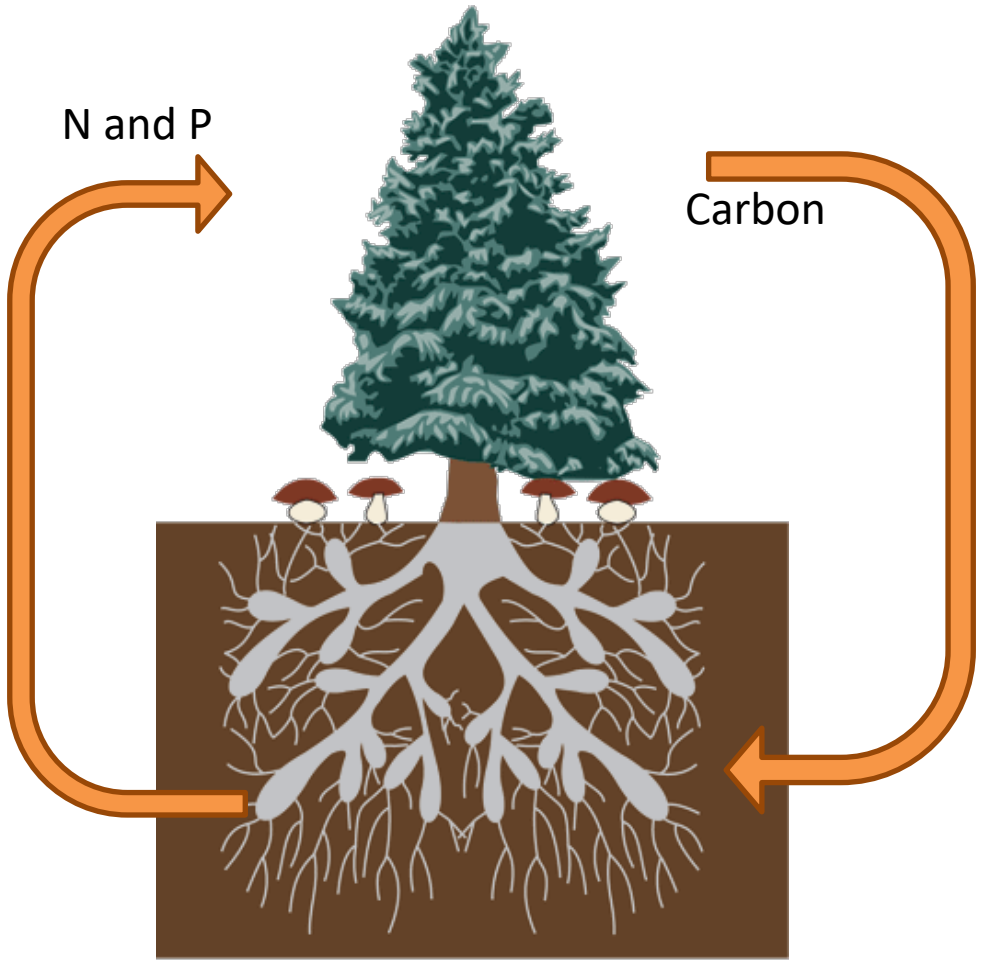
Investigating fungal communities in Icelandic soils

Christine Palmer, PhD
Fulbright-NSF Arctic Research Scholar
Associate Professor of Biology
Castleton University, Vermont USA

Mycorrhizal fungi



Mycorrhiza

"fungus" "root"

~90% of plant species

Ashley Lang

Mycorrhizal fungi

Arbuscular endomycorrhizal (AM)

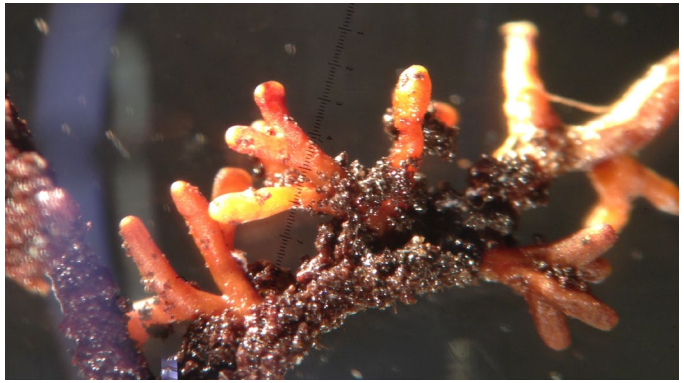
Grow INTO root cell, obligate

Tropical/temperate

~80% of all plants

Acer
Juniperus

Ectomycorrhizal (ECM)



