Are mycorrhizal fungi shared between trees recolonizing a post-mine site?

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RESEARCH COMMITTEE..

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Introduction

Effects of mining activities on the boreal forests…

- Affect the terrestrial biodiversity of Canadian boreal forests through changes in the
  - -Landscape structure
  - -Stand structure
  - -Age distribution
  - -Species composition

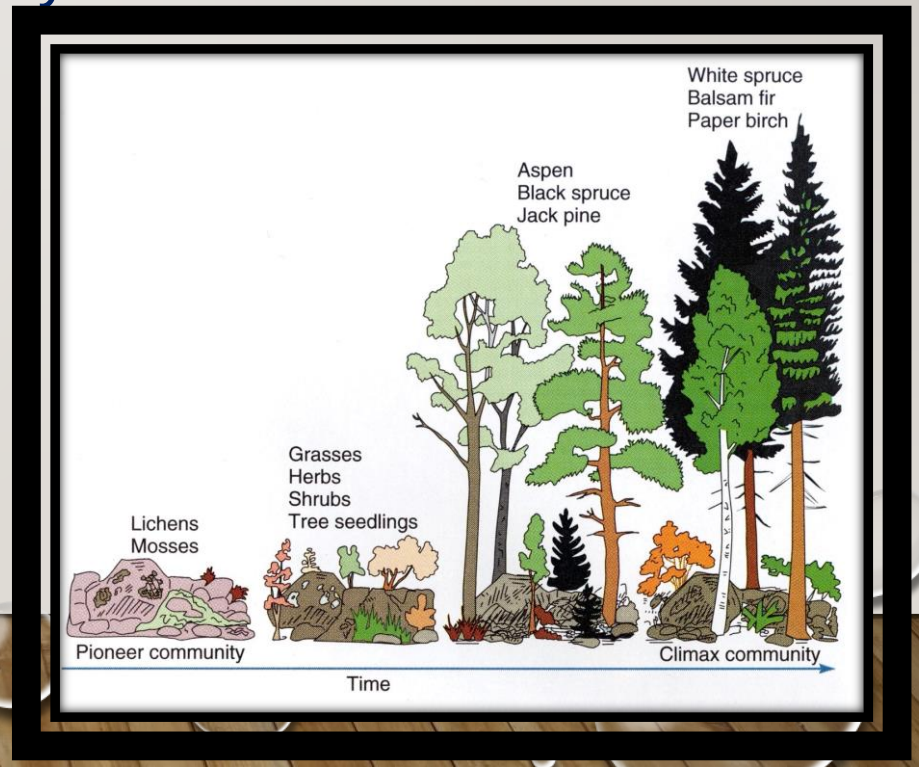
- More than 10,000 mining explorations.
- 2200 mining sites in Quebec.
- 1300 abandoned mining sites.
- 14 182 ha of mining-d lands in Quebec .
- 3307 ha have been restored by 2000.

- Cause soil infertility and soil toxicity through drastic changes in the soil properties.
- The abandoned mining sites in the boreal region are recent surface deposits, poor in organic matter and nitrogen.

Source: https://www.borealbirds.org/threats-canadian-boreal-forest

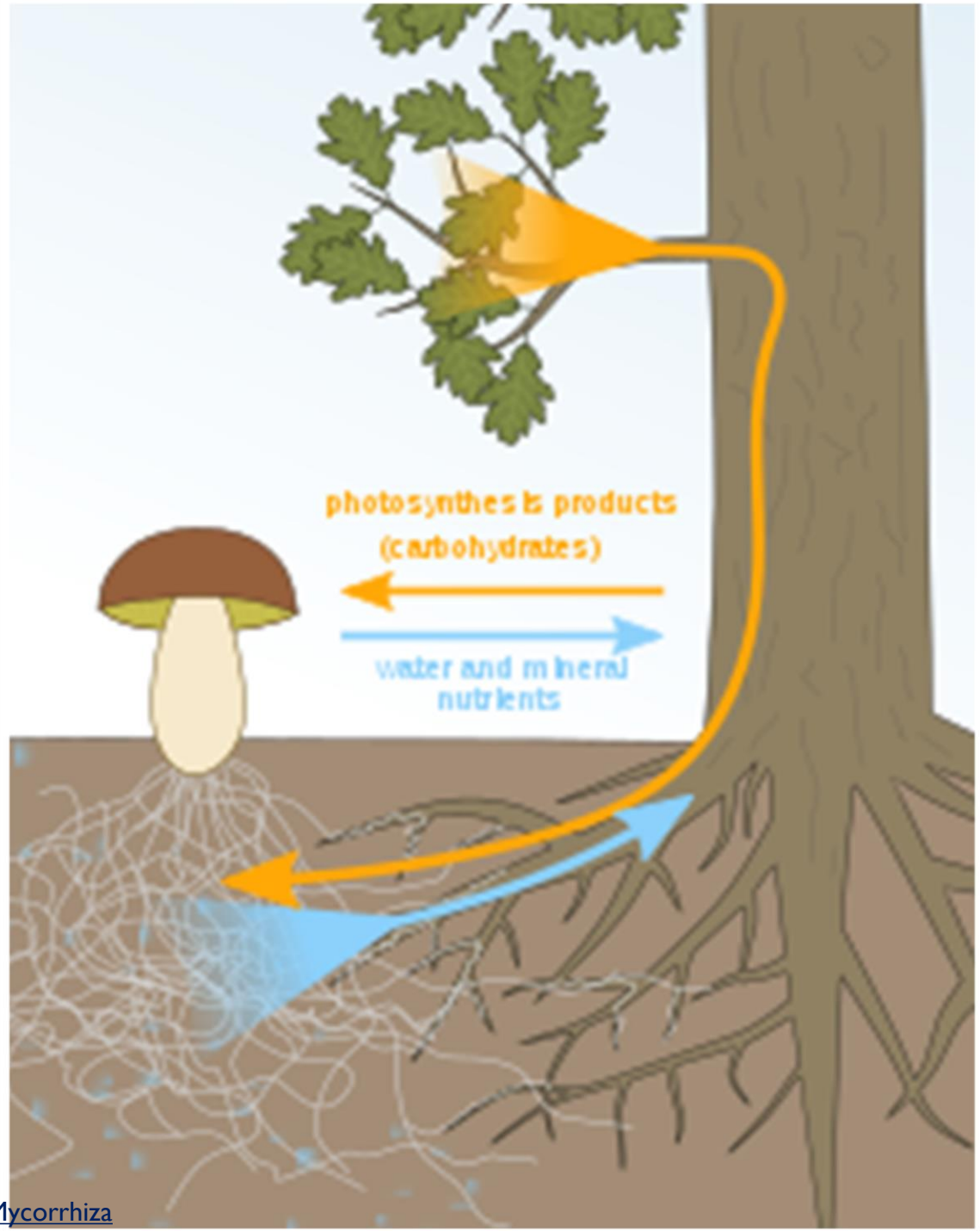
Regeneration of boreal forests in post-mining sites…

- Surface mineral extractions create suitable substrates for primary succession.
- Colonization occurs in these types of post-mining lands through dispersal of seeds over long or short distances by plant species from neighboring forests.
- Slowly colonized by lower plants, herbs and eventually with trees.



