MYCORRHIZAL FUNGAL COMMUNITY OF POPLARS GROWING UNDER EXTREME ENVIRONMENTAL CONDITIONS

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INTRODUCTION

Mycorrhiza

❖Mutualistic association between fungi and higher plants

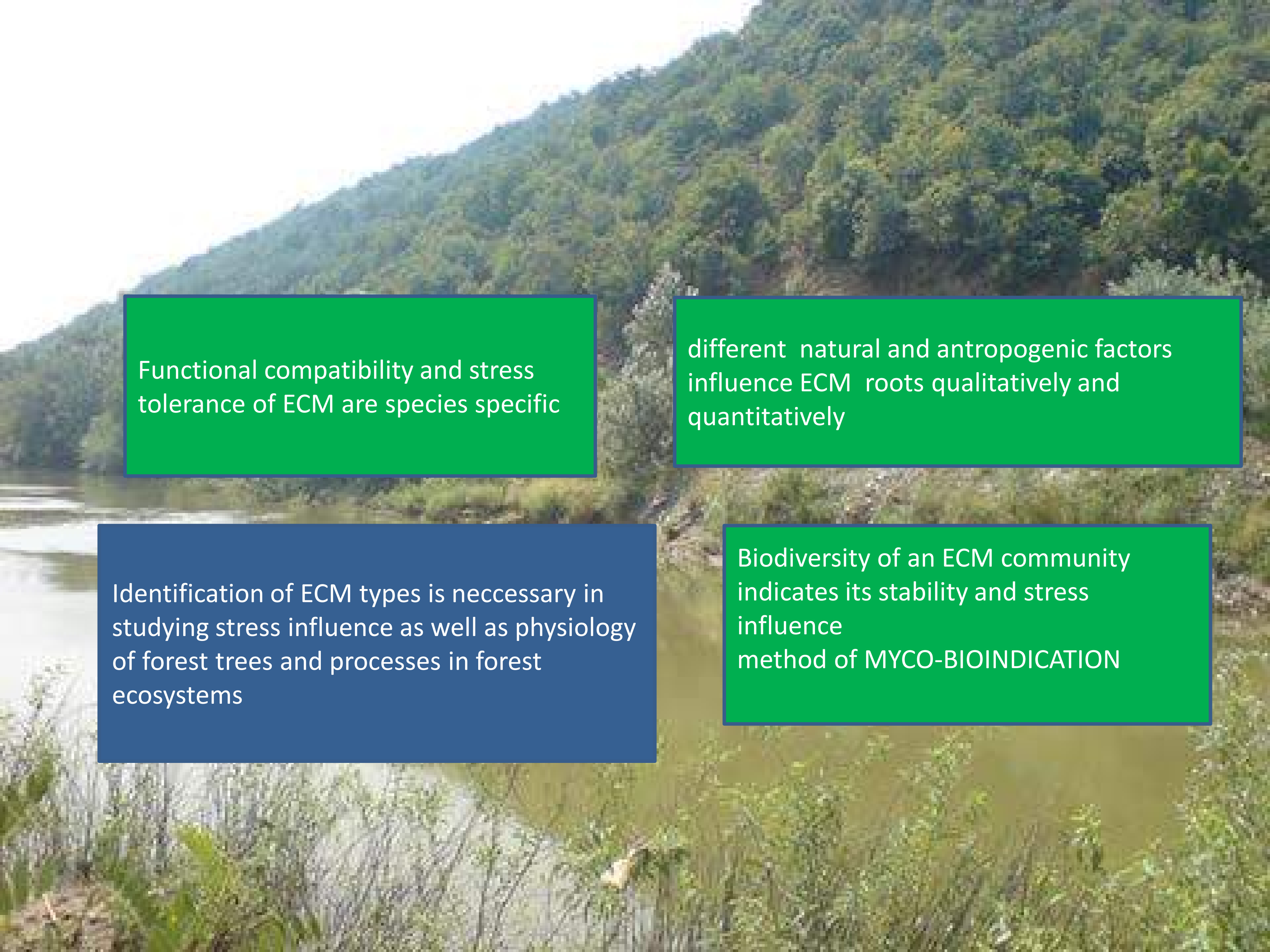
➢ Normal state for most plants in most ecological condition

