

# Introduction to Metacoder

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BIO9905MERG1\_V21

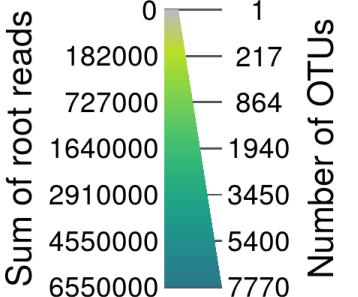
06.05.21

Actinobacteria

Proteobacteria

Firmicutes

Nodes

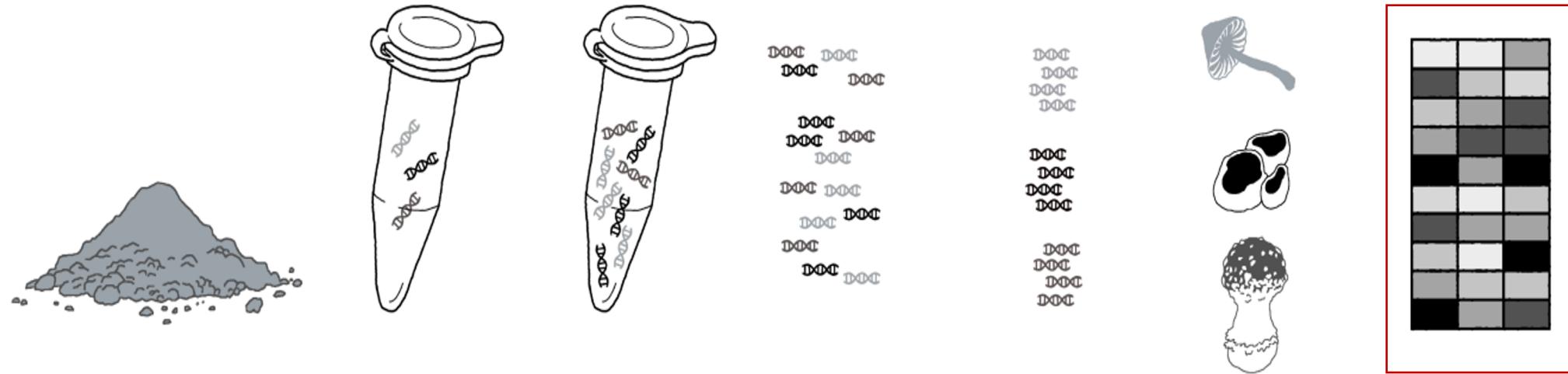


Actinobacteria

Proteobacteria

Firmicutes

# How to visualize the taxonomic data from your metabarcoding study?

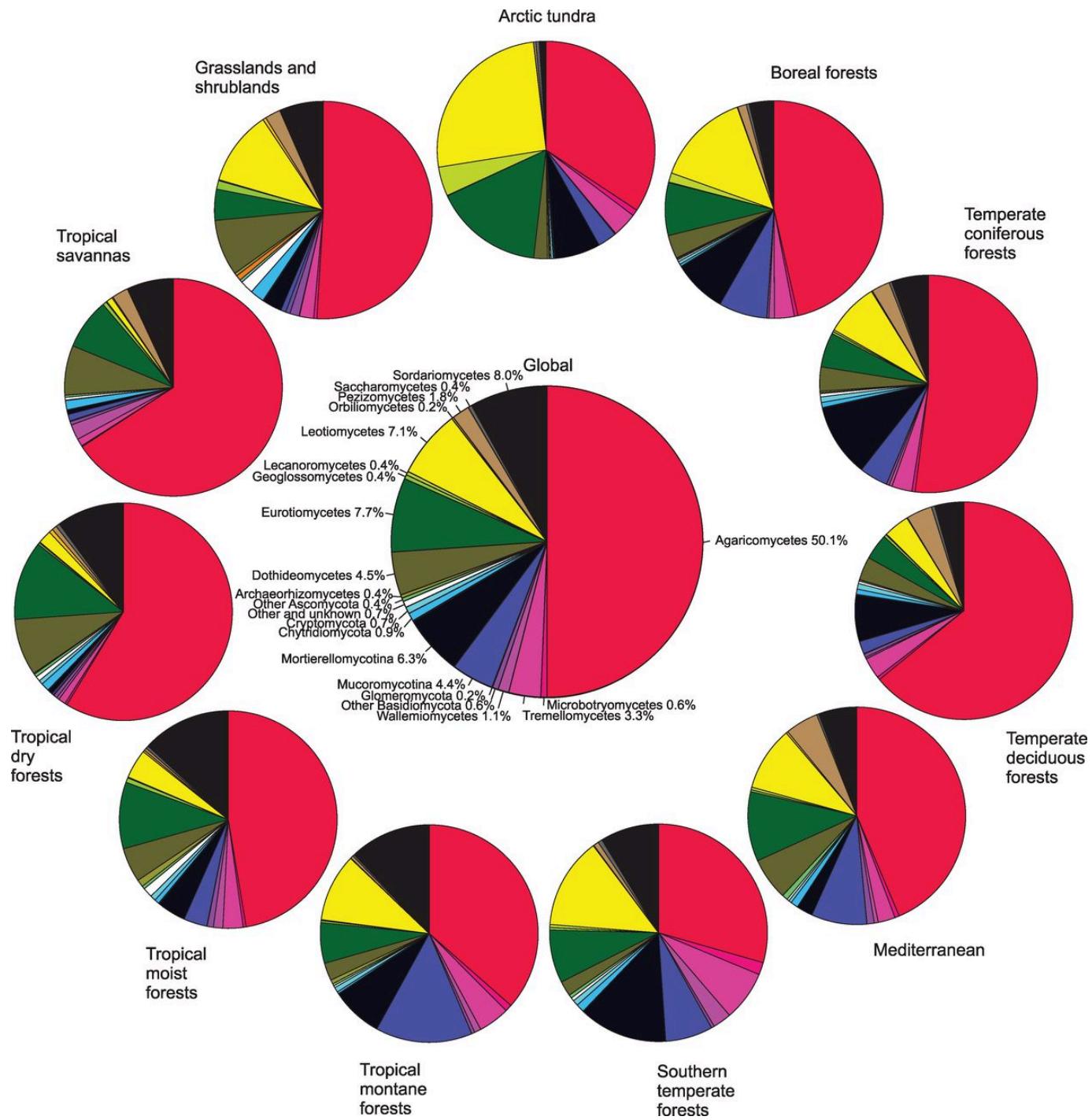


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OTU1576	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo	
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OTU2014	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	5	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo	
OTU0282	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	6	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo	
OTU1577	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo	
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OTU0957	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo	
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OTU1192	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	30	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo
OTU0771	2	0	0	0	3	0	0	0	3	0	4	0	0	0	0	0	54	k_Fungi;p_Ascomycota;c_Archaeorhizomycetes;o_Archaeorhizomycetales;f_Archaeorhizo