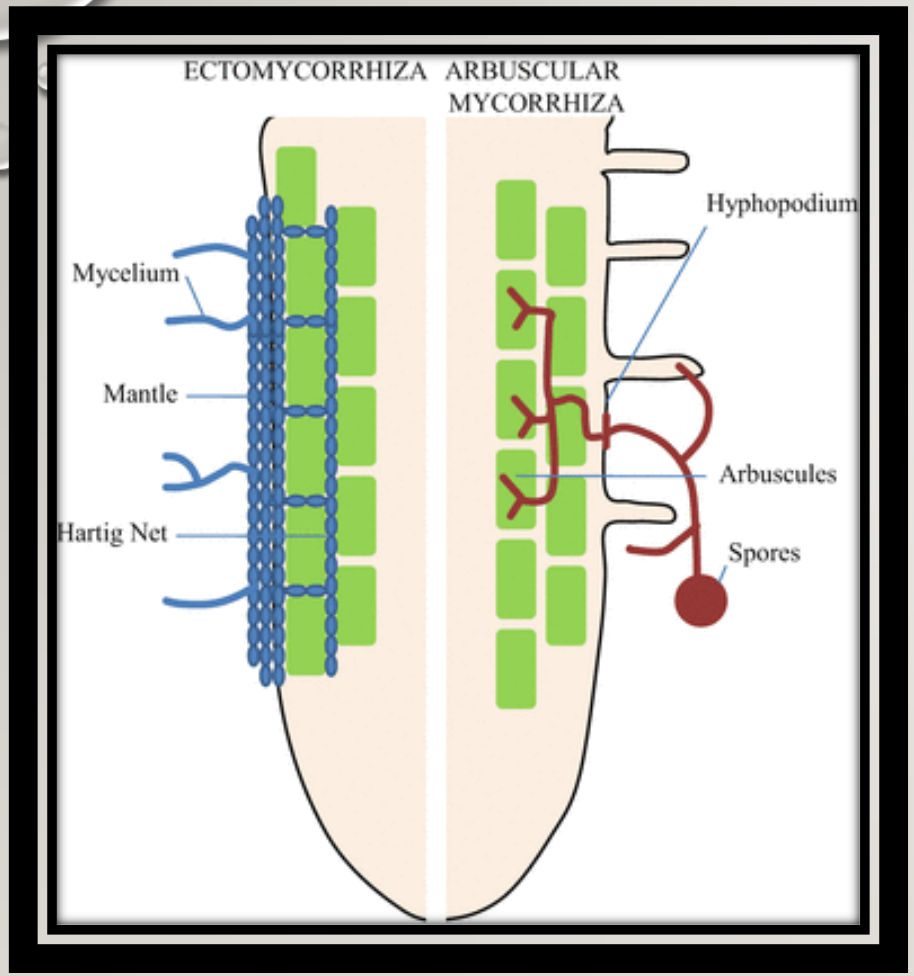
Mycorrhizal symbiosis as a determinant…

- Under nutrient-poor environments, mycorrhizae play a key role in facilitating the survival and growth of host plants through the uptake and distribution of plant nutrients.
- Facilitates the mutual exchange of resources where fungi supply limited nutrients to plants whereas plants provide assimilates such as carbon to fungi.



https://en.wikipedia.org/wiki/Mycorrhiza

Ectomycorrhizal (ECM) and Arbuscular (ABS) mycorrhizal fungi….



