Fine-scale partitioning among plant roots and soil fungi associated with changes in mycorrhizal dominance

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15/09/2021

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Institut de recherche en biologie végétale

UNIVERSITÀ DEGLI STUDI DI MILANO

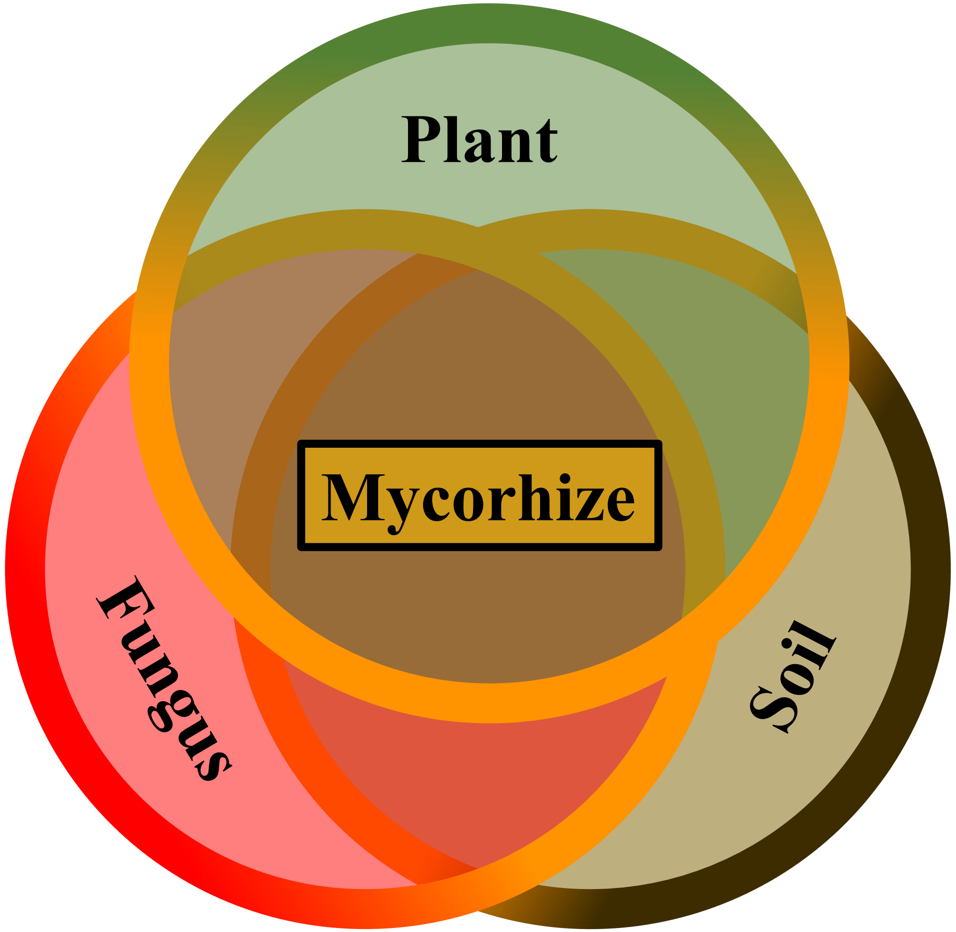
Outline of my endeavor into "microbial" ecology

- Introduction on my study system and the ecological research question

- Navigating the labyrinth of eDNA metabarcoding and its challenges
  - Challenge #1
  - Challenge #2
  - Challenge #3
  - Challenge #4
  - Challenge #...

- Results!

What about mycorrhizae?



D. Read

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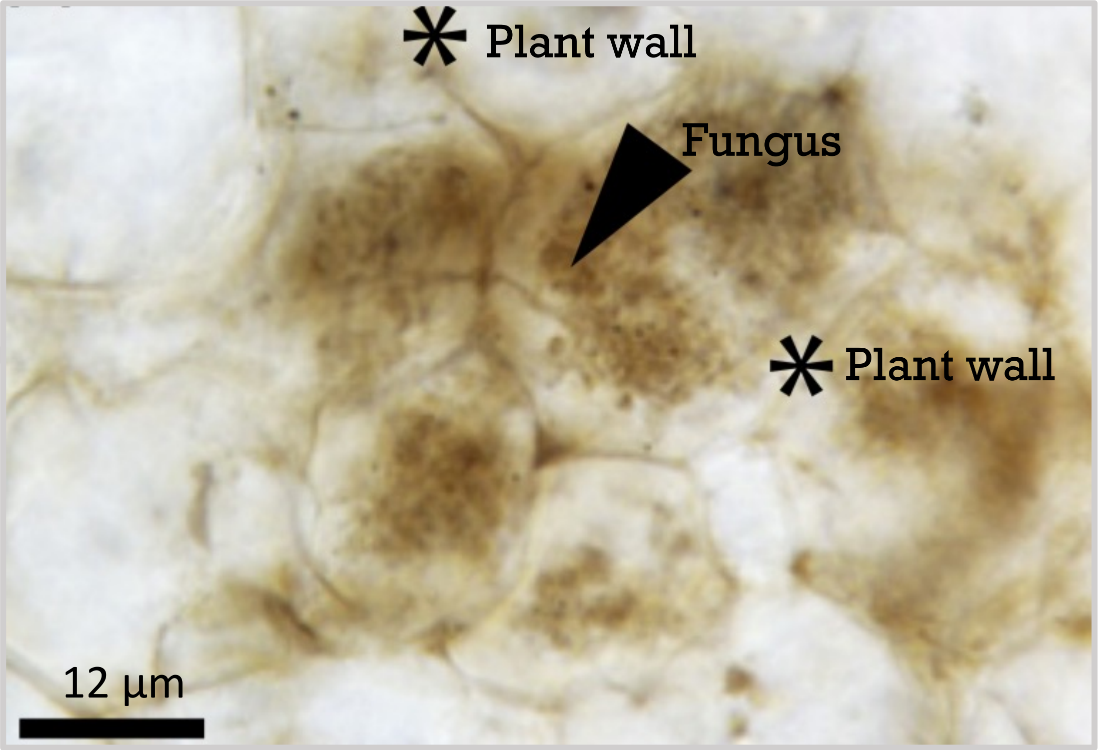

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Fossil of a 400 million year old mycorrhizal root (Taylor et al. 1995 *Mycologia*)

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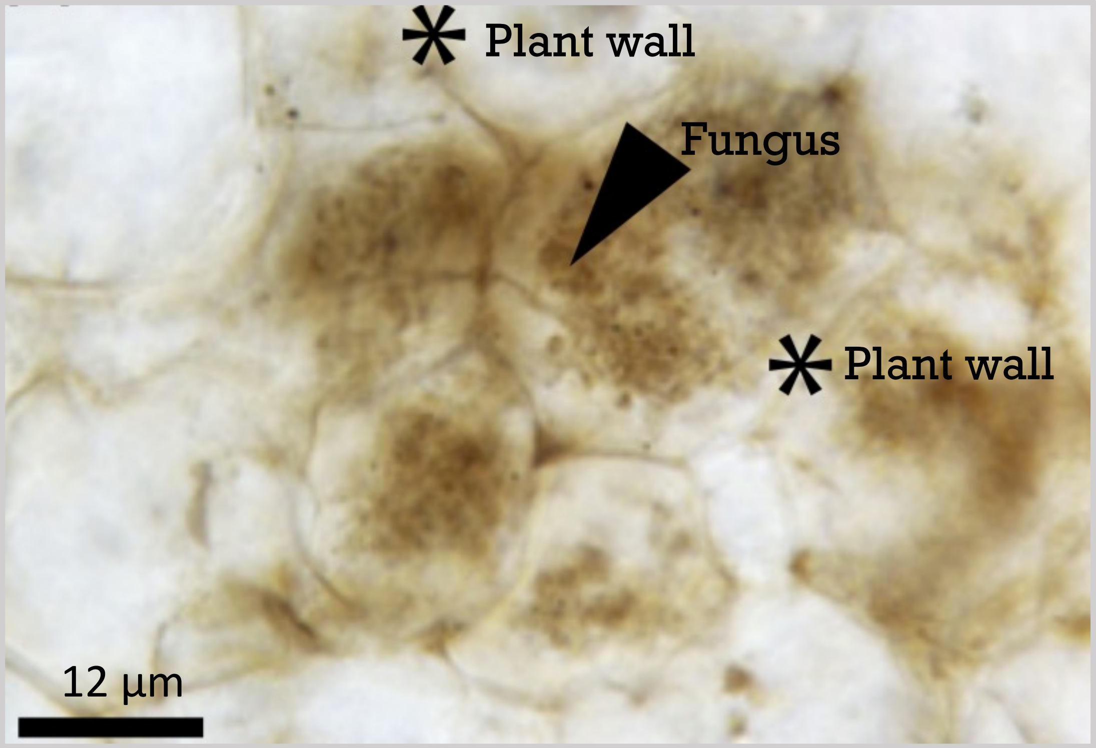

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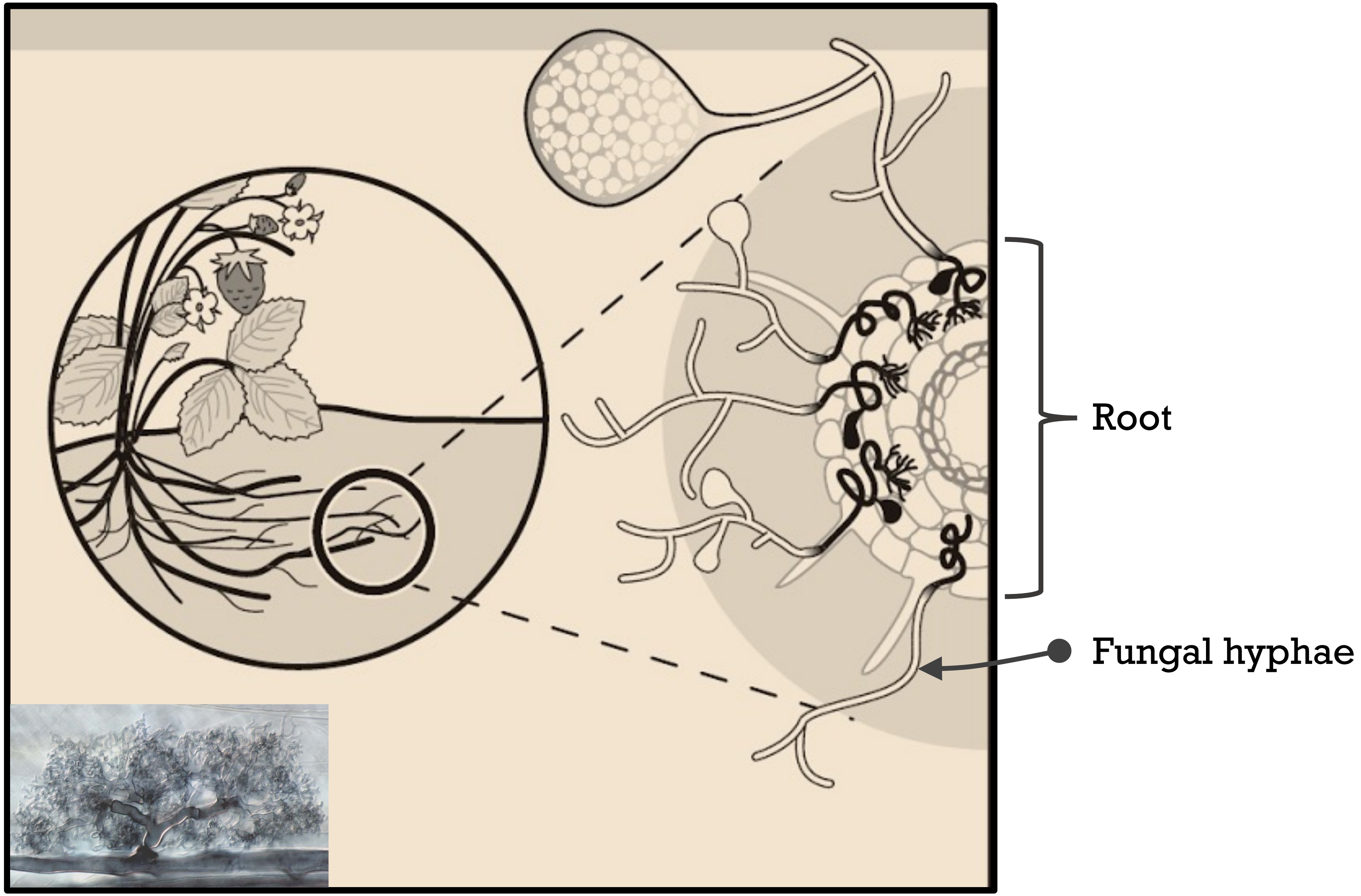
Fossil of a 400 million year old mycorrhizal root (Taylor et al. 1995 *Mycologia*)

« The symbiosis that made life on land. »

(Sélosse 2020, *Pour la Science*)