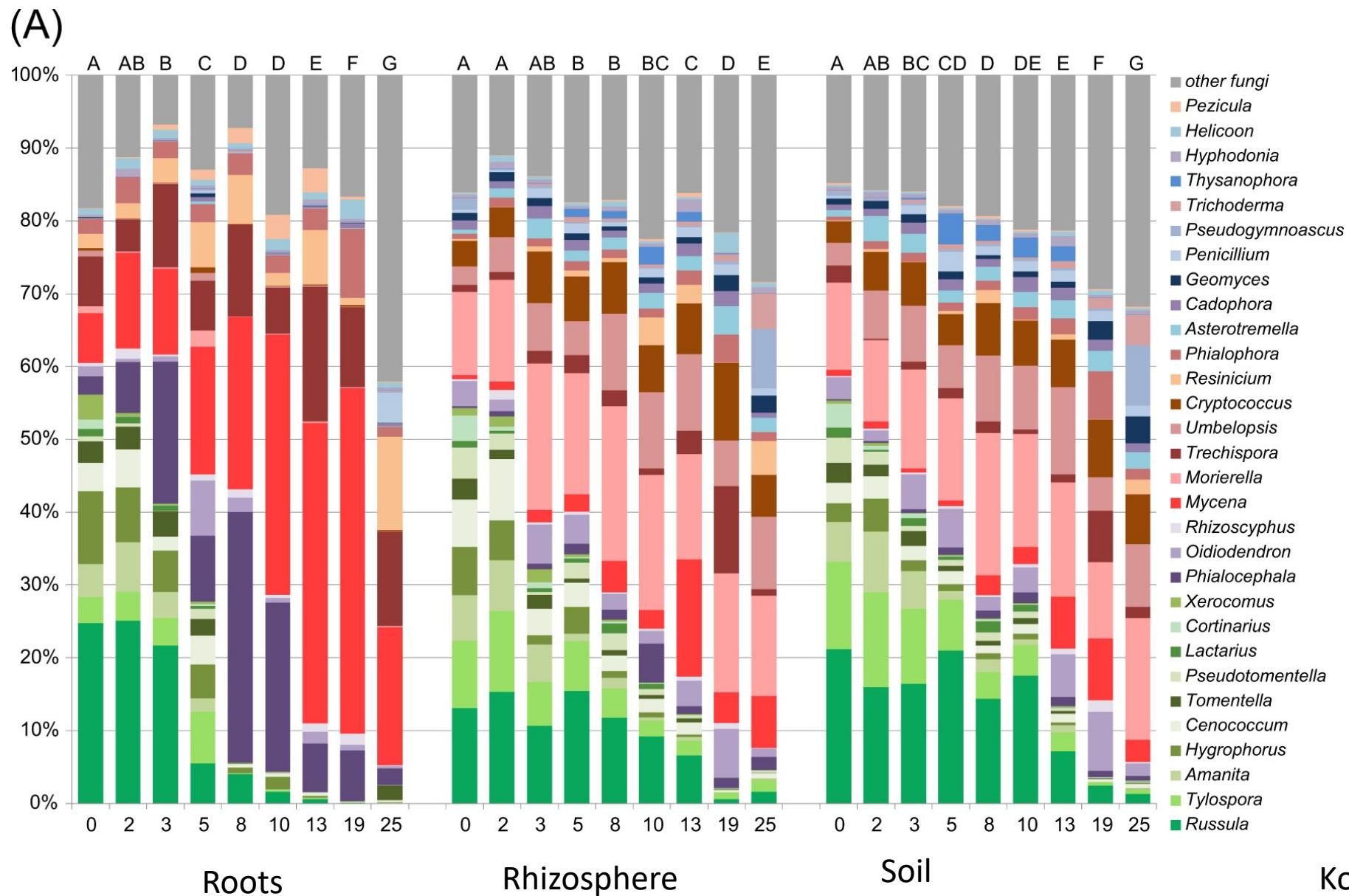
SampleID	Sample	Type	Location	Temperat	Precipitat	pH	N	C	C:N	Free_ergc	Total_erg
DB04	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB05	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB06	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB07	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB08	Bistorta	Plantroot	Arh	low	semi-wet	0.385	0.396	0.602	0.742	0.518	0.395
DB09	Bistorta	Plantroot	Arh	low	semi-wet	0.385	0.396	0.602	0.742	0.518	0.395
DB10	Bistorta	Plantroot	Arh	low	semi-wet	0.385	0.396	0.602	0.742	0.518	0.395
DB14	Bistorta	Plantroot	Ulv	alpine	dry	0.663	0.626	0.485	0.402	0.693	0.554
DB15	Bistorta	Plantroot	Ulv	alpine	dry	0.663	0.626	0.485	0.402	0.693	0.554
DB16	Bistorta	Plantroot	Ulv	alpine	dry	0.663	0.626	0.485	0.402	0.693	0.554
DB20	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB21	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB22	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB26	Bistorta	Plantroot	Alr	mid	dry	0.513	0.46	0.629	0.69	0.638	0.487
DB27	Bistorta	Plantroot	Alr	mid	dry	0.513	0.46	0.629	0.69	0.638	0.487
DB28	Bistorta	Plantroot	Alr	mid	drv	0.513	0.46	0.629	0.69	0.638	0.487