Grow ON root cell

Temperate/boreal/arctic

~3% plants: trees/shrubs

Populus
Sorbus
Salix

Pinus
Picea
Abies
Larix

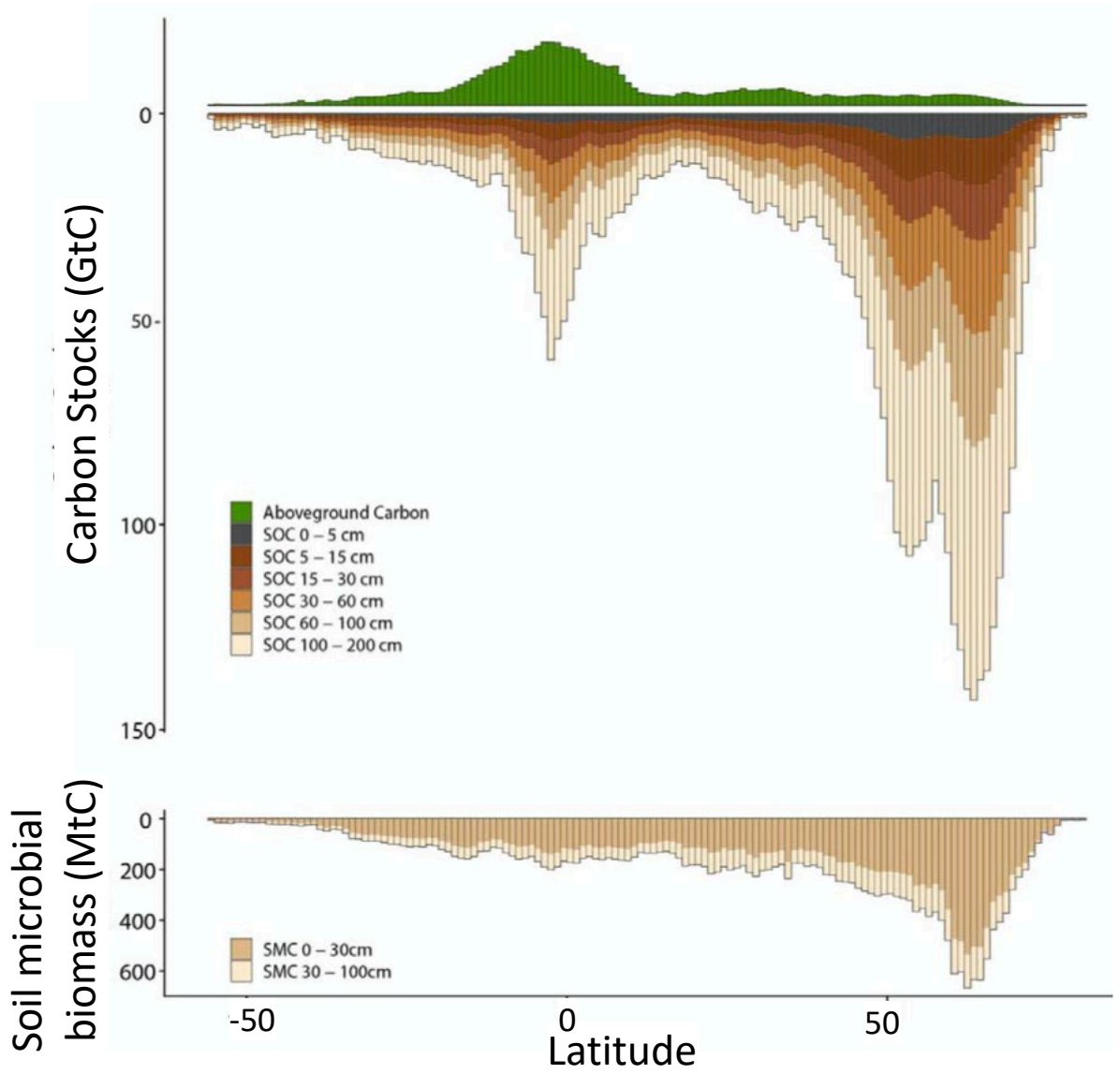
Betula
Fagus
Quercus

Mark Brundrett

Mycorrhizal fungi: importance

- Enhanced supply of nutrients
  - Nitrogen (ECM)
  - Phosphorus (AM/ECM)
  - Carbon (ECM)
  - K, Ca, Cu, Zn, Fe (AM/ECM)
- Water uptake/drought tolerance (AM/ECM)
- Protection against pathogens/predation (AM/ECM)
- Carbon storage (ECM)