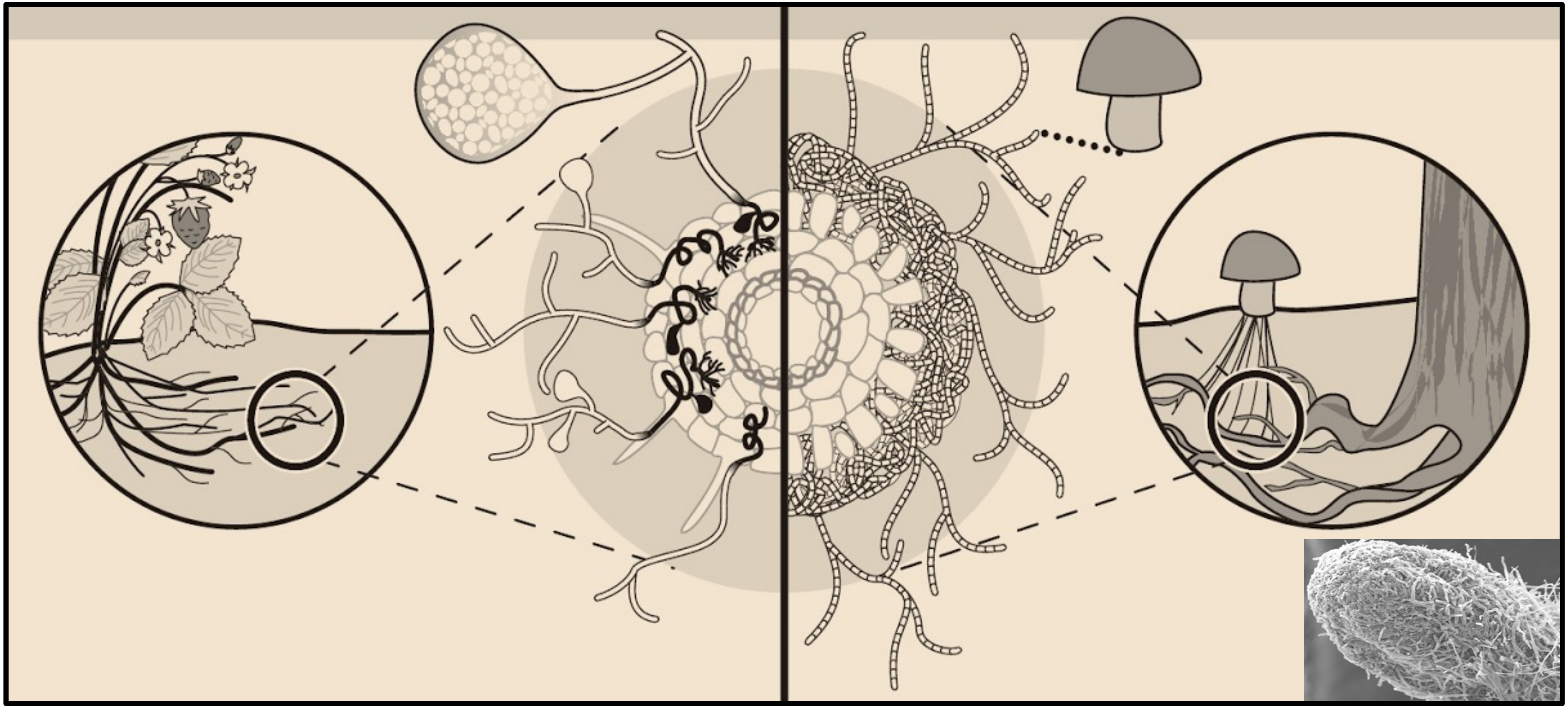
Arbuscular Mycorrhiza (AM)

Ectomycorrhiza (EcM)



Modified from Fortin et al. 2015, *Les mycorhizes: L'essor de la nouvelle révolution verte*

Arbuscular Mycorrhiza (AM) Ectomycorrhiza (EcM)



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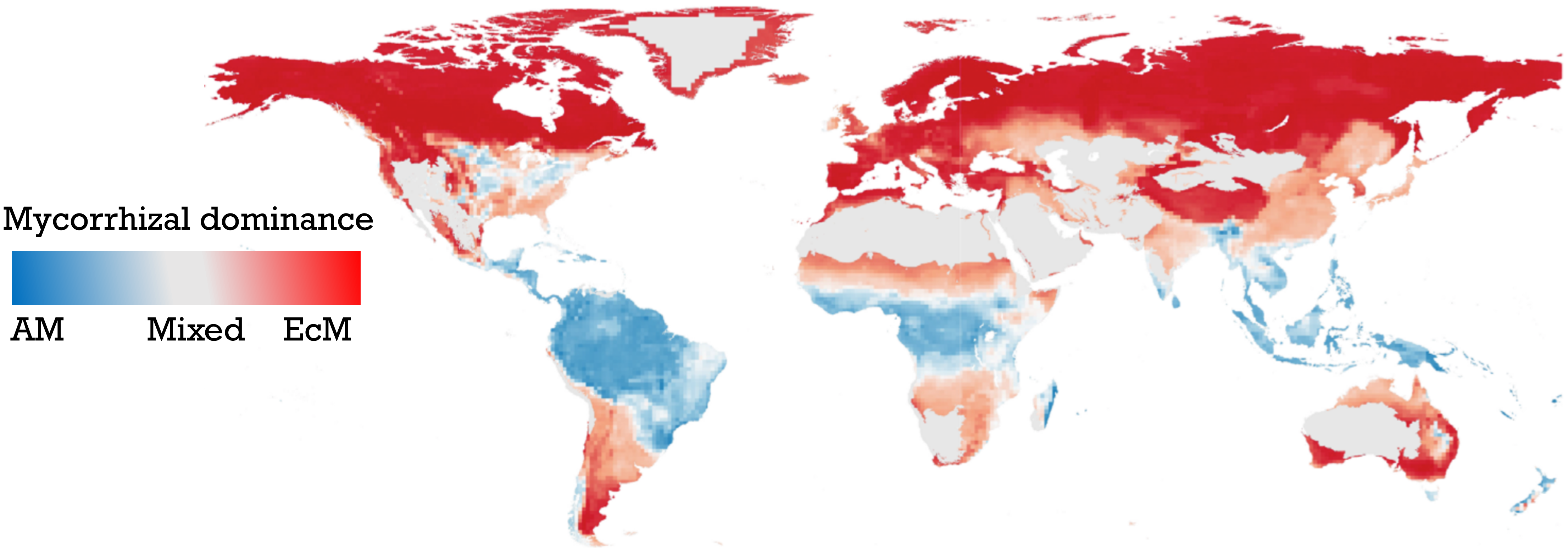
Mycorrhizal distribution

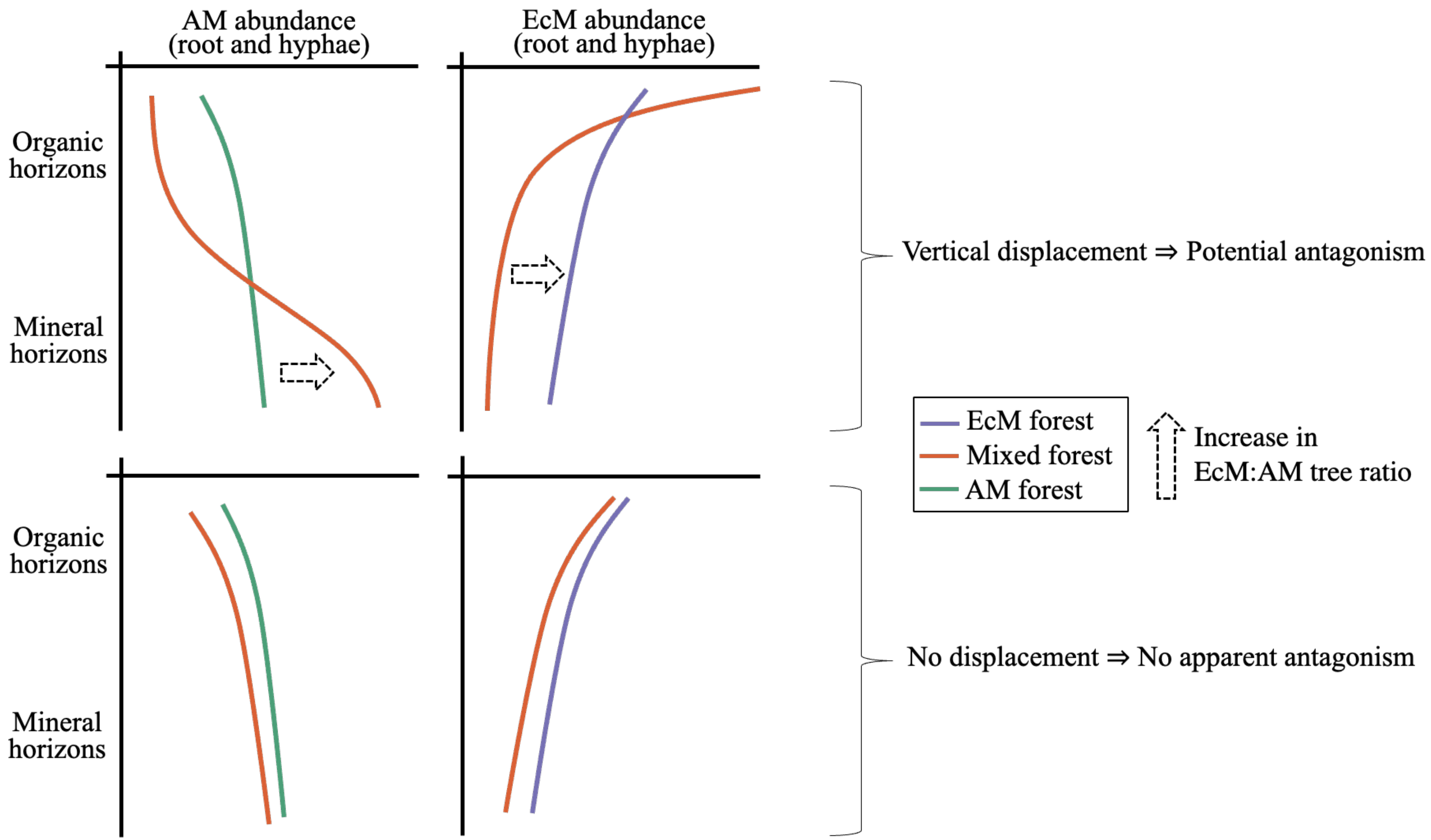


- From this distribution pattern it has been hypothesized that AM and EcM symbiosis have **antagonist relationships** (Smith & Read, 2008 *Mycorrhizal symbiosis*; Tedersoo et al. 2020 *Science*)

Modified from Crowther et al. 2019 *Science*

Main hypothesis to be tested

Antagonism between AM and EcM symbioses within the soil profile



Carteron et al. *In preparation*

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1. Mycorrhizal abundance can be divided into 3 individual components (Soudzilovskaia et al., 2017 *Biogeography of mycorrhizal symbiosis*):
   - The intensity of root colonization by fungal symbionts
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   - The abundance of fine roots of plant symbionts

Solution?

Navigating the labyrinth

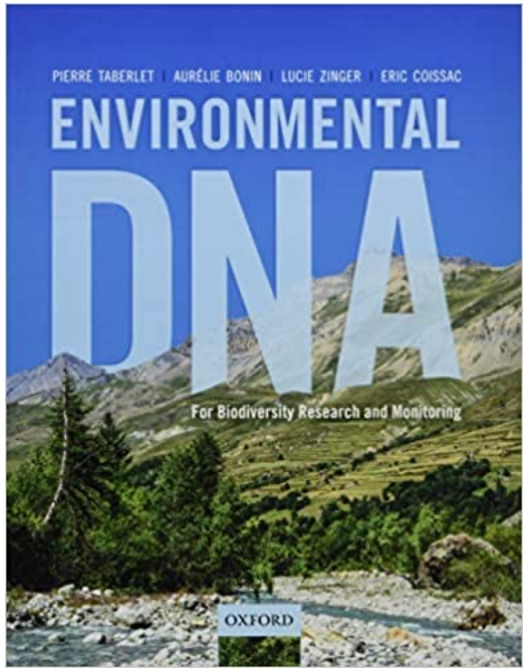
New Phytologist

Research

Methods

Navigating the labyrinth: a guide to sequence-based, community ecology of arbuscular mycorrhizal fungi

Miranda M. Hart[1], Kristin Aleklett[1], Pierre-Luc Chagnon[2], Cameron Egan[1], Stefano Ghignone[3], Thorunn Helgason[4], Ylva Lekberg[5], Maarja Öpik[6], Brian J. Pickles[1] and Lauren Waller[7]



Navigating the labyrinth

Challenge #1: Terminology

OTU?
MOTU?
ZOTU?
ASV?

