



Growing  
**ideas**  
through  
**networks**

# Experiences and practices from COST actions

Scientific and added values

Dr Jelena Lazarević



Funded by the Horizon 2020 Framework Programme  
of the European Union

## Participation in COST actions improved scientific knowledge and work through:

- ✓ Meetings with scientists from different countries
- ✓ Exchange of experiences and knowledge
- ✓ Improvement of techniques and skills in laboratory work
- ✓ Presentation and visibility of achieved scientific results....

...I have better results, which are more competitive (and visible)

- ✓ Improved international collaboration



Photo I. Perić

**Dr Jelena Lazarević**

University of Montenegro  
**Biotechnical faculty**  
Center for Forestry

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[enalazarevic@ucg.ac.me](mailto:enalazarevic@ucg.ac.me)

- ✓ **FP1102: Determining Invasiveness and risk of Dothistroma (DIAROD) (2011-2015)**  
29 countries, Montenegro's participation (NNC): since 2013
- ✓ **FP1103: Fraxinus dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK) (2012-2016)**  
39 countries were involved  
Montenegro's participation (NNC) since 2013
- ✓ **FP1305: Linking belowground biodiversity and ecosystem function in European forests (BioLink) (2014-2018)**  
33 countries were involved  
Montenegro's participation (NNC) since 2015
- ✓ **FP1401 - A global network of nurseries as early warning system against alien tree pests (Global Warning) (2015-2019)**  
39 countries were involved  
Montenegro's participation (NNC) since 2015

➤ Domain FPS Forest, their products and services

- ✓ **CA17133 - Implementing nature based solutions for creating a resourceful circular city (2018-2022)**

## **COST Action networking tools**

- ✓ MC meetings
- ✓ WG meetings & conferences
- ✓ Workshops & Training schools
- ✓ STSM
- ✓ Collaborative projects and publications

## WG meetings & Conferences

Lazarević J.(2015): **Application of autochthonous fungi and forest soil for seedling mycorrhization - trials in Montenegro**, Soil Biological Communities and Aboveground Resilience, COST Action FP1305 BioLink: *Linking belowground biodiversity and ecosystem function in European forests*, Proceedings of the 3rd Annual Meeting, Rome, 17-19 November 2015, 54

Lazarević J., Keča N. (2016): **Basic physiological characteristics of *in vitro* cultures of ectomycorrhizal isolates from SE Montenegro**, The meeting of COST Action FP1305 BioLink: Linking soil biodiversity and Ecosystem function in European forests "Belowground biodiversity and global change", Průhonice, Czech Republic, 24-26 October 2016.

Lazarević J., Menkis A. (2017): **Belowground fungal biodiversity associated with endemic *Pinus heldreichii* in high altitude Montenegrin forests**, 7th international Symposium on physiological processes in roots of woody plants -Woody root 7 jointly with Annual meeting of European network Cost Action FP1305 Biolink: Linking belowground biodiversity and ecosystem functions in European forests , June 26-29, 2017, Tartu, Estonia, Book of Abstracts, page 81.

Lazarević, J., Topalović, A., Menkis, A. (2018): **Fungal biodiversity associated with fire-disturbed *Pinus heldreichii* forest soils in Montenegro**. Soil biodiversity and European woody agroecosystem FP1305 Biolink Cost Action Annual Meeting, Granada, 14-16 March, pp. 83-84. ISBN 978-88-97655-03-9

**FP1305: Linking belowground biodiversity and ecosystem function in European forests (BioLink) (2014-2018)**



Conference in Granada, March 2018

**Training School on Molecular Detection and Population Genetics of Dothistroma Needle Blight Pathogens , 3- 7 March 2014, Uppsala, Sweden**



**DIAROD training workshop**, trainers and participants, March 2014 - Sweden  
Participant from MNE: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Lazarević J.(2015): **Dothistroma needle blight in different pine forests in Montenegro**, COST Action FP1102: Determining Invasiveness And Risk Of Dothistroma, DIAROD, **Management Committee meeting, workshop and final meeting**, 6 – 8 October 2015, Kraków, Poland

Lazarević J., Davydenko K. , Menkis A. (2018): **Dothistroma needle blight and other needle fungi of native conifers in Montenegro** , **The 15<sup>th</sup> International Phytotechnology Conference**, University of Novi Sad, 1-5 October 2015, Book of abstract, page 17., **invited speaker**

## SHORT TIME SCIENTIFIC MISSIONS

### FP1102: Determining Invasiveness And Risk Of Dothistroma (DIAROD)

COST-STSM-FP1102-16414 :

STSM Topic: **Learning molecular techniques to identify DNB in field samples**

Participant: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Host: Dr Hanna Milberg, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Period: 08.03-30.04. 2014

COST-STSM-FP1102-26562:

STSM Topic: **Identification of DNB from field samples/occurrence of DNB at high altitude pine forests**

Participant: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Host: Dr Audrius Menkis, Department of Forest Mycology and Plant Pathology, SLU, Sweden

Period: 1.06-30.06.2015

### FP1103: Fraxinus dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK)

COST-STSM-FP1103-32332

STSM Topic: **Learning molecular techniques for genetical characterization and phylogeny of Ascomycetes-example of genus *Hymenoscyphus***

Participant: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Host: Dr Audrius Menkis, Department of Forest Mycology and Plant Pathology, SLU, Sweden

**FP1305: Linking belowground biodiversity and ecosystem function in European forests (BioLink)**

COST-STSM-ECOST-STSM-FP1305-150117-080732

STSM Topic: **Identification and genetic characterization of fungi from fire-disturbed forest soils with special reference to *Pinus heldreichii***

Participant: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Host: Dr Audrius Menkis, Department of Forest Mycology and Plant Pathology, SLU, Sweden

**FP1401 - A global network of nurseries as early warning system against alien tree pests (Global Warning)**

STSM reference number 41430

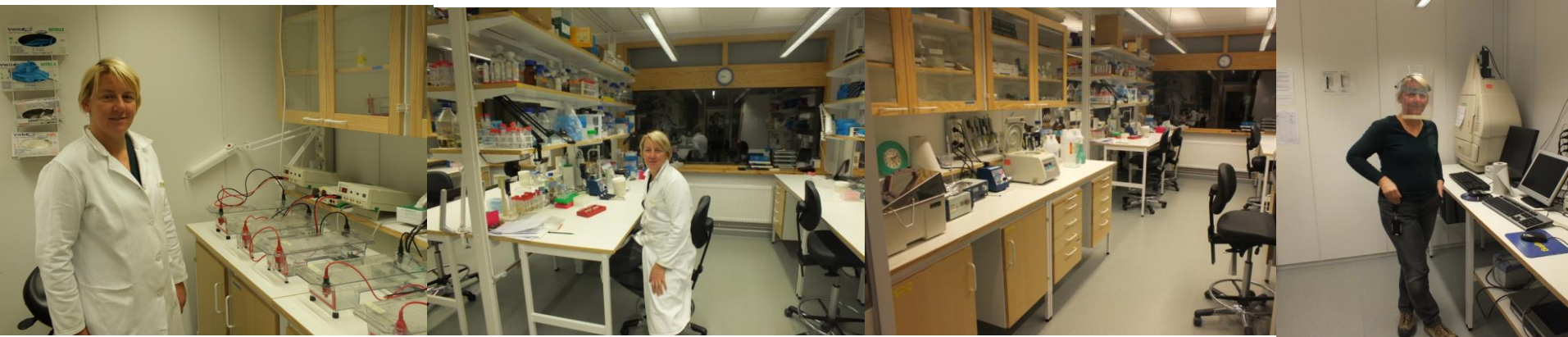
STSM Topic: **Invasive fungi in the phyllosphere of the principal tree species in Montenegro**