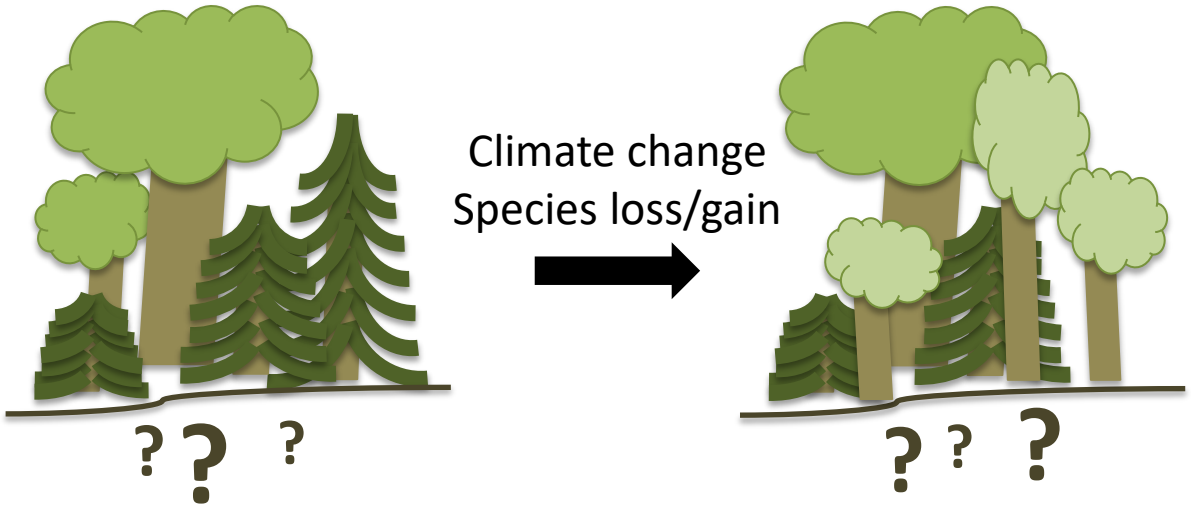
Mycorrhizal fungi: importance



Crowther et al., Science 365, eaav0550 (2019)

Mycorrhizal fungi: study systems

New England/VT/NH

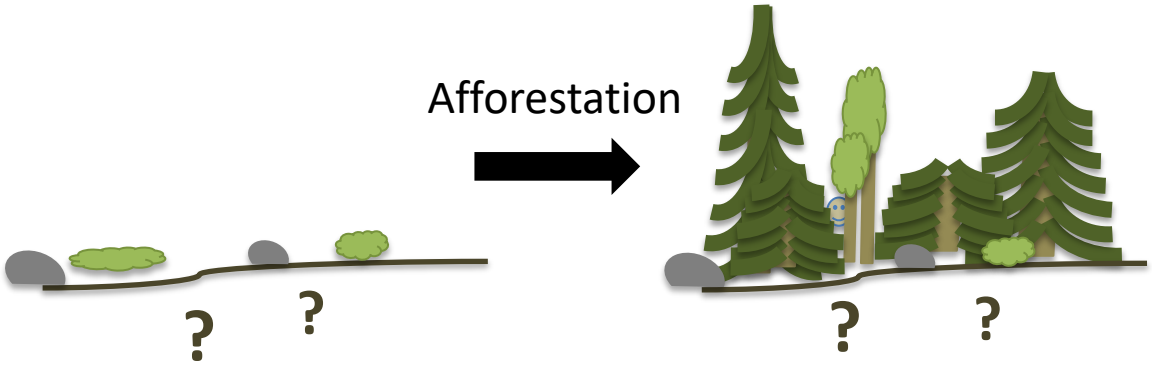


Community composition

Cryptic species ID

Morphotype/molecular ID species guide

Iceland



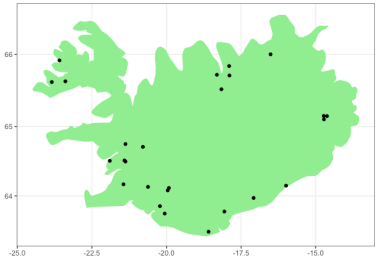
ECM dynamics in "primary succession"

Community composition

Early vs late stage (colonize vs sustain)

Previous Tools: morphotype/colonization, baiting/culturing, RFLP, Sanger sequencing

1) Collect soil samples



4) Sequence

2) Isolate DNA

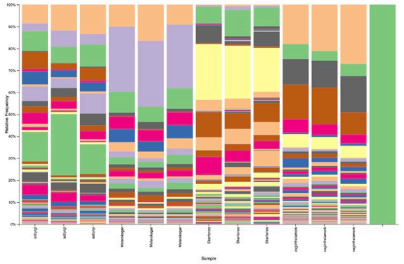
5) Bioinformatics/sequence analysis

```
qiime dada2 denoise-paired\
   --i-demultiplexed-seqs demuxtrimcore.qza\
   --p-trim-left-f 0 --p-trim-left-r 0\
   --p-trunc-len-f 0 --p-trunc-len-r 0\
   --p-n-threads 18\
   --o-denoising-stats dnstrimITS\
   --o-table tabletrimITS\
   --o-representative-sequences rep-seqstrimITS
```

3) Amplify “barcodes”