Navigating the labyrinth

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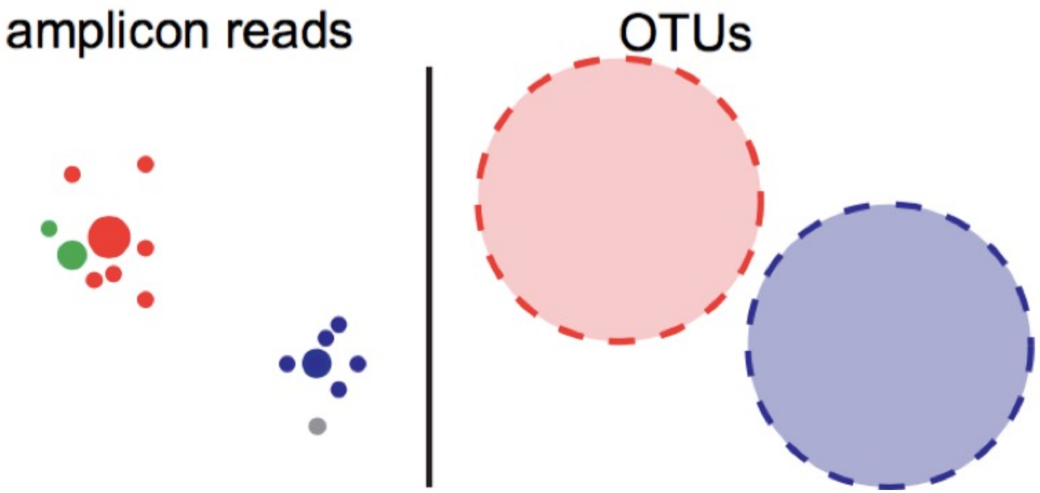
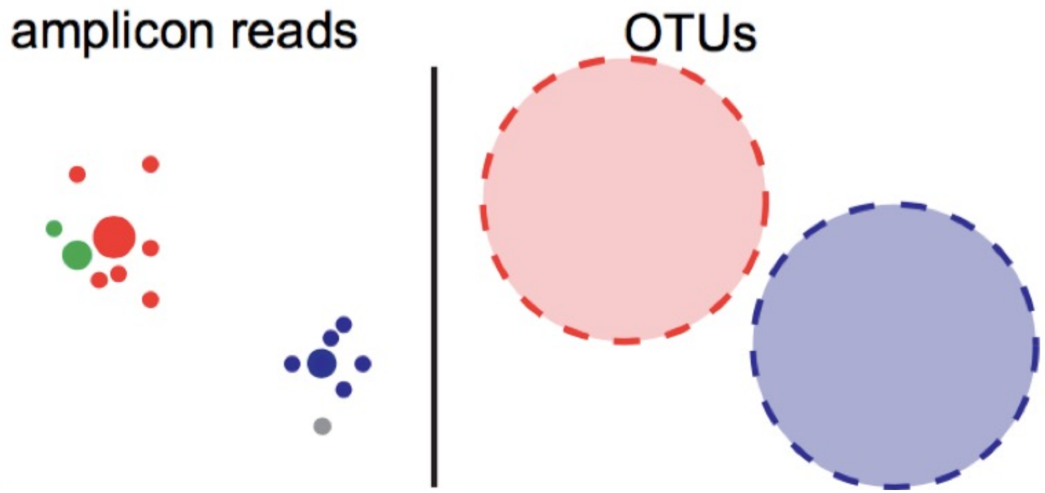


Navigating the labyrinth

Challenge #1: Terminology

OTU = Operational Taxonomic Units
MOTU = Molecular OTU
ZOTU = zero-radius OTU
ASV = Amplicon sequence variants
Oligotypes, ESV, etc.



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- Can corresponds to different approaches

Navigating the labyrinth

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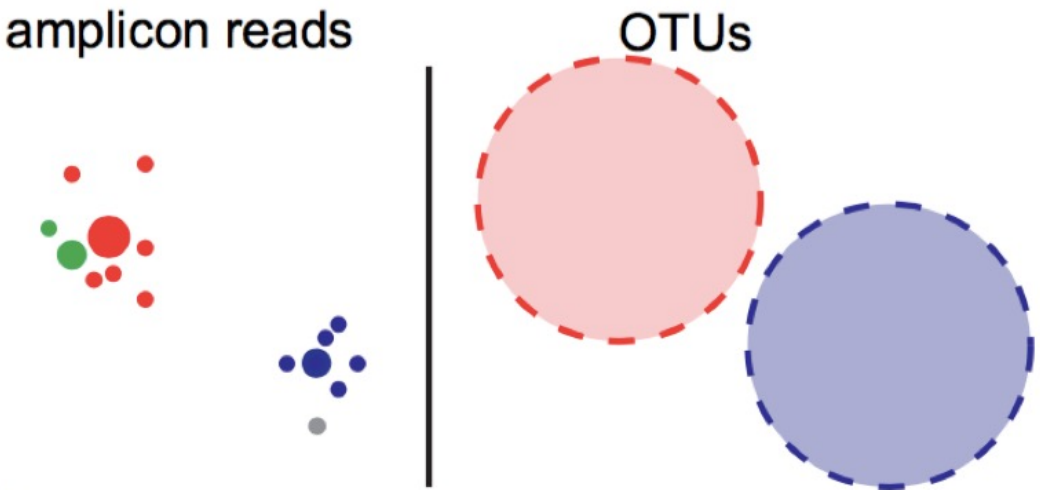

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Challenge #2: Which sequencing plateform?

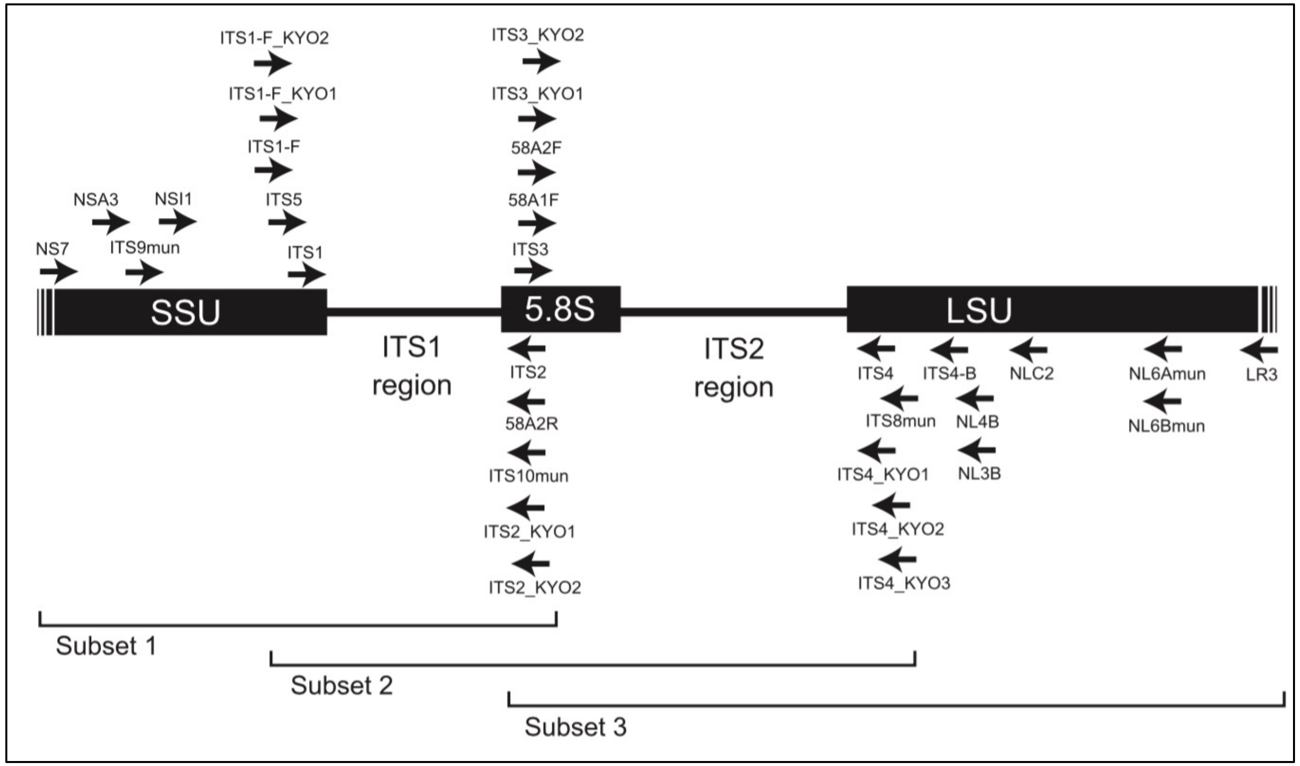
- Illumina MiSeq 2 x 300 bp

Challenge #3: Choice of primers/markers

<u>For general fungal amplification:</u>

→ ITS3_KYO2: GATGAAGAACGYAGYRAA
position 2029–2046

← ITS4: TCCTCCGCTTATTGATATGC
position 2390–2409



(Toju et al. 2012 Plos One)

Navigating the labyrinth

Challenge #3: Choice of primers/markers

For general fungal amplification:

→ ITS3_KYO2: GATGAAGAACGYAGYRAA
position 2029–2046

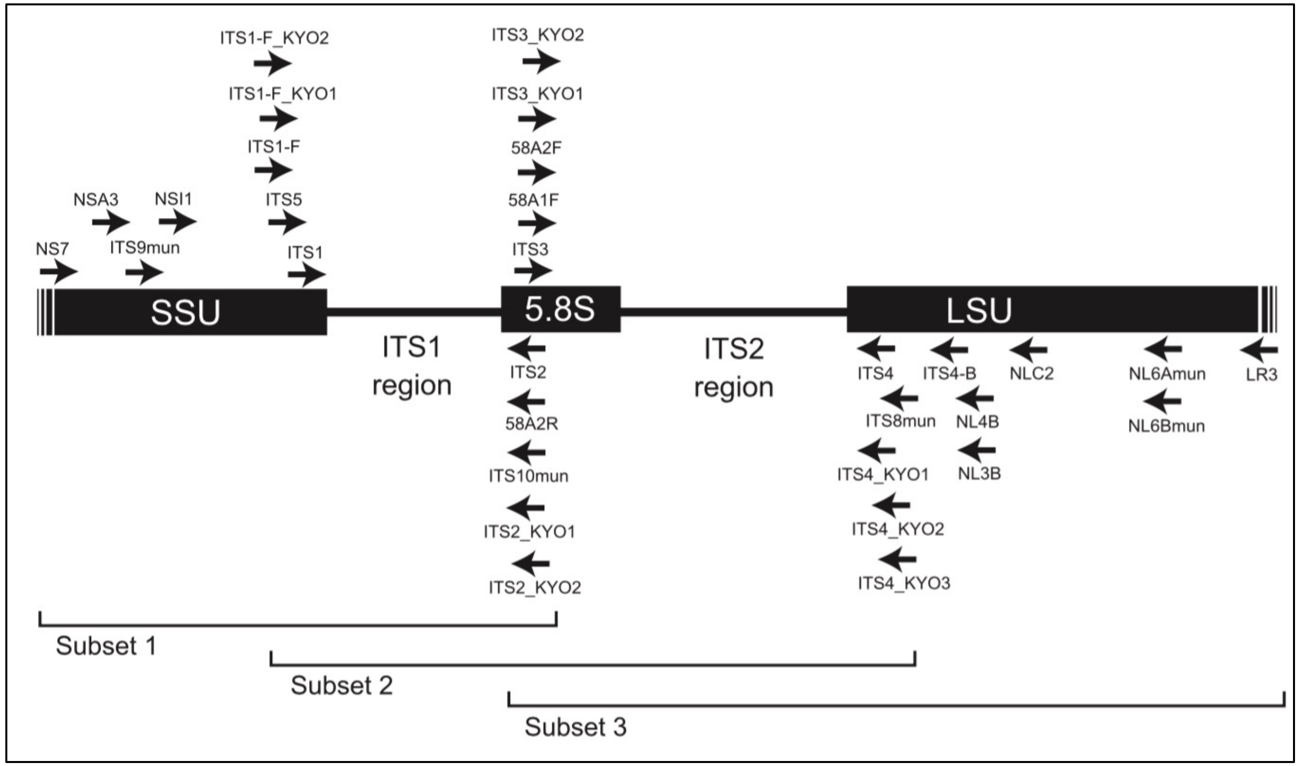
← ITS4: TCCTCCGCTTATTGATATGC
position 2390–2409

For Glomeromycetes:

LSU: nested PCR with SSUmAf-LSUmAr
then LSUD2f-CS1-LSUmBr-CS2

For plants:

Large subunit of RuBisCO: rbcLa_f-rbcLa_r



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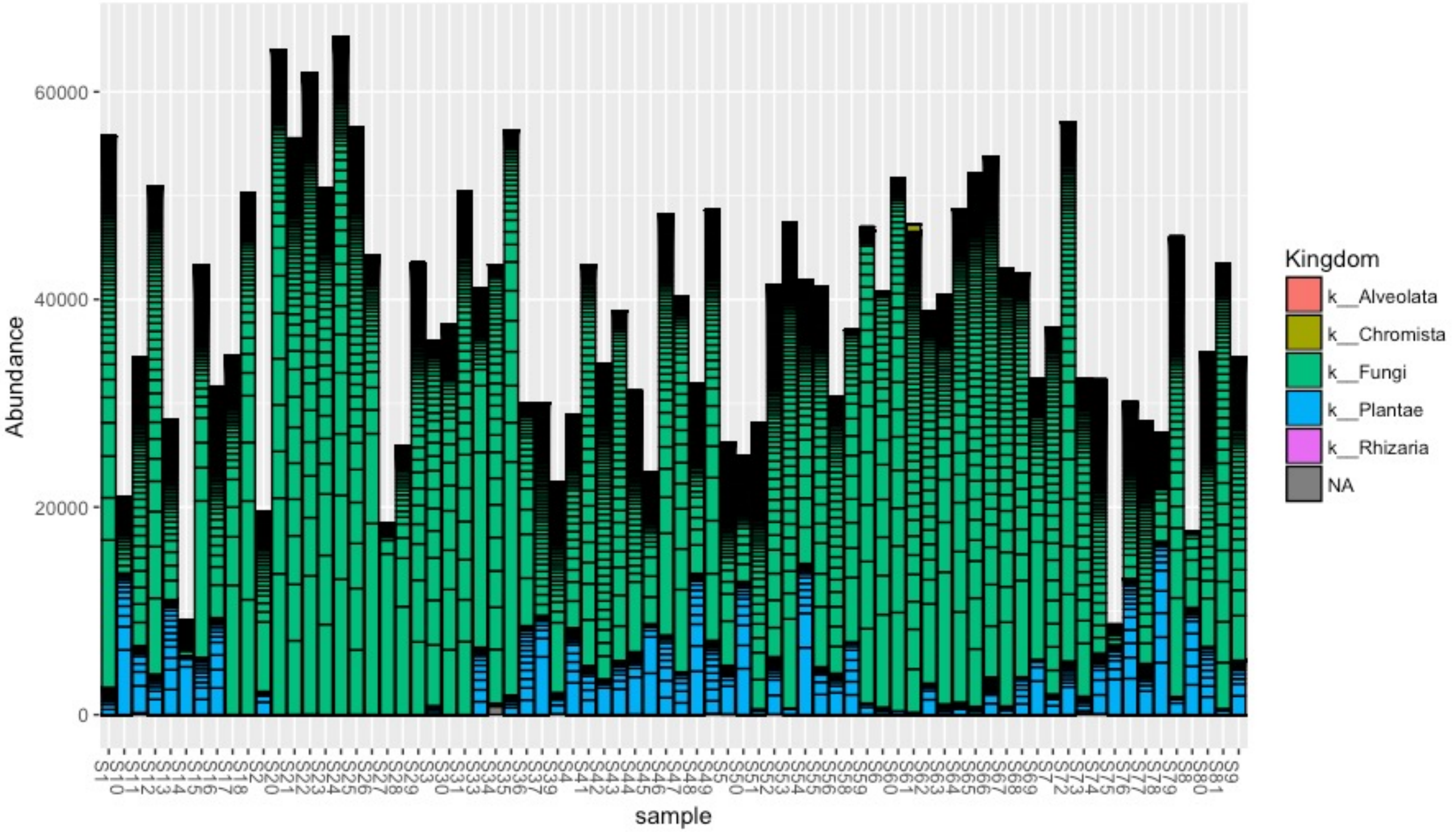
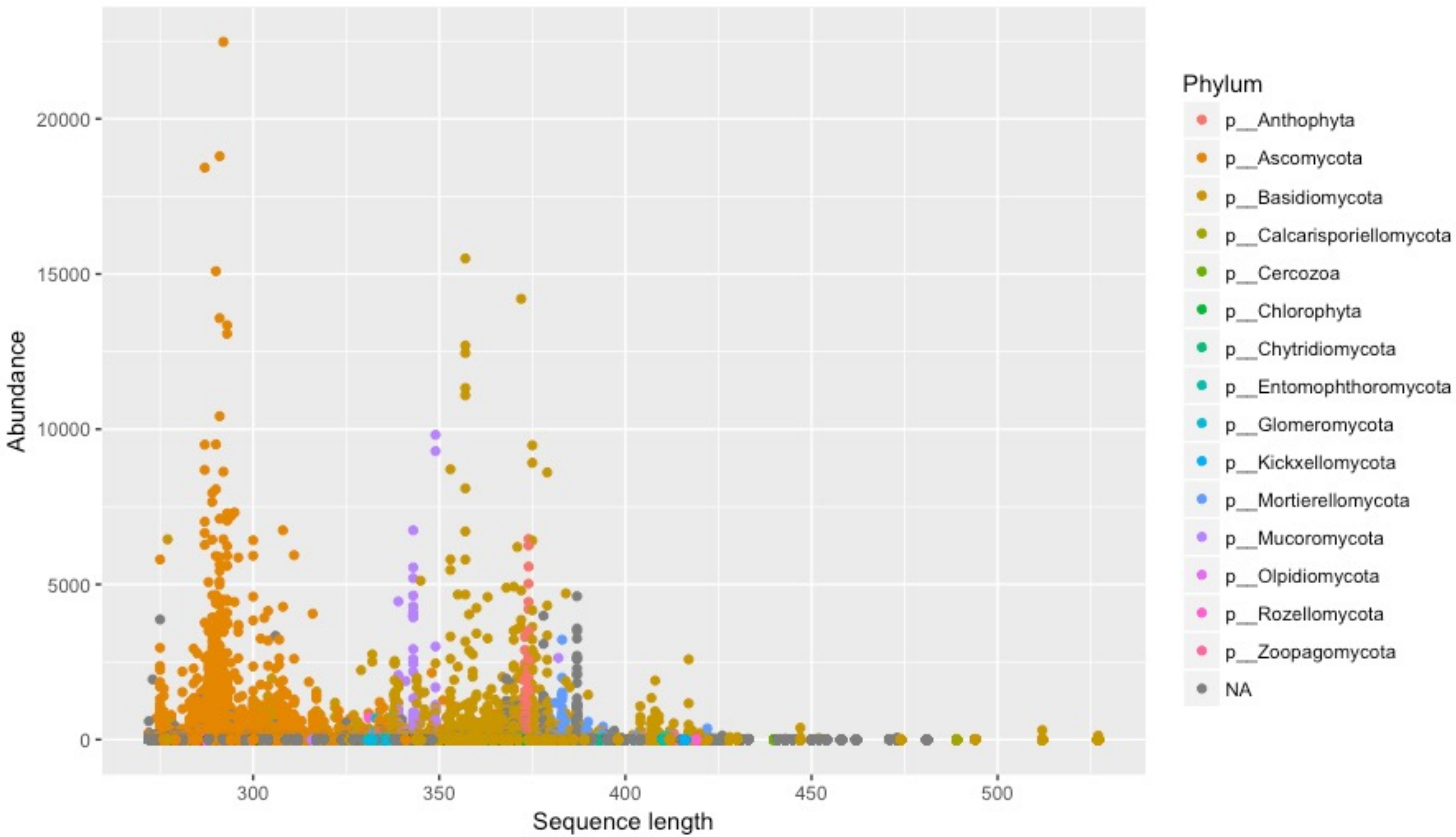
Choice of primers: Primer specificity

Example for fungal ITS



Choice of primers: Expected sequence length

Example for fungal ITS



Navigating the labyrinth

Challenge #4: Which pipeline?