## OTU table & metadata

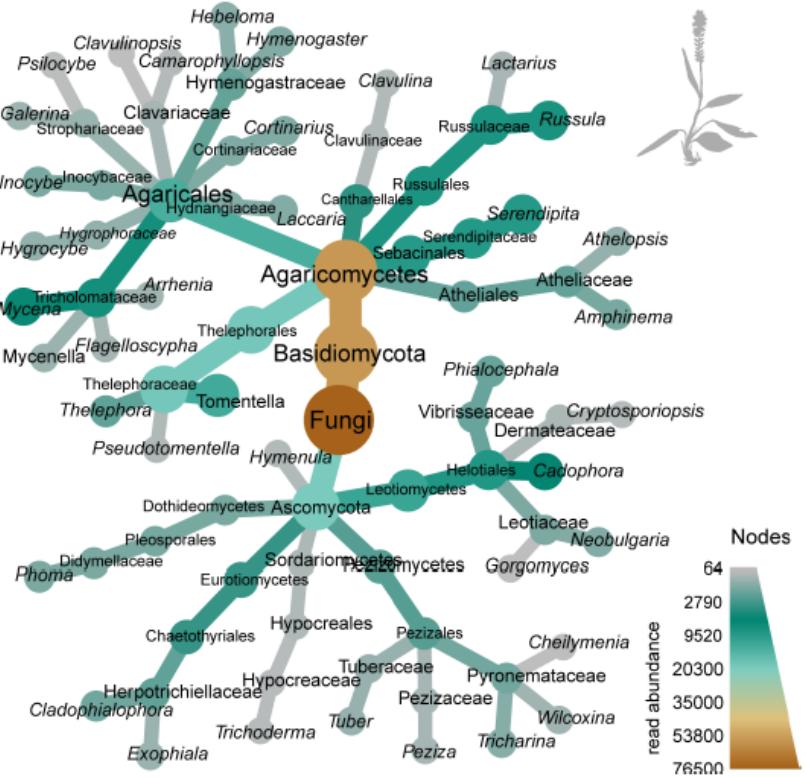
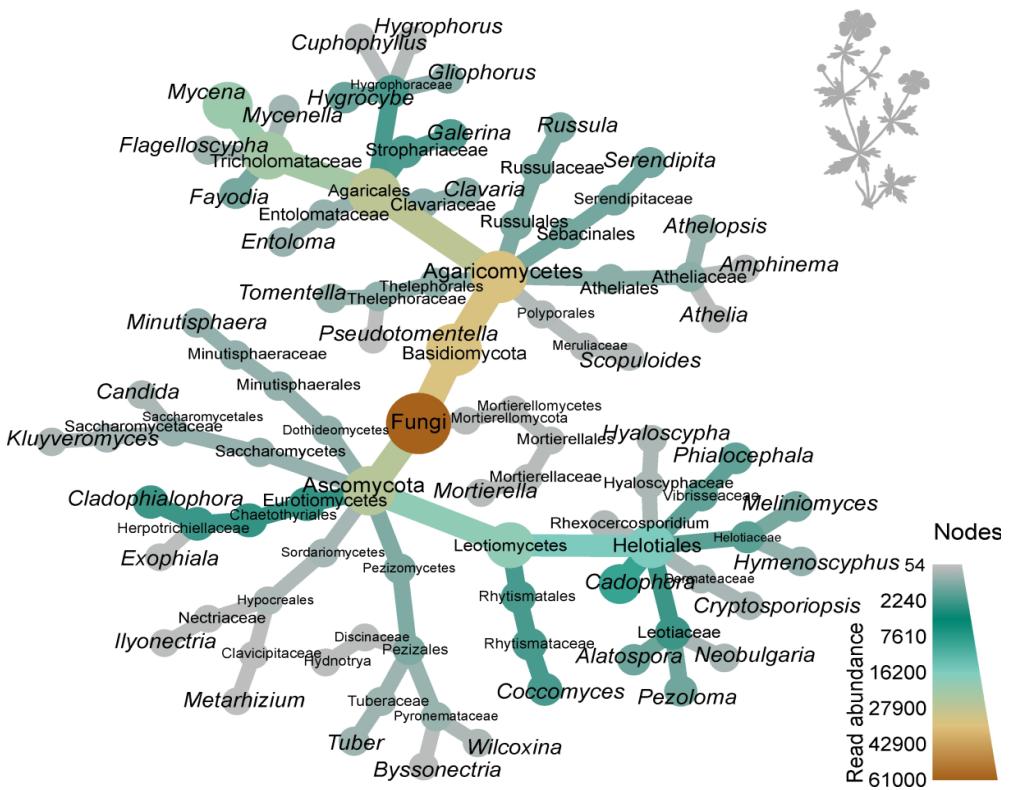
Piechart?

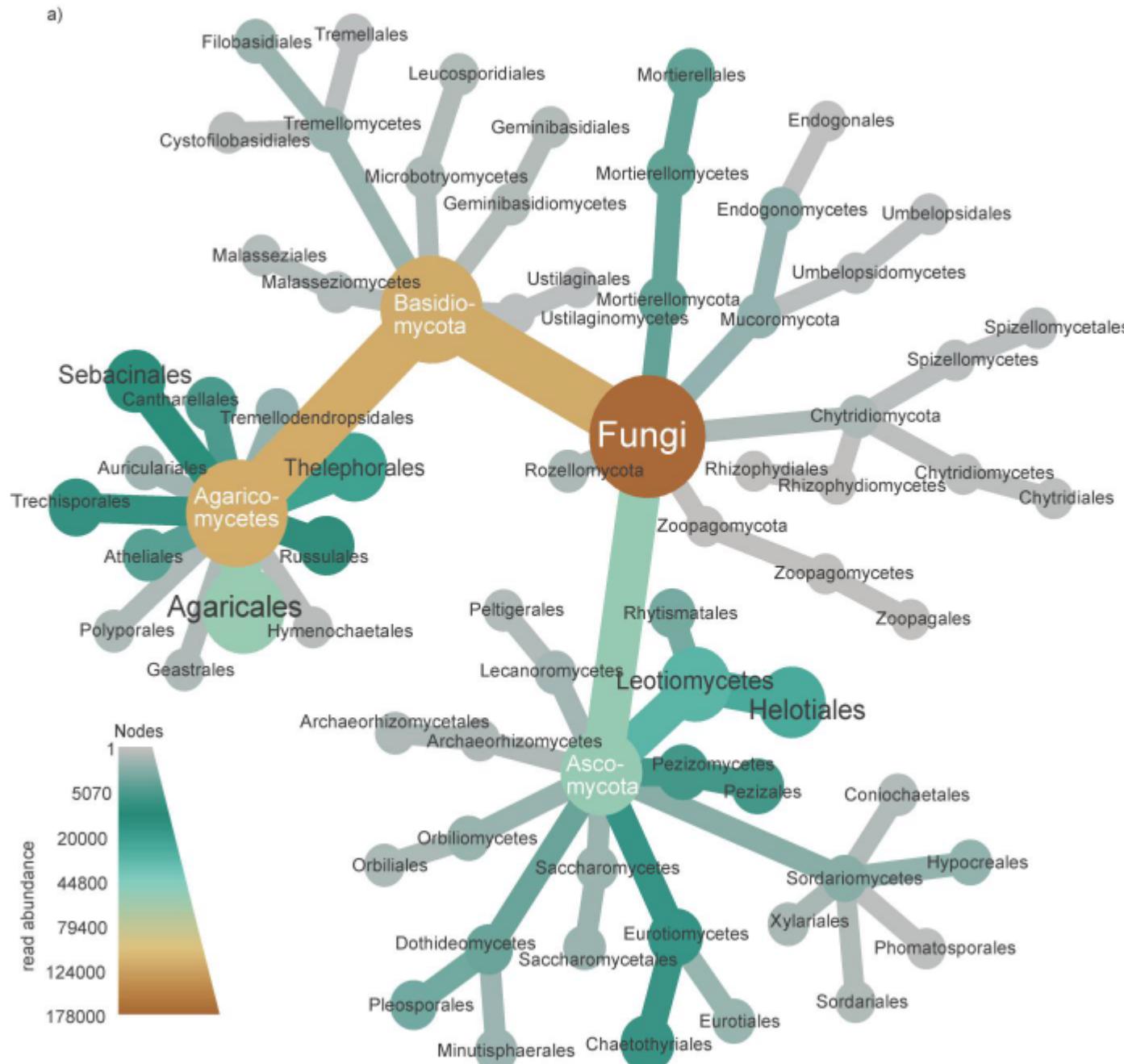


# Barchart?



# Heat trees?





## Heat trees with Metacoder

Metacoder uses the phylogenetic backbone of your taxonomic data to construct heat trees

=NOT phylogenetic trees!

