from 40 surface-sterilized segments of lateral roots resulted in 247 fungal sequences or 6.2 per root segment on average.

Our observations did not reveal any external signs (fruitbodies) of *H. annosum s.s.* infections in the investigated stands. The examination of the superficial root systems of the 37 declining pines around the sample site and fungal isolations from the wood samples did not show a presence of the root rot disease in spite of the fact that some root samples demonstrated a presence of *H. annosum s.s*. (Table 1).

The most dominant fungi from the infested trees of *P. sylvestris* were *Dactylonectria macrodidyma* (4.98%), *Acremonium* sp (4.52%), *Cladosporium cladosporioides* (4.07%) from Ascomycota and *Heterobasidion annosum* s.s. (4.07%) from Basidiomycota.

Table 1

Occurrence and relative abundance of fungal taxa from infested *Pinus sylvestris*, %

Fungal taxa (functional group)*	Genbank accession number	Trees infested by *H.annosum* s.s			Total
		Root (visu-ally infected)	Root (healthy looking)	Wood	
Ascomycota					
Acremonium sp. (P, S)	HF680219.1	1.14	13.79	25.00	4.52
Acephala applanata (E)	JN091527.1	2.84	3.45	0.00	2.71
Alternaria alternata (P, S)	KU663948	0.00	0.00	6.25	0.45
Aspergillus cervinus (FF)	AB025458.1	2.27	0.00	0.00	1.81
Aspergillus versicolor (FF)	KU663952	0.00	6.90	0.00	0.90
Bionectriaceae sp. (P, S, FF)	KU663955	2.84	0.00	0.00	2.26
Chaetomium sp. (P, S, FF)	KU663958	3.41	0.00	0.00	2.71
Chalara sp. (P, S)	KU663959	0.00	6.90	0.00	0.90
Cladosporium cladosporioides (P, FF)	KU663962	5.11	0.00	0.00	4.07
Cladosporium herbarum (P, FF)	MH865203.1	0.00	0.00	12.5	0.90
Cladosporium sp. (P, S, E, FF)	MH865203.1	3.98	0.00	0.00	3.17
Dactylonectria macrodidyma (P)	KU663971	6.25	0.00	0.00	4.98
Gliocladium sp. (P, S, E, FF)	KY359203.1	0.00	3.45	0.00	0.45
Gibberella avenacea (P, S)	KU663968	3.98	0.00	0.00	3.17
Mariannaea elegans (P, S, E, FF)	KU663974	0.00	0.00	12.5	0.90
Metapochonia bulbillosa (BCA)	MH876089.1	4.55	0.00	0.00	3.62
Ogataea neopini (S, FF)	KC768085.1	0.00	0.00	6.25	0.45
Ophiostoma piceae s.l. (P, S, E)	JX444603.1	0.00	3.45	6.25	0.90
Ophiostoma sp. s.*l.* (P, S, E, FF)	MK371461.1	0.00	0.00	12.5	0.90
Oidiodendron chlamydosporicum (S, P, FF)	NR_111032.1	2.84	0.00	0.00	2.26
Penicillium brevicompactum (S, E, FF)	KF156318	2.84	0.00	0.00	2.26
Penicillium citreonigrum (S, E, FF)	KU663988	0.00	6.90	0.00	0.90
*Penicillium roquefort*i (S, E, FF)	KU663990	3.41	0.00	0.00	2.71
Pezicula eucrita (P, S, E)	MT156284	0.00	6.90	0.00	0.90
Phialocephala sp. (P, S, E, FF)	KF156325	1.70	0.00	0.00	1.36
Phoma macrostoma (P, S)	KF156326	4.55	0.00	0.00	3.62
Phomopsis sp. (P, S, N)	KU663994	0.00	6.90	0.00	0.90
Pseudogymnoascus roseus(S, E, FF)	OL989270.1	3.98	0.00	0.00	3.17
Rhizoctonia sp. (P, S, E, FF)	KU663995	0.00	6.90	0.00	0.90
Sordaria sp (P, S, E, FF)	MH860578.1	3.41	3.45	0.00	3.17
Trichoderma asperellum (S, E, BCA)	KU664000	13.98	0.00	0.00	3.17
Trichoderma harzianum (S, E, BCA)	FG342170.1	3.41	6.90	0.00	3.62
Trichoderma viride (S, E, BCA)	JF440620.1	2.27	0.00	0.00	1.81
Unidentified Helotiales HH79	KC768103	4.55	0.00	0.00	3.62
Unidentified Pezizales	KU664004	1.70	0.00	0.00	1.36
Basidiomycota					
Ceratobasidium bicorne (P)	KF156330	2.27	3.45	0.00	2.26
Fomitopsis pinicola (WDF)	KU663967	3.98	0.00	0.00	3.17
Heterobasidion annosum s.s. (WDF)	KC768081	3.41	10.34	0.00	4.07
Hebeloma sp..(ECM)	KU663969	1.14	0.00	0.00	0.90
Hyphoderma setigerum (ECM)	KU663970	6.25	0.00	0.00	0.45
Phlebiopsis gigantea (WDF, BCA)	KU663992	0.00	0.00	12.5	0.90
Thelephora sp.(S, AM)	WIUZ02000018	2.27	0.00	0.00	1.81
Mucoromycotina					
Mucor sp. (S, FF)	KU663977	3.41	3.45	0.00	3.17
*Mucor fragili*s . (S, FF)	KU663976	3.98	0.00	0.00	3.17
Umbelopsis isabelline . (S, FF)	AB199612.1	0.00	3.45	0.00	0.45
Unidentified culture *Mucor*-like		4.55	3.45	0.00	4.07
No. of OTU(operational taxonomic unit)		176	29	16	221
Shannon-Weaver diversity index		3.48	3.07	3.22	3.95
Simpson diversity index		0.97	0.96	0.93	0.98

