Microbiome: Pooled analysis fungi

Indoor dust bacterial and fungal microbiota composition and allergic diseases: a scoping review

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```
R version 4.2.2 Patched (2022-11-10 r83330)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 20.04.6 LTS

Matrix products: default

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] parallel  stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] report_0.5.8        pals_1.7            RColorBrewer_1.1-3
 [4] phyloseq_1.42.0     vegan_2.6-4         lattice_0.22-5
 [7] permute_0.9-7       lubridate_1.9.2     forcats_1.0.0
[10] stringr_1.5.0       dplyr_1.1.2         purrr_1.0.1
[13] readr_2.1.4         tidyr_1.3.0         tibble_3.2.1
[16] ggplot2_3.4.2       tidyverse_2.0.0     BiocManager_1.30.22
```

Description

A selection of studies included in the scoping review that had available raw sequence data and a comparable dust collection method were identified to retrieve sequence data to undergo processing in the same pipeline, as a proof of concept that pooled analyses could offer insight into the composition of the indoor dust microbiome and to identify potential needs and difficulties for the performance of such pooled microbiome analyses. The type of indoor environment was restricted to dwellings as this was the most common type of indoor environment. A total of **3 studies**(Amin et al., 2022; Fakunle et al., 2023; Vestergaard et al., 2018) following the electrostatic dust collector (EDC) method and **2 studies**(Adams et al., 2020; Hickman et al., 2022) using the petri dish method were selected due to the known comparability of these sample collection methods.(Adams et al., 2015) Additionally, previously unpublished data from a study sampling Dutch households with the EDC method was also included. Of these, 5 studies reported bacterial indoor dust sequences, whereas 4 reported the fungal microbiome.

This report includes the processing and analysis of **fungal ITS** sequences.

Nigerian(Fakunle et al., 2023) and Dutch (unpublished) households

Dutch and Nigerian indoor dust samples follow similar methods of collection through EDC and were processed and analyzed together in the laboratory. These samples have been sequenced for the 16S rRNA combined V5-V6 hypervariable region for bacteria, and the ITS1 region between the 18S and 5.8S rRNA subunits for fungi. After processing, these were compared against the SILVA (bacteria) and UNITE datasets to determine the microbial composition of the samples.

The Dutch samples are from a cross-sectional study aiming to assess environmental factors as determinants of the indoor house dust microbial composition. The study population consisted of households located in Utrecht and surrounding areas of whose inhabitants were invited between April and May 2017 to participate in a survey in which diverse household characteristics, socioeconomic data, and health status variables were collected, as well as airborne dust samples from the household. Participants were instructed to place electrostatic dust collectors at a height of at least 1.25 meters above floor level in the main living room. Out of 600 homes selected to represent different housing characteristics, to which invitations were sent, seventy-nine ultimately provided the answered questionnaires and household dust samples. Only one adult per household provided responses to the questionnaire including their health status and was responsible for collecting and delivering the samples.

The Nigerian samples are from a case-control study of the relationship between indoor airborne dust microbiome and childhood lower respiratory tract infections.(Fakunle et al., 2023)

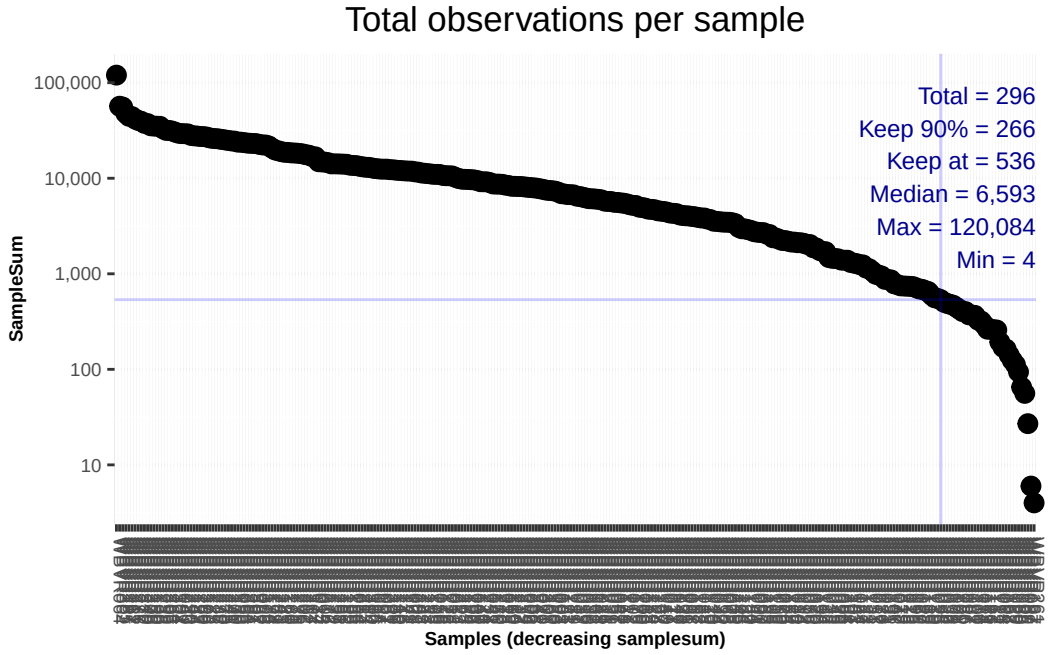
Phyloseq object containing both NL and NG samples + controls:

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13145 taxa and 317 samples ]
sample_data() Sample Data:       [ 317 samples by 55 sample variables ]
tax_table()   Taxonomy Table:    [ 13145 taxa by 7 taxonomic ranks ]
```