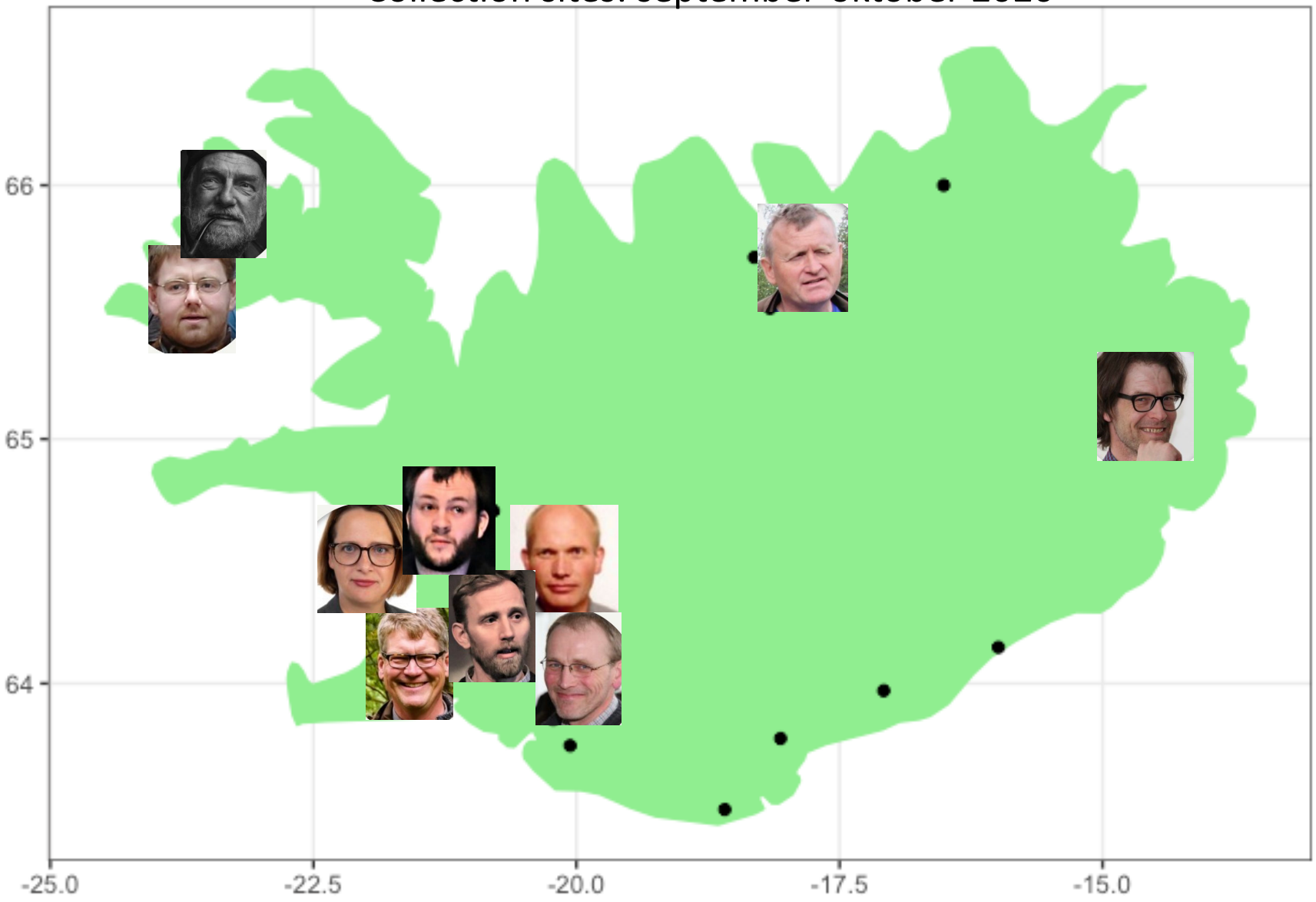
6) Community composition

(taxonomic + functional)



Collect soil samples

Collection sites: september-október 2020



Forest types:

B. pubescens (downy birch)
P. contorta (lodgepole pine)
P. sitchensis (Sitka spruce)
P. trichocarpa (black cottonwood)
L. sibirica (Siberian larch)
+mixtures

Forest age:

Young vs “old”

Habitat type:

Eroded/ash vs. vegetated/forested
(including gradients)
± lupine
(barren sites only)

Planting conditions:

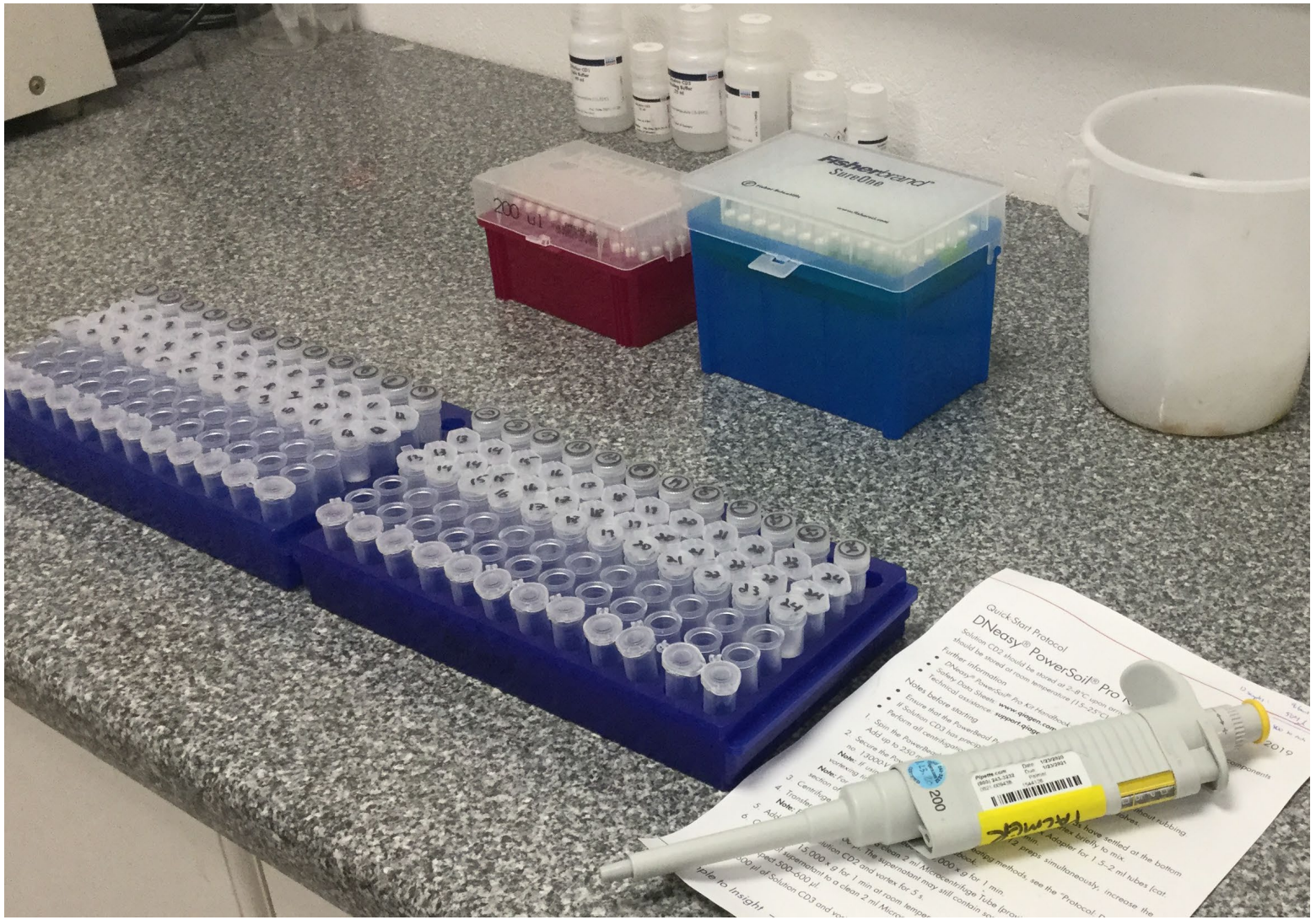
± forest soil/inoculant
(~30yr plot w/Úlfúr)
± fertilizer
(Mosfellsheiði site)

Isolate DNA from soil samples

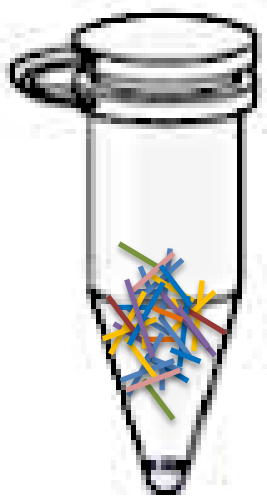
Break cells open with physical and chemical forces

Bind DNA to membrane and wash to remove impurities

Extract purified DNA



Amplify “barcodes”



BARCODE:

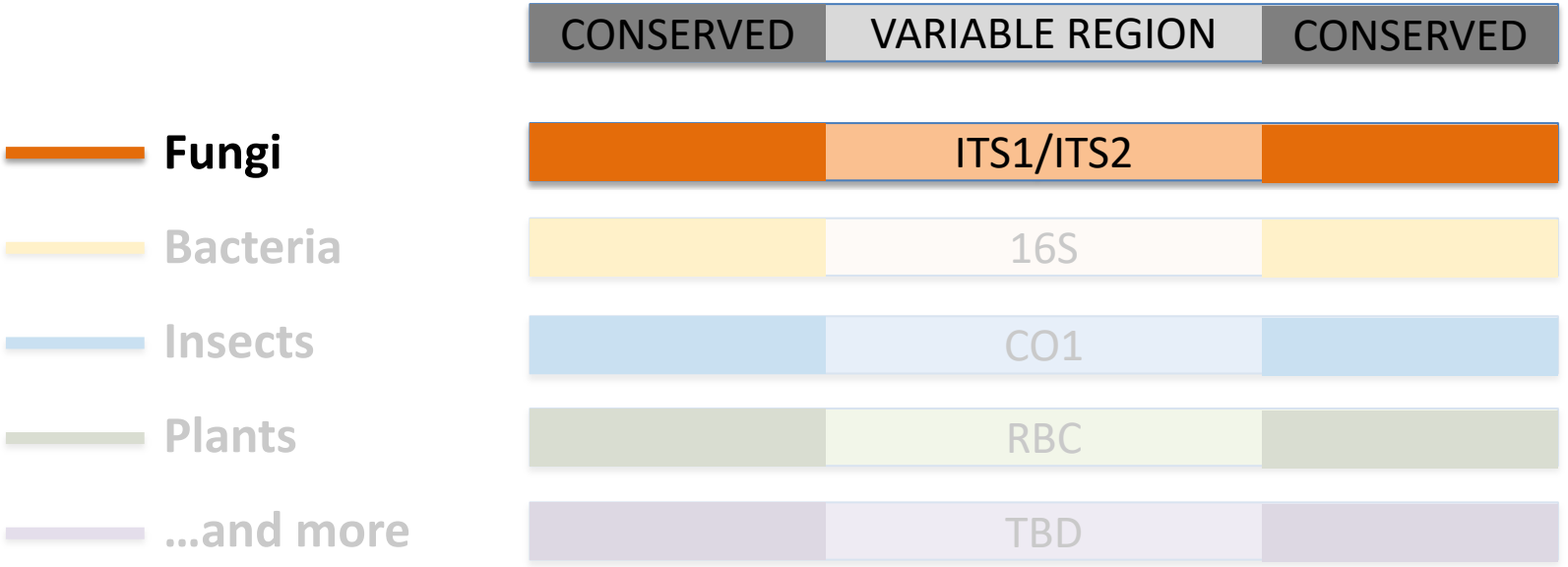
specific region of DNA used to distinguish species