➢Poplars are dually colonized with ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM)

http://www.waldwissen.net/wald/baeume_waldpflanzen/oekologie/wsl_mykorrhiza_lebensgemeinschaft/index_EN

- Mycorrhizas are of crucial importance for functioning of forest ecosystems
- Influence sustainability, productivity and vitality of forest
- Mycelium of mycorrhizal fungi links forest trees and ground vegetation with decompozers and nutrients in forest soil
- Individual trees, of same or different species are conected in space and time with hyphae of mycorrhizal fungi making COMMON MYCELIAL NETWORK

http://www.botanicalgarden.ubc.ca/potd/2010/03/mycorrhizal_networks.php



Functional compatibility and stress tolerance of ECM are species specific

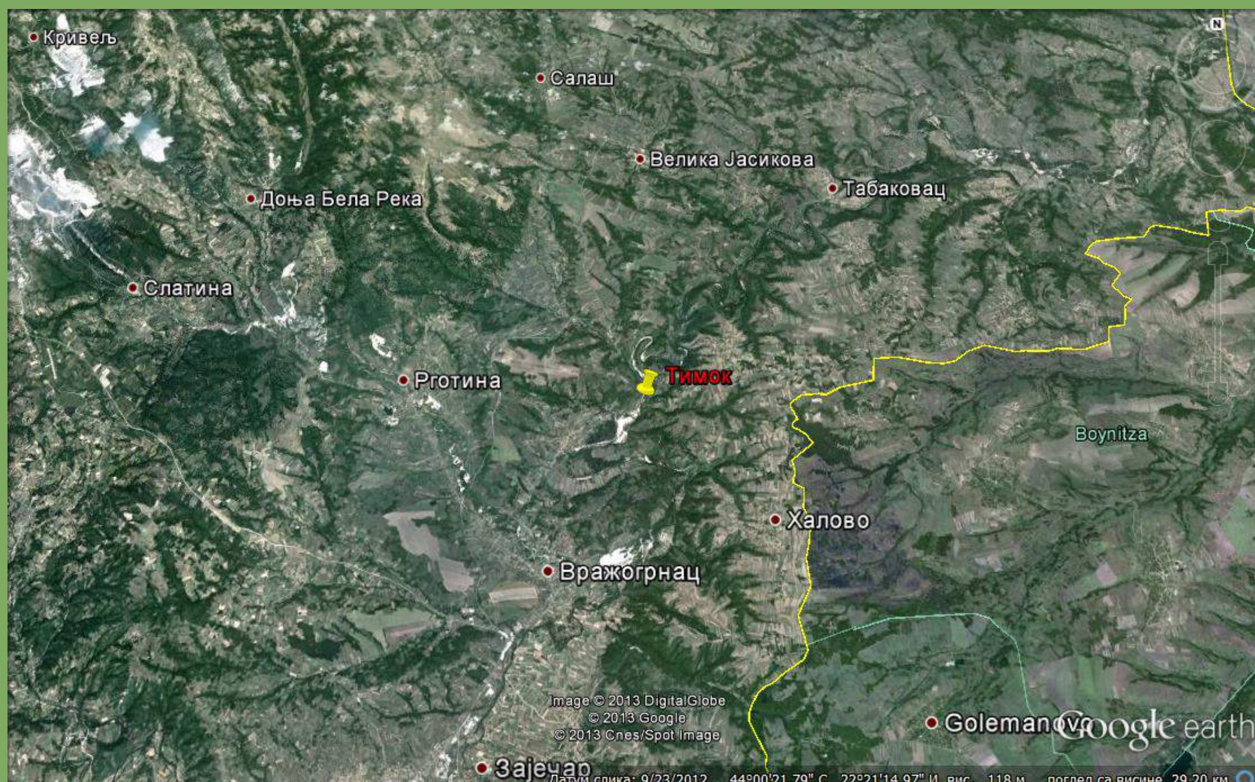
different natural and antropogenic factors influence ECM roots qualitatively and quantitatively