Only residential households were kept, since this was similarly done with retrieved samples from studies captured in the literature review to reduce the number of samples to process. This was done under the assumption that quality control has already been done for all published and unpublished studies.

Phyloseq object containing both NL and NG samples, after removing controls:

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 13145 taxa and 296 samples ]
sample_data() Sample Data:       [ 296 samples by 55 sample variables ]
tax_table()   Taxonomy Table:    [ 13145 taxa by 7 taxonomic ranks ]
```



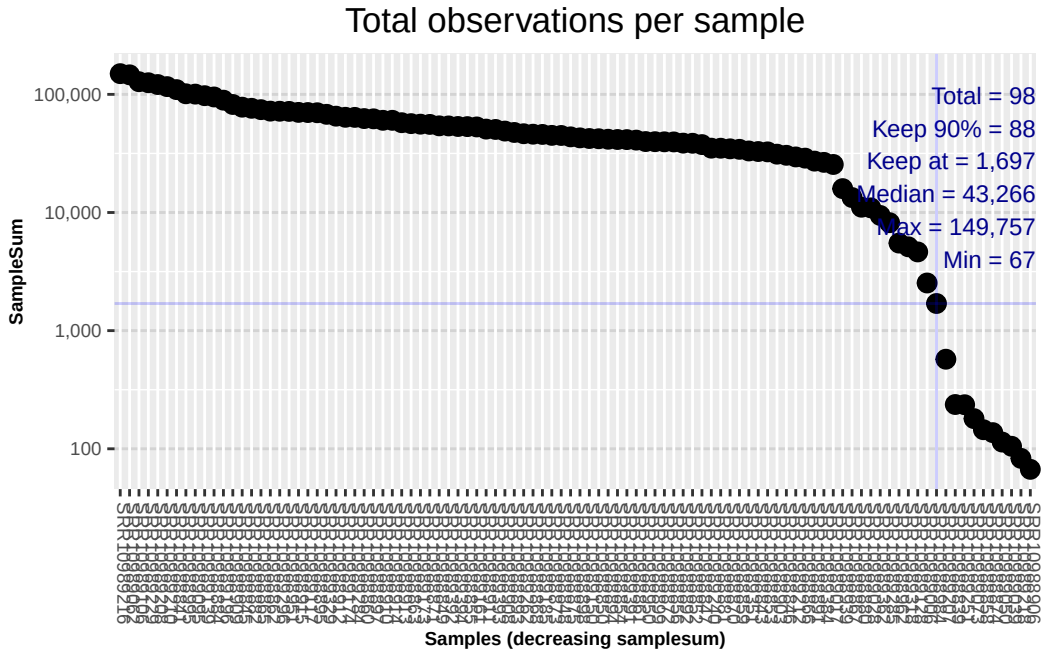
Since there is limited metadata from other studies that would allow comparison by additional environmental determinants or health outcome, this analysis will be limited to a comparison by the country of origin of the samples.

USA households (Adams et al., 2020)

PRJNA603120

Fungal phyloseq object:

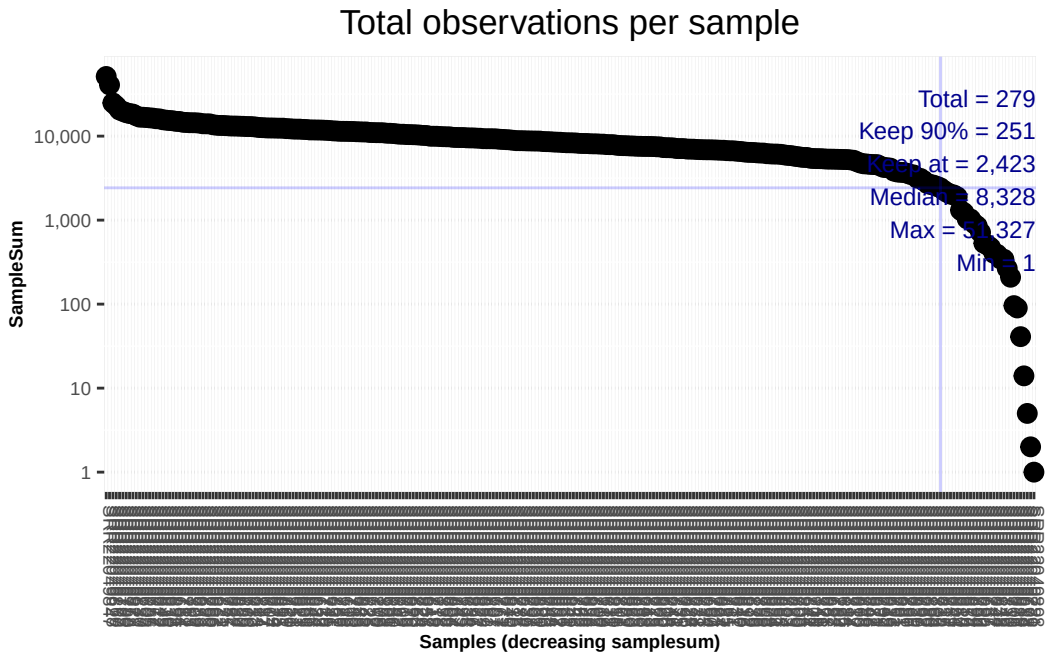
```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 1110409 taxa and 98 samples ]
sample_data() Sample Data:       [ 98 samples by 1 sample variables ]
tax_table()   Taxonomy Table:    [ 1110409 taxa by 8 taxonomic ranks ]
```



Finnish households (Hickman et al., 2022)

PRJNA892469