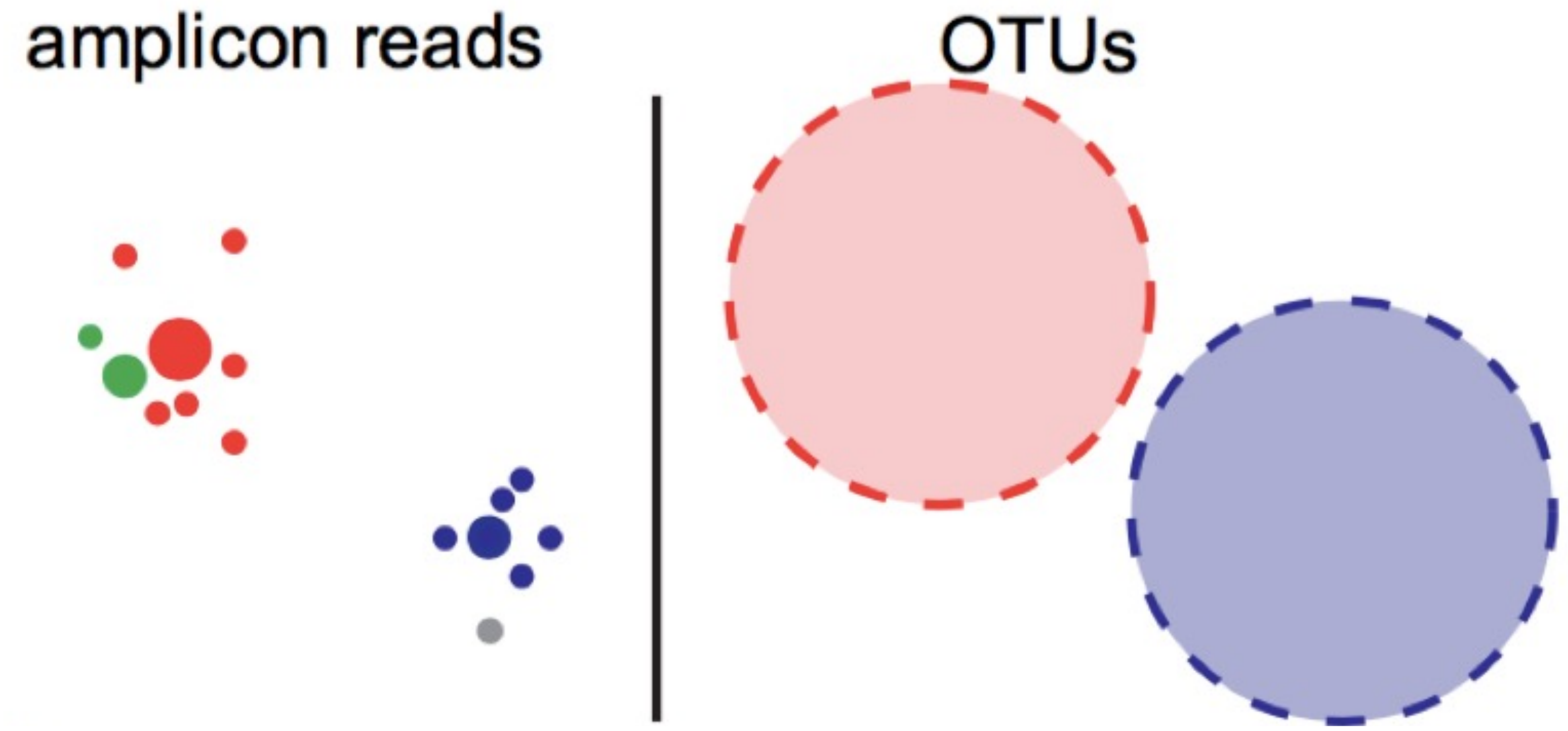
DADA2

http://benjjneb.github.io/dada2/tutorial.html

Callahan et al. 2016 *Nature Methods*

Navigating the labyrinth

The idea behind DADA2



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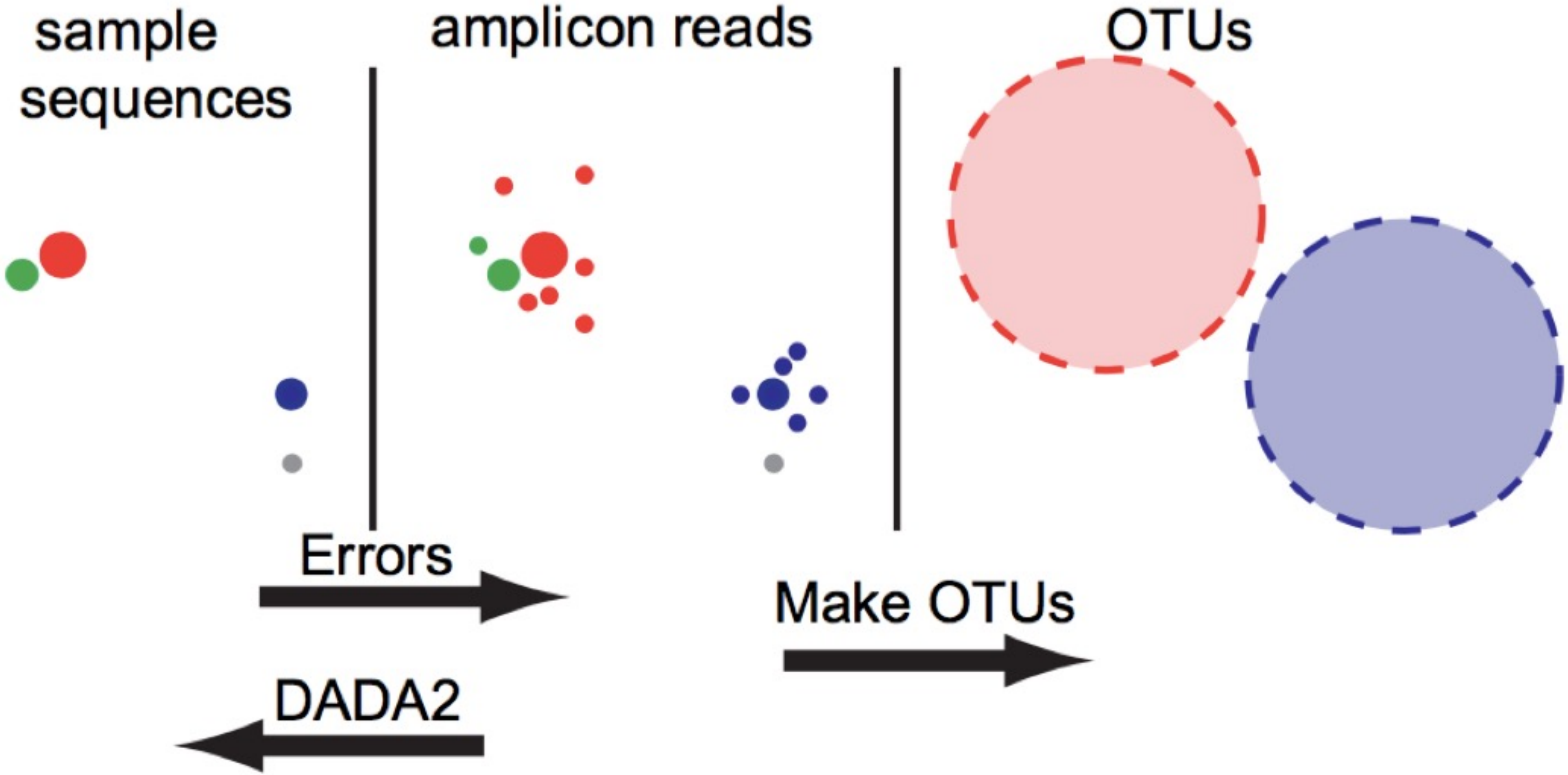
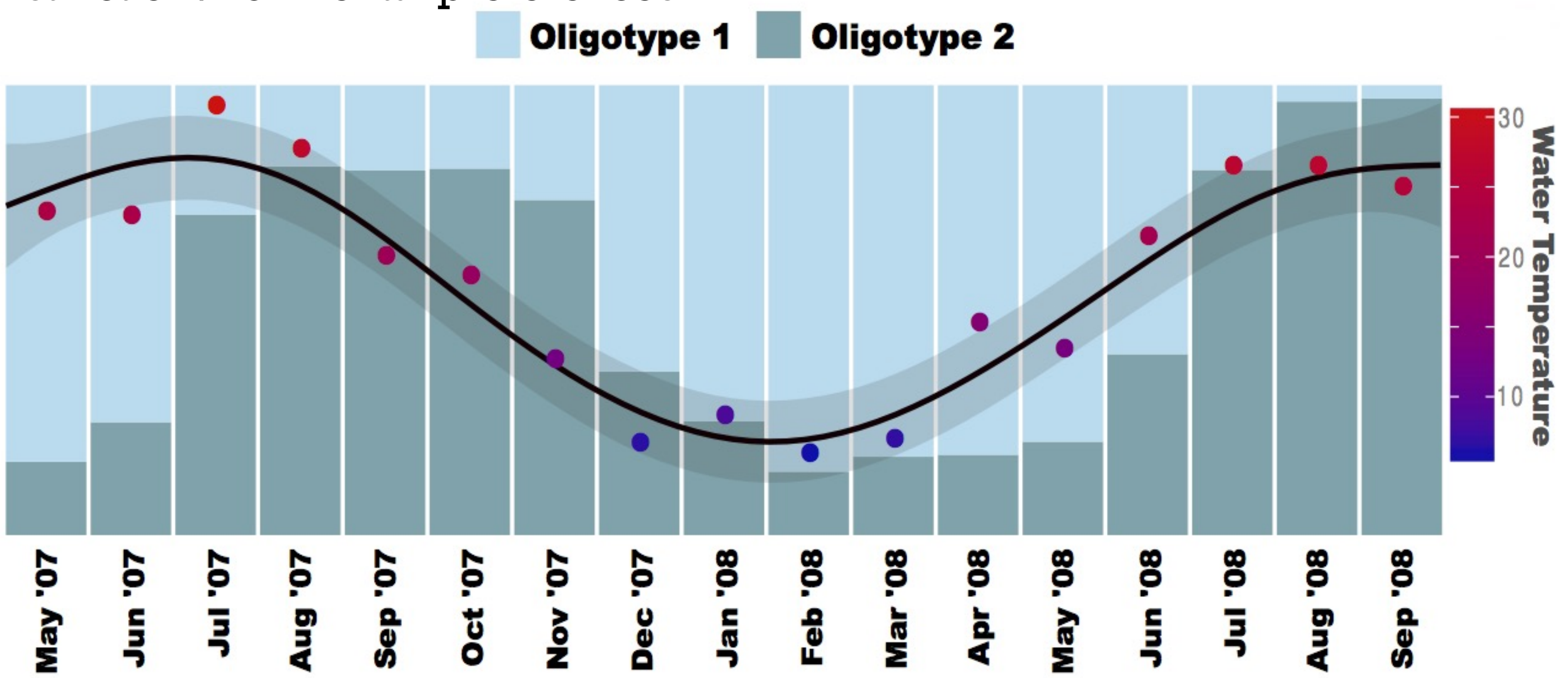


Challenge #5: To cluster or not to cluster?

In favor of NOT clustering:

➢ **Improved taxonomic resolution**

e.g. differentiate between pathogenic and non-pathogenic lineages, discriminate between strains that have distinct environmental preferences



Eren et al. 2013. MEE

Challenge #5: To cluster or not to cluster?

In favor of NOT clustering:

- **Improved taxonomic resolution**

e.g. differentiate between pathogenic and non-pathogenic lineages, discriminate between strains that have distinct environmental preferences

- **ASVs as consistent labels**

A single sequence for all members of a variants

Sequences within each ASV are identical to one another.

Different datasets are more readily compared against one another.

Navigating the labyrinth

Challenge #5: To cluster or not to cluster?

In FAVOR of clustering:

- **Intra-genomic heterogeneity**

Half of bacteria have more than one rRNA operon (Pei et al. 2010) with some bacteria having >10 rRNA operons in a single genome.

Fungi have high intra-isolate nucleotide variation

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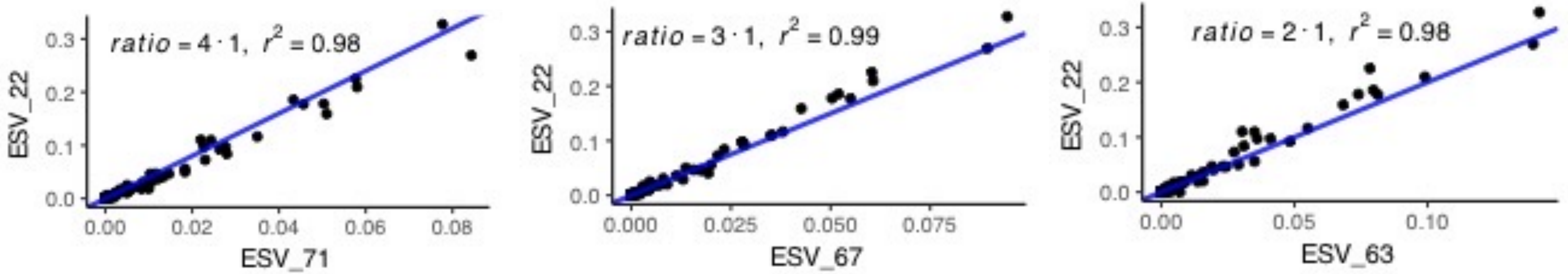
Solution? A single taxon split into multiple ASVs, abundance of those ASVs would be highly correlated



Challenge #5: To cluster or not to cluster?

In FAVOR of clustering:

- **Intra-genomic heterogeneity**

- **Too much diversity**

But not always true

Solution? Possible to cluster afterward

- **Sensitivity to data quality**

Discriminate between PCR or sequencing errors and ‘real’ biological variation

Solution? Error modeling…

The idea behind DADA2

➢ Core "denoising" algorithm

Model the errors in Illumina-sequenced amplicon reads

Quantifies the rate at which an amplicon read is produced from a sample sequence as a function of sequence composition and quality

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Model the errors in Illumina-sequenced amplicon reads

Quantifies the rate at which an amplicon read is produced from a sample sequence as a function of sequence composition and quality

```
dadaFs <- dada(derepFs, err=errF, multithread=TRUE)
```

```
dadaRs <- dada(derepRs, err=errR, multithread=TRUE)
```

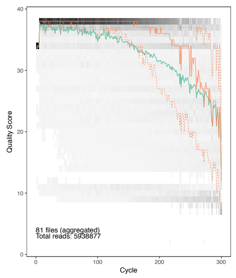
➢ The math behind

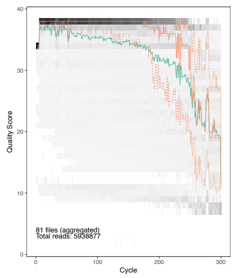
See Callahan et al. 2016 *Nature Methods*

$$p_A(j \rightarrow i) = \frac{1}{1 - \rho_{\mathrm{pois}}(n_j \lambda_{ji}, 0)} \sum_{a=a_i}^{\infty} \rho_{\mathrm{pois}}(n_j \lambda_{ji}, a)$$

DADA2 pipeline

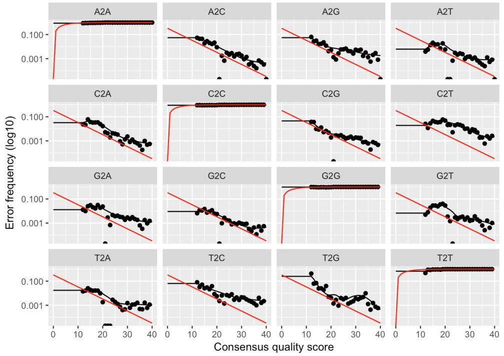
```
library(dada2)

out <- filterAndTrim(fnFs, filtFs, fnRs, filtRs,
                     maxN=0,
                     truncLen = c(290,290),
                     maxEE=c(3,3),
                     truncQ=6,
                     rm.phix=TRUE,
                     trimLeft=c(18,20),
                     compress=TRUE,
                     multithread=TRUE)
```





DADA2 pipeline

```
# Learning the error model from the data
errF <- learnErrors(filtFs)
errR <- learnErrors(filtRs)
```




```
# Infering the sequence variants all the samples
dadaFs <- dada(derepFs,
               err=errF,
               pool=TRUE)
dadaRs <- dada(derepRs,
               err=errR,
               pool=TRUE)
```

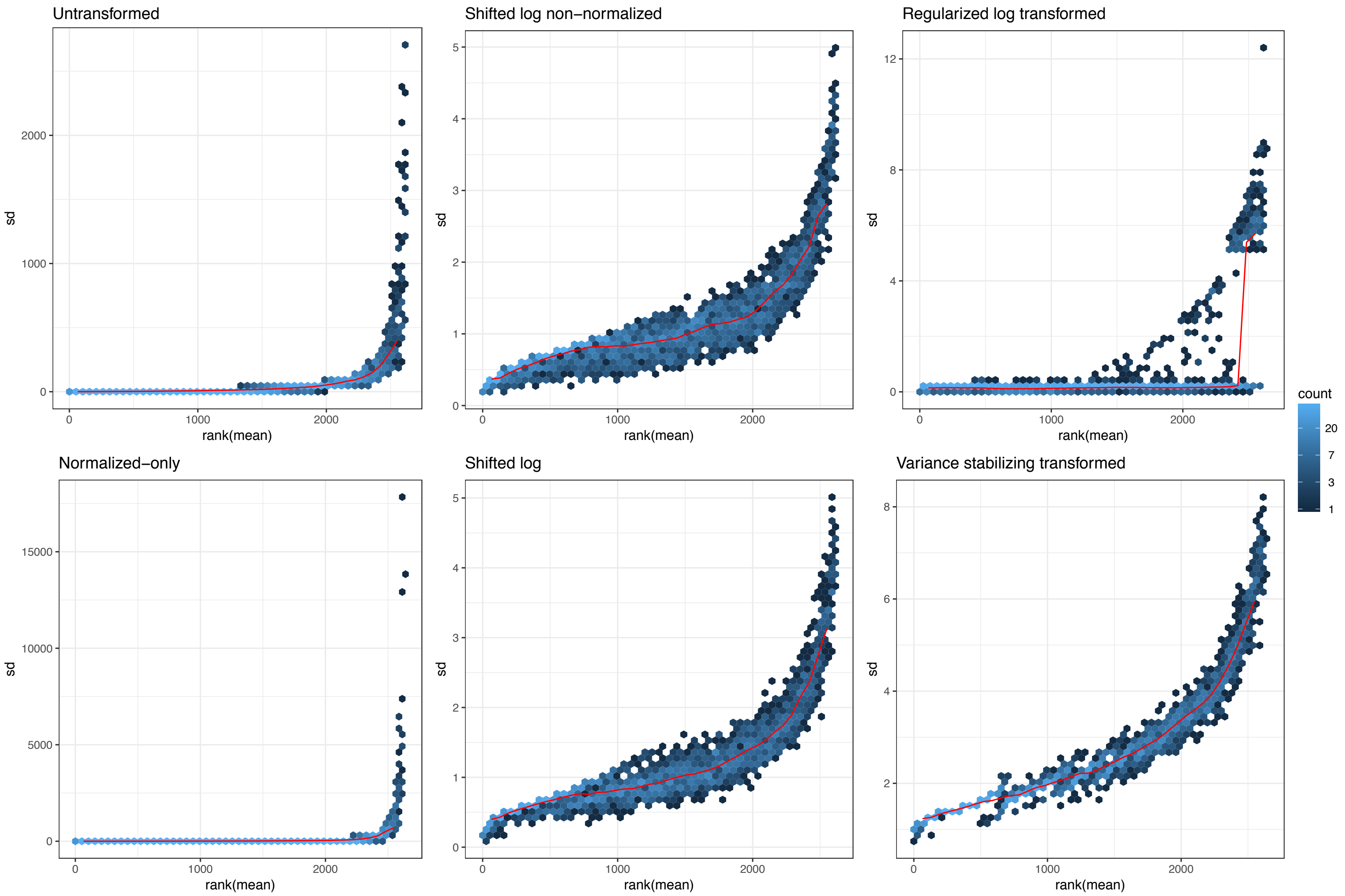
It quantifies the rate at which an amplicon read is produced from a sample sequence as a function of sequence composition and quality

DADA2 pipeline

```
# Merging
mergers <- mergePairs(dadaFs, derepFs, dadaRs, derepRs,
                    minOverlap = 12,
                    maxMismatch = 0,
                    returnRejects = FALSE,
                    propagateCol = character(0),
                    justConcatenate = FALSE,
                    trimOverhang = FALSE)

# Construct sequence table
seqtab <- makeSequenceTable(mergers)

# Removing chimeras
seqtab.nochim <- removeBimeraDenovo(seqtab,
                    method="pooled“)

# Assigning Taxonomy
taxa.paired <- assignTaxonomy(seqtab.nochim,
                    “UNITEreferencedatabase",
                          minBoot = 80)
```

Navigating the labyrinth

Challenge #6: Transformation the data for stabilizing variance inflation?