Identification of ECM types is neccessary in studying stress influence as well as physiology of forest trees and processes in forest ecosystems

Biodiversity of an ECM community indicates its stability and stress influence
method of MYCO-BIOINDICATION

•Production of copper in Bor mine complex (Eastern Serbia) represents a considerable source of environmental pollution

•Soils from a large area in the riverland of the river Timok were contaminated by flotation tailing which was composed of pyrite (FeS_2) with minor amounts of other sulphides and heavy metals



Position of locality „Timok“

The aim of this study was to analyse ectomycorrhizal fungal community of poplars (*Populus* spp.) growing on pyrite tailings contaminated site near the river Timok and root length colonization with ectomycorrhizal, arbuscular mycorrhizal and dark septated endophytic fungi

Site “Timok” (photo Katanić, 2010)

MATERIAL AND METHODS

•ECM roots were isolated from 10 soil samples collected in July 2010 in riverland of the river Timok, located about 20 km from Zaječar town

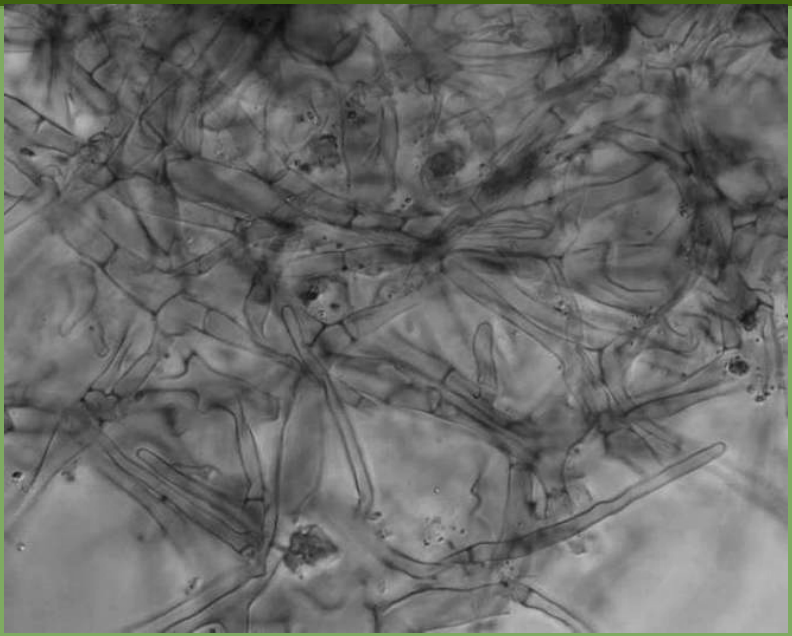
•A soil corer of 274 ml volume and reaching 18 cm depth was used for taking standardized soil core samples (Kraigher 1999).

•identification of the fungal partner in ectomycorrhiza was achieved by combining morphological and anatomical descriptions with molecular methods

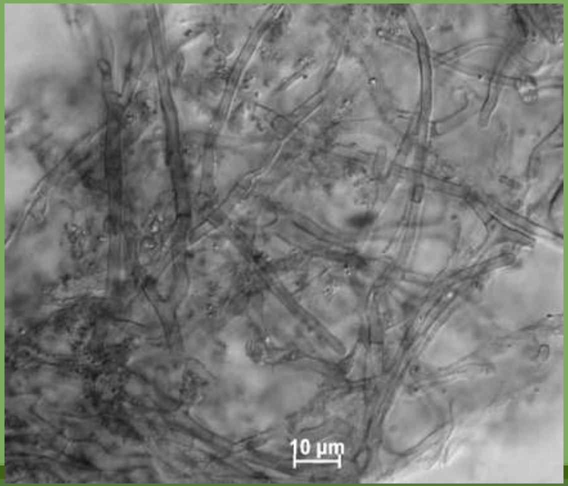
• morphological and anatomical methods - binocular Olympus SZX 12 and microscope Olympus BX 51 (enlargement 100-2000x), according to published descriptions (Agerer, 1987-2008; Agerer et al., 2001-2006 DEEMY, 2013) and according methodology given in Agerer (1991) and Kraigher (1996)