```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 4789 taxa and 279 samples ]
sample_data() Sample Data:       [ 279 samples by 1 sample variables ]
tax_table()   Taxonomy Table:    [ 4789 taxa by 8 taxonomic ranks ]
```



Merge phyloseq objects

Check if rank names are equal

```
[1] "Kingdom"      "Phylum"       "Class"        "Order"        "Family"
[6] "Genus"        "Species"      "GenusSpecies"
```

```
[1] "Kingdom"      "Phylum"       "Class"        "Order"        "Family"
[6] "Genus"        "Species"      "GenusSpecies"
```

```
[1] "Kingdom" "Phylum"  "Class"   "Order"   "Family"  "Genus"   "Species"
```

The GenusSpecies rank is missing in the NL and NG data. I will remove it and check if they are not equal:

```
[1] TRUE
```

Merge into a single phyloseq object

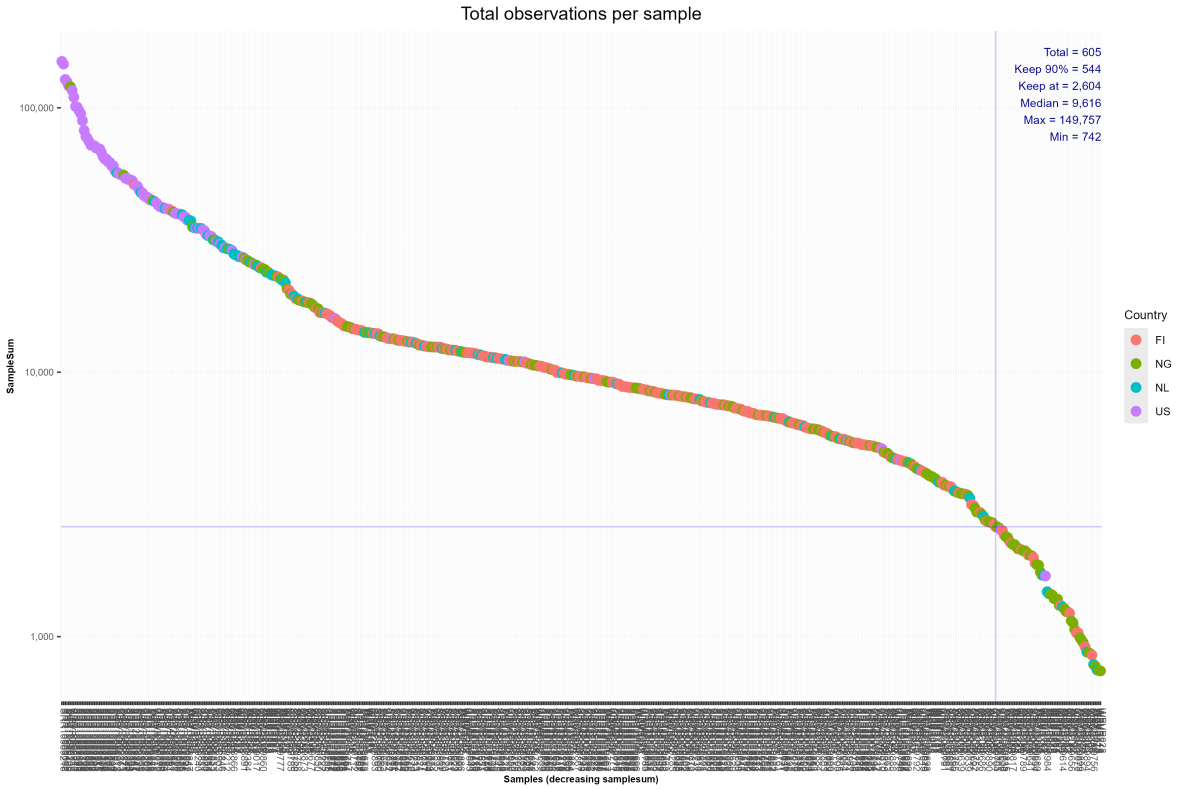
Save unrarefied data:

```
saveRDS(
  fungi,
  paste0(psfolder,"/fungi_combined.rds")
)
```

Sample sums plot:

```
sample_sum_plot(fungi, color = "Country", percentKeep = 90)

ggsave(
  filename = "sample_sum_plot.png",
  path = figfolder,
  width = 12,
  height = 8,
  units = "in",
  dpi = 300
)
```



I will keep 90% of samples.

```
saveRDS(
  fungi,
```

```
  paste0(psfolder,"/fungi_keep.rds")
)
```

The construction of rarefaction curves was done with the following script:

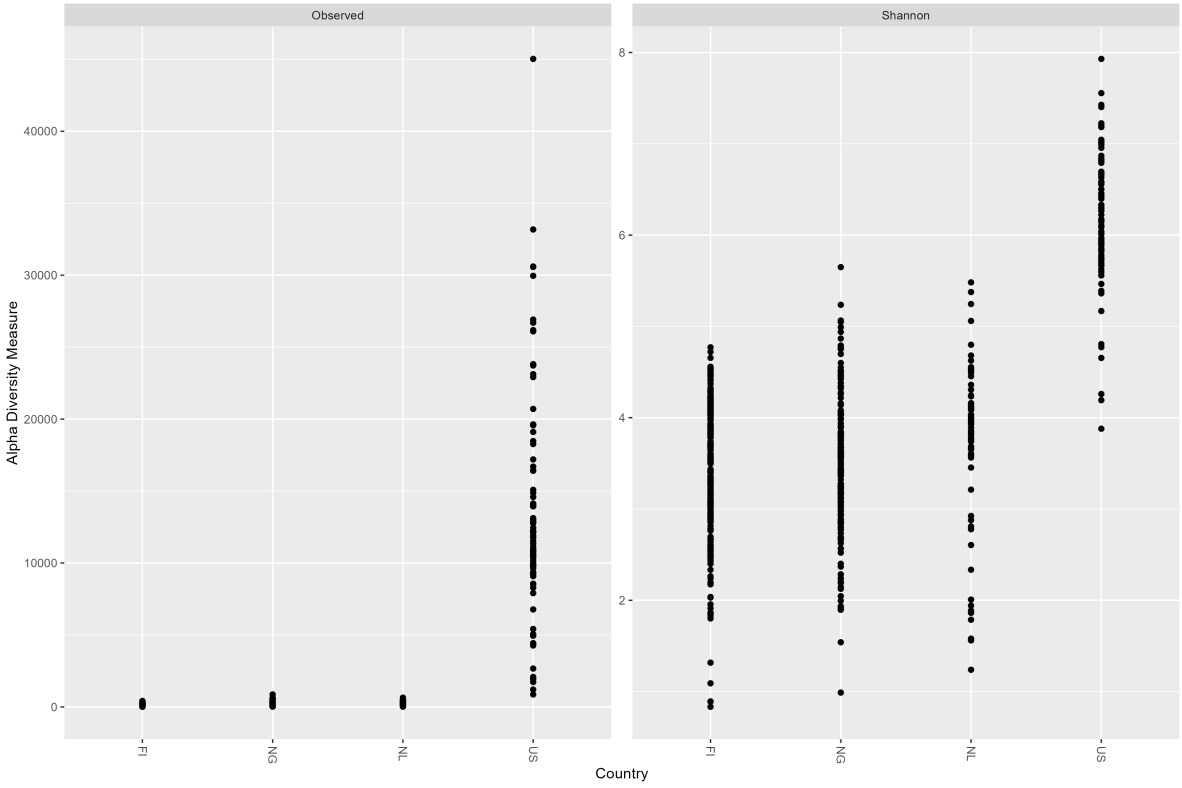
```
source("scripts/rarefaction_plot_fungi.R")
```

I will rarefy at 2,500 which leads to loosing close to ~10% (n=57) of samples.