-short (~100-500bp)

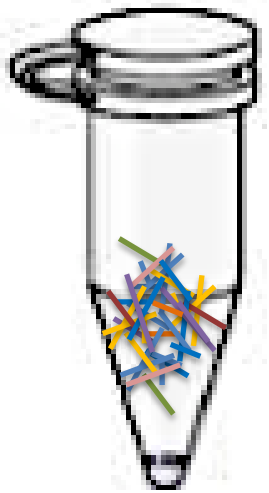
-different region for fungi vs bacteria vs plants vs animals

-often found in multiple copies (chloroplast, mitochondria, ribosomal DNA)

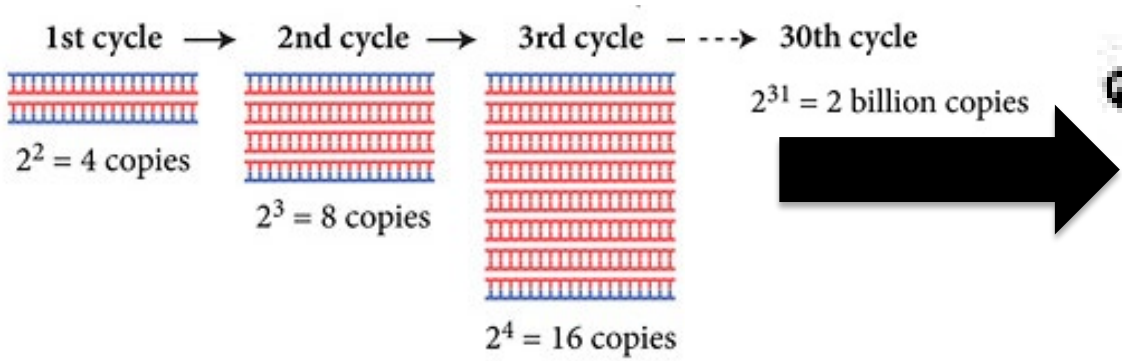
-widely used in research communities

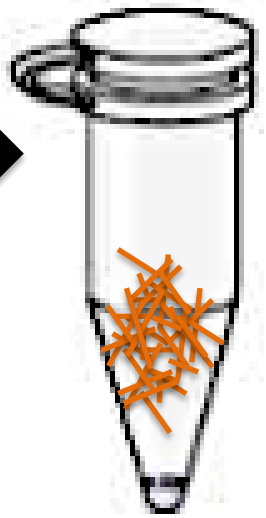


Amplify “barcodes”: PCR



+ ITS1/ITS2 primers (fungi)
+ other PCR reagents

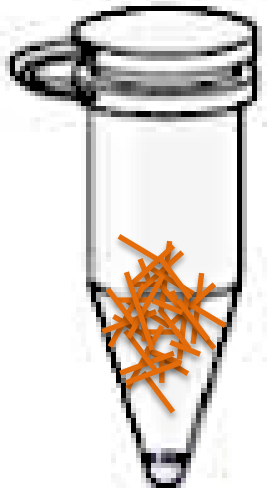




All DNA regions
All organisms

Barcode only
Fungi only

Sequence DNA



Barcode only
Fungi only

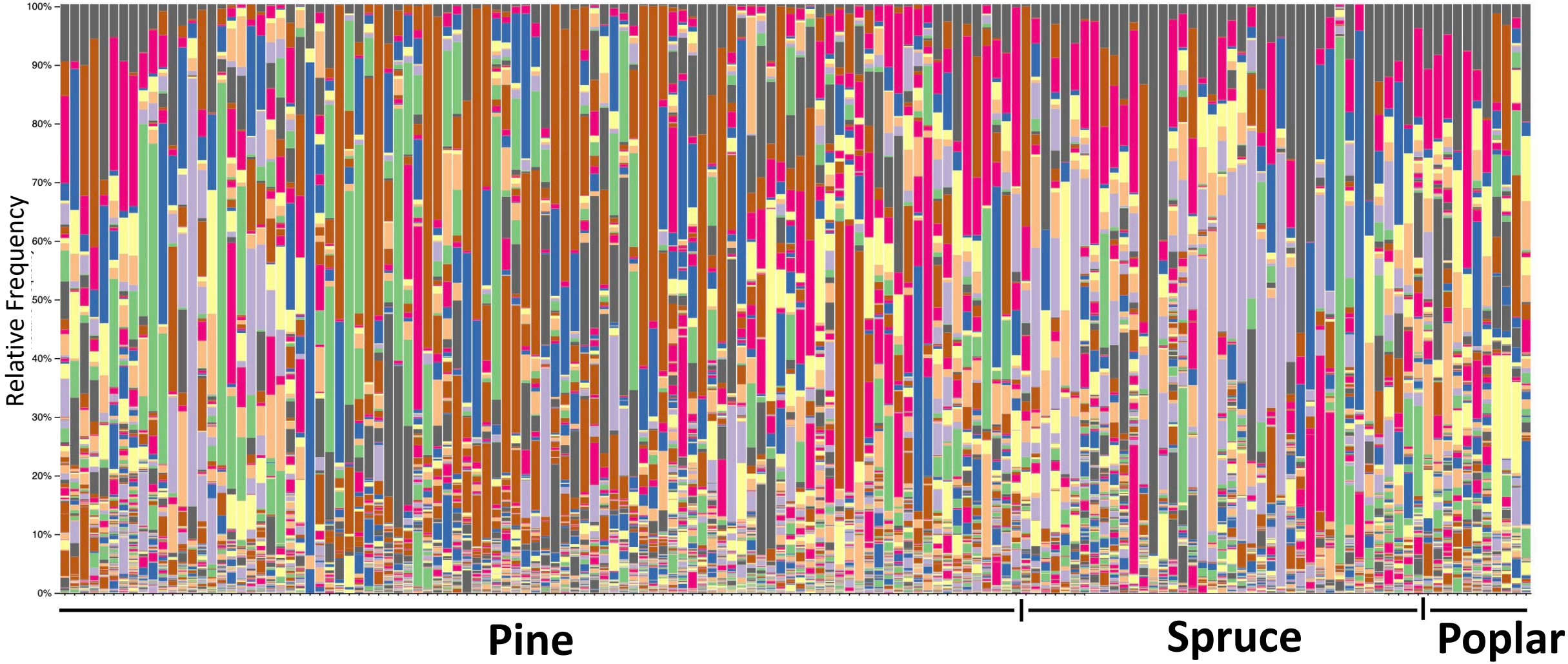
DNA sequence reads

Bioinformatics/sequence analysis

ATTCATGACATAGACATCCATAGATAAGCATAACGATACTATAACGATGACGATGACGTAGACGCATCAGTAACGATGATGAGGAGTATACAGATGATTTAGGACAGAGAGCATAGACGATGACGATGACGATGACGATGTAAACGATGATGAGGAGTATACAGATGATTTAGGACAGAGAGCATAGACGATGACGATGACGATGACGATGTA

```
###import reference dataset for assigning taxonomy (used UNITE, downloaded tar.gz from site)
#unzip and untar files
gunzip -c sh_qiime_release_04.02.2020.tar.gz | tar xvf -

#import files
qiime tools import \
  --type FeatureData[Sequence]   \