Depict statistics for every taxon in a taxonomy using color and size

- Nodes (circles) scaled and colored according to read abundance
- Edges (branches) scaled and colored by read abundance

# Barcharts vs Heat trees with metacoder

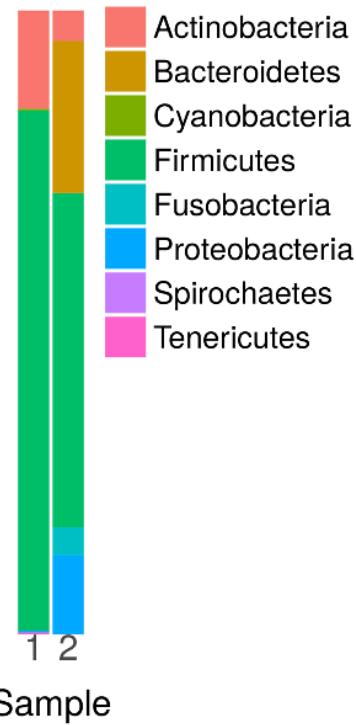


Fig 2. from Foster *et al* 2017

Heat trees allow for a better understanding of community structure than stacked bar charts

# Heat trees with metacoder

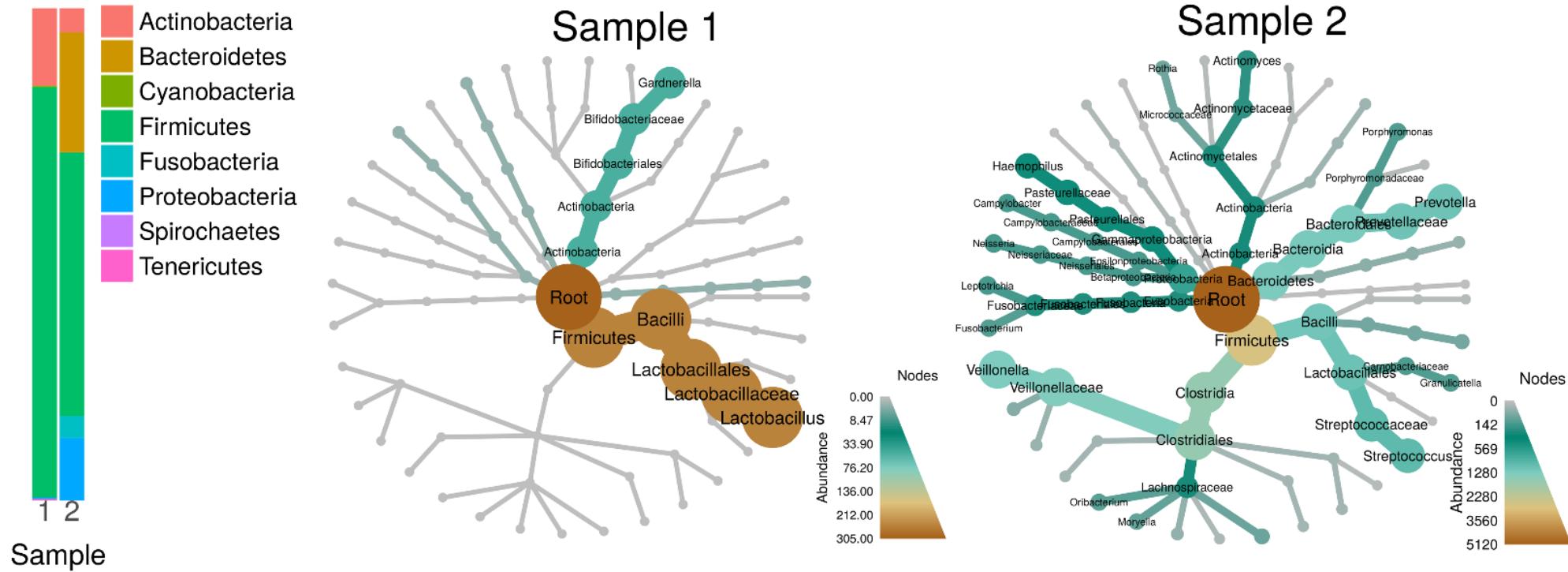


Fig 2. from Foster *et al* 2017

Heat trees allow for a better understanding of community structure than stacked bar charts

## Metacoder: An R package for visualization and manipulation of community taxonomic diversity data

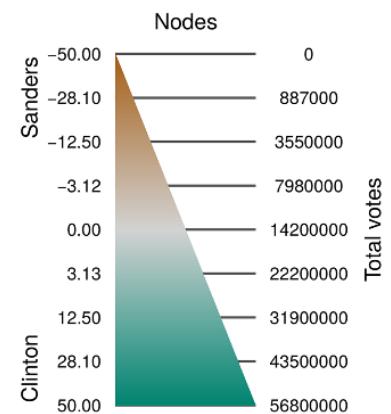
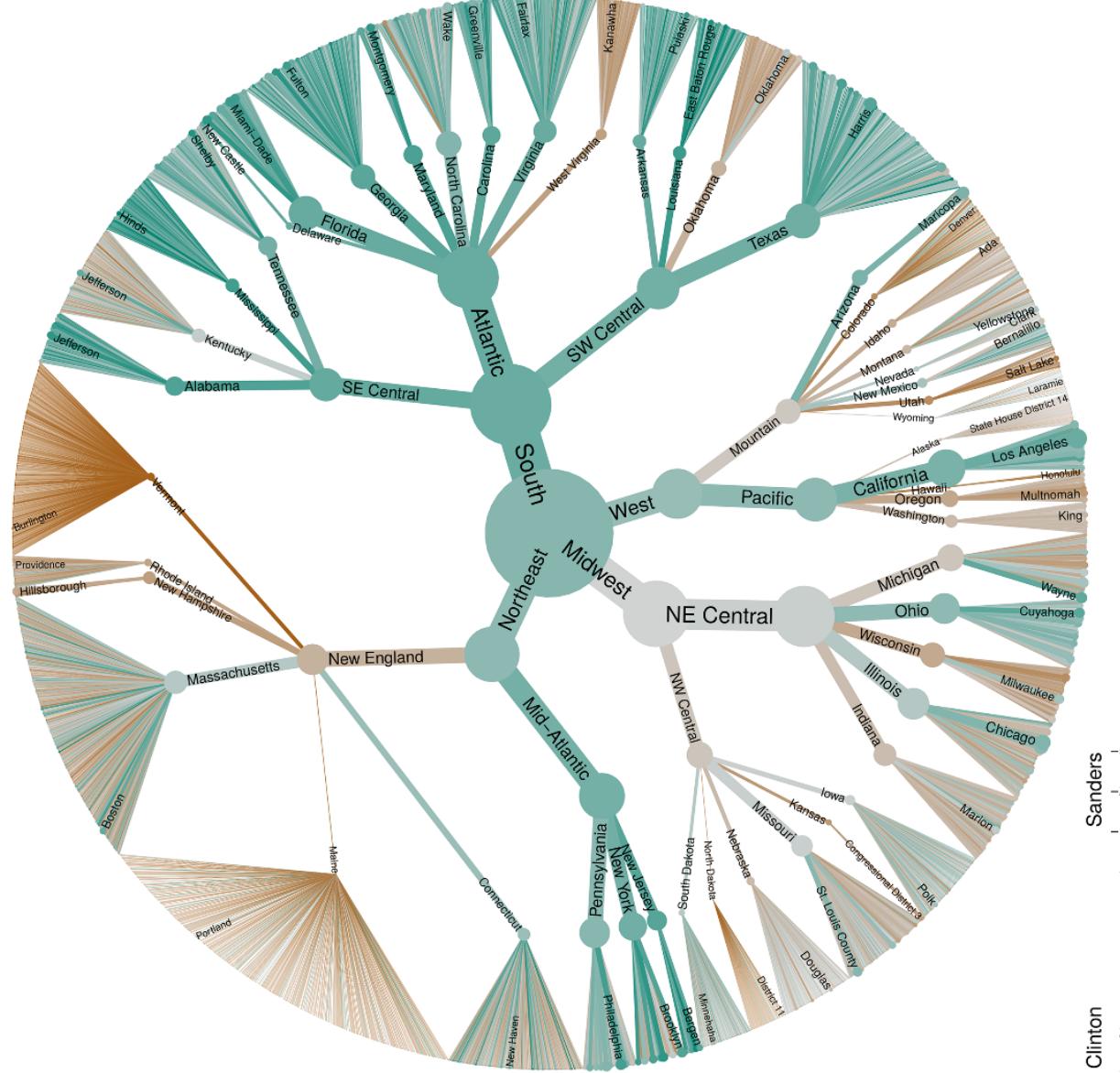
Zachary S. L. Foster, Thomas J. Sharpton, Niklaus J. Grünwald 