```
source("scripts/rarefy_fungi.R")
```

After multiple attempts to rarefy, I was not able to do it. Thus, I will continue with the analyses on the unrarefied data.

Alpha diversity



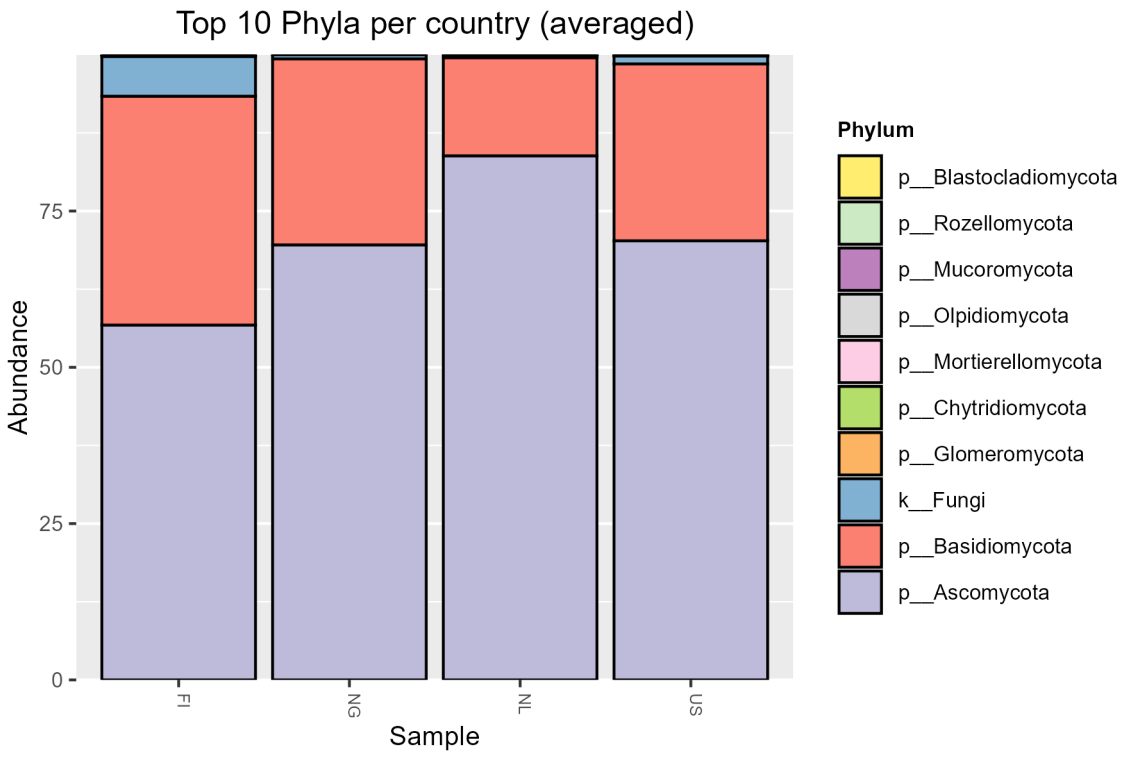
Relative abundance

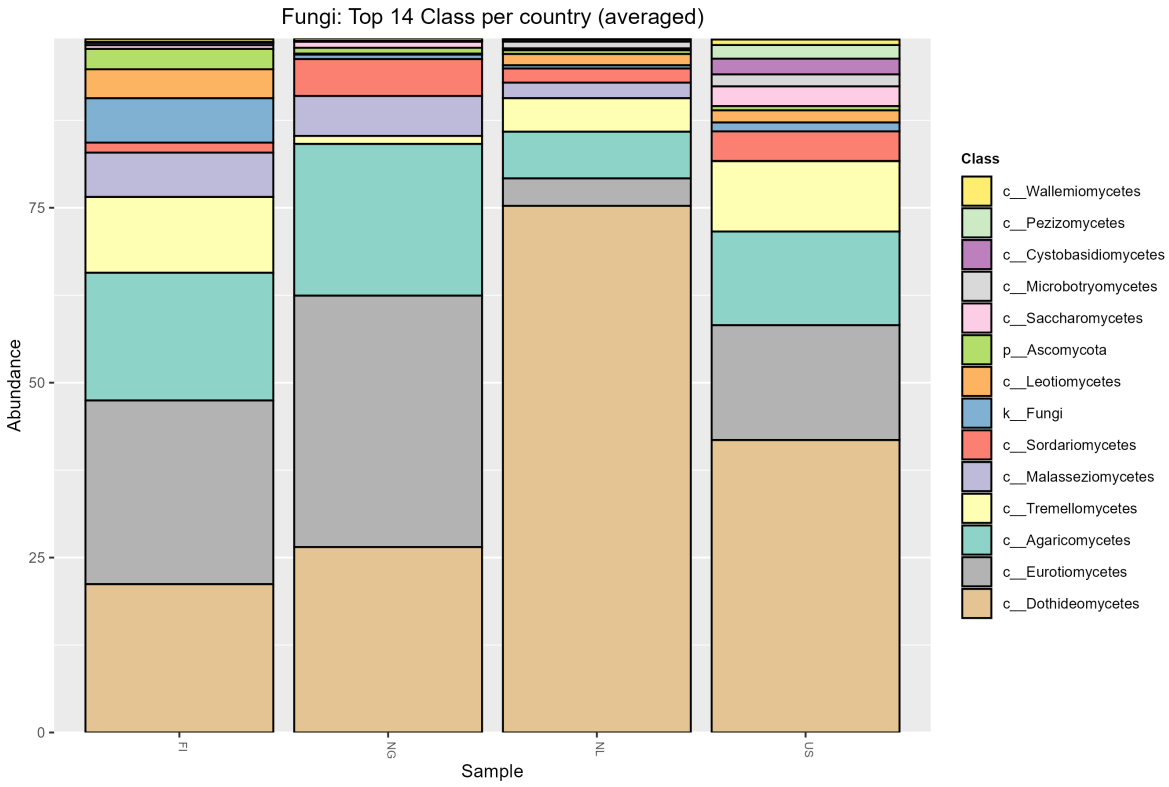
Convert to relative abundance with the following script:

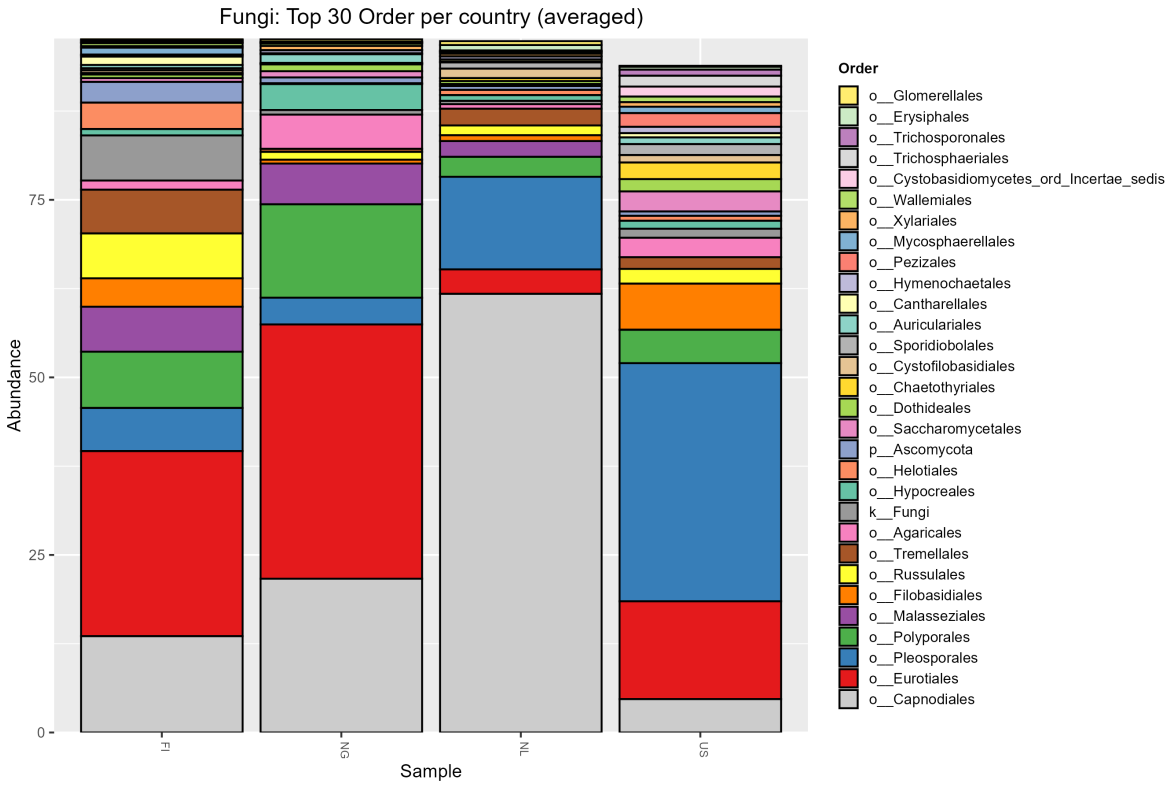
```
source("scripts/relative_abundance_fungi.R")
```

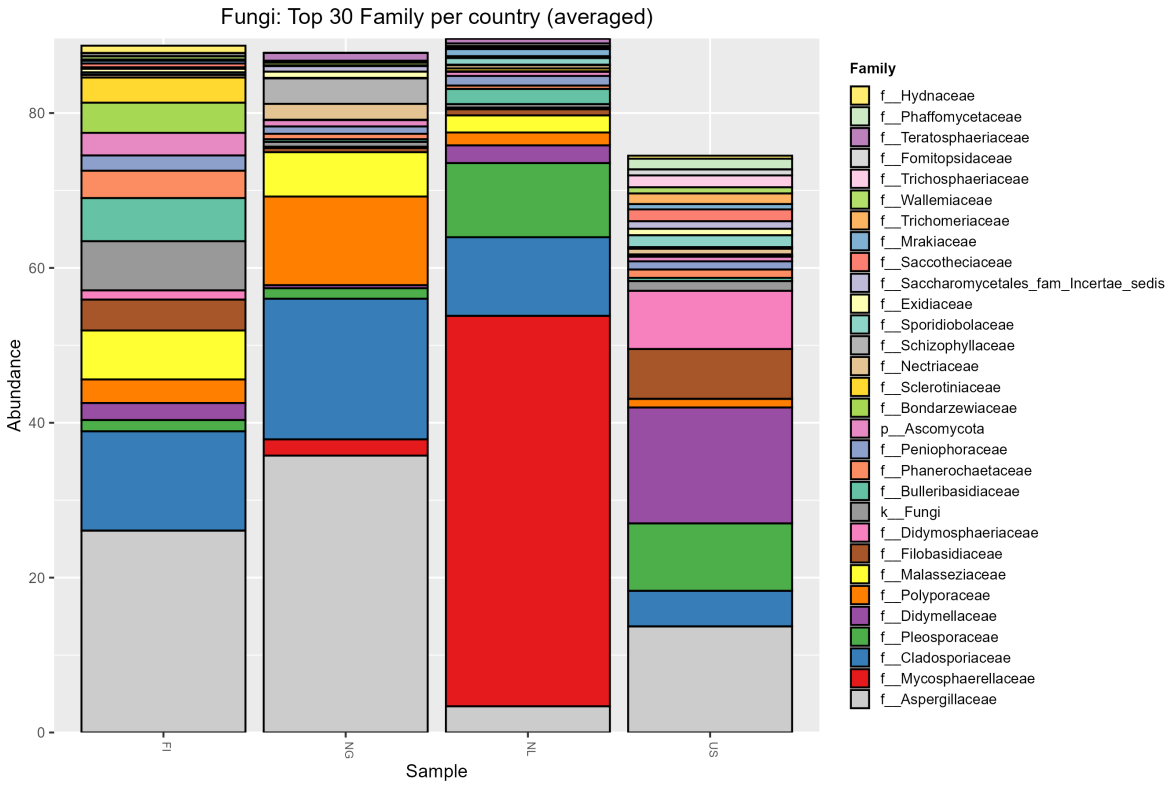