•Based on the presence and abundance of emanating elements, ECM types were also classified into the exploration types proposed by Agerer (2001).

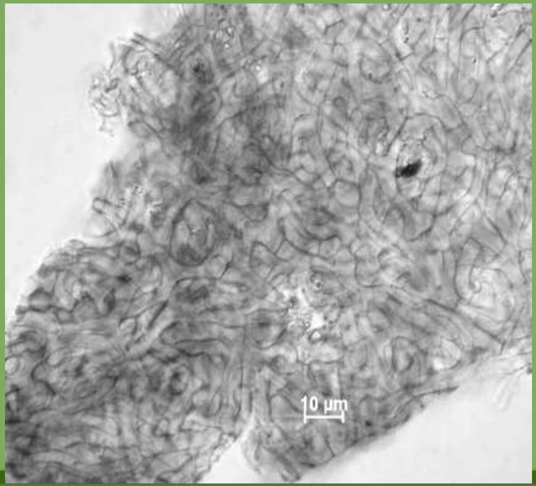
Thelephora terrestris: ectomycorrhiza



cystidia on the surfice of the mantle



a



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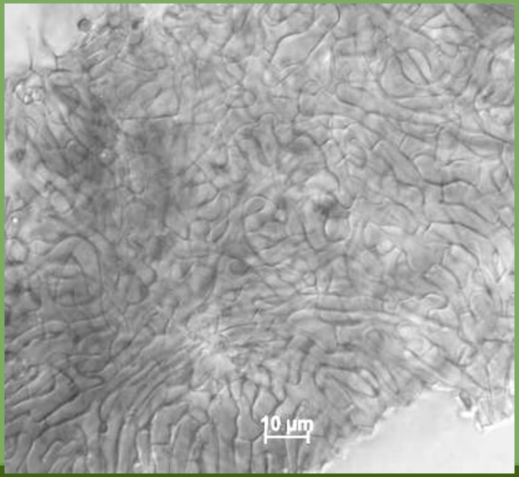


c

Thelephora terrestris – cross section through the sample: a –surface of the mantle b – outer mantle; c – inner mantle

• molecular identification with PCR amplification of the ITS regions within nuclear rDNA with ITS 1f and ITS 4 primer pair and sequencing (Gardes & Bruns 1993, Martin 2000)