  --output-path UNITE_dynamic_2020.02_seqs.qza   \
  --input-path  sh_qiime_release_04.02.2020/sh_refs_qiime_ver8_dynamic_04.02.2020.fasta

  qiime tools import   \
    --type FeatureData[Taxonomy]   \
    --input-format HeaderlessTSVTaxonomyFormat \
    --output-path UNITE_dynamic_2020.02_tax.qza  \
    --input-path  sh_qiime_release_04.02.2020/sh_taxonomy_qiime_ver8_dynamic_04.02.2020.txt

###train the classifier (this is a slow step)
qiime feature-classifier fit-classifier-naive-bayes\
   --i-reference-reads UNITE_dynamic_2020.02_seqs.qza\
   --i-reference-taxonomy UNITE_dynamic_2020.02_tax.qza\
   --o-classifier UNITE_dynamic_2020.02_classifier.qza
##################################classifying################


##copy classifier from previously made folder
cp ~/HB_ITS/ITStest_202001/UNITE_dynamic_2020.02_classifier.qza .

###taxonomic assignment using UNITE
  qiime feature-classifier classify-sklearn\
   --i-classifier UNITE_dynamic_2020.02_classifier.qza\
   --i-reads rep-seqstrimITS.qza\
   --p-n-jobs 18\
   --o-classification taxonomytrimITS.qza
```