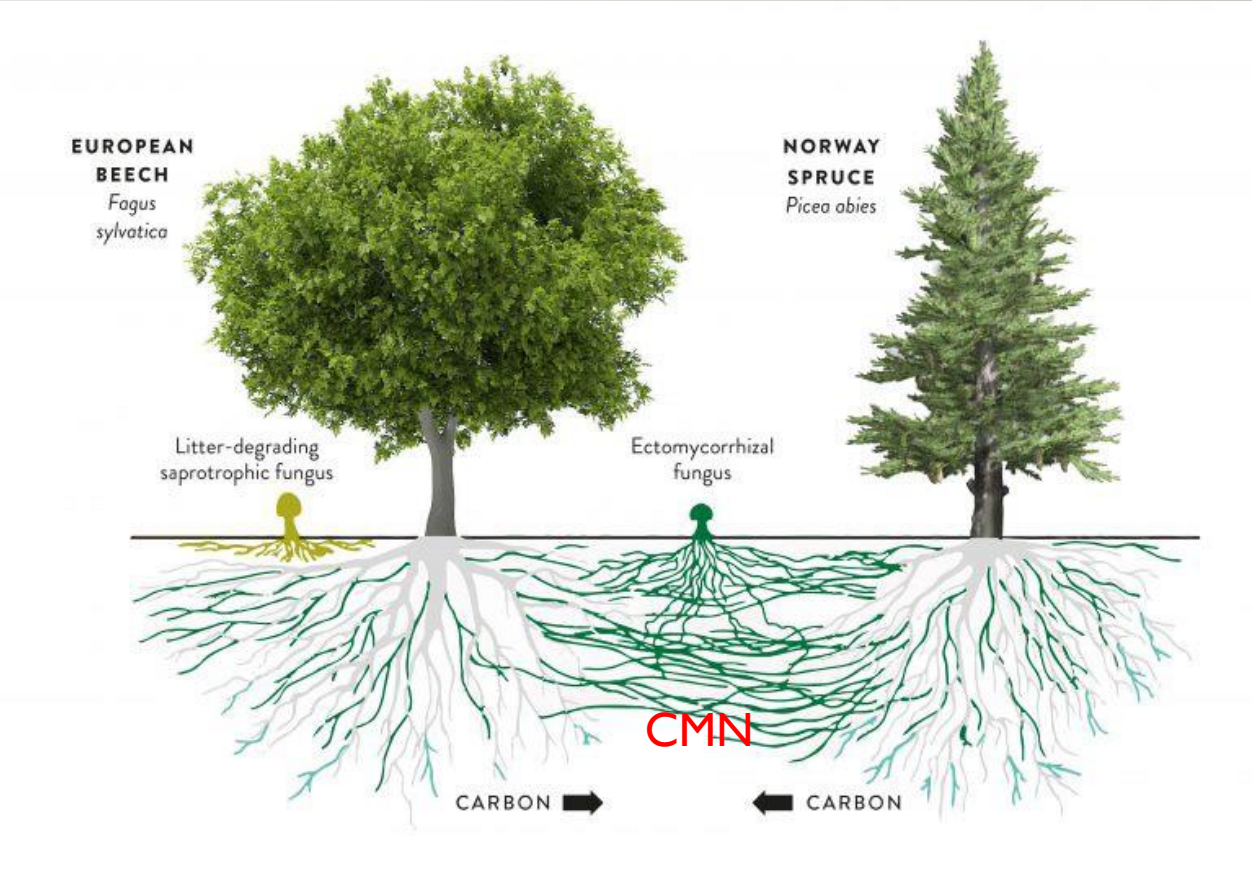
ECM Host Plant Families

...Mycorrhizal networks…

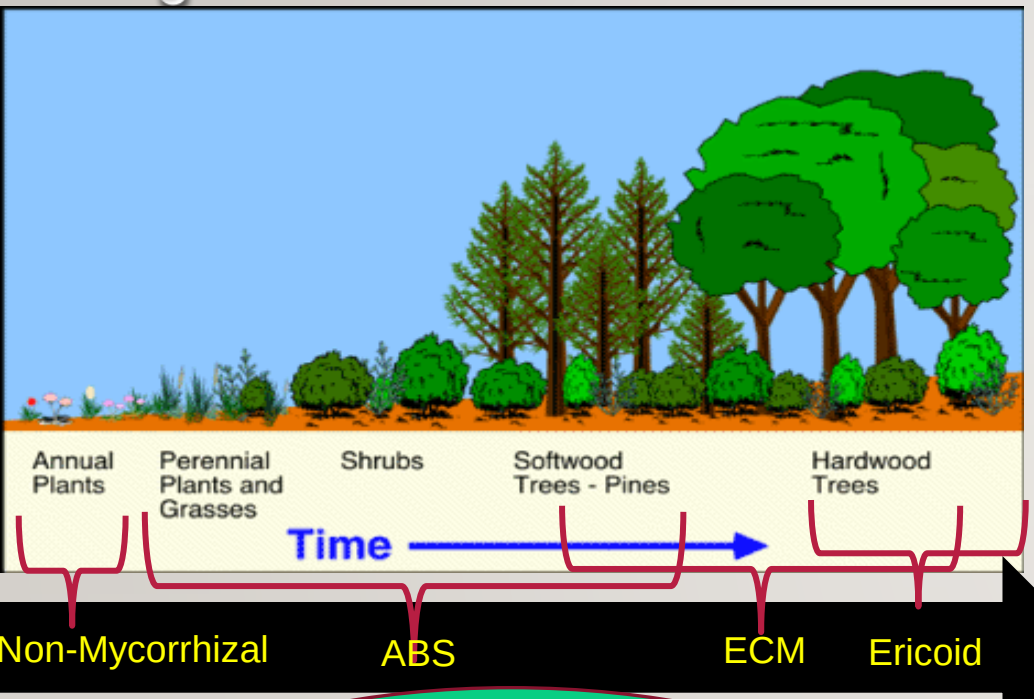
Mycelia of mycorrhizal fungi produce common mycorrhizal networks (CMNs) by colonizing roots of neighboring trees and seedlings.

https://thekidshouldseethis.com/post/the-wood-wide-web-how-trees-secretly-talk-to-and-share-with-each-other

- Facilitate the uptake and transportation of nutrients and other resources among plants.
- The networking complexity of a CMN can range from two plant species connected by one fungal species to several plants of different species with various fungal species.



Do mycorrhizae determine the plant community assemblage in successional forest ?



- Mycorrhizal colonization ⟷ Dominant plant community
- Boreal forests are dominated by ECM associated plants.
- ECM colonization + interspecific competition determine plant community assemblage.
- Diversity of a CMN shape of the coexistence of plant species since it define the types of resources provided to their respective hosts.
- Through this asymmetry, CMNs ultimately affect the coexistence of plant species in particular ecosystems by enhancing or suppressing their growth and survivorship.

CMNs → mutualistic plant-plant interactions

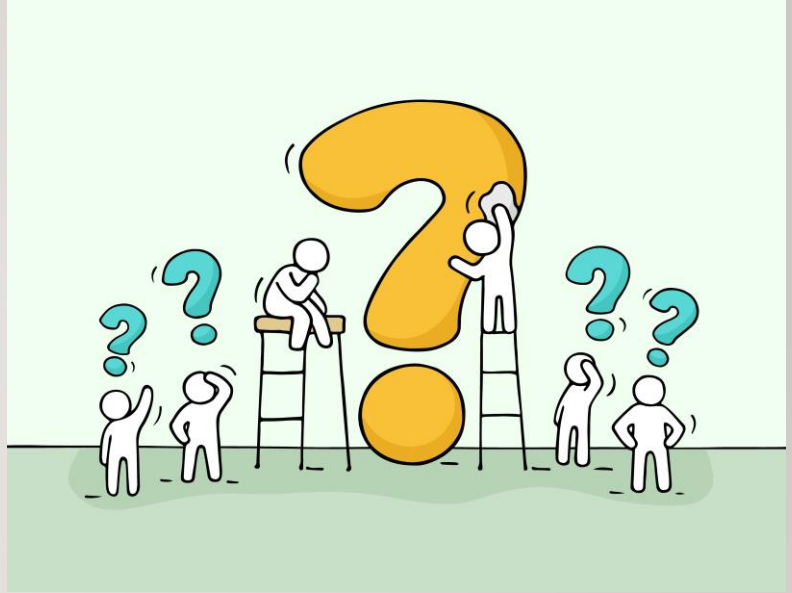
Non-shared → Neutral interactions

Minimizie the antagonistic interactions

Objectives

1. Determine which intraspecific and interspecific interactions affect plant growth and survival at this early succession stage on a former mining site.
2. Determine diversity, distribution and sharing of existing mycorrhizae species.
3. If evidence of facilitation is found in objective 1, determine whether this facilitation is due to mycorrhizal networks or due to other factors.

Questions



1. What is the fungal richness and abundance at the site, overall and by fungi type?
2. Does fungal richness and abundance change with the species identity and size of the host?
3. How does mycorrhizal fungi community composition vary across host species?
4. Does the fungal composition vary between plots and spatially within each a plot?

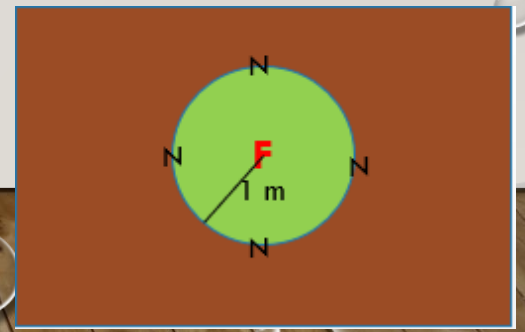
Methodology

Study sites..

- Mine tailings site of the Beattie Gold Mine (operated from 1933 to 1957) near Lake Duparquet in north-west Quebec.
- Lake Duparquet and surrounding forests are dominated by balsam fir followed by black spruce, white spruce and paper birch.

Sampling of plants…

- Plants mapped in two 15x15 m^2 plots in 2018.
- All woody plants 10 cm or taller were identified to the species.
- Their position was recorded to the centimeter.
- Height and diameter at breast height (DBH), only for plants > 2 m tall) were noted.
- Focal plants and neighboring plants were selected within at 1-meter radius.



Plots	Total	Focals (F)	Neibhors (N)	Species								
				Balsam Popler	Paper Birch	White Spruce	Cedar	Willow	Dwarf birch	Black Spruce	*Larix* sp.	*Cornus* sp.
West	168	39	129	F- 18 N- 35	F- 10 N- 41	F- 03 N- 01	F- 08 N- 00	F-00 N- 51	F-00 N- 01	F-00 N- 00	F-00 N- 00	F-00 N- 00
East	249	62	187	F- 12 N- 18	F- 17 N- 91	F- 27 N- 08	F- 03 N- 01	F- 00 N- 53	F- 00 N- 00	F- 00 N- 13	F- 00 N- 02	F- 00 N- 01
Mycorrhizal status recorded according to the literature				ECM	ECM	ECM	AM	AM and ECM	ECM	ECM	ECM	Dark Septate Fungi

- **Sampling of plants and soil…**
  - Growth parameters were taken-
    - Height.
    - Annual bud scar length (for 2016,2017 and 2018).
    - Root collar diameter (For all focals and neighbors).
  - Root sampling for mycorrhizae- For all focals and neighbors.
  - 42 soil samples (each with around 500 g) were collected from two sites.