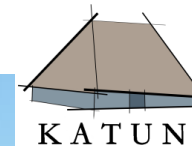
Participant: Dr Jelena Lazarević, University of Montenegro, Biotechnical faculty

Host: Dr Audrius Menkis, Department of Forest Mycology and Plant Pathology, SLU, Sweden



**Department of Forest Mycology and Plant Pathology,  
Swedish University of Agricultural Sciences, Uppsala, Sweden**





**University of Montenegro:**

**Biotechnical faculty**

Historical Institute of Montenegro

Faculty of Tourism and Hotel  
management

**National commercial entities:**



HM Durmitor, Žabljak  
The Old House, Podgorica

○ Intertehna, Berane

**International RTD entities:**



University of Ljubljana - Biotechnical faculty (Slo)



Swedish Univ. of Agricultural Sciences - Dept.  
of Forest Mycology and Plant Pathology (S)



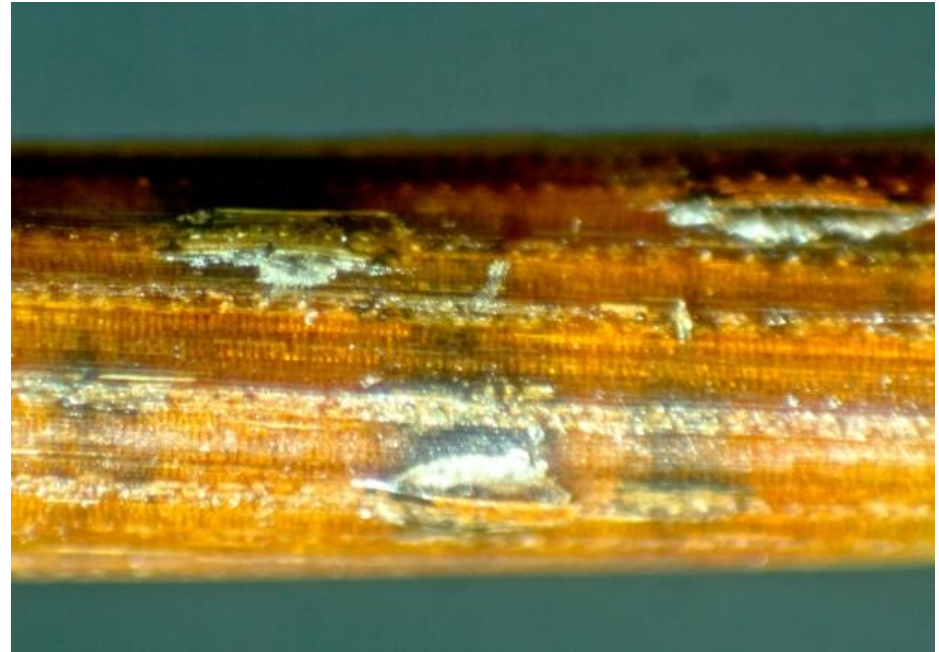
CNR, Institute for Technologies Applied  
to Cultural Heritage(I)



University of Basilicata (I)

- ✓ Learning molecular techniques to identify DNB in field samples
- ✓ Identification of DNB from field samples: Occurrence of DNB at high altitude pine forests

More than **350** samples from different pine forests from Montenegro were analyzed



- Looking for symptoms in pine forests under different ecological conditions

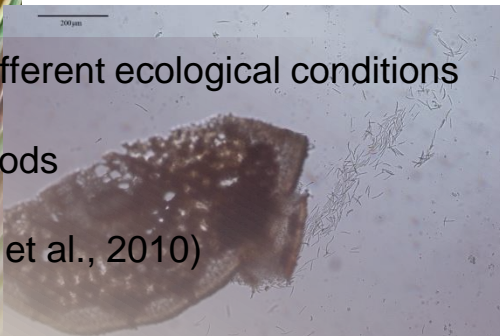
- symptoms were confirmed by molecular methods

- **PCR with species specific primers**

- *D. septosporum*: **Dstub 2-F & Dstub2-R** (loos et al., 2010)