Community composition: taxonomic diversity



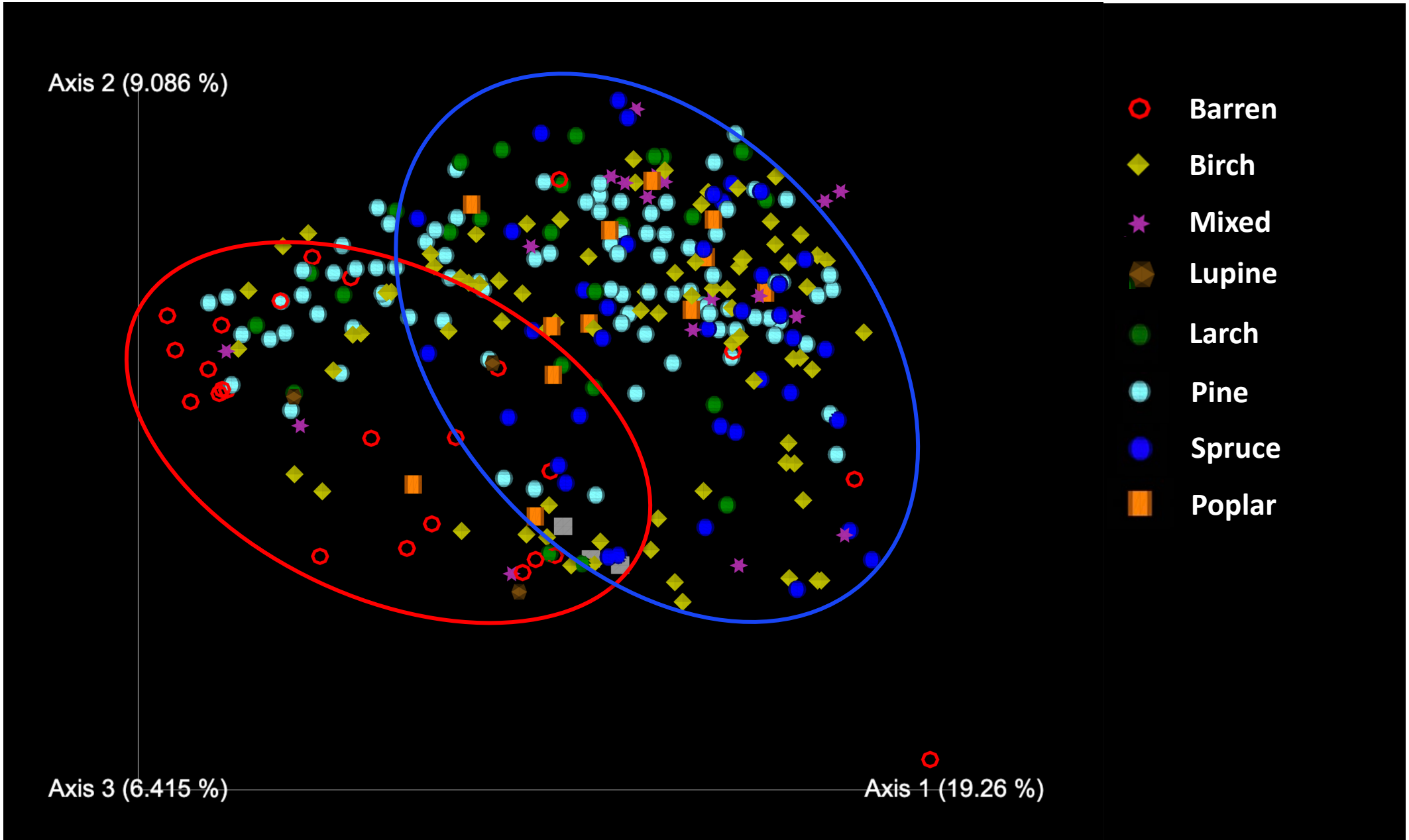
High taxonomic diversity across:

- Forest type
- Forest age (new, mid, established)
- Site

Some specificity:
between tree type and fungal species

Many mycorrhizal species represented

PCA: Analyzing multi-dimensional data

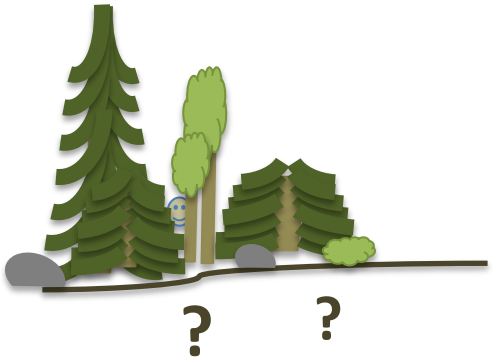


Ongoing work

Fungal composition:

Fungal diversity (# species, evenness, functional guilds)

Patterns across forest type, age of forest, treatments

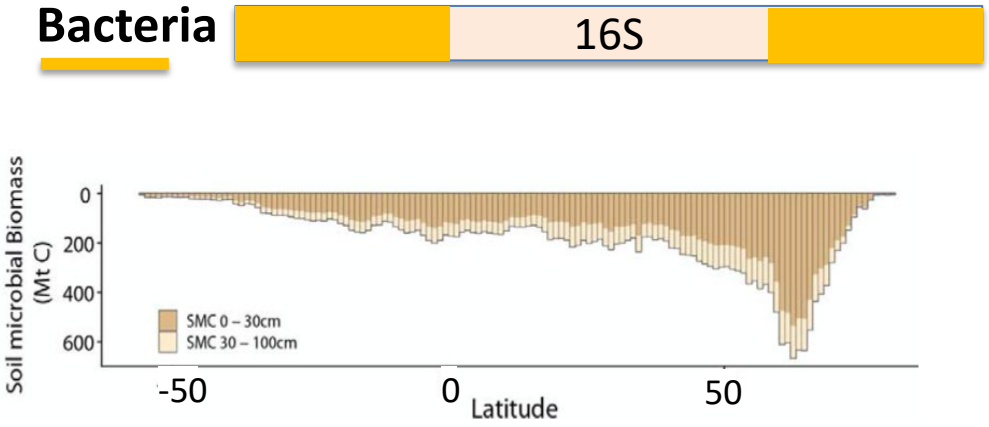


Microbial composition:

16S/microbial community composition

-Nitrogen cycling/fixation

-Carbon cycling



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…og margir fleiri!

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