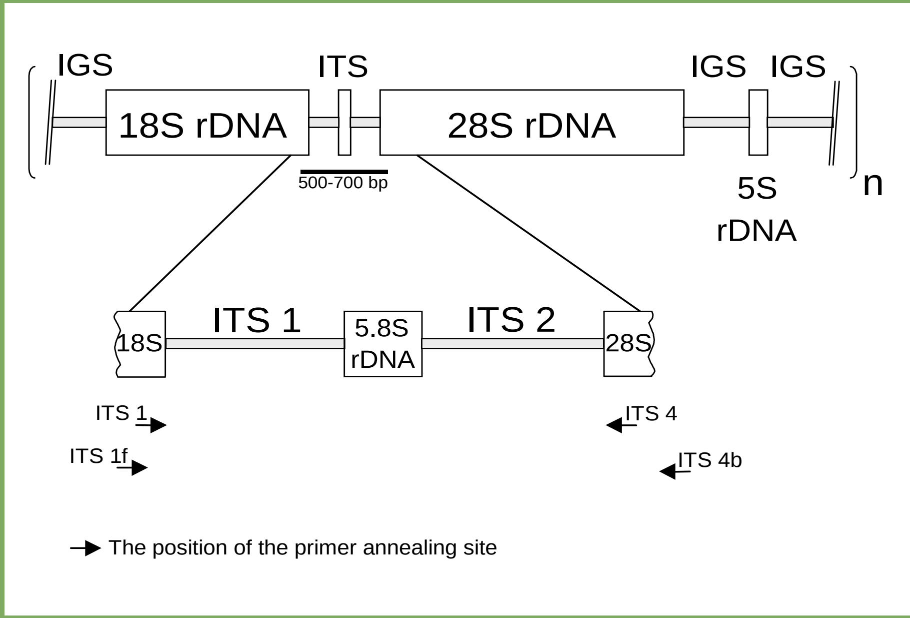


Fig. 1: Grebenc & Kraigher 2009

ROOT COLONIZATION WITH ECTOMYCORRHIZAL, ARBUSCULAR MYCORRHIZAL AND DARK SEPTATED ENDOPHYTIC FUNGI

- Roots were extracted from soil, washed and after clearing in KOH were **stained with trypan blue** according to the protocol given by Kormaník and McGraw (1982) and modified by Karlinski *et al.* (2010).

- Root length colonization with ectomycorrhizal, arbuscular mycorrhizal and dark septated endophytic fungi was estimated using the **intersection method** according to McGonigle *et al.* (1990).

- Three subsamples were made from every root sample and a minimum of 100 line intersections per subsamples was scored for the presence of ectomycorrhizal, arbuscular mycorrhizal and dark septated endophytic fungi.

- Resuts were presented as a percentage of root length colonized (% RLC).

STATISTICAL ANALYSE OF ACCURANCE OF ECM TYPES IN SOIL SAMPLES

- Number of different ECM types
- Number of vital, old nonturgescent and nonmycorrhizal fine roots in the sample
- Ratio of particular ECM type on the locality
- Ratio of vital ECM roots in sample

- **Species richness index**
- $d=(S-1) / \log(10) N$

S= Number of ECM types in the sample
N= Number of all vital mycorrhizal roots in the sample

- **Shannon-Weaver-index**

$H=C/N(N*\log N-\sum n_{i*} \log n_i)$

C = 2,3 (coefficient of correction),
N = number of all vital mycorrhizal roots in the sample
n_i = number of mycorrhizal roots of particular ECM type

- **Teoretical maximal Shannon-Weaver index**

$H_{max} = \ln S$

S = Number of ECM types in the sample

- **Equitability**

$e = H/\log S$

H = S.-W.- index, S = Number of ECM types in the sample

- **Evenness**

$J = H/H_{max}$

H = S.-W.- index s, H_{max} = Teoretical maximal Shannon-Weaver

- **Berger-Parker index of evenness**

$BP = 1-(N_{max}/N)$

N_{max} = number of the most abundant ECM type in the sample
N = number of all vital mycorrhizal roots in the sample

RESULTS

Physico - chemical properties of the soil from Timok locality

Table 1. Granulometric composition of soil from Timok locality

Coarse sand (%)	Fine sand (%)	Dust (%)	Clay (%)	Total sand (%)	Total clay (%)	Texture class
8,2	83,2	3,0	5,6	91,4	8,6	sand

Table 2. pH value in water and KCl-u, content of $CaCO_3$, carbon, nitrogen and C/N ratio on locality Timok

pH in H_2O	pH in KCl	$CaCO_3$	Carbon	Nitrogen	C/N
4,91	4,74	1,67	4,71	0,06	78,34

RESULTS

Physico-chemical properties of the soil from Timok locality

Table 3. Concentrations of heavy metals in soil from Timok locality (MAA of heavy metals according to the National legislation)

Heavy metal	Concentration (ppm)	Maximum allowed amounts (MAA) in the soil
Cr	28,3	100
Ni	14,4	50
Cd	3,3	3,0
Pb	83,4	100
Cu	896,9	100
Mn	147,5	/
Fe	58141,21	/
Zn	413,9	300