The figures were generated with the following script:

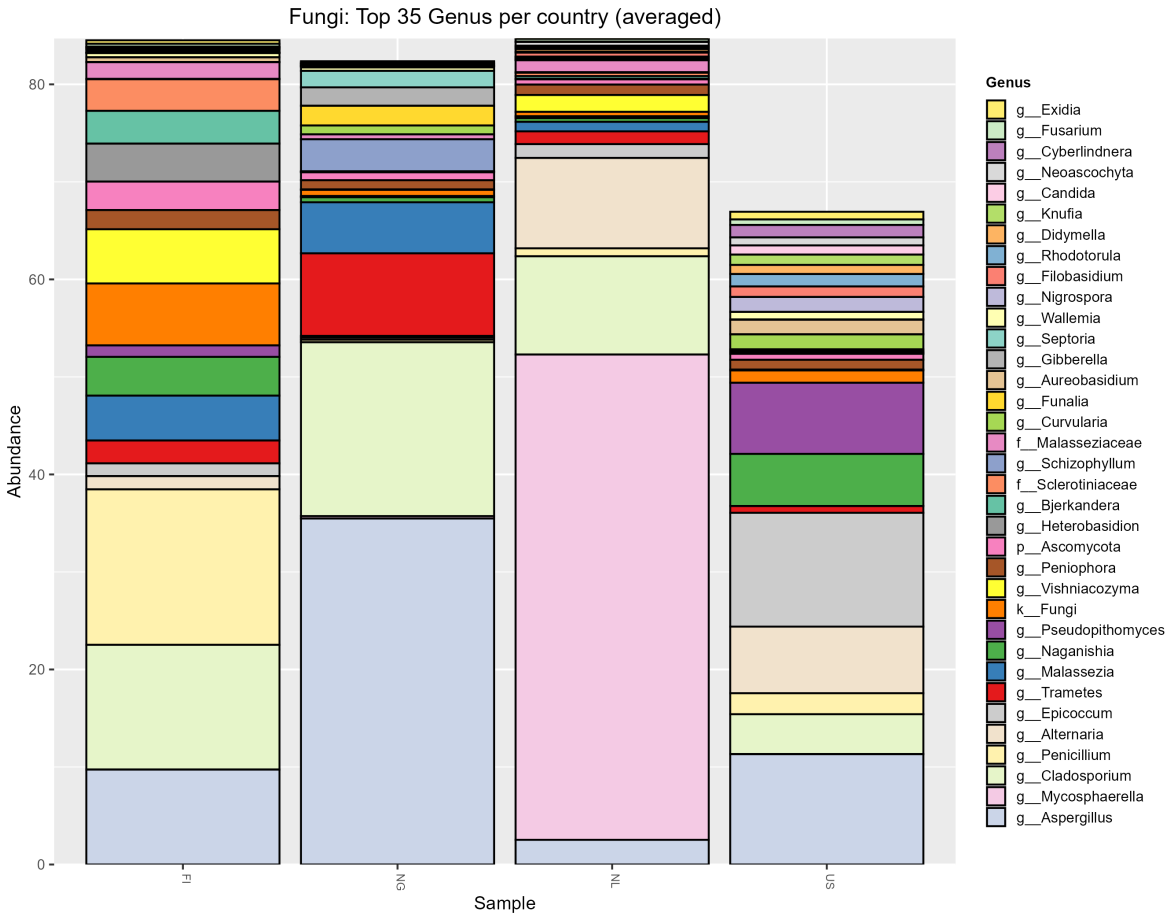
```
source("scripts/relative_abundance_plots_fungi.R")
```




Fungi: Top 14 Class per country (averaged)
Abundance
0
25
50
75
FI
NG
NL
US
Sample
Class
c__Wallemiomycetes
c__Pezizomycetes
c__Cystobasidiomycetes
c__Microbotryomycetes
c__Saccharomycetes
p__Ascomycota
c__Leotiomycetes
k__Fungi
c__Sordariomycetes
c__Malasseziomycetes
c__Tremellomycetes
c__Agaricomycetes
c__Eurotiomycetes
c__Dothideomycetes


Fungi: Top 30 Order per country (averaged)