An R package for parsing, **plotting** and manipulating large taxonomic datasets

# What can you do with metacoder?

- Summing read counts/relative abundance per taxon
- Converting counts to proportions and rarefaction of counts using `vegan`
- Comparing the abundance of groups of samples per taxon
- Combining data for groups of samples
- Simulated PCR (primer specificity and coverage of taxonomic groups)
- Converting common microbiome formats for data and reference databases into the objects defined by the `taxa` package.
- Converting to and from the `phyloseq` format and the `taxa` format

**But most important: data visualization with heat trees!**



**Metacoder can be used with any type of data that can be organized hierarchically**

Fig. 6. Foster et al 2017

# Relationship with other packages

- Metacoder can do similar things to e.g. phyloseq
- Uses the flexible data types defined by taxa
- Plotting is using ggplot2 – graphics
- Your data is organized in tibbles  
(Tidyverse)  
→ easy to use in other analyses

```
> print(obj)
<Taxmap>
1310 taxa: aab. Fungi, aac. Ascomycota ... byj. Rozellomycota_sp, byk. Fungi_sp
1310 edges: NA->aab, aab->aac, aab->aae ... bdh->byi, bdi->byj, bdj->byk
2 data sets:
  tax_data:
    # A tibble: 1,361 x 119
      taxon_id OTUID DB04 DB05 DB06 DB07 DB08 DB09 DB10 DB11 DB12 DB13 DB14 DB15 DB16 DB20
      <chr>   <chr> <dbl> <dbl>
    1 bdk     OTU0778 0     0     0     0     0     0     0     0     0     0     0     0     0     0     0
    2 bdk     OTU1150 0     0     0     0     0     0     0     0     0     0     0     0     0     0     0
    3 bdk     OTU1576 0     0     0     0     0     0     0     0     0     0     0     0     0     0     0
    # ... with 1,358 more rows, and 106 more variables: DB21 <dbl>, DB22 <dbl>,
    # DB26 <dbl>, DB27 <dbl>, DB28 <dbl>, DB32 <dbl>, DB33 <dbl>, DB34 <dbl>,
    # DB38 <dbl>, DB39 <dbl>, ...
  class_data:
    # A tibble: 9,527 x 5
      taxon_id input_index tax_rank name          regex_match
      <chr>       <int>   <chr>   <chr>          <chr>
      1 aab         1       k       Fungi        k_Fungi
      2 aac         1       p       Ascomycota  p_Ascomycota
      3 aao         1       c       Archaeorhizomycetes c_Archaeorhizomycetes
    # ... with 9,524 more rows
  0 functions:
> |
```

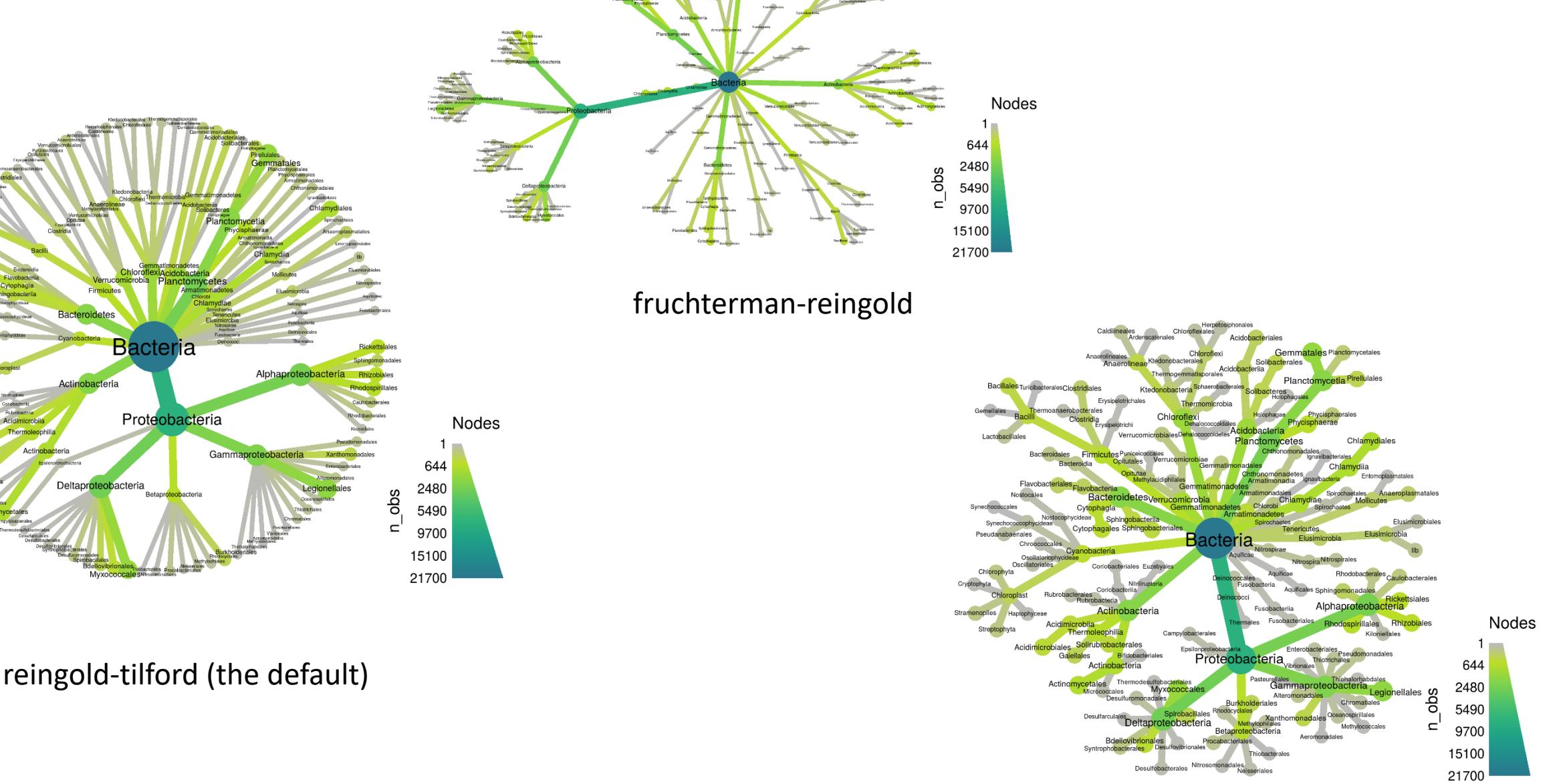
# Visualization of «standard» metabarcoding datasets