RESULTS

Table 4. Total values of number of ECM types, ratio of vital ECM roots and diversity indices at investigated site Timok

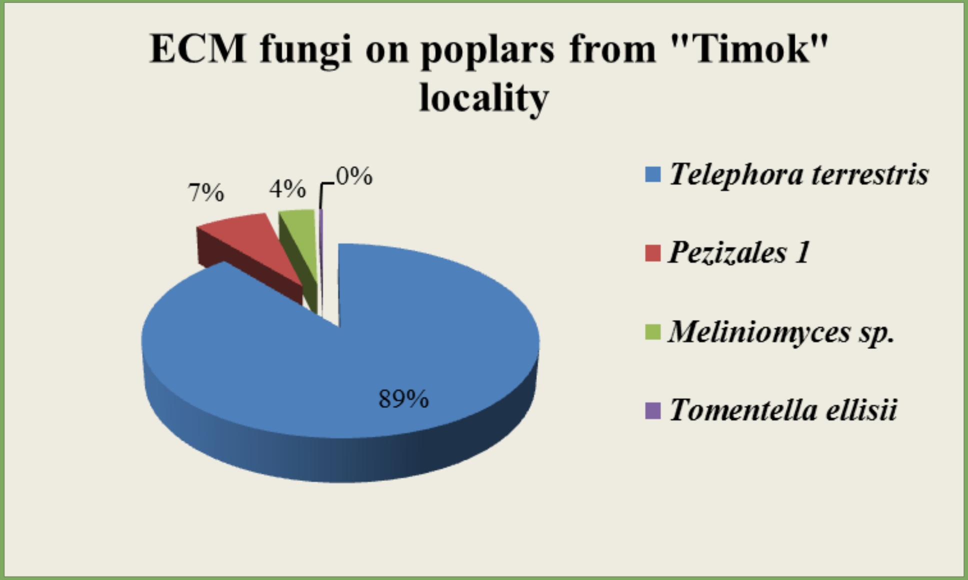
Number of ECM types	**4**
Ratio of vital mycorrhizal roots	**16,2**
Species richness index (d)	**0,80**
Shannon-Weaver index	**0,43**
Equitability (J)	**0,31**
Eveness (e)	**0,71**
Berger-Parker index	**0,11**

Table 5. Average values per sample (±st. erorr) for number of ECM types, number of vital ECM roots, Old, nonturgescent and nonmycorrhizal roots, number of all roots, Ratio of vital mycorrhizal roots and diversity indices

Number of ECM types	**1,4±0,16**
Number of vital mycorrhizal roots	**550,6±184,6**
Old, nonturgescent and nonmycorrhizal roots	**2853,6±892,2**
Number of all roots	**3404,2±946,4**
Ratio of vital mycorrhizal roots	**19,7±5,0**
Species richness index (d)	**0,144±0,06**
Shannon-Weaver index	**0,138±0,07**
Equitability (J)	**0,199±0,10**
Eveness (e)	**0,461±0,23**
Berger-Parker index	**0,045±0,03**

RESULTS

Fig. 2 ECM fungi community structure on poplars from „Timok“ locality



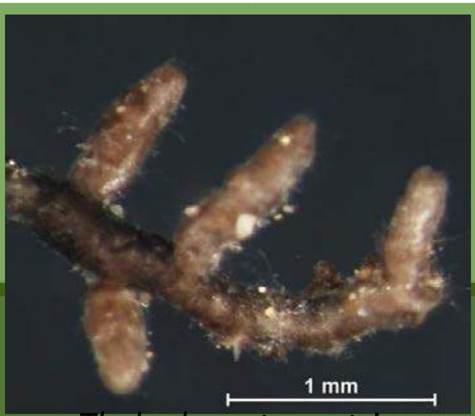
T. terrestis belongs to the **MEDIUM DISTANCE EXPLORATION TYPE** and consequently this exploration type dominated ectomycorrhizal community.