*P – plant pathogen, S – saprotroph, ECM – ectomycorrhizae, WDF – wood decay fungi, AM – arbuscular mycorrhiza, E – endophytes, FF – filamentous fungi, indoor and soil fungi, decaying organic matter, BCA – biocontrol agent, N – unknown.

Dactylonectria macrodidyma (Hallen, Schroers & Crous) L. is a worldwide distributed fungus, a part of the *Nectriaceae*, a family containing important plant pathogens (Probs et al. 2019). This species is soil-borne and associated with black foot disease infecting grapevine and other plant roots and stem bases from soil-borne inoculum that remains in the soil after infected host plants have been removed (Probst et al. 2019). *D. macrodidyma* causes different symptoms of black foot disease including a reduction in root biomass and root hairs with sunken and necrotic lesions (Halleen et al. 2006). Severe necrosis of the root system results in stunting, wilting, leaf chlorosis, browning, and leaf drop prior to death (Parkinson et al. 2017). It can also affect *Abies* sp., *Picea glauca*, *Pinus* sp., and other trees and shrubs (Probst et al. 2019). *Acremonium* sp. and *Cladosporium cladosporioides* are both one of the most common environmental fungi to be isolated worldwide (Peršoh et al. 2010). These species belong to the most frequently encountered species, in both outdoor and indoor environments. They are also the most common species reported as indoor contaminants occasionally linked to health problems as other airborne spores of fungal species such as *Alternaria, Aspergillus*, *Penicillium*, and *Chaetomium* are found throughout the world (Peršoh et al. 2010). In the present study, the majority of the taxa identified were common saprotrophic or endophytic fungi. Among these, three *Thrichoderma* species were also the frequently detected fungus found in both root groups. *T. viride* has previously been known as an antagonist of several pathogens and many biocontrol agents including different Trichoderma species (Menkis et al. 2005).

Phoma macrostoma was also among the most common fungi found in visually healthy damaged roots. *P. macrostoma* was recently described as pathogenic to many dicotyledonous plant species but non-pathogenic to monocots (Bailey et al. 2011). The herbicidal activity of *P. macrostoma*, shown since to result from the secretion of phytotoxic compounds, macrocidins, did not affect monocotyledonous species, is being investigated as a bioherbicide for the control of dandelion and other broadleaved weeds of turfgrass.