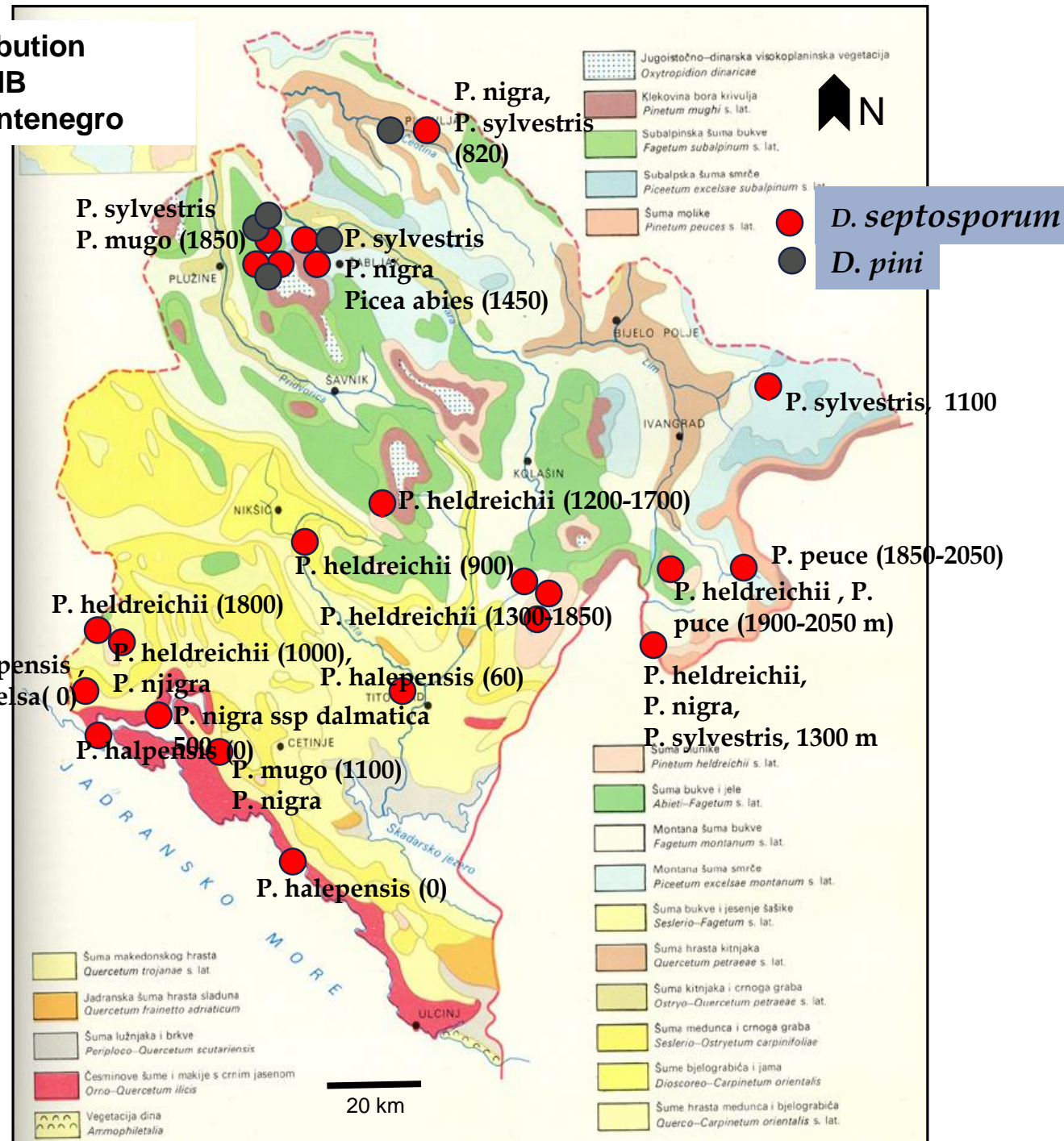
- *D. pini*: **Dptef-F & Dptef-R** (loos et al., 2010)

to determine which species of *Dothistroma* is present in Montenegro



**FP1102: Determining Invasiveness and risk of Dothistroma (DIAROD)**

# Distribution of DNB in Montenegro



## Results:

- 10 pines + spruce
- new hosts
- first records
- new Mt. areas/climates
- *D. pini*... in Europe

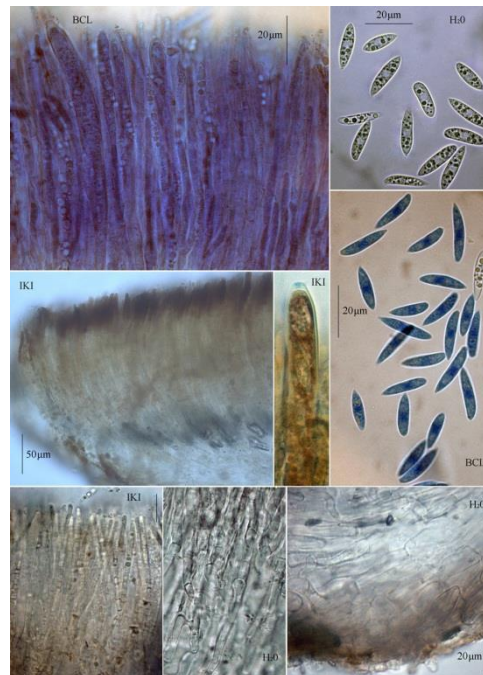
STSM: Learning molecular techniques for genetical characterization and phylogeny of Ascomycetes-example of genus *Hymenoscyphus*

**FP1103: Fraxinus dieback in Europe: elaborating guidelines and strategies for sustainable management (FRAXBACK)**

- ✓ Molecular characterisation of different (10) *Hymenoscyphus* species from mycological collection (exicates) and from freshly collected material, with different molecular markers
- ✓ During FRAXBACK action, health status of *Fraxinus excelsior*, *F. angustifolia* and *F. ornus* were under observation, and estimated in different parts of Montenegro.



Photo B. Perić



*H. fructigenus* & *H. rokebyensis*

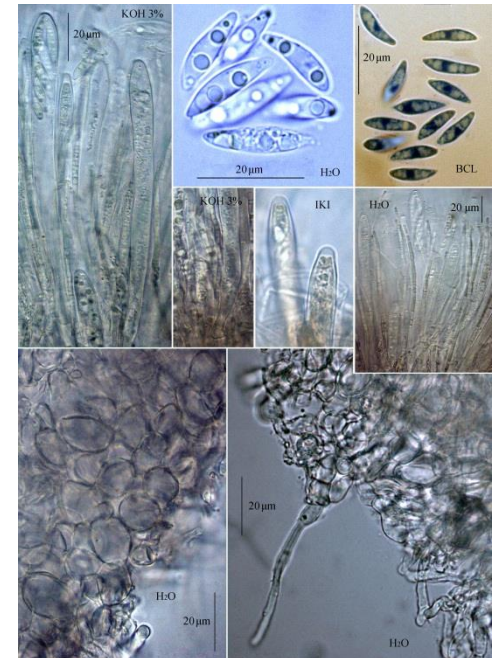


Photo B. Perić



**FP1305: Linking belowground biodiversity and ecosystem function in European forests**

**STSM Topic: Identification and genetic characterization of fungi from fire-disturbed forest soils with special reference to *Pinus heldreichii***

Fungal biodiversity associated with fire-disturbed *Pinus heldreichii* forest soils in Montenegro


Jelena Lazarević<sup>1</sup>, Ana Topalović<sup>1</sup>, Audrius Menkis<sup>2</sup>





## Forest fires are among the most devastating events in forest ecosystems

- Forest fires are the major threat to forests of *P. heldreichii* in Montenegro.
- about 25 % of forest area covered by *P. heldreichii* in SE Montenegro were damaged by forest fires in the last 10-15 years.
- 98% of all forest fires in MNE are caused by man