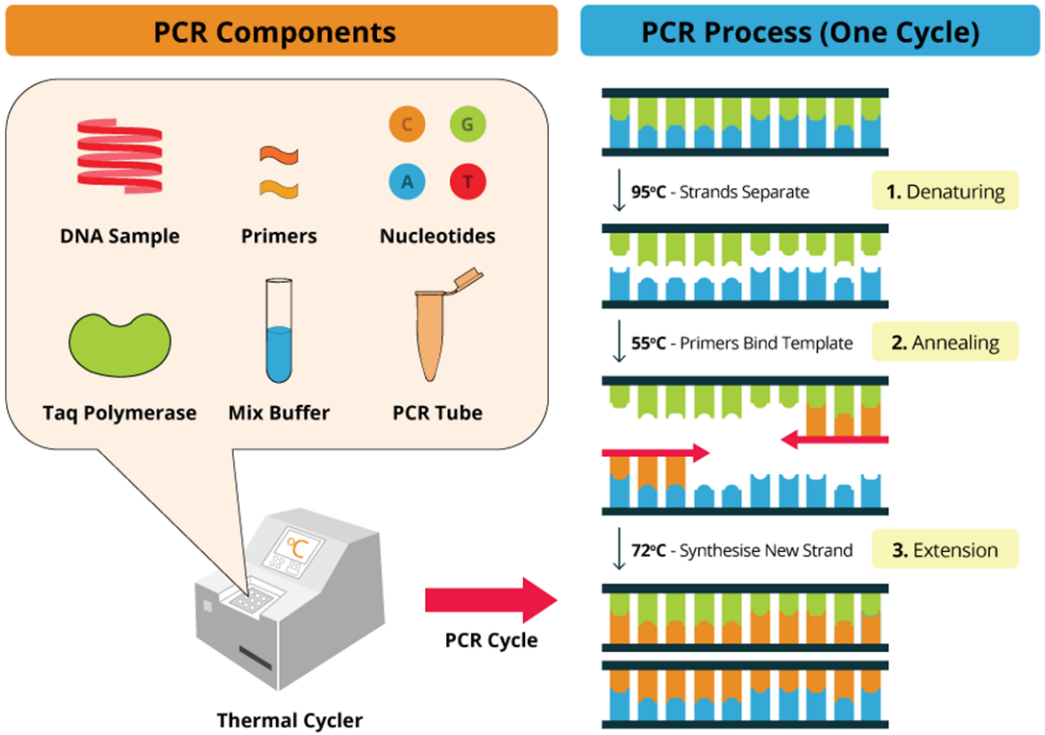
Identification of existing mycorrhizae species…

DNA extraction….

- ❑ DNA were extracted from,
  - Focal plants
  - Neighboring plants
  - Soil – Nucleo Spin kit

Power Soil kit

(Nagati, Roy et al. 2018)



PCR...

- ❑ ITS1 region was amplified using forward and reversed primers.
- ❑ 431 samples were placed to PCR with 49 controls as 26 without samples and PCR mix (blanks) and 23 only with PCR mix.
- ❑ Library construction with PCR performed samples.

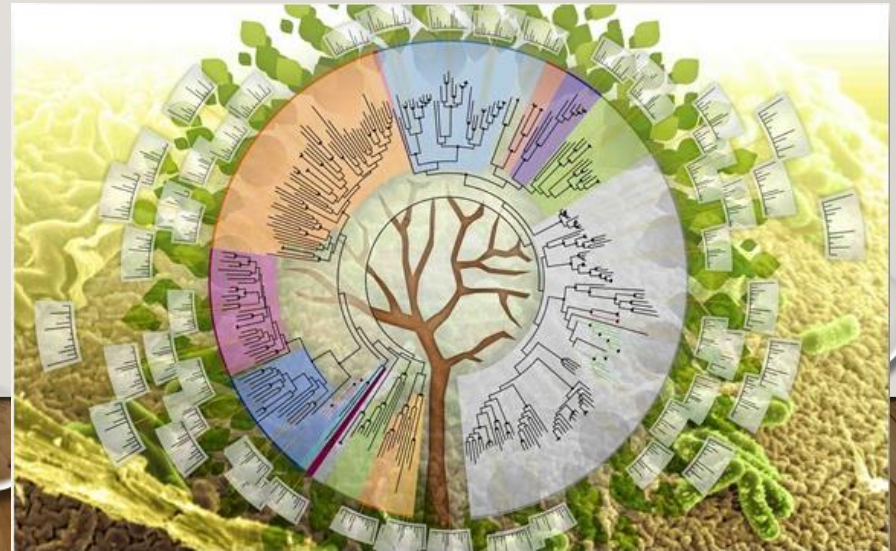
Illumina metabarcoding...

- ❑ GENOTOUL sequencing platform
  - TruSeq Nano PCR-free kit
  - Paired-end sequencing technology
  - Chemistry V2

https://upload.wikimedia.org/wikipedia/commons.png

Bioinformatics analysis…

- Using OBITools package and Unix commands.
- Application of metabarcoding- to obtain taxa occurring in each sample.
- Sequence clustering using OBITool Sumaclust at 97% identity.
- Taxonomic identification with Genbank.
- The most similar sequence was stated for each OTU (Operational Taxonomic Unit).
- Assign OTUs to a taxonomic rank- OBITool Ecotag function.
- OTUs were assigned to a trophic status - FUNGuild software.



Statistical Analysis…

- All analyses were done in R.
- Fungal richness and abundance analysis.
- Calculation of dissimilarity matrices and principal coordinate analysis (PCoA) for fungal community data using Bray-Curitis and Hellinger distances.
- PERMANOVA → significant differences in composition between two plots and between species.
- Mantel test → correlation between dissimilarity of fungal communities and physical distance between host plants (in each plot).
- Multipatt test → Indicator species analysis.
- Co-occurrence analysis.

Results and Discussion

Amplified fungal sequences by host species and plot…..

Host	plot	No. of amplified individuals	Number of individuals	Percentage of amplification %
Paper birch	East	40	111	36
Paper birch	West	24	51	47
Balsam poplar	East	8	30	27
Balsam poplar	West	20	54	37
Willow	East	13	53	25
Willow	West	22	51	43
White spruce	East	20	35	57
White spruce	West	2	4	50
Cedar	East	2	4	50
Cedar	West	6	8	75

Fungal Diversity….