Although it was not known to occur in forest before, *Phoma* spp. were previously reported from diseased roots of forest nursery seedlings (Menkis et al. 2005). The presence of *P. macrostoma* in roots of *P. sylvestris* may suggest that this fungus is adapted to a wide range of hosts and habitats. Its common occurrence in roots of healthy-looking trees may further suggest that *P. macrostoma* is latent in living roots. Furthermore, several other pathogenic fungi including *Phomopsis* sp., *Rhizoctonia* sp., *Gibberella avenacea* were detected. These are mainly known as facultative parasites but could also live endophytically. It was suggested previously that the pathogenic behavior of such fungi may change depending on the environmental conditions and/or health status of the plants (Menkis et al. 2005).

Also, slow-growing fungi from Basidiomycota were detected, among them *Heterobasidion annosum* s.s. (4.07%), *Fomitopsis pinicola* (3.17%), *Ceratobasidium bicorne* (2.26%), and *Thelephora* sp. (1.81%) were the most common, while *Hebeloma* sp., *Phlebiopsis gigantea*, and *Hyphoderma setigerum* were less common (0.9, 0.9 and 0.45% respectively). *Hyphoderma setigerum,* Thelephora sp. and *Hebeloma* sp are ectomycorrhizal fungi that are known worldwide as dominating in forest nurseries (Menkis et al. 2005, Stenström et al. 2014) but they may be outcompeted by indigenous ectomycorrhizal fungi in field. *Heterobasidion annosum* s.s. is the root and butt rot pathogen that brings about large economic losses to the forest sector in the Northern Hemisphere (Oliva et al. 2017). *Phlebiopsis gigantea* has been widely used as the biocontrol fungus against the root and butt rot disease of conifers caused by *Heterobasidion annosum*. Stump treatment with *P. gigantea* is therefore the preferred alternative measure to control and manage the spread of the infection. The fungal preparation is commercially marketed as Rotstop® in Scandinavia, PG suspension® in UK and PG IBL® in Poland (Oliva et al. 2017). *Fomitopsis pinicola* is a wood decay fungus and brown rot species of *Polyporales.* This species represents a functionally important component of forest ecosystems due to their involvement in nutrient cycles, in the formation of specialized but important ecological niches, in the regeneration of forests, and in the improvement of soil quality. Fungal diversity, species richness, as well as the presence of

certain species, may all be used as indicators of forest health. A number of wood decay fungi have been identified as positive indicators for the creation and conservation of habitats for wildlife (Vasaitis 2013).

Data on fungal communities associated with root systems of non-infected *P. sylvestris* are shown in Table 2.

Table 2

Occurrence and relative abundance of fungal taxa from non-infested Pinus sylvestris, %

Fungal taxa (functional group)*	Genbank accession number	Root samples	Wood samples	Total
Ascomycota				
Acremonium sp. (P, S)	HF680219.1	5.56	21.05	8.79
Bionectriaceae sp. (P, S, FF)	KU663955	11.11	0.00	8.79
Chaetomium fusiforme(P, S, FF)	MT028042.1	1.39	10.53	3.30
Cladosporium sp. (P, S, E, FF)	KU663964	6.94	0.00	5.49
Cytospora sp. (P, S, E)	MK912135.1	5.56	0.00	4.40
Ophiostoma piceae s.l. (P, S, E)	JX444603.1	2.78	10.53	4.40
*Penicillium roquefort*i (S, E, FF)	KU663990	9.72	0.00	7.69
Penicillium spinulosum (S, E, FF)	KF156323	12.50	0.00	9.89
Pseudeurotium sp (S, E, FF)	HM589261.1	9.72	0.00	7.69
Rhizoctonia sp. (S, P, FF)	KU663995	6.94	0.00	5.49
Trichoderma sp (S, FF, BCA)	ON969989.1	4.17	15.79	6.59
Unidentified Ascomycota175244	KU664003	9.72	26.32	13.19
Basidiomycota				
Heterobasidion annosum s.s. (WDF)	KC768081	4.17	0.00	3.30
Hebeloma sp..(ECM)	KU663969	6.94	0.00	5.49
Unidentified Basidiomycota FG139	KU664005	2.78	15.79	5.49
No. of OTU		72	19	91
Shannon-Weaver diversity index		1.02	1.14	1.95
Simpson diversity index		0.91	0.88	0.93

*P – plant pathogen, S – saprotroph, ECM – ectomycorrhizae, WDF – wood decay fungi, AM – arbuscular mycorrhiza, E – endophytes, FF – filamentous fungi, indoor and soil fungi, decaying organic matter, BCA – biocontrol agent, N – unknown.