- OTU table
- Taxonomy for each OTU
- (Sample metadata)

OTUid	DS31	DS32	DS34	DS37	DS38	DS39	DS47	DS48	DS50	DS52	DS54	DS55	DS58	DS60	DS61	size	taxonomy
OTU0778	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1576	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0517	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0729	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0958	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0730	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	3 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0249	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0788	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU2041	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU2014	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	5 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0282	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	6 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1577	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
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OTU0726	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
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OTU0576	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	14 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0766	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
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OTU0731	0	0	0	1	4	4	0	0	0	0	0	0	0	0	0	0	17 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1072	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0061	0	0	0	0	0	0	0	0	0	0	0	0	8	1	0	0	23 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0768	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0	0	29 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0636	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1192	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	54 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0771	2	0	0	0	3	0	0	0	3	0	4	0	0	0	0	0	55 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0440	3	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	56 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0770	0	0	0	0	0	0	27	10	17	0	0	0	0	0	0	0	58 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU2015	0	0	0	0	0	0	0	0	0	0	0	0	54	0	0	0	62 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU0518	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
OTU1218	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0	0	65 k_Fungi;p_Ascomycota;o_Archaeohizomycetes;o_Archaeohizomycetales;f_
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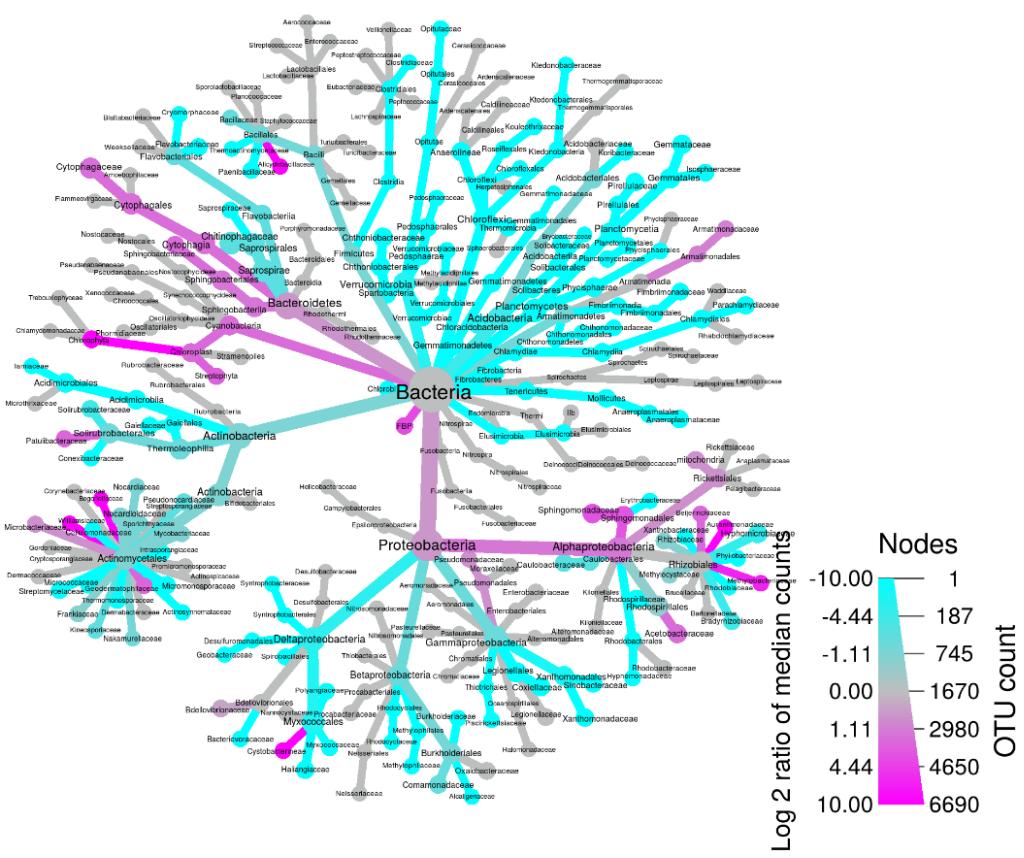
SampleID	Sample	Type	Location	Temperat	Precipit	pH	N	C	C:N	Free_erg	Total_erg
DB04	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB05	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB06	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
DB07	Bistorta	Plantroot	Ves	mid	wet	0.718	0.367	0.223	0.414	0.192	0.524
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DB09	Bistorta	Plantroot	Arh	low	semi-wet	0.385	0.396	0.602	0.742	0.518	0.395
DB10	Bistorta	Plantroot	Arh	low	semi-wet	0.385	0.396	0.602	0.742	0.518	0.395
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DB15	Bistorta	Plantroot	Ulv	alpine	dry	0.663	0.626	0.485	0.402	0.693	0.554
DB16	Bistorta	Plantroot	Ulv	alpine	dry	0.663	0.626	0.485	0.402	0.693	0.554
DB20	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB21	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB22	Bistorta	Plantroot	Lav	alpine	semi-dry	0.744	0.422	0.739	0.774	0.343	0.497
DB26	Bistorta	Plantroot	Air	mid	dry	0.513	0.46	0.629	0.69	0.638	0.487
DB27	Bistorta	Plantroot	Air	mid	dry	0.513	0.46	0.629	0.69	0.638	0.487
DB28	Bistorta	Plantroot	Ram	mid	semi-wet	0.56	0.406	0.686	0.769	0.26	0.572
DB39	Bistorta	Plantroot	Ram	mid	semi-wet	0.56	0.406	0.686	0.769	0.26	0.572
DB40	Bistorta	Plantroot	Ram	mid	semi-wet	0.56	0.406	0.686	0.769	0.26	0.572
DB44	Bistorta	Plantroot	Vik	low	semi-dry	0.194	0.784	0.422	0	0.499	0.52
DB45	Bistorta	Plantroot	Vik	low	semi-dry	0.194	0.784	0.422	0	0.499	0.52
DB46	Bistorta	Plantroot	Fau	low	semi-dry	0.194	0.784	0.422	0	0.499	0.52
DB50	Bistorta	Plantroot	Fau	low	dry	0.56	0.574	0.673	0.597	0.371	0.436
DB51	Bistorta	Plantroot	Fau	low	dry	0.56	0.574	0.673	0.597	0.371	0.436
DB52	Bistorta	Plantroot	Fau	low	dry	0.56	0.574	0.673	0.597	0.371	0.436