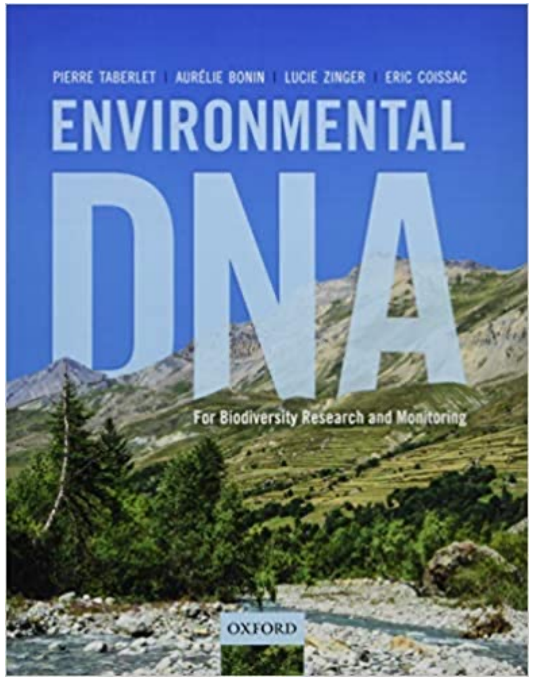
DESeq
Love et al. 2014
Genome Biology

Navigating the labyrinth

Many more challenges…

My two cents:

- ❑ Follow workshops
- ❑ Strong review of literature
- ❑ Take into account local expertise
- ❑ Ask questions



New Phytologist
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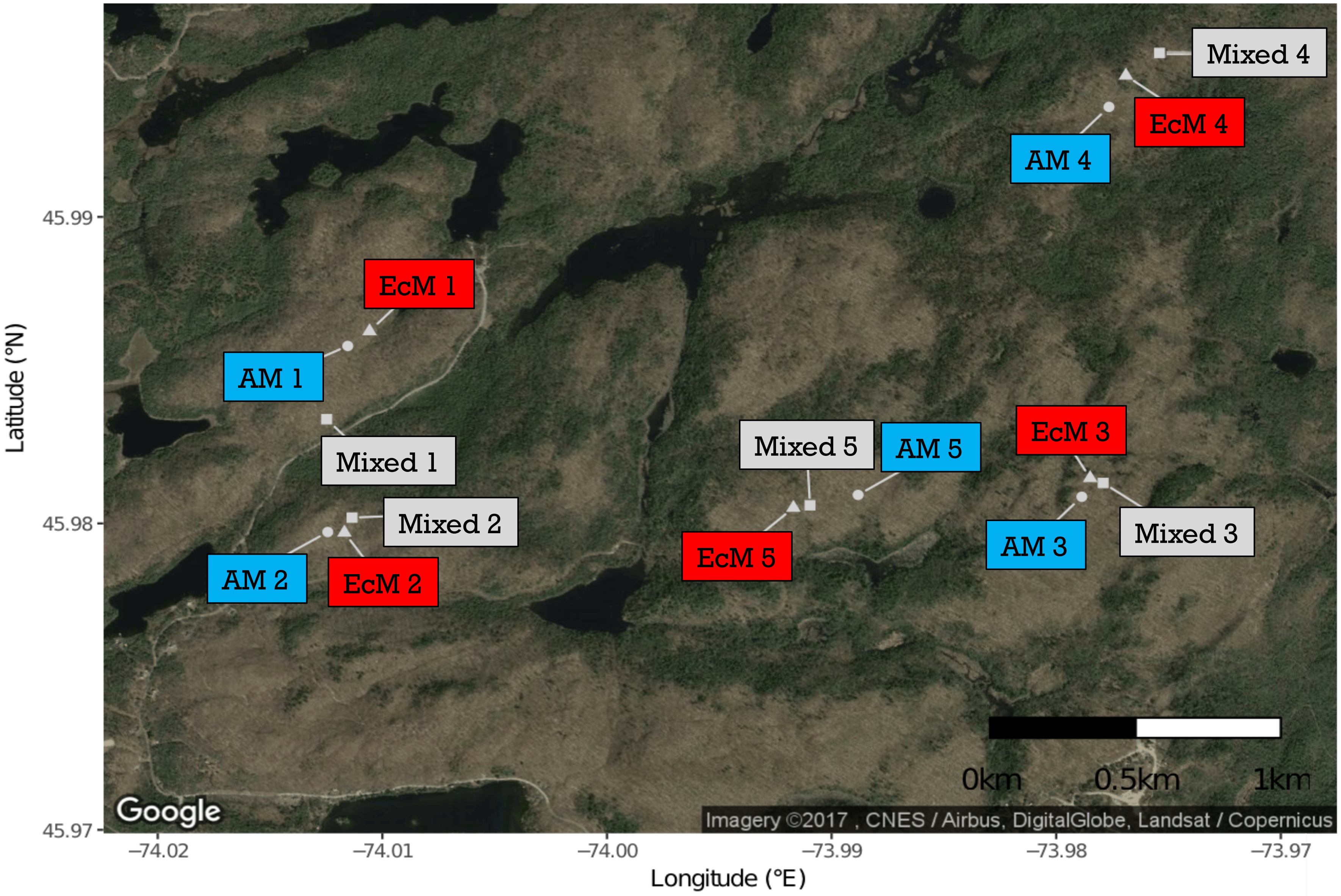
Solution = eDNA metabardoding!

2. Natural sites where AM and EcM symbioses are co-occurring

Solution = sampling design!

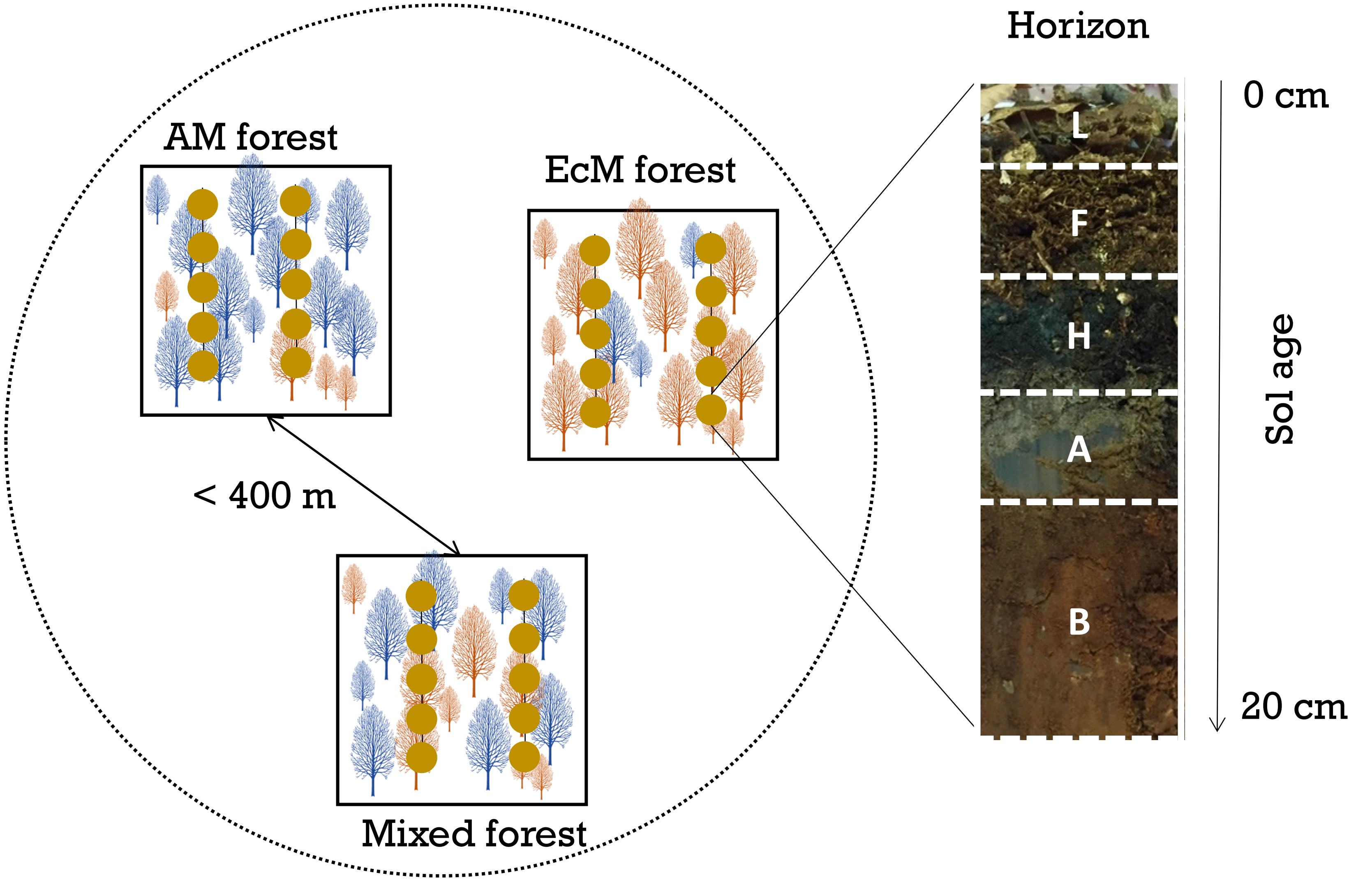
Sampling design



- Station de biologie de l'Université de Montréal, QC, Canada
- 15 permanent plots (dominated by AM, mixed or EcM)
- Limiting variations in:
  - ✓ Climate
  - ✓ Parent material
  - ✓ Historical events

Carteron et al. 2020 *Microb Ecol*

Sampling design



- ↘ pH
- ↘ Nutrient availability
- ↘ Organic matter

Amplification et sequencing:

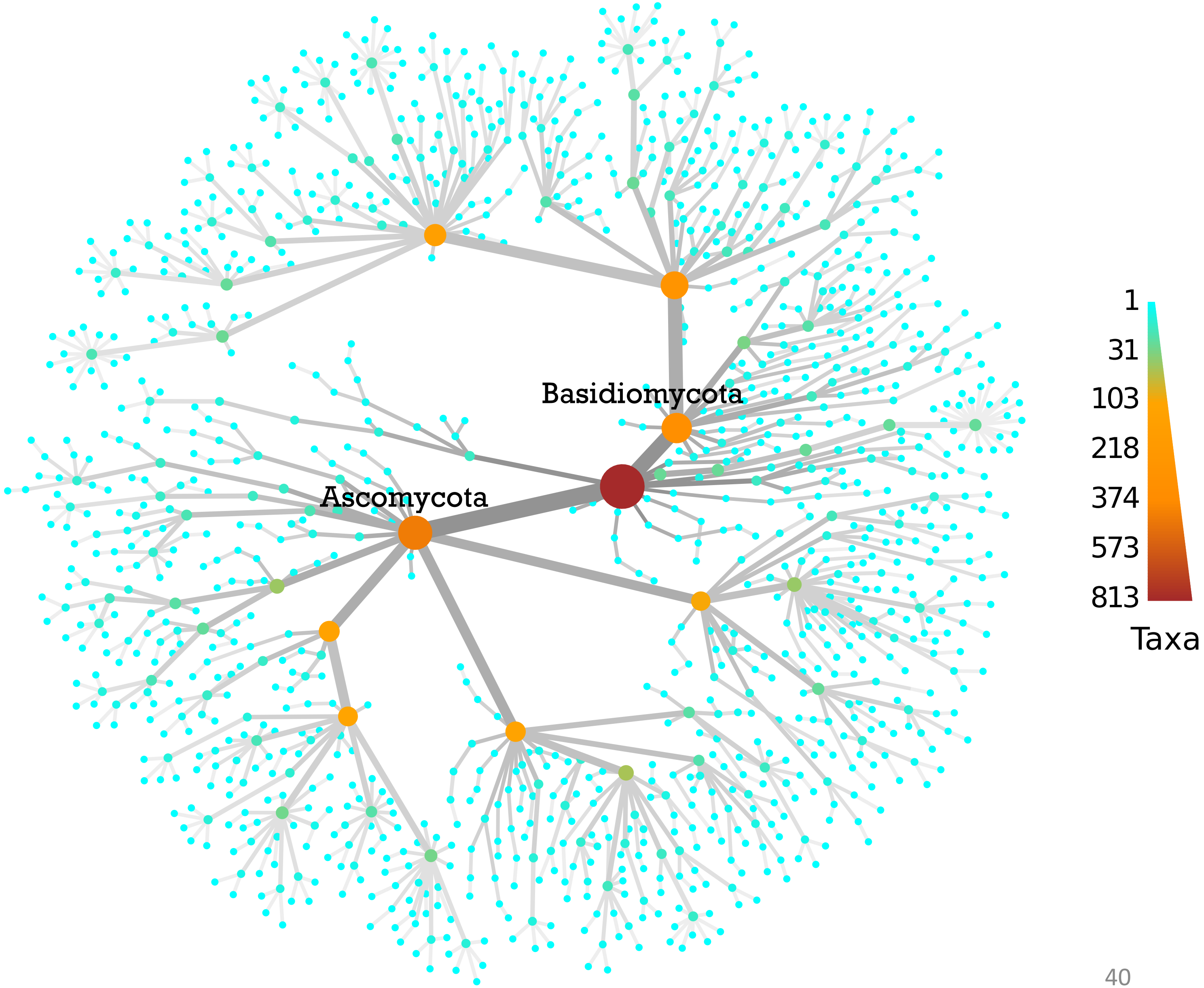
- Fungi (ITS)
- Glomeromycota (LSU)
- Plants (rbcL)