The amplification from root and wood segments of non-infested *P. sylvestris* was successful for 76 % of root and wood segments, respectively, resulting in 1–3 different amplicons from each of them. The separation as well as the ITS rRNA sequencing of individual amplicons of *P. sylvestris* resulted in 91 high-quality sequences representing 16 and 6 distinct fungal taxa, respectively for root and wood segments. Among the detected fungi, the most dominant were Unidentified Ascomycota175244 (13.19%), *Penicillium spinulosum* (9.89%), *Acremonium* sp. (8.79%), and *Bionectriaceae* sp. (8.79%).

Direct sequencing also yielded several unidentified taxa, among them Unidentified Ascomycota175244 was the most common. Comparison of the ITS rRNA sequence of these fungi with available sequences in the Genbank database showed that previously it was detected by direct sequencing in mycorrhizal roots of pine trees (Menkis et al. 2005). This suggests that unidentified species are probably unculturable but have a broad geographic distribution and are commonly associated with roots of conifers, and therefore can be important to the health of the plants though the taxonomic affiliation and ecology of this fungus remain largely unknown.

Species accumulation curves (Fig. 1) were used to depict the relationship between the cumulative number of species found and the sampling intensity (Colwell & Coddington1994). SACs were calculated using STATISTICA® 7.0 (StatSoft, Inc., Tulsa, OK, USA). In this case, additional sampling is needed to compare species richness in particular for visually diseased root samples from infested trees (RIT) and visually healthy root samples from infested trees (RHIT): accumulation curves show whether we sampled enough (the curves do not flatten to asymptote and they are still gradually climbing up) (Fig. 1).

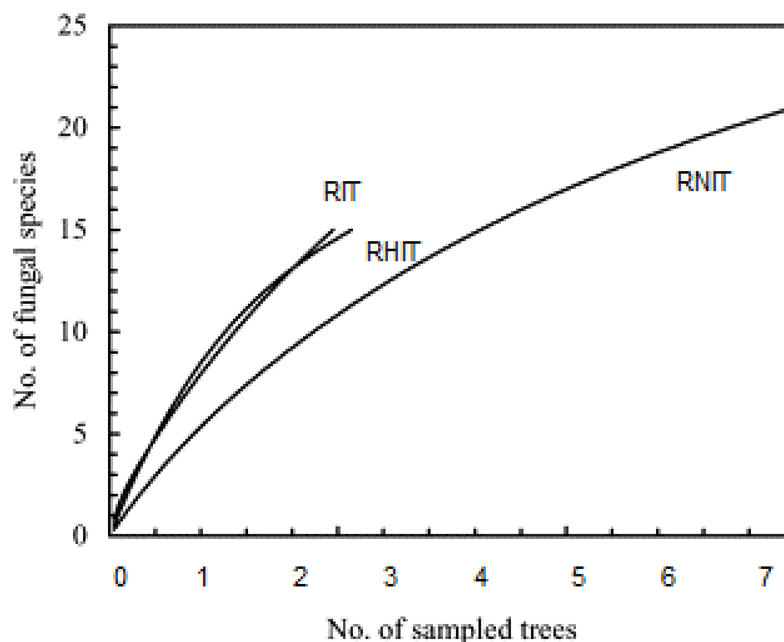

Fig. 1 – Increase in species richness in visually diseased root samples from infested trees (RIT), visually healthy root samples from infested trees (RHIT), and root samples from non-infested trees (RNIT) as a result of sampling more trees. Species accumulation curves were calculated according to Colwell and Coddington (1994)

A preliminary assessment of the fungal community allowed identifying 46 different taxa isolated from infected *P. sylvestris* and 15 taxa from non-infected *P. sylvestris*. Furthermore, a chi-square test showed a significant difference in the richness of fungal taxa between the infected and non-infected trees ($p \leq 0.05$). Pooling of all taxa detected by direct sequencing showed the presence of 49 distinct fungal taxa of which 31 (58.5 %) could be identified to taxon level and 17 (32.1 %) to genus level while 5 (9.4%) remain unidentified (data not shown). Seven taxa were common to both infested and non-infested trees while eight taxa were different. Among the group, the total number of detected taxa varied between 4 and 8 in non-infested *P. sylvestris* and between 12 and 32 in infested *P. sylvestris*. At that, a comparison by the chi-square test showed significant differences ($p \leq 0.05$) in the richness of fungal taxa among the trees (data not shown).