# Standard heat tree



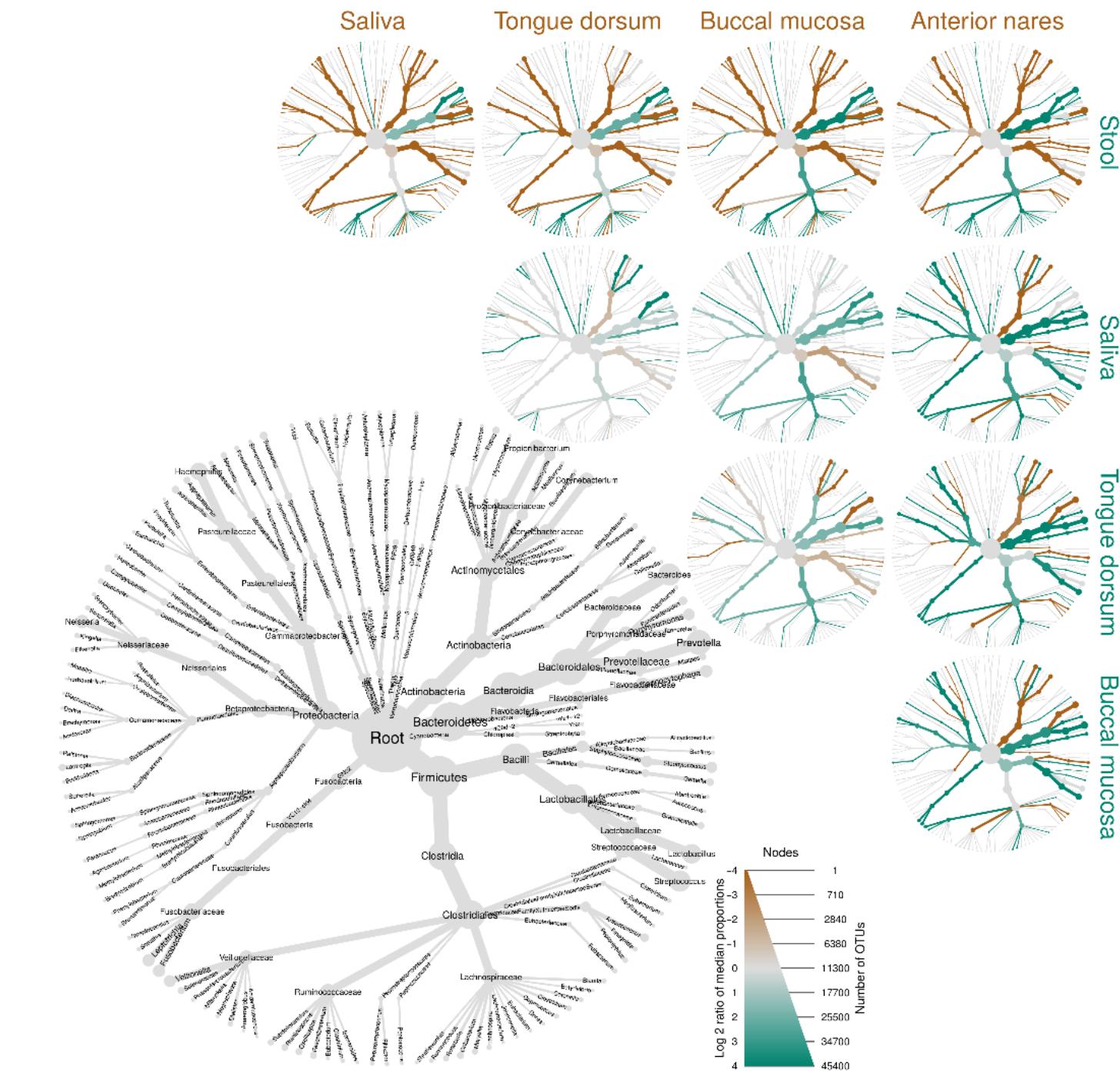
# Comparing two treatments

# leaf vs root samples



compare\_groups()

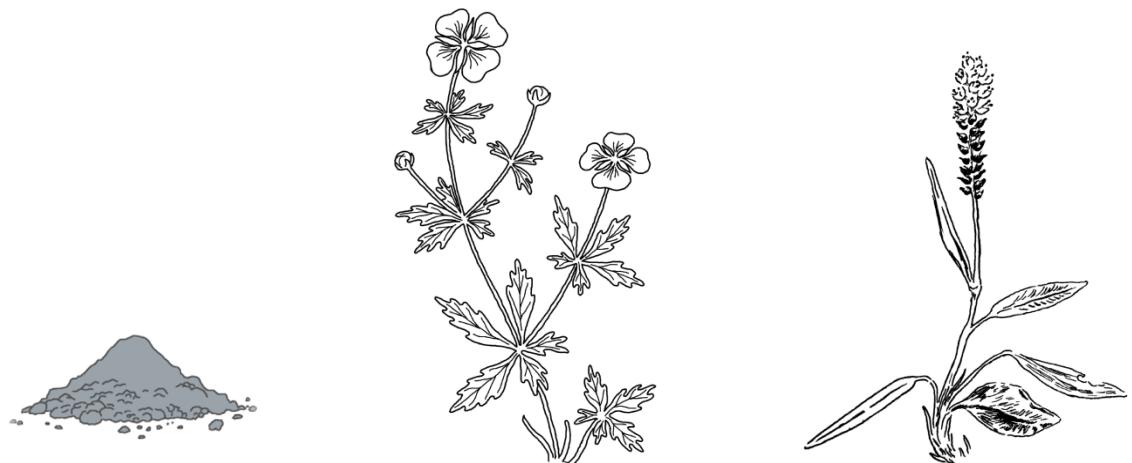
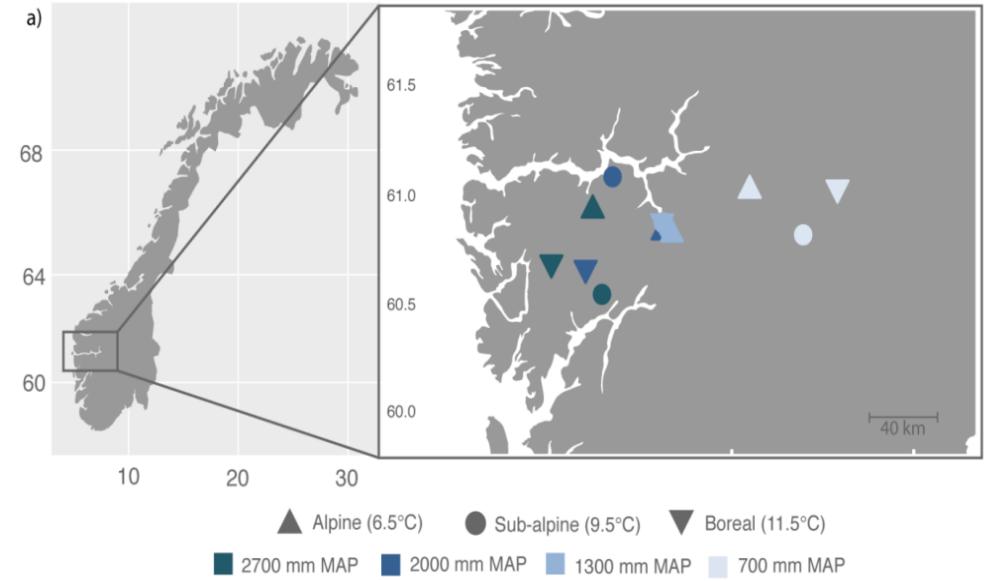
# Comparing three or more treatments