Carteron et al. 2020 *Microb Ecol*

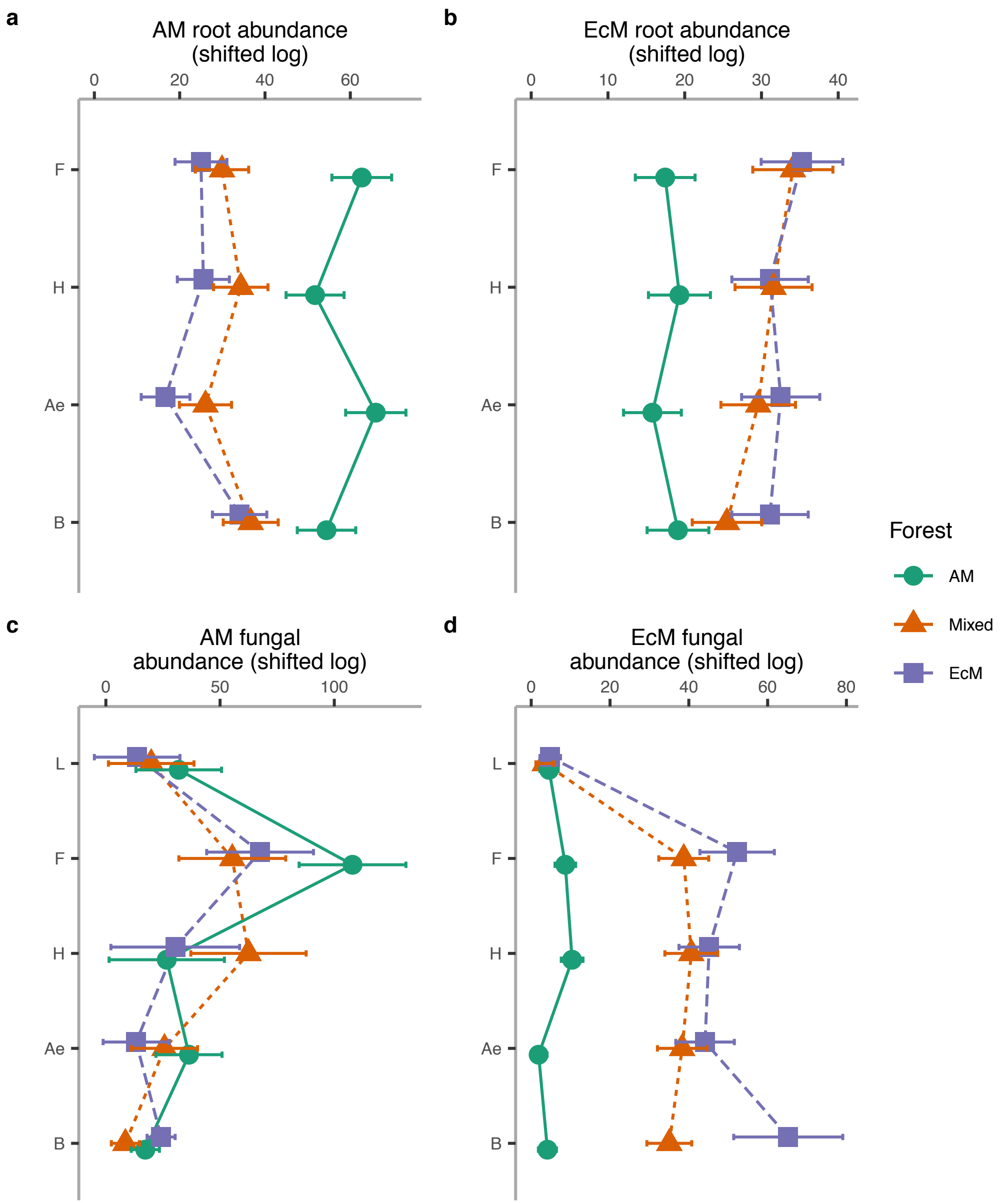
Results and Discussion

Fungal community (ITS)

- ✓ 2,865,791 sequences
- ✓ 88.9% fungal origin
- ✓ Grouped in 813 taxa and 7 phylums



Root and fungal distribution (from sequence data)



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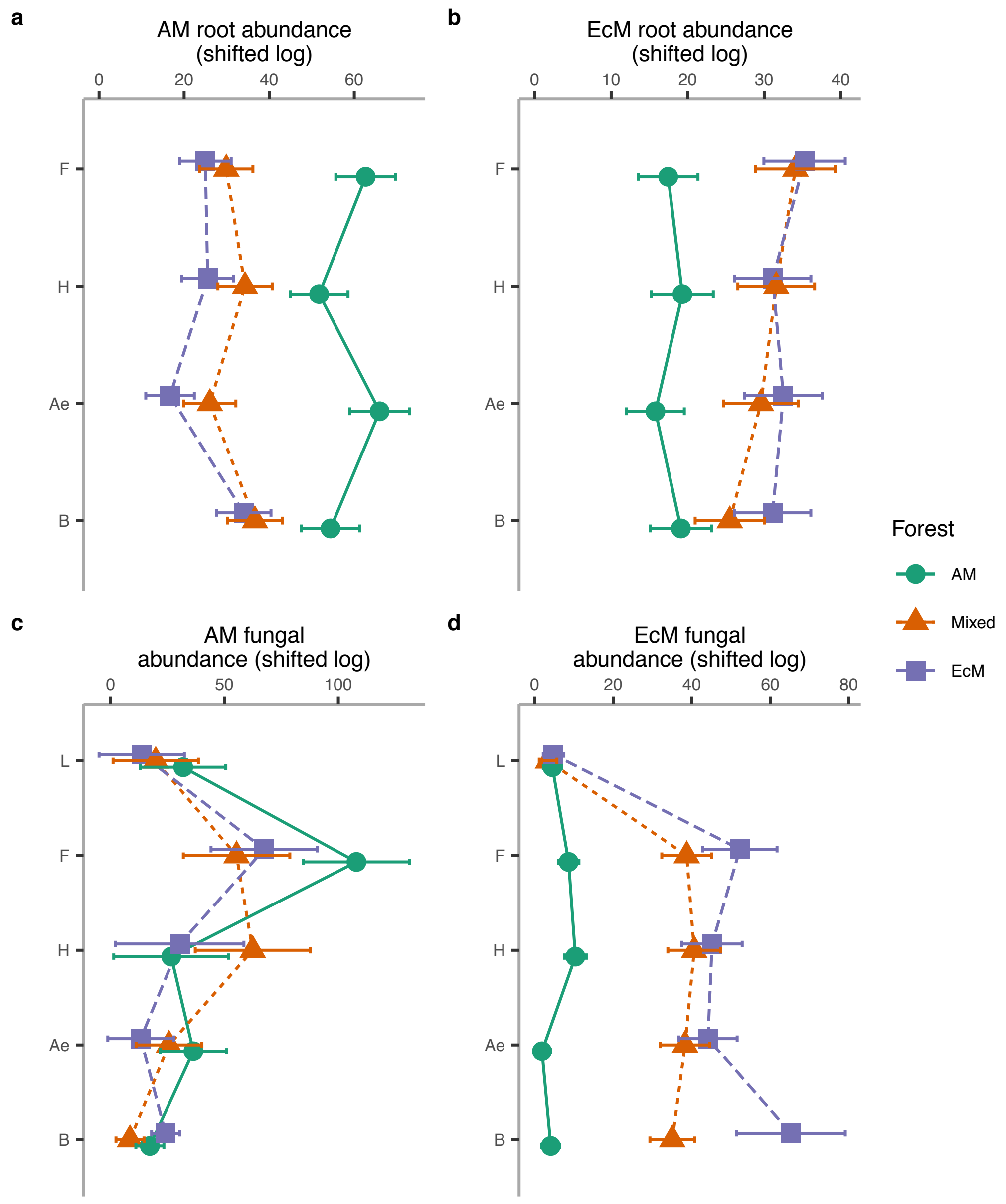
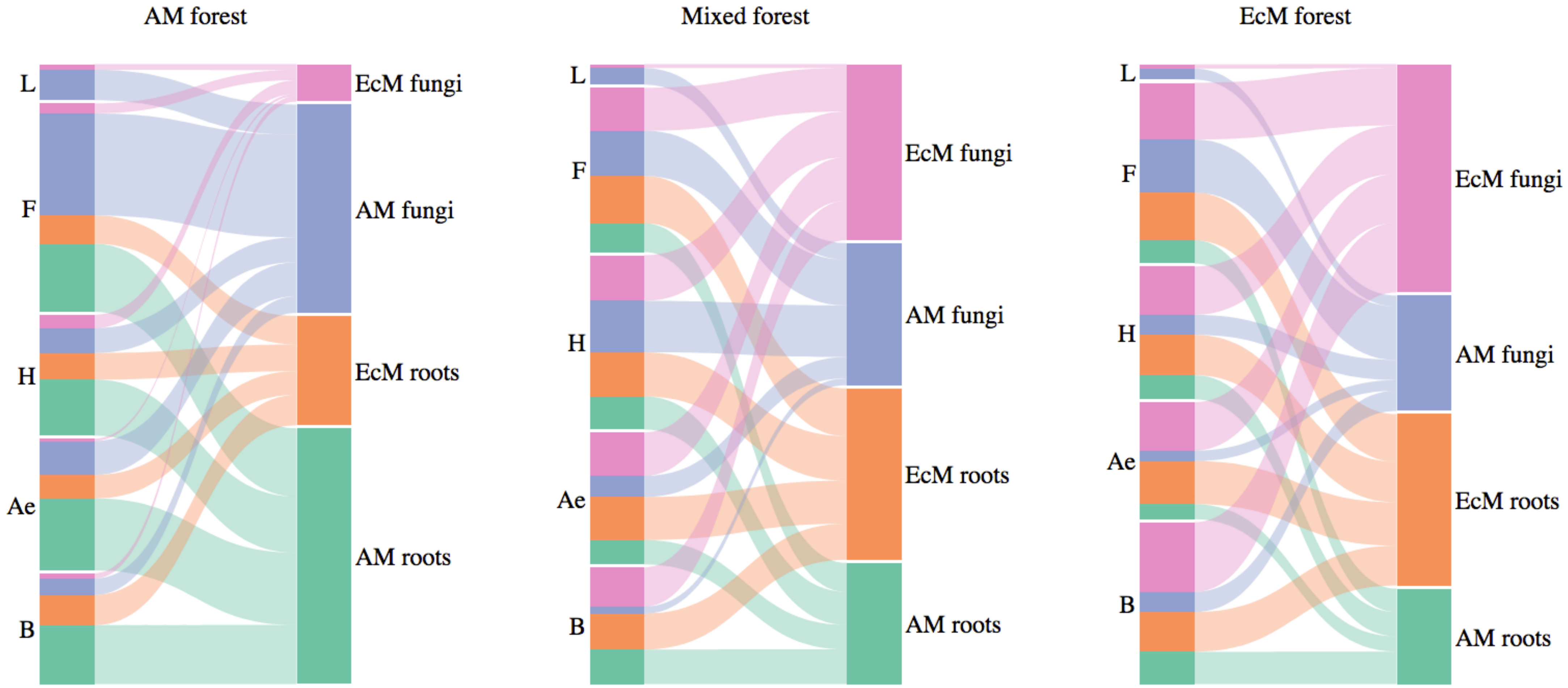


- <u>Approach</u>: Comparison of distribution with shifted-log data and sequence abundance summed by sample
- AM fungal very variable (data not great?)
- EcM fungi and root are abundant in EcM <u>and</u> mixed plots
- But no apparent antagonism

Root and fungal network

Approach: Network analysis using sequence abundance as a proxy of mycorrhizal abundance