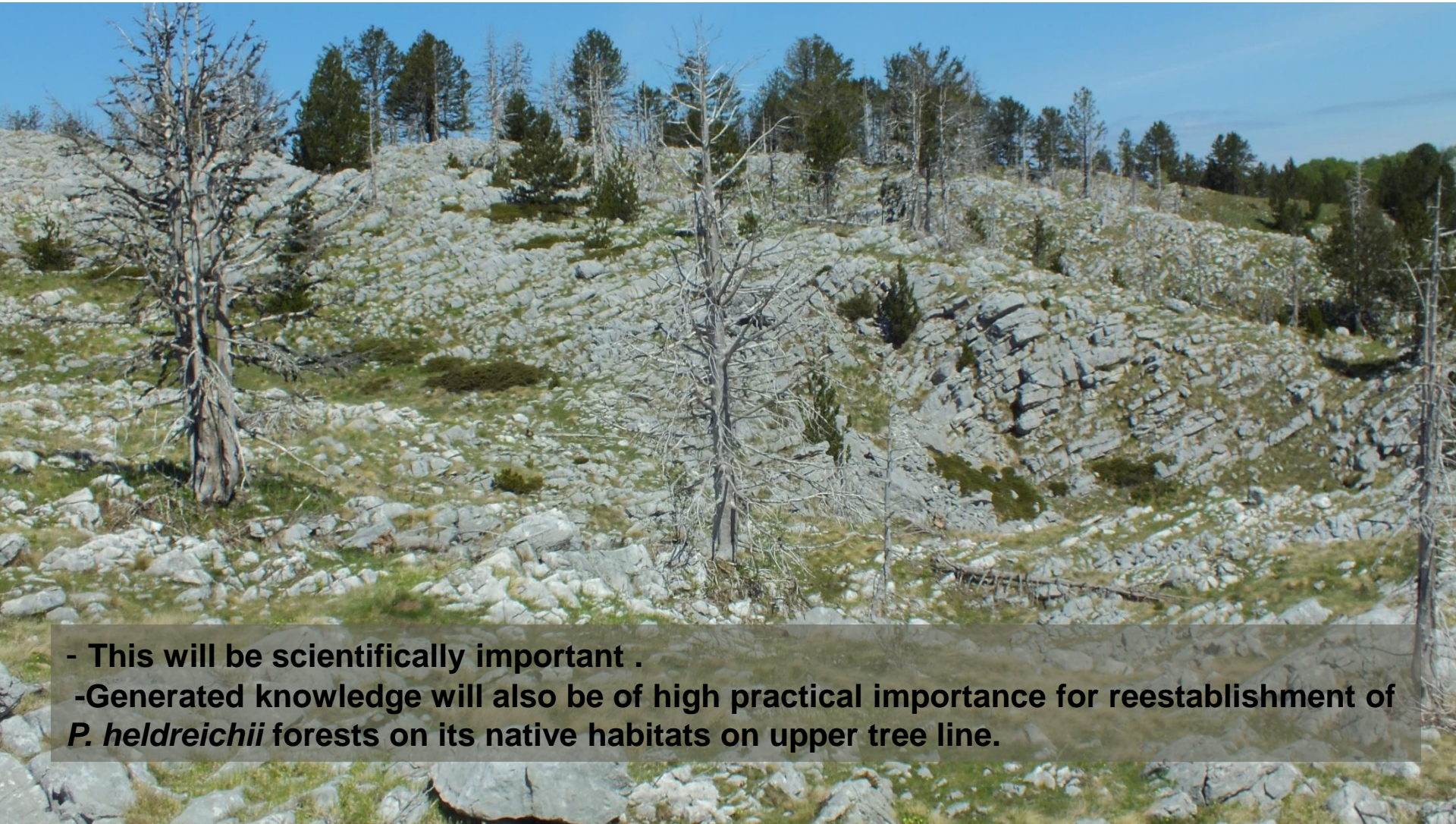
- 
- A landscape photograph showing a mountain slope. The foreground is rocky with sparse vegetation. The middle ground features a dense forest of tall, thin trees, many of which are dead and white, interspersed with some green trees. The background shows a steep, rocky mountain face with patches of green vegetation. A dark red text box is overlaid on the right side of the image.
- (some other) forests need more than 70 years to recover after fire, while here ...



**Little is known about the succession of soil fungi following a forest fire.**

Aim of research :

**Getting knowledge on fungal succession, composition and structures of fungal communities in forest soils after the fire**

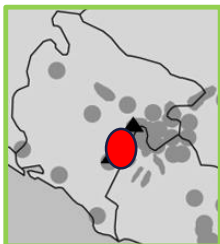


- This will be scientifically important .
- Generated knowledge will also be of high practical importance for reestablishment of *P. heldreichii* forests on its native habitats on upper tree line.

Field\_sampling : **June 2016**



Less than 1 year after fire

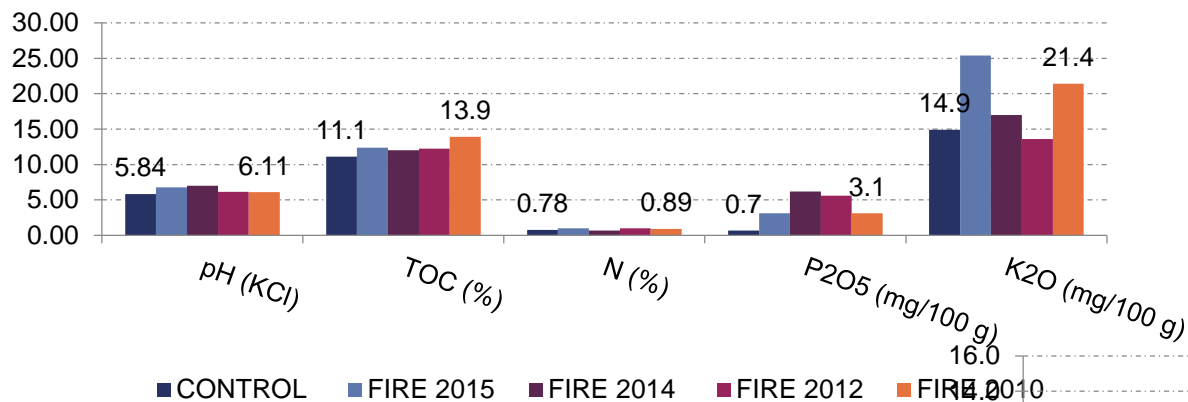


- **Stravče**– forest fire in **2015**  
O- burned litter  
A- soil (kalkomelanosol)
- **Hum Orahovski** –forest fire in **2014**  
A-soil (kalkomelanosol)
- **Kastrat** –forest fire in **2012**  
O- burned litter  
A- soil (kalkomelanosol)
- **Treskavac** –forest fire in **2010**  
O- burned litter  
A- soil (kalkomelanosol)
- Control (without fire) : **Kucka korita** - A- soil (kalkomelanosol)  
**Sovrh** –A-soil (regosol)
- Roots /plantlets from control sites