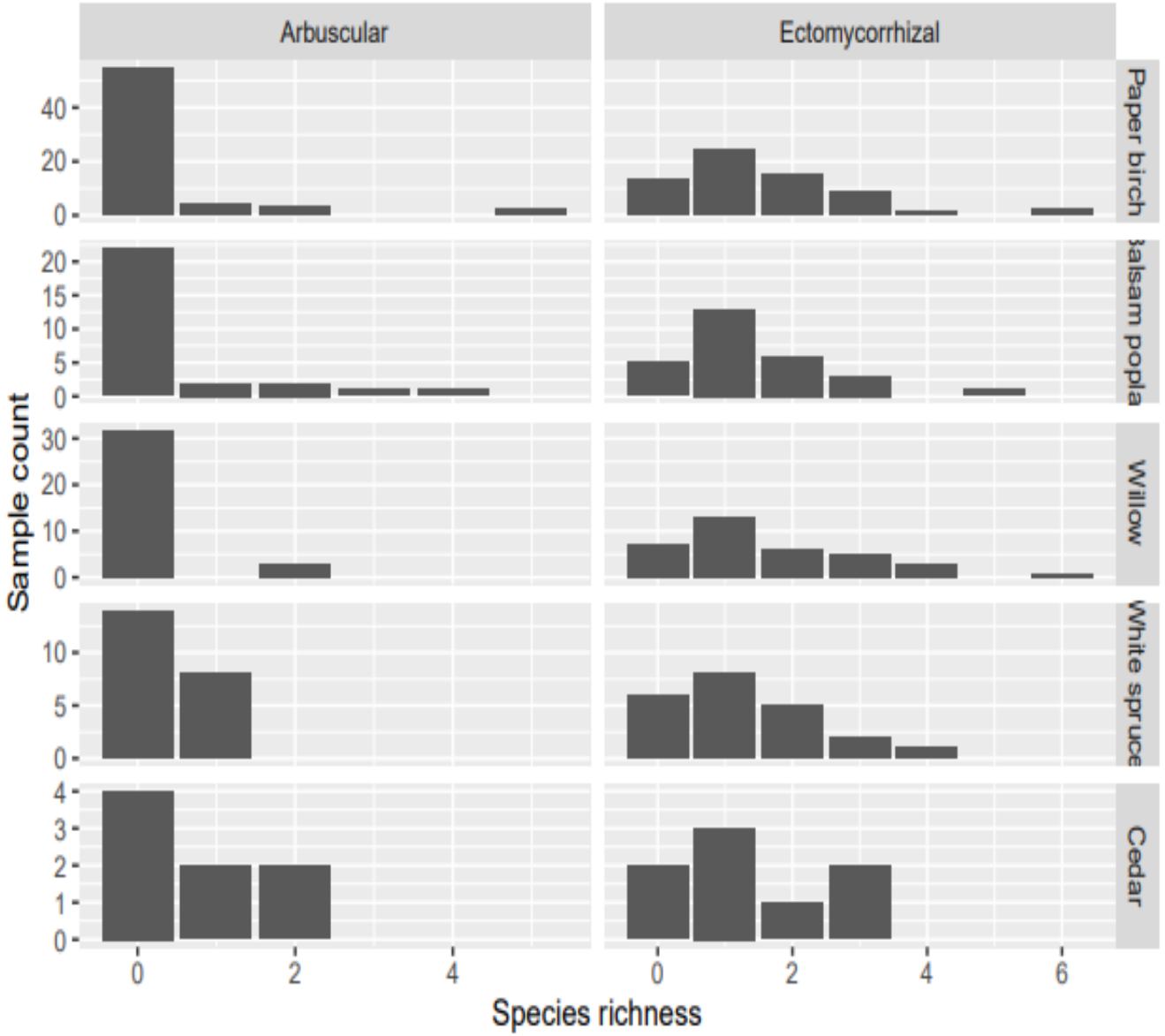
1. What is the fungal richness and abundance at the site, overall and by fungi type?

Mean species richness (S) and number of reads (N, i.e. abundance) by host species and plot for ectomycorrhizal (ECM), arbuscular mycorrhizal (ABS), saprotrophic (sapro) and plant pathogenic (patho) fungi.

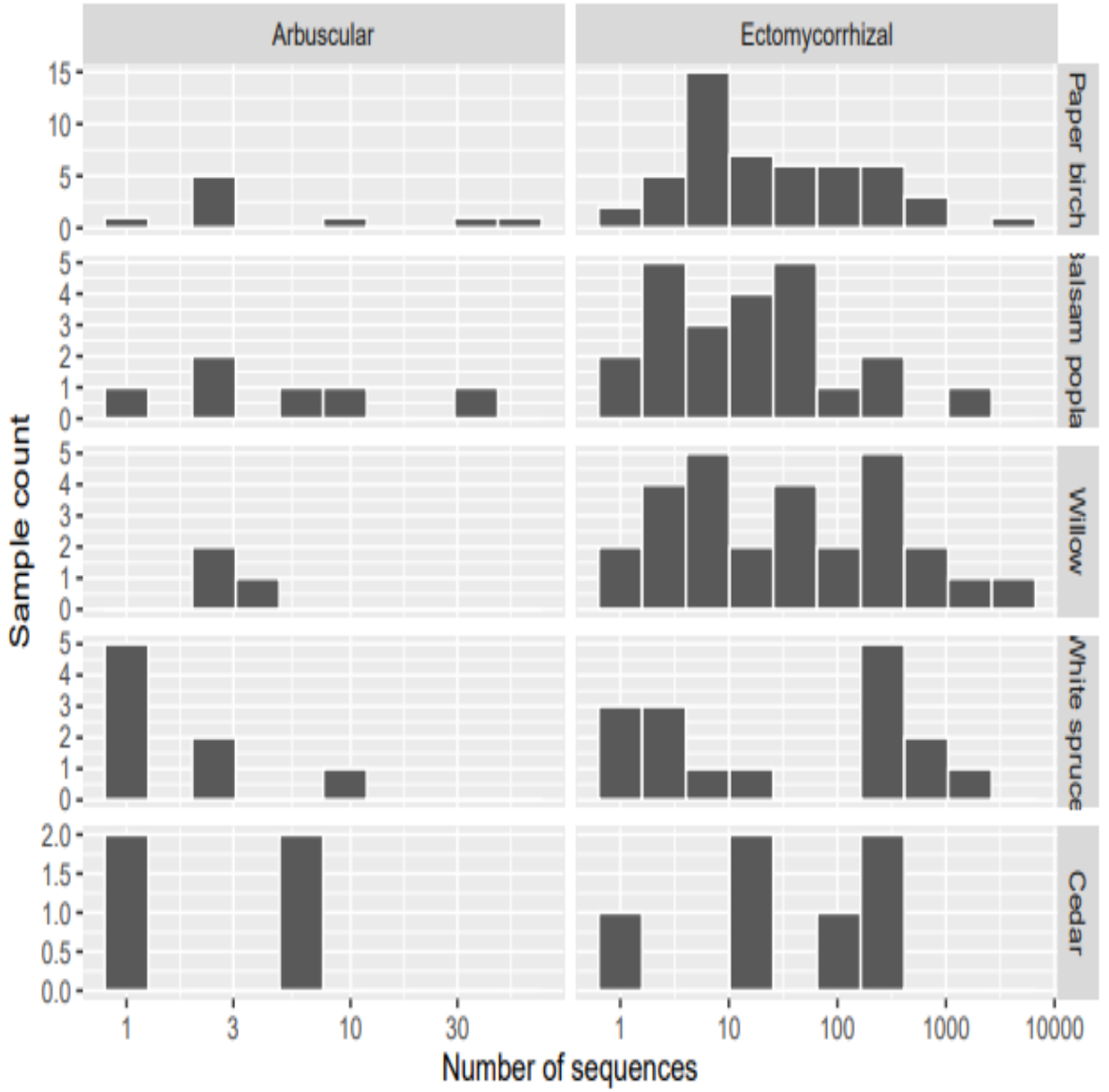
Host species	S_ECM	S_ABS	S_sapro	S_patho	N_ECM	N_ABS	N_sapro	N_patho
Paper birch	1.5	0.3	4.2	3.0	107.6	1.9	154.0	179.0
Balsam poplar	1.4	0.5	4.5	3.9	70.0	2.4	171.5	152.7
Willow	1.7	0.2	4.6	3.2	238.2	0.3	408.7	181.8
White spruce	1.3	0.4	5.0	3.4	250.0	0.8	511.0	119.9
Cedar	1.4	0.8	3.1	1.2	88.1	1.8	23.2	7.9