Table 6. Ratio of number of ECM types and ratio of ECM root number of Ascomycota and Basidiomycota on „Timok” locality

Ratio of the number of ECM types belonging to Ascomycota/ Basidiomycota	ratio of ECM roots from Ascomycota and Basidiomycota groups (%)
2:2	10,6:89,4

Table 7. Identified ECM fungi on the basis on the similarities with sequences given in the internet basis GenBank and UNITE and phylogenetical analyses

ECM type	Accession numbers of three best shot in GenBank and % of match and % of identity	Accession numbers of three best shot in UNITE and % of match and % of identity	Morphological-anatomical identifiication	Phylogenetical analysis
***Meliniomyces* sp.**	Meliniomyces sp. KC007335.1 99% 99%; Uncultured Hebeloma JQ724056.1 99% 99%; Uncultured Helotiales DQ273322.1 99-98%	Mollisia benesuada | EstoniaUDB003038; Crocicreas furvum | Lithuania UDB003037; Niptera dilutella | Estonia UDB003005	/	/
Thelephora terrestris	Thelephora terrestris JQ711980.1 100-100%, HM189965.1 Thelephora terrestris 100-100%; Thelephora terrestris HQ406822.1 100-100%	Thelephoraceae | Slovenia UDB008264 100%; Thelephora terrestris | Estonia UDB003348 LOCKED by Urmas Kõljalg; Thelephora terrestris | Estonia UDB003346 99%	*Thelephora terrestris*	*Thelephora terrestris*
Tomentella ellisii	Tomentella ellisii DQ068971.1 100% 99%; Uncultured Thelephoraceae JN704829.1 100% 99%; Uncultured ectomycorrhiza (Tomentella) clone EU700261.1 97% 99%	Tomentella ellisii | Italy UDB016490 95%; Tomentella ellisii | Estonia UDB000219 96%; Tomentella ellisii | Finland UDB011603 LOCKED by Irja Saar	/	*Tomentella ellisii*
Pezizales 1	Uncultured Pezizales clone P1_Contig_0290 JN704819.1 100% 99%; Uncultured ectomycorrhizal fungus clone Riv-5 EF484935.1 100% 99%; Uncultured ectomycorrhizal fungus clone unk1350 GU553372.1 100% 99%	Sphaerosporella brunnea | Finland UDB000994 94%; Otidea alutacea | Estonia UDB011428 98%; Rhizina undulata | Finland UDB016153 96%	/	/



Thelephora terrestris

Pezizales

Tomentella ellisii

POPLARS ROOT COLONIZATION WITH ECM, AM AND END FUNGI

Table 8. Average values (±st. error) of poplar root colonization with ectomycorrhizal, arbuscular mycorrhizal and dark septated edophytic fungi at "Timok" locality

	Number of fungal structures
Vesicles	0
Arbuscules	0
Hyphae	0
Coils	0
AM	0
ECM	32,72±6,22
END	3,64±0,84
Other hyphae	23,56±13,07
Empty roots	114,12±5,95
% RLC AM	0
% RLC ECM	18,19±3,47
% RLC END	2,23±0,50
%Other hyphae	11,80±5,65
AM/ECM	0

CONCLUSIONS

- The main cause for extreme conditions at the pyrite tailings contaminated site near the river Timok are the unfavourable water-air properties (texture) of analysed soil, low pH and contamination with heavy metals (Cu and Zn)

- Only four different ectomycorrhizal fungi were found and identified (*Thelephora terrestris* and *Tomentella ellisi,* Meliniomyces and Pezizales)

- Type *Thelephora terrestris* made up 89% of all ectomycorrhizal roots on studied locality.

- Species richness index and Shannon-Weaer diversity index were 0.80 and 0.43 respectively.

- No AM fungal structures were found

- Overall species diversity of ECM at site contaminated with pyrite tailings was low

Thank You for Your attention

Acknowledgements

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