In all the root rot-infested stands, the root health had not a profound impact on fungal colonization (the chi-squared test, $p < 0.0001$). All comparisons between the visually healthy and visually decayed roots from root rot-infested trees were not statistically significant (the chi-squared test, $p < 0.21$). All comparisons between the infested and non-infested trees were highly statistically significant (the chi-squared test, $p < 0.0001$).

The chi-square test showed no significant difference in the richness of fungal taxa detected between the tree species ($p > 0.05$). Different stands were colonized by distinct fungal communities (see Fig. 1), and post-hoc PCAs on separate stands indicated that the relationship between a community composition and forest health was strongest in the Basidiomycota group and marginally insignificant in the Ascomycota group (Fig. 2).

Fungal community composition was significantly affected by forest health both at the species and genera/orders levels, with a higher explanatory power at genera/orders level (see Tables 1, 2, Fig. 2) (canonical correspondence analysis, PCA, with Monte Carlo permutations (without permutations within plots). Significance remained after removal of the two outliers (see Fig. 2). Therefore, the fungal community associated with root was significantly different for healthy and infested stands.

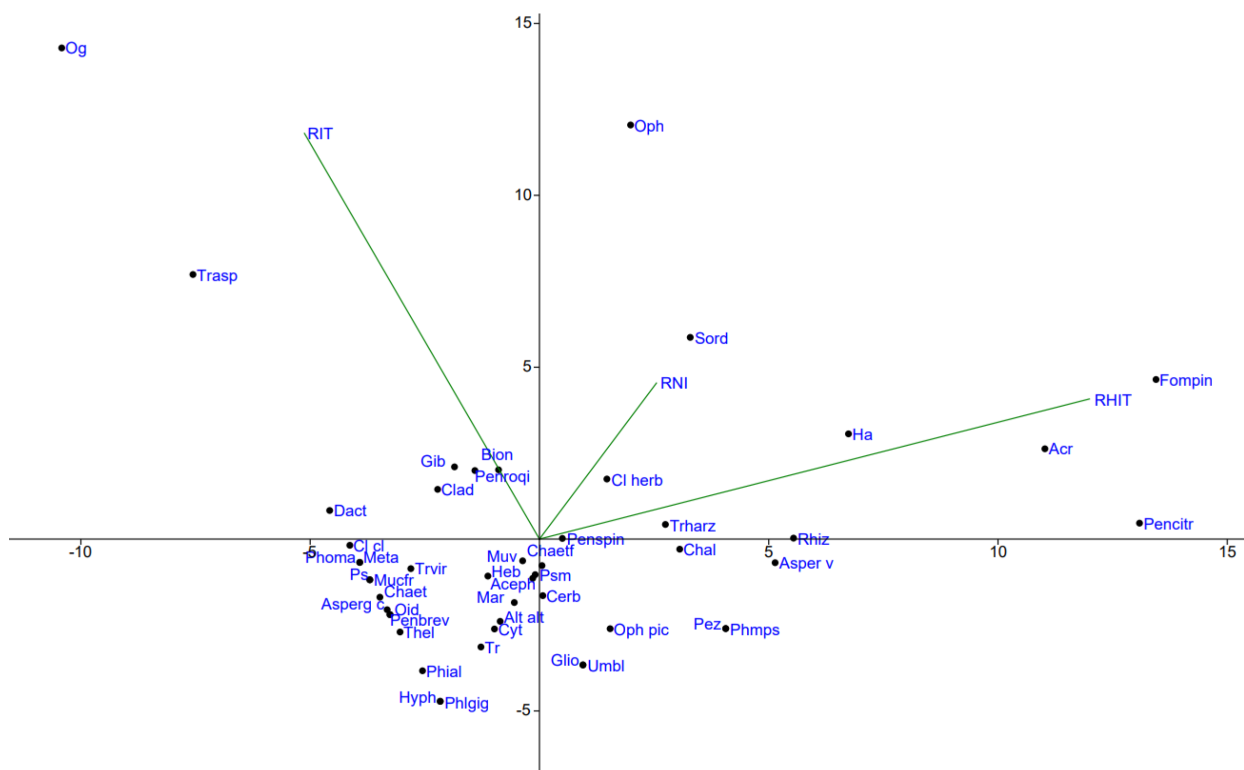

Fig. 2 – Principal components analysis showing an association between different fungal species and individual samples of this study: RIT – from the visually diseased root from infested trees; RHIT – from the visually healthy root from infested trees; RNI – root from non-infested trees. Taxonomic names correspond to a position in the ordination (centred) and their font size corresponds to a relative abundance as indicated in the upper right corner of the diagram and Tables 1 and 2 (*Acremonium* sp. – Acr, *Acephala applanate* – Aceph; *Alternaria alternata* – Alt alt; *Aspergillus cervinus* – Asperg c; *Aspergillus versicolor* – Asper v; *Bionectriaceae* sp. – Bion; *Chaetomium* sp. – Chaet; *Chaetomium fusiforme* – Chaetf; *Chalara* sp. – Chal; *Cladosporium cladosporioides* – Cl cl; *Cladosporium herbarum* – Cl herb; *Cladosporium* sp. – Clad; *Cytospora* sp. – Cyt; *Dactylonectria