- Mycorrhizal richness and abundance distribution

Richness

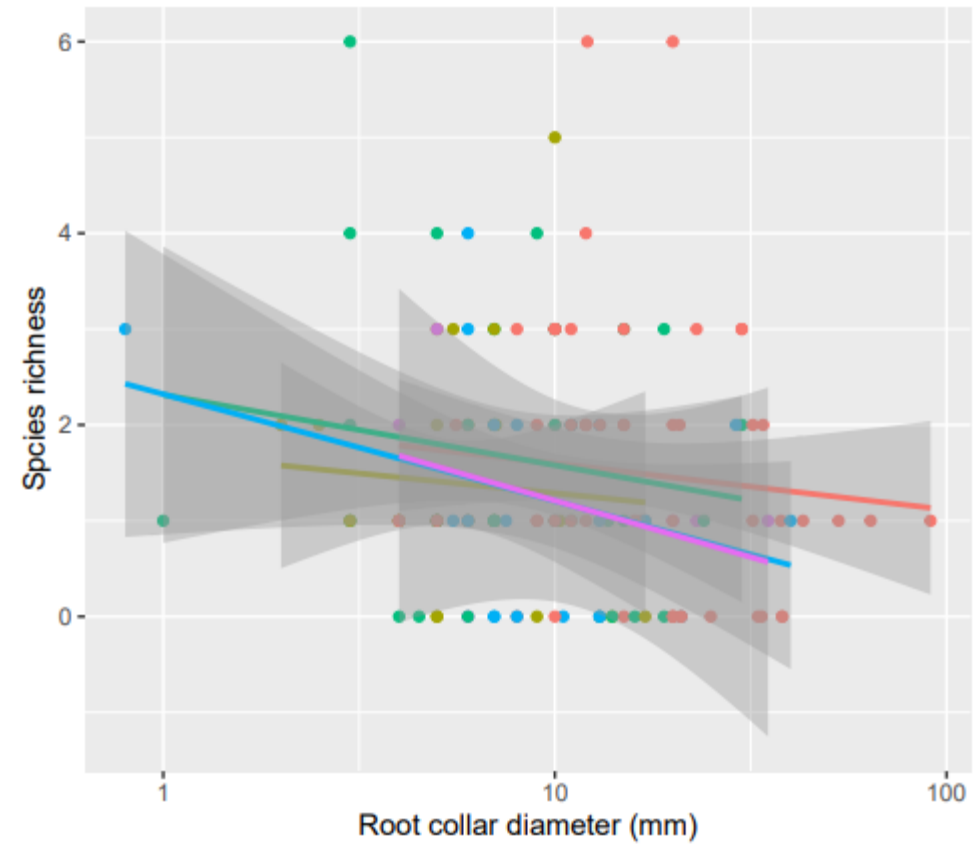


Abundance

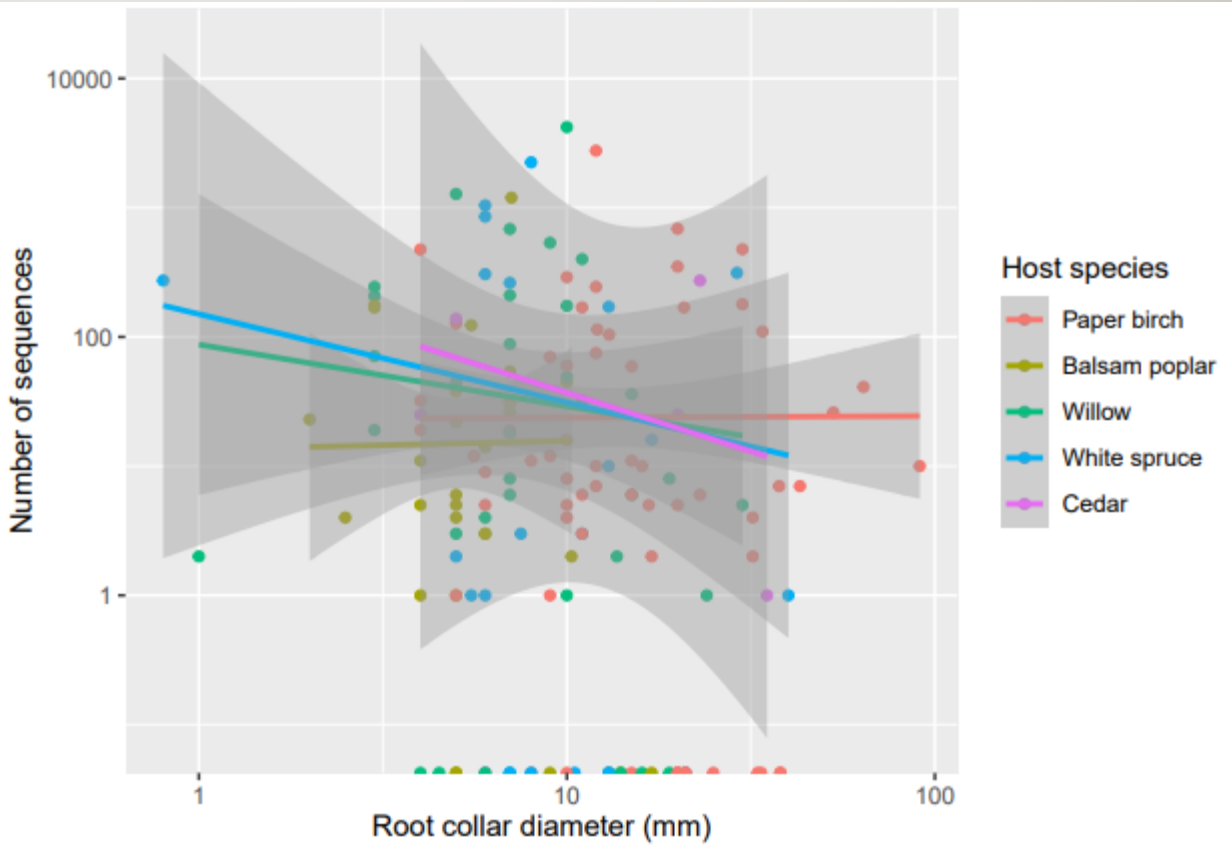


2. Does fungal richness and abundance change with the species identity and size of the host?

ECM richness vs. root collar diameter



ECM abundance vs. root collar diameter

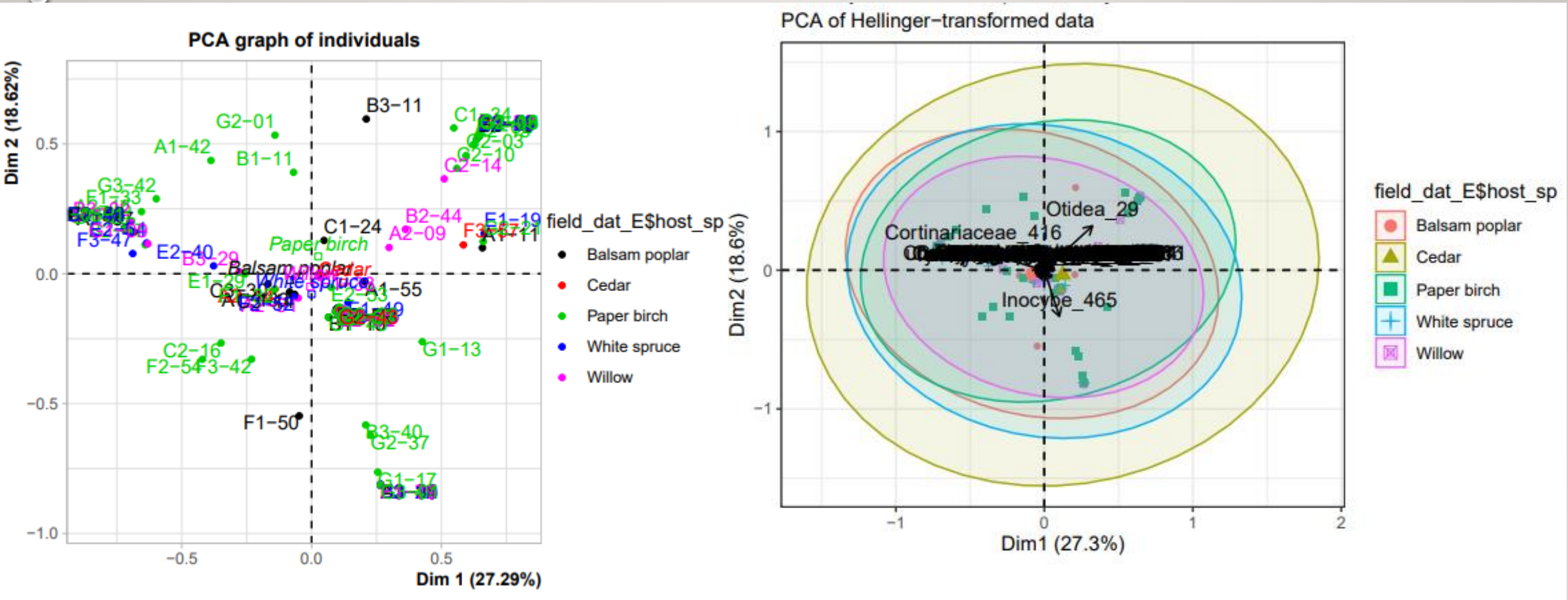


- ECM richness decreases with increasing root collar diameter for all five species.