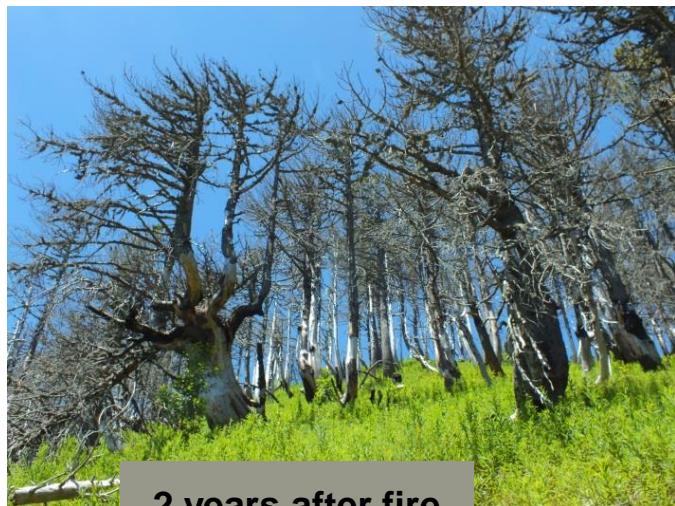
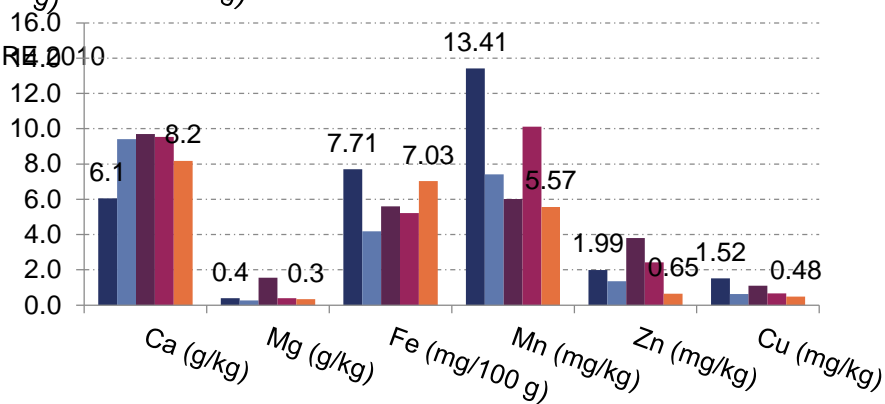
- 5 replicates per locality  
(20-50 m between replicates)

# Soil chemical characteristics- A horizont

## pH, TOC, and primary macronutrients



## secondary elements and microelements



2 years after fire



4 years after fire



6 years after fire

- **Direct isolation of genomic DNA from soil samples**

- CTAB extraction protocol (additional cleaning with Wizard DNA purification kit , PROMEGA

- NucleoSpin Soil kit, Macherey-Nagel**

- **PCR amplification**

- gITS7 (8bpIDtag- G T G A R T C A T C G A R T C T T T G)

- ITS4 (8bpIDtag-TCCTCCGCTTATTGATATGC)

- (Ihrmark et al., 2012).

- **High-throughput PacBio sequencing**

for the purpose of fungal community studies, different samples were amplified with different **barcoded** fungal specific primers

**Sequence analysis**

SeqMan Pro version 12.0.0

BioEdit version 7.0.5.2

GenBank (<https://blast.ncbi.nlm.nih.gov>.)

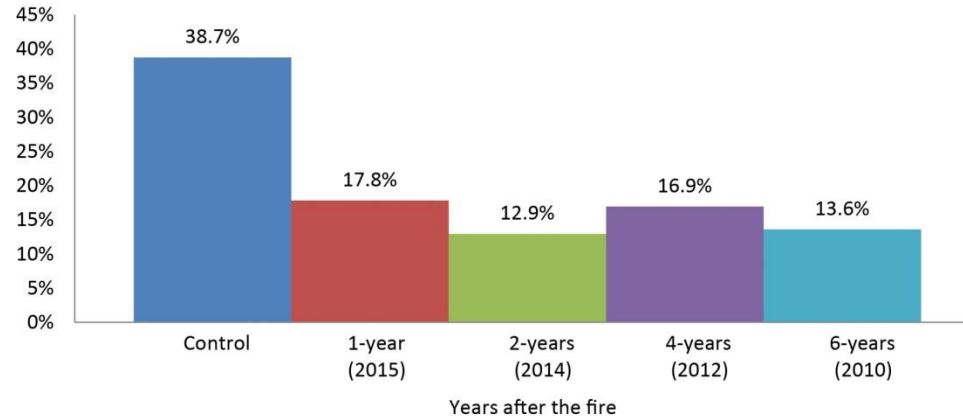
The criteria for species identification:

- sequence coverage >80%
- similarity to genus level 94-97%
- similarity to species level **98-100%**

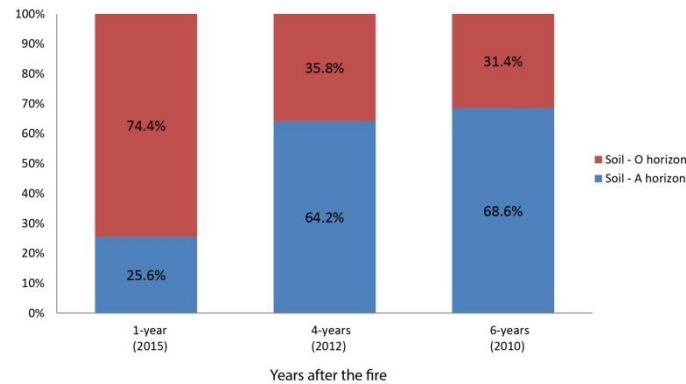
- Sequencing resulted in 10,105 high quality sequences



Relative abundance of high-quality sequences



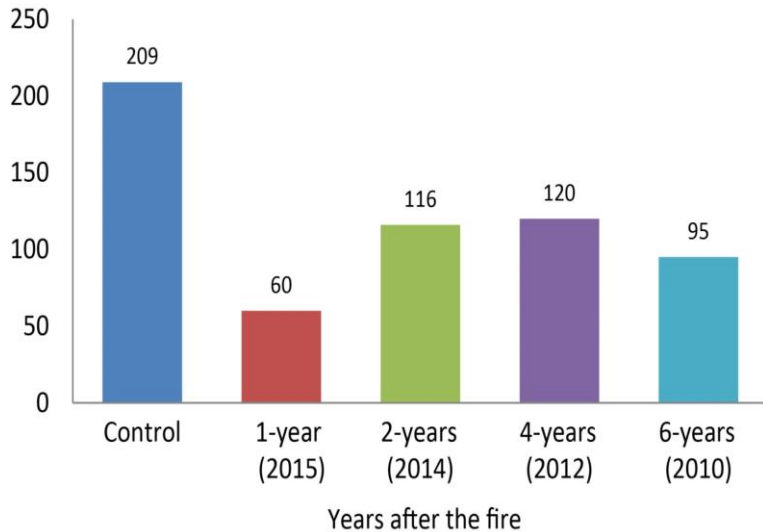
Distribution of high-quality sequences in soil horizons