macrodidyma* – Dact; *Gliocladium* sp. – Glio; *Gibberella avenacea* – Gib; *Mariannaea elegans* – Mar; *Metapochonia bulbillosa* – Meta; *Ogataea neopini* – Og; *Ophiostoma piceae* s.l. – Oph pic; *Ophiostoma* sp. s.l. – Oph; *Oidiodendron chlamydosporicum* – Oid; *Penicillium brevicompactum* – Penbrev; *Penicillium citreonigrum* – Pencitr; *Penicillium roqueforti* – Penroqi; *Penicillium spinulosum* – Penspin; *Pezicula eucrita* – Pez; *Phialocephala* sp. – Phial; *Phoma macrostoma* – Phoma; *Phomopsis* sp. – Phmps; *Pseudogymnoascus roseus* – Ps; *Pseudeurotium* sp. – Psm; *Rhizoctonia* sp. – Rhiz; *Sordaria* sp – Sord; *Trichoderma asperellum* – Trasp; *Trichoderma harzianum* – Trharz; *Trichoderma viride* – Trvir; *Trichoderma* sp. – Tr; *Ceratobasidium bicorne* – Cerb; *Fomitopsis pinicola* – Fompin; *Heterobasidion annosum* s.s. – Ha; *Hebeloma* sp. – Heb; *Hyphoderma setigerum* – Hyph; *Phlebiopsis gigantea* – Phlgig; *Thelephora* sp. – Thel; *Mucor* sp. – Muv; *Mucor fragilis* – Mucfr; *Umbelopsis isabelline* – Umbl

Overall species richness and evenness are significantly lower in non-infested stands as indicated by significant Mantel statistics (r = 0.46, P = 0.02 and r = 0.46, P = 0.03, respectively, for infested and non-infested stands). In contrast, pathogenic fungal richness and evenness in the infested stands increased significantly compared with non-infested ones. For richness, the relationship was significant also with the two groups of wood samples excluded.

Both decomposition and nutrient cycling in forest soils are mainly driven by fungi. Our study revealed the two predominant fungal phyla, Ascomycetes and Basidiomycetes, which cover a wide range of ecological functions, reaching from saprotrophs to parasitic pathogens and mutualistic symbionts. The key functional groups for C and N cycling in forest ecosystems are litter and wood-decomposing saprotrophs and root-associated mutualistic fungi. Functional groups were segregated significantly between sampling groups, confirming a preference for fungi to be associated with certain ecological niches, as root-associated taxa were predominantly closely associated with different sampling groups. For both forest stands, Mantel statistics indicated no significant correlation between forest health and community matrices (r = -0.4, P = 0.9), and data were not