- ECM abundance is slightly increased with increasing root collar diameter for Paper birch and Balsam poplar species.

Fungal Community Analysis….

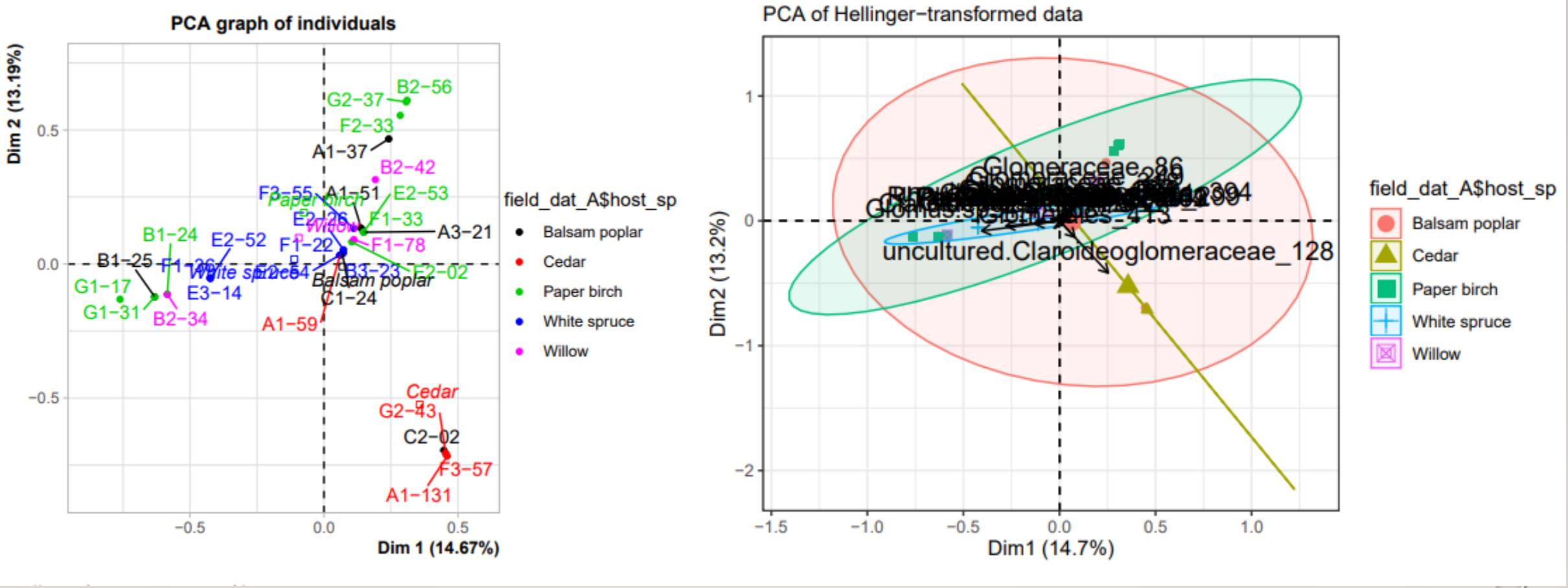
3. How does mycorrhizal fungi community composition vary across host species?

Ordination for ECM - 124 trees, 52 OTUs



- No distinct clusters observed for plant species- ECM are shared between plant species.
- Ellipses are not distinct- ECM are not host-specific (PERMANOVA $R^2 = 0.06$).