Carteron et al. *In prep*

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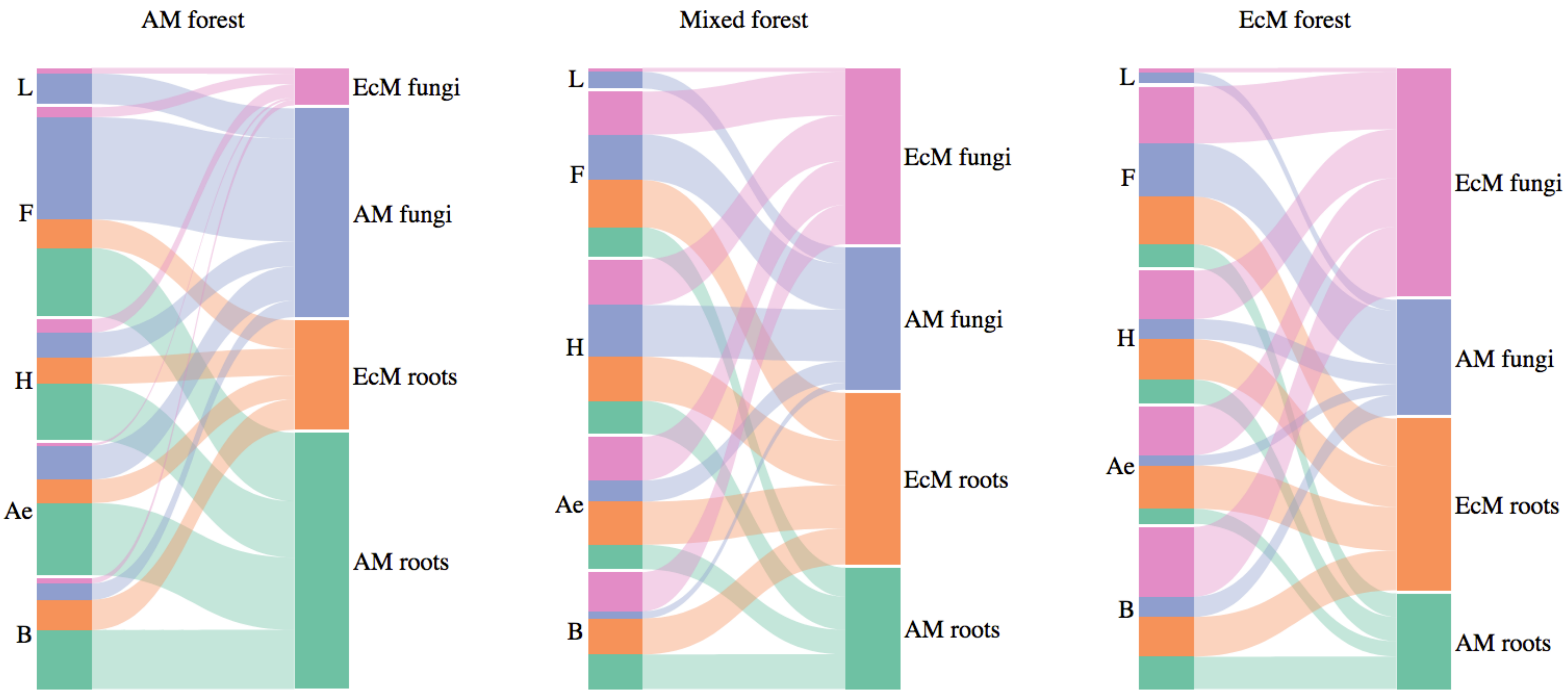
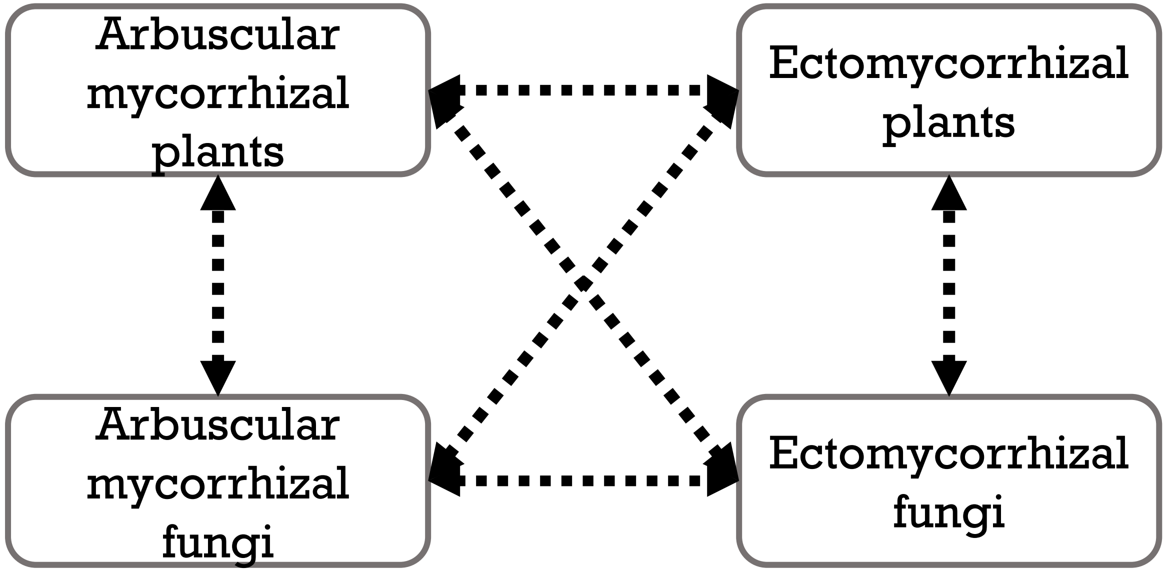


- ➢ Patterns of dominance as expected
- ➢ But no apparent antagonism between AM and EcM
- ➢ Mycorrhizal fungi colonize L (broader niche than usually expected?)

Carteron et al. *In prep*

Root and fungal co-variance

Approach: Permutation analysis to test the strength of the relationship among groups using the Monte-Carlo method on the sum of eigenvalues of the co-inertia analysis



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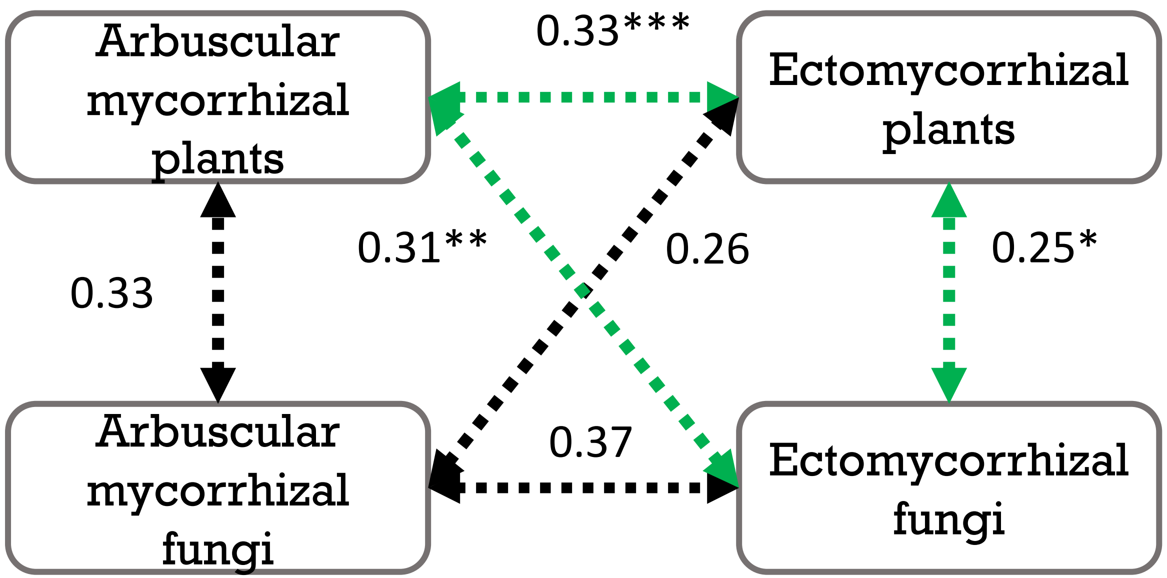
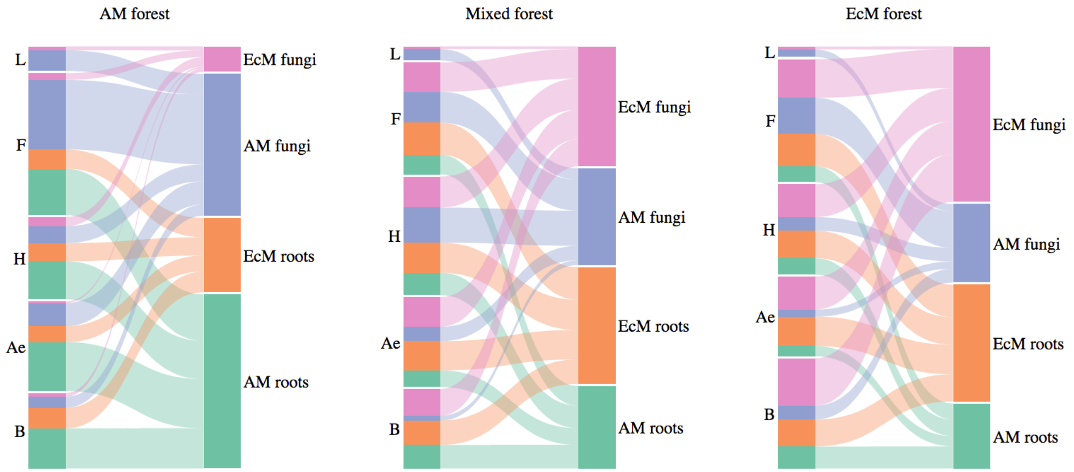


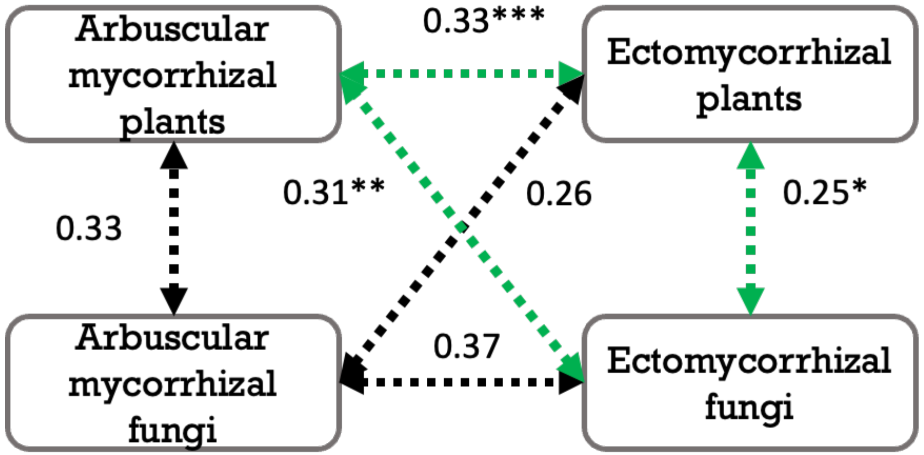
No apparent antagonism between AM and EcM symbioses?

Carteron et al. *In prep*

Main points

- No apparent antagonism between AM and EcM symbioses
- Important to take into account the vertical distribution including organic and mineral horizons
- Hyphae in the soil are clearly not only present where roots are
- AM fungi are abundant in organic horizons, present in L and highest "abundance" in F (but issue with LSU marker?)





NEXT -> DADA2 Tutorial

https://alexiscarter.github.io/metab/

https://alexiscarter.github.io/metab/Dada_script_ES.html

https://alexiscarter.github.io/metab/Dada_script_EN.html

ALEXIS.CARTERON@UNIMI.IT

Data source and manipulation

Group	Fungi	AM Fungi	Plant roots
Sampling	Composite soil samples from soil core, particles < 2 mm	Composite soil samples from soil core, particles < 2 mm	Fine roots" (< 2 mm diameter) from composite soil samples
DNA extraction	PowerSoil MoBio kit	PowerSoil MoBio kit	Adapted CTAB protocol
Marker for amplification	Internal transcribed spacer ITS3_KYO2-ITS4	Large Subunit (nested PCR with SSUmAf-LSUmAr then LSUD2f-CS1-LSUmBr-CS2)	Large subunit of RuBisCO rbcLa_f-rbcLa_r
Sequencing	Illumina MiSeq 2x250 bp (~1/3 run)	Illumina MiSeq 2x250 bp (~1/3 run)	Illumina MiSeq 2x250 bp (~1/3 run)
Denoising	dada2 (1.4) pipeline, link: https://doi.org/10.5281/zenodo.3631982	dada2 (1.4) pipeline	dada2 (1.4) pipeline
Taxonomy assignment	Using RDP classifier and UNITE database (version 8.1 release 2/2/2019)	LSU training set #11 https://doi.org/10.5281/zenodo.835855	Customized database derived from the BOLD system http://www.boldsystems.org

Data source and manipulation

Group	Fungi	AM Fungi	Plant roots
Threshold	Singletons and doubletons excluded (keep ASV with total sum > 2)		
Transformation	Initial step for normalization: Shifted log transformation For combined analysis: Relative abundance by groups of organisms		
analysis NMDS	Sorensen (presence/absence) index Bray-Curtis index		
Groups of interest	EcM fungi, saprotrophs	Glomeromycota (phylum)	AM plant, EcM plant (using info at genus level)