further analyzed. It can be explained by small sample sizes of trials (see Fig. 1), and short study duration (one year). Therefore, it is necessary to continue the study and increase the sample size to enable explaining the difference in fungal communities. Many studies of root-associated communities included a few environmental factors and wide soil sampling, and soil indicators can also explain 71.4 % of the variation in functional groups (Hagenbo et al. 2018, Davydenko et al. 2020, Zhao et al. 2020). As litter decomposers, free-living saprotrophs dominate fungal communities (see Tables 1, 2) presumably by suppressing the ingrowth of root-associated biotrophs. The mycorrhizal fungi were found to be associated with roots of infested and non-infested trees; however, small sampling did not enable assessing the overall richness of mycorrhizal fungi and their functional differences in forest ecosystems. There are different forms of mycorrhization, which can be intracellular in arbuscular and ericoid mycorrhiza, extracellular, as in ectomycorrhiza, or an intermediate form that occurs for certain host plants in different conditions and have differed in morphotype at a genus level.

Conclusions. Fungal culturing from 10 surface-sterilized wood cores resulted in 21 fungal cultures, 2.1 per wood segment. Direct sequencing from 40 surface-sterilized segments of lateral roots resulted in 247 fungal sequences or 6.2 per root segment on average. The most dominant fungi from infested trees of *Pinus sylvestris* were *Dactylonectria macrodidyma* (4.98 %), *Acremonium* sp. (4.52 %), *Cladosporium cladosporioides* (4.07 %) from Ascomycota and *Heterobasidion annosum* s.s. (4.07 %) from Basidiomycota, while for non-infested group Unidentified Ascomycota175244 (13.19 %), *Penicillium spinulosum* (9.89 %), *Acremonium* sp. (8.79 %), *Bionectriaceae* sp. (8.79%) were most common. In all the root rot-infested stands, the root health had not a profound impact on fungal colonization. All comparisons between the visually healthy and visually decayed roots from root rot-infested trees were not statistically significant while differences between the infested and non-infested trees were highly statistically significant. The fungal community composition was significantly affected by forest health both at the species and genera/orders levels, with higher explanatory power at the genera/orders level. The dominant root-associated fungi were expected to be ectomycorrhizal fungi, providing “positive plant-soil feedbacks” and plant pathogenic fungi in particular for infested stands.

However, the most common fungal groups associated with roots of *P. sylvestris* were saprotrophic fungi that are opportunistic, aerial, or soilborne fungal pathogens. Some ectomycorrhizal fungi had been found and they show strong environmental adaptability and can establish relatively stable symbiotic relationships with hosts. Undermining the health affects the diversity and community structure of fungi by changing the supply of ectomycorrhizal nutrients and changes in the pathogenic community; however, this hypothesis should be carefully examined and proved. Moreover, additional sampling is required.

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ЗМІНИ В ГРИБНИХ УГРУПОВАННЯХ, ПОВ'ЯЗАНИХ З КОРЕНЕВОЮ СИСТЕМОЮ ДЕРЕВ *PINUS SYLVESTRIS*, УРАЖЕНИХ КОРЕНЕВИМИ ГНИЛЯМИ

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Кореневі гнилі, викликані грибами *Heterobasidion annosum* s.l. та іншими дереворуйнівними грибами, уражують підземну й надземну частини сосни звичайної (*Pinus sylvestris* L.). Властивості ґрунту та рослинного покриву змінюються в часі, і ці зміни, ймовірно, також впливають на розвиток хвороби й викликають зміни грибних угруповань лісового ґрунту та асоційованих із кореневою системою грибів. У цьому дослідженні ми спробували оцінити видовий і функціональний склад грибних угруповань у насадженнях *P. sylvestris*, уражених кореневими гнилями, й дослідили кореляції між таксономічним складом грибів кореневої системи і фізіологічним станом дерев. Ураження кореневими гнилями значною мірою впливало на чисельність і різноманітність грибів. Під час розвитку захворювання в складі грибних угруповань виникли зміни від домінування сапротрофних грибів до панування ектомікоризних і патогенних видів грибів. Наші результати дають змогу зробити припущення, що збереження біологічного різноманіття в грибному угрупованні кореневої системи може певною мірою впливати на підтримання нормального фізіологічного стану дерев або навіть на стійкість до кореневої гнилі, причому зберігається здатність грибів до переробки органічних поживних речовин, однак для підтвердження цієї гіпотези необхідні подальші дослідження.

К л ю ч о в і с л о в а : сосна звичайна, *Heterobasidion annosum* s.l.

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