Ordination for ABS 30 trees, 20 OTUs



A distinct cluster observed for Cedar plants – A ABS dependent species

Indicator Species Analysis…

Predicting the diversity of other species or communities within an area…

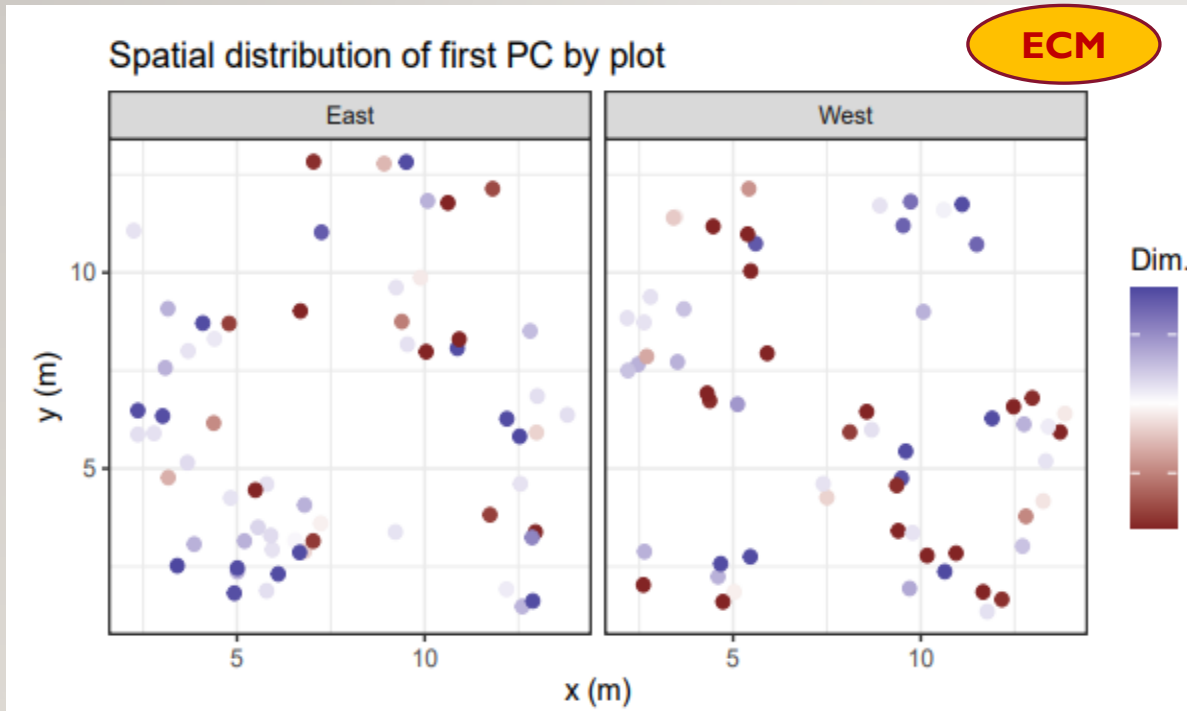
Mycorrhizae species	Guild	Associated host plant group	Stat	P value
Inocybe.umbrinella_186	ECM	Cedar	0.361	0.024
uncultured.Piloderma_258	ECM	Cedar	0.308	0.048
uncultured.Claroideoglomeraceae_128	ABS	Cedar	0.758	0.005

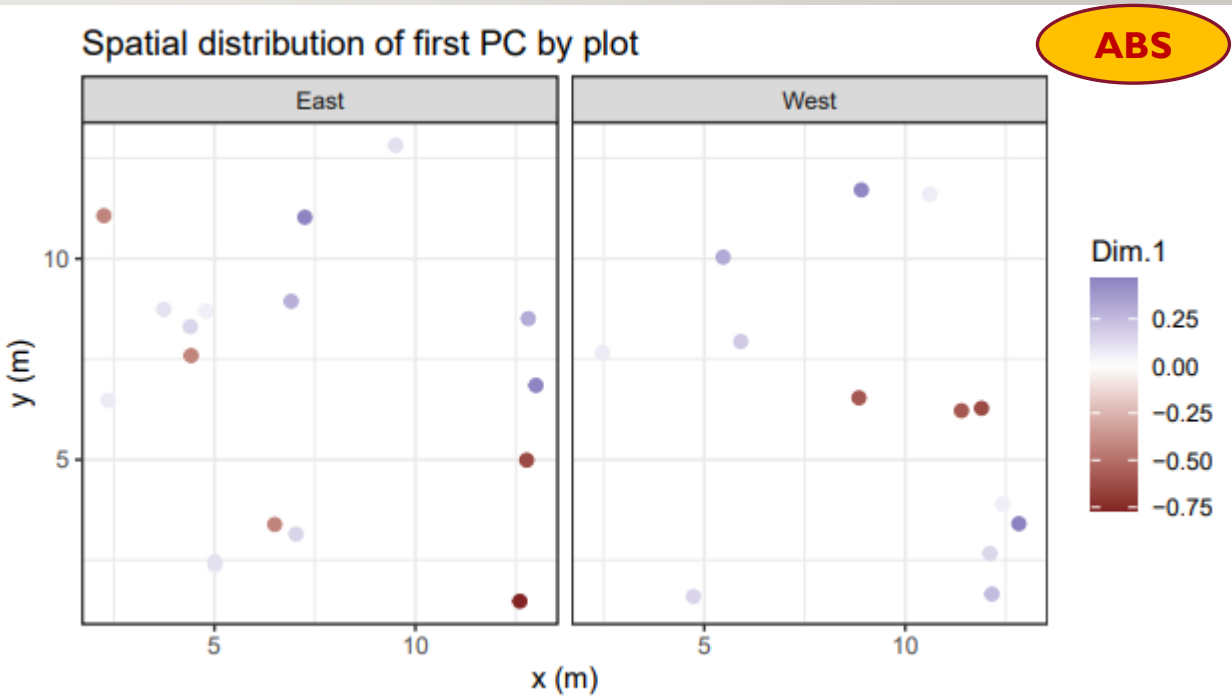
4. Does the fungal composition vary between plots and spatially within each a plot?

❖ Between plots- PERMANOVA test…

- PERMANOVA test reveals that there is no significance difference between fungal composition with plots (east and west plot) for both ECM and ABS.
- Only species for ABS is significant.

❖ Within each a plot- Mantel test…





- Similar values seem to be spatially grouped.
- There is a correlation between distance and community similarity with a Mantel test.

Co-occurrence Analysis…

Summary

- The distribution of richness is similar between host species.
- When plants getting bigger and older, ECM richness tends to be decreased.
- ECM are not host-specific. That means they are shared between host plant species.

ACKNOWLEDGEMENT

- **Philippe Marchand**, Forest Recharch Institute, University of Quebec in Abitibi-Témiscamingue
- **Nicole Fenton**, Forest Recharch Institute, University of Quebec in Abitibi-Témiscamingue
- **Melanie Roy**, Evolution and Biological Diversity laboratory
- **Sophie Manzi**, Evolution and Biological Diversity laboratory
- ***Benoit Lafleur,*** Forest Recharch Institute, University of Quebec in Abitibi-Témiscamingue
- **All the field assistants**
- **Bryology lab members**

CRSNG NSERC

Fonds de recherche Nature et technologies Québec

Hecla QUÉBEC

NEMASKA LITHIUM

Acknowledgement

Grand Council of the Crees (Eeyou Istchee)
Grand Conseil des Cris (Eeyou Istchee)

Cree Nation Government
Gouvernement de la Nation Crie

stornoway

PIKOGAN

MINE CANADIAN MALARTIC

AGNICO EAGLE

OURANOS

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