=heat tree matrix

# Case study (for R)

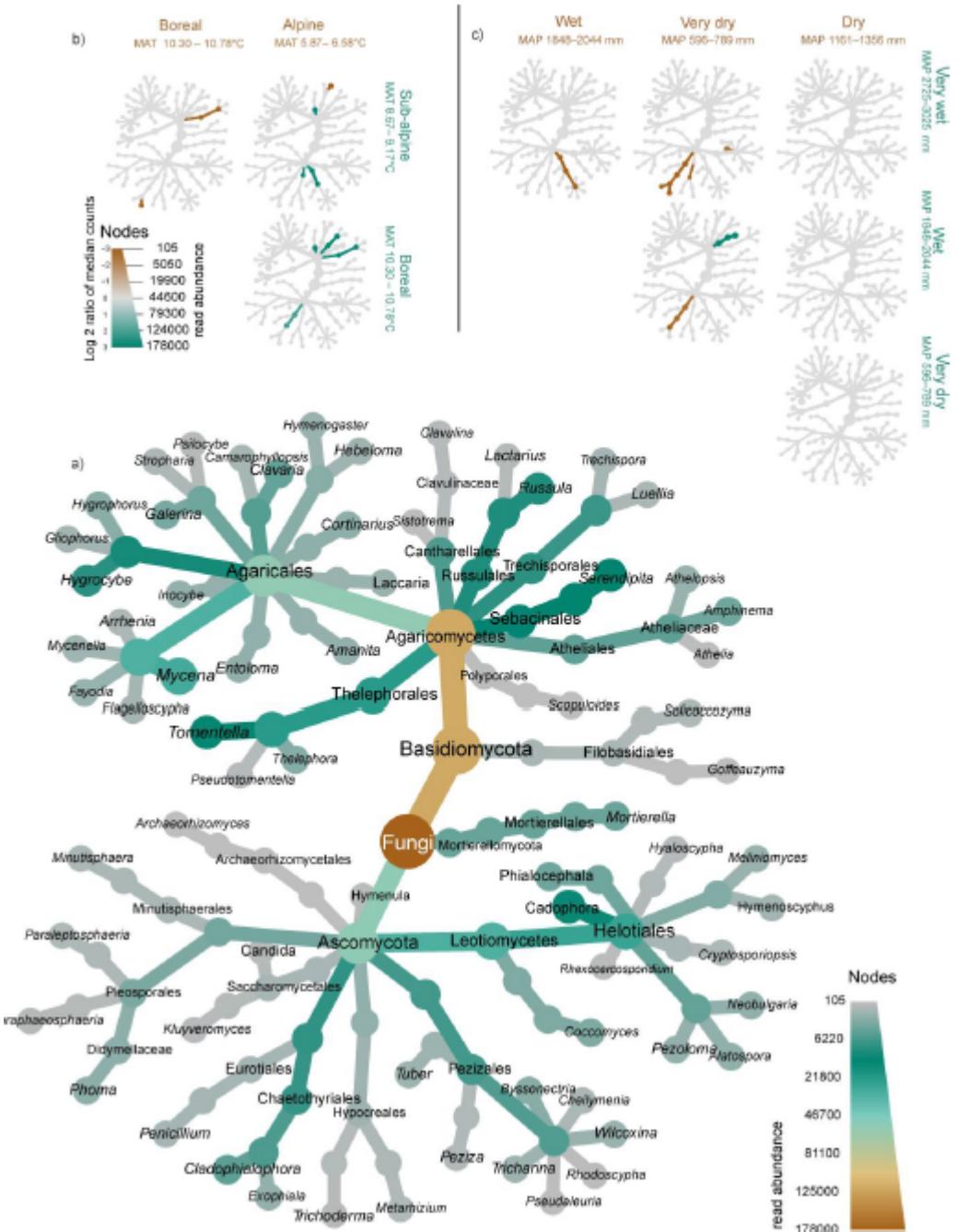
- Three sample types collected over a climate gradient in seminatural grasslands in West Norway
- The dataset has been reduced and modified – here we will only look at how to make heat trees and compare differences in sample type



Soil  
«All» fungi

Potentilla erecta  
AM or non-  
mycorrhizal fungi

Bistorta vivipara  
EcM fungi



# The dataset

Three files:

1. The script called: `IntroMetacoder.R`
2. The OTU table including taxonomic assignment  
`(rarotutable_small.txt)`
3. Sample data containing information for each sample (`sampledata.txt`)

These will be available at the github pages if you wish to try this yourself

# References:

Foster ZSL, Sharpton TJ, Grünwald NJ (2017) Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLOS Computational Biology* 13(2): e1005404.  
<https://doi.org/10.1371/journal.pcbi.1005404>

Kohout P, Charvátová M, Šturssová M. *et al.* (2018) Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *ISME J* 12: 692–703 <https://doi.org/10.1038/s41396-017-0027-3>

Tedersoo L, Bahram M, Põlme S, *et al* (2014) Global diversity and geography of soil fungi. *Science* 346 (6213): 1052-1063. DOI: 10.1126/science.1256688

Thoen (2019) Functional versatility and diversity in the plant root mycobiome. Series of dissertations submitted to the Faculty of Mathematics and Natural Sciences, University of Oslo.

Metacoder documentation: [https://grunwaldlab.github.io/metacoder\\_documentation/index.htm](https://grunwaldlab.github.io/metacoder_documentation/index.htm)