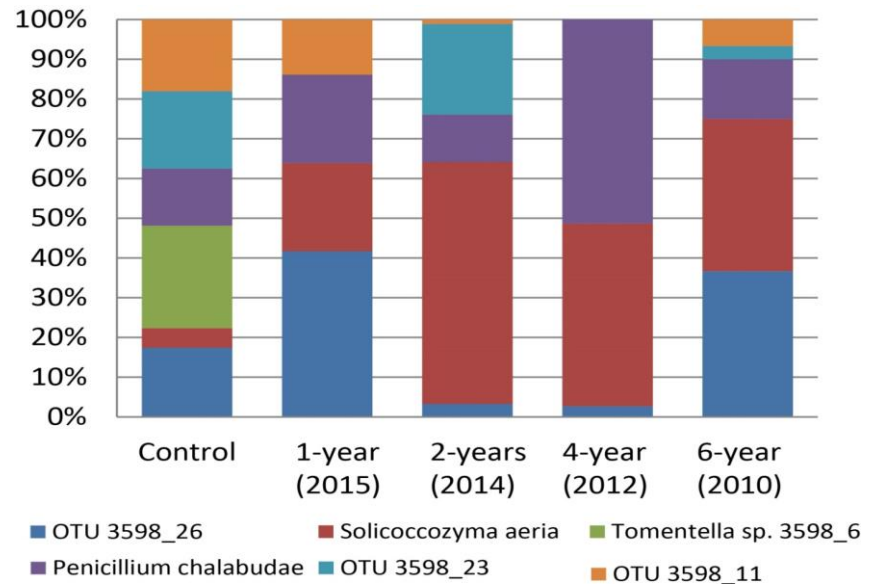
**Fire results in the loss of biodiversity and shift in the fungal community structure.**

- Assembly showed the presence of **848** OTUs (at 98% similarity level)
- majority of dominant fungal species are unidentified
- majority of all fungal species are not identified at species (even genus) level

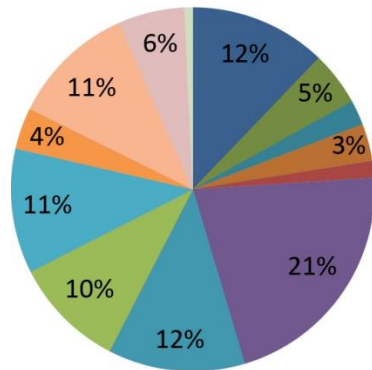
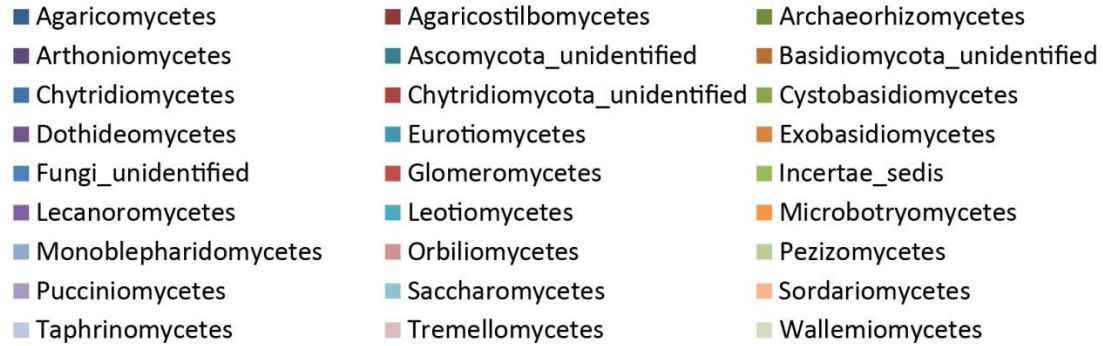
### Richness of fungal OTUs in soil A-horizon



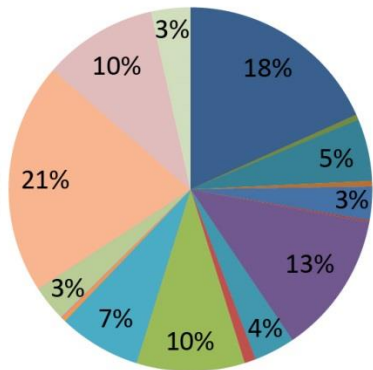
### Abundance of the six most common OTUs in soil A-horizon



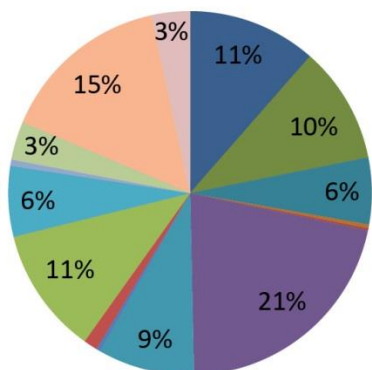
# Composition of fungal community in soil A-horizon



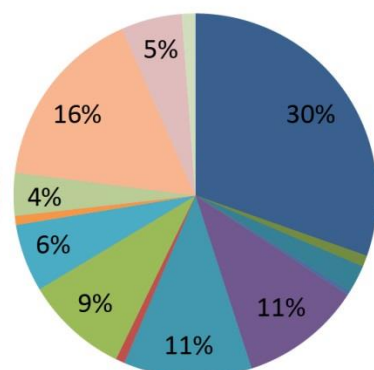
1-year  
(2015)



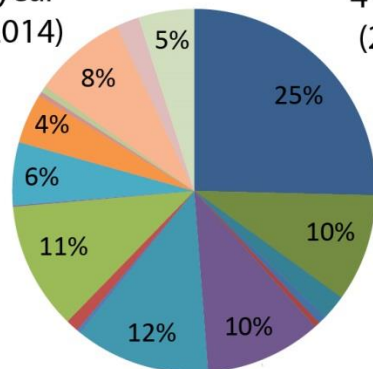
2-year  
(2014)



4-year  
(2012)



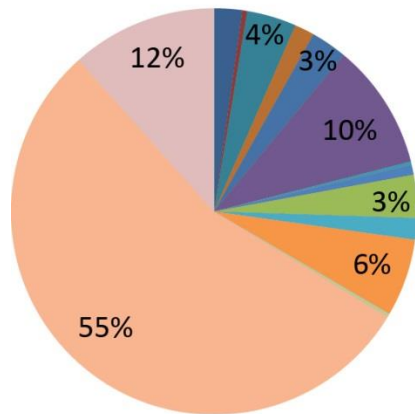
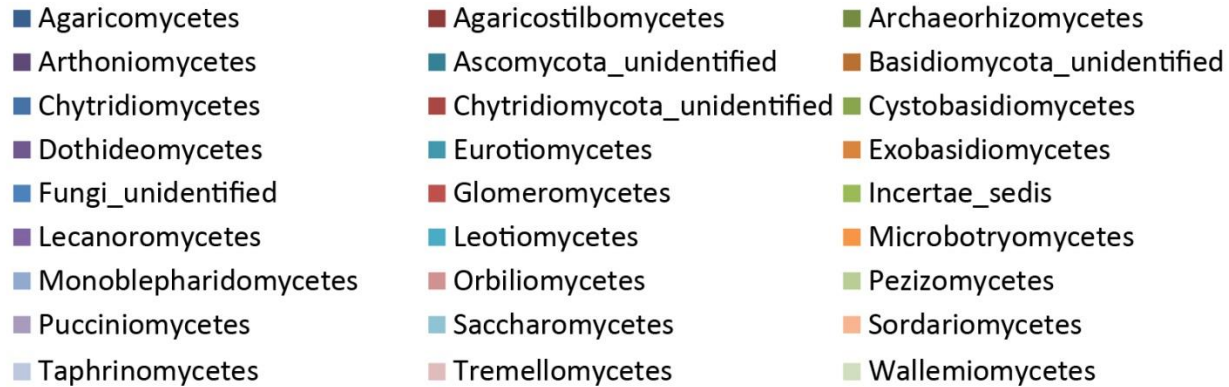
6-year  
(2010)



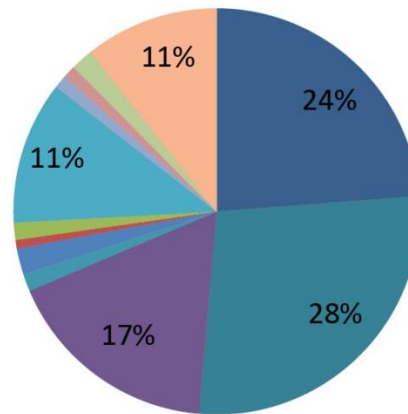
Control  
(2016)

**Up to 6 years is needed for  
*Agaricomycetes* to recover  
after the forest fire**

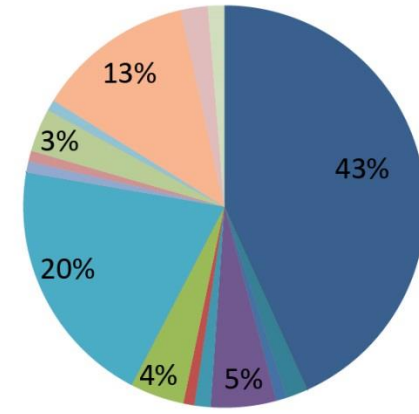
## Composition of post-fire fungal community in O-burned litter layer



1-year  
(2015)



4-years  
(2012)



6-years  
(2010)



FP1401 - A global network of nurseries as early warning system against alien tree pests (Global Warning)

STSM Topic: **Invasive fungi in the phyllosphere of the principal tree species in Montenegro**

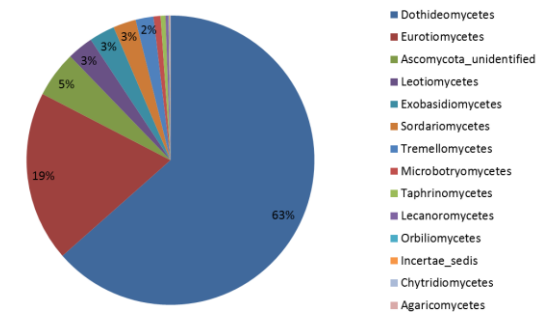
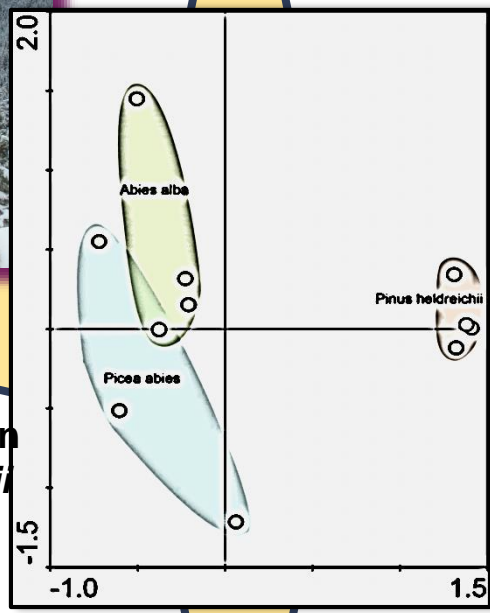
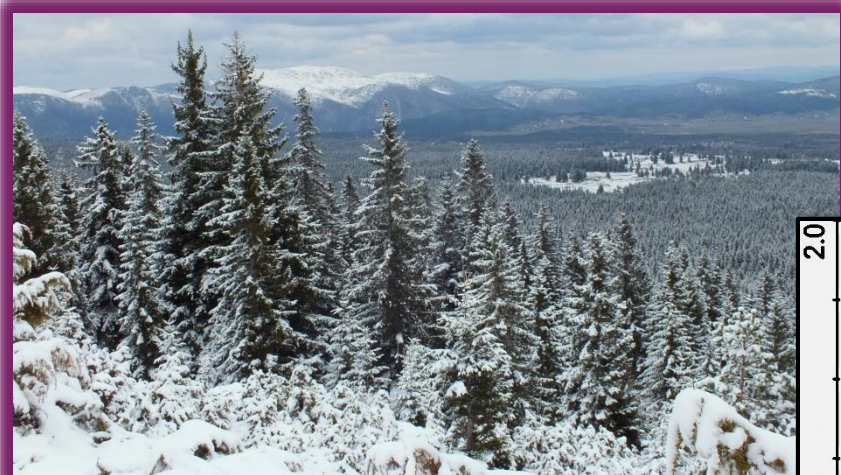
**The aim** of this study was to investigate fungi in healthy-looking needles and leaves of native tree species.

**Widescale sampling:**

- ✓ 5 principal tree species: *Fagus sylvatica*, *Abies alba*, *Picea abies*, *Pinus heldreichii* and *Pinus peuce*
- ✓ 3 representative localities for each species
- ✓ 50 trees on each locality (50 m between)

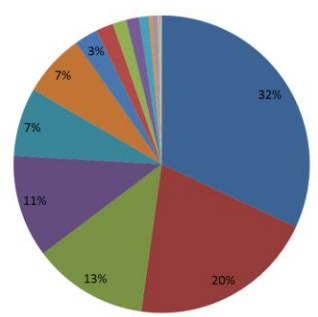
*P. peuce*, Prokletije



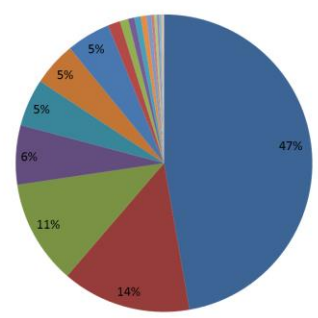


**Composition of fungal community in needles of *Pinus heldreichii***

**Ordination diagram of fungal communities in needles of *P. abies*, *A. alba* and *P. heldreichii***



**Composition of fungal community in needles of *Picea abies***



**Composition of fungal community in needles of *Abies alba***



## FP1202: Strengthening conservation: a key issue for adaptation of marginal/peripheral populations of forest trees to climate change in Europe (MaP-FGR)

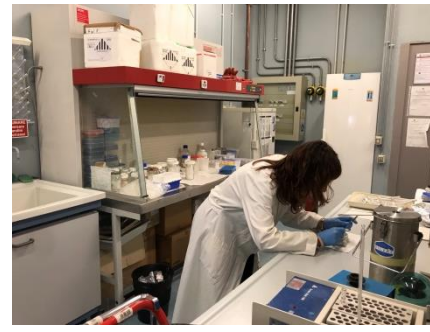
COST-STSM-FP1202-32290

STSM Topic: **Learning molecular techniques for genetic characterization of forest tree populations- example of *Pinus heldreichii***

Participant: **Ivana Stojanović, M.Sc** , University of Montenegro, Biotechnical faculty

Host: **Giovanni Giuseppe Vendramin**, National Research Council, **Institute of Biosciences and BioResources**, Florence(IT),

Period: 01.03-31.03. 2016.



## Lessons learned - what is important for successful STSM (Message for young researchers )

- ✓ You are **selecting & proposing** the **title** and the frame of your work, so explain it first to your possible host, and then to COST action coordinators (through application form);
- ✓ It is not possible to stay always and persistently with your favorite themes from your master or PhD thesis, so **be flexible and open for new research topics**;
- ✓ **Responsible** and **dedicated work** is appreciated (in lab, but also in other cases), as well as reliable **communication**;
- ✓ Sometimes, you will be asked, or you will need just to apply for already proposed call for STSM (Hosts are looking for applicants/ topics are already proposed – special tasks needed for the action);
- ✓ In other cases, you will be able to develop your own small project, which fits in general topic of the action.

# Collaborative projects and publications

## WG meeting:

- ✓ Presentation of the topic by R. Drenkhan (EE)
- ✓ Presentation about geodatabase by P. Vahalik, GIS expert (CZ)

Dothistroma interactive map:

<http://arctgis.mendelu.cz/monitoring/>

- ✓ Discussion about criteria for creating database
- ✓ Presentations, discussions and comments from participants

**Participants sending reports and data** (records) by e-mail (deadlines are defined)

## New WG meeting(s).....

**Meeting dedicating to publishing strategy** (selected participants),

- ✓ first draft were already prepared,
- ✓ we passed through all fragments/sections of work (for several publications);
- ✓ tasks for further work were delegated.

**Final drafts and manuscript** for communication delivered via **e-mail**.



- ✓ Bulman L., Bradshaw R., Fraser S., Martín-García J., Barnes I., Musolin D., La Porta N., Woods A., Diez J., Koltay A., Drenkhan R., Ahumada R., Polaković-Pajnik L., Queloz V., ; Piskur B., Doğmuş-Lehtijärvi H. T., Chira D., Tomešová-Haataja V., Georgieva M.; Jankovsky L., Anselmi N., Markovskaja S., Papazova I., Sotirovski K., Lazarević J., Adamčíková K., Boroń P., Bragança H., Vettraino AM., Selikhovkin A., Bulgakov T., Tubby K. (2016). "**A worldwide perspective on the management and control of Dothistroma needle blight**", **Forest Pathology 46/5**: 472-488 DOI: 10.1111/efp.12305
  
- ✓ Lazarević J. , Davydenko K., Millberg H. , 2017. **Dothistroma Needle Blight on High Altitude Pine Forests in Montenegro**, **Baltic Forestry** vol 23 (1) Special issue: Advances in ash dieback research, and some other invasive diseases of trees, 294-302.
  
- ✓ Lazarević J., Davydenko K., Millberg H., 2015: **Incidence of *Dothistroma septosporum* in different pine forests in Montenegro**, *Mycologia Montenegrina XVII* (2014) 2015: 119-131
  
- ✓ Lazarević J., Davydenko K. , Menkis A. (2018): **Dothistroma needle blight and other needle fungi of native conifers in Montenegro** , 2018, **The 15<sup>th</sup> International Phytotechnology Conference, University of Novi Sad**, 1-5 October 2015, Book of abstract, page 17., **invited speaker**

## Global patterns in insects and fungi of dormant twigs of native and exotic congeneric tree species

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**30 countries, 45 institutions**

We performed a global study aimed at detection of potential insect pests and fungal pathogens on congeneric native and exotic woody plant species.

- ✓ Sampling was done simultaneously in **30 countries** around the world following a common sampling protocol.
- ✓ The plant species from genera ***Pinus***, ***Fagus*** and ***Quercus*** ( Northern hemisphere) and ***Podocarpus***, ***Nothofagus*** and ***Eucalyptus*** Southern hemisphere).
- ✓ Sampling was done in January/February 2018 on the Northern hemisphere and in June/July 2018 on the Southern hemisphere, to respect seasonal phenomena.
- ✓ At each location twenty 50 cm long twigs were collected from each tree species and kept in containers with water at room temperature.
- ✓ Emerged insects were collected and identified to species or morphospecies level based on morphology and DNA barcoding of the mtDNA COI region.
- ✓ Fungal DNA was extracted from pooled buds, twig parts and needles for species identification using a metabarcoding approach based on the rDNA ITS region.
- ✓ Fungi were also isolated from collected material and cultivated...

Pairs from Montenegro:

***Quercus robur* & *Quercus rubra*** (Loc. Podgorica)

***Myrthus communis* & *Eucalyptus globulosus*** (loc. Podgorica and Bar)



*Some ther collaborations, comes after COST conferences....*

**with: Prof dr Stefano Grego, World Agricultural Heritage Foundation**



*Introductory presentation:*

***Culture, Agriculture and Innovation for Sustainable Development***

at **KATUN** PROJECT FINAL CONFERENCE: “*THE KATUNS PROJECT RESULTS AND FUTURE OF THE MONTENEGRIN KATUNS*” *THE KATUNS – HOW TO MOVE FORWARD?*



A group of KATUN team members with guests on Mt. Durmitor, Sedlo, 28. September 2017

**Application for Horizont 2020 project** (call : SC5-21-2016-2017 TOPIC : *Cultural heritage as a driver for sustainable growth Scope b) Heritage-led rural regeneration.* )

**“Maintenance of Mountain Heritage in Europe for Sustainable Use of its Natural Environment, for PROtection and Promotion of the Mountains' Cultural-Historical Traditions, for a Vlable tourism, and for RESilience to Climate and Land Use Changes – PROVIRE”**, with very active involmnet of our team.

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