Abundance
0
25
50
75
FI
NG
NL
US
Sample
Order
o__Glomerellales
o__Erysiphales
o__Trichosporonales
o__Trichosphaeriales
o__Cystobasidiomycetes_ord_Incertae_sedis
o__Wallemiales
o__Xylariales
o__Mycosphaerellales
o__Pezizales
o__Hymenochaetales
o__Cantharellales
o__Auriculariales
o__Sporidiobolales
o__Cystofilobasidiales
o__Chaetothyriales
o__Dothideales
o__Saccharomycetales
p__Ascomycota
o__Helotiales
o__Hypocreales
k__Fungi
o__Agaricales
o__Tremellales
o__Russulales
o__Filobasidiales
o__Malasseziales
o__Polyporales
o__Pleosporales
o__Eurotiales
o__Capnodiales


Fungi: Top 30 Family per country (averaged)
Abundance
0
20
40
60
80
FI
NG
NL
US
Sample
Family
f__Hydnaceae
f__Phaffomycetaceae
f__Teratosphaeriaceae
f__Fomitopsidaceae
f__Trichosphaeriaceae
f__Wallemiaceae
f__Trichomeriaceae
f__Mrakiaceae
f__Saccotheciaceae
f__Saccharomycetales_fam_Incertae_sedis
f__Exidiaceae
f__Sporidiobolaceae
f__Schizophyllaceae
f__Nectriaceae
f__Sclerotiniaceae
f__Bondarzewiaceae
p__Ascomycota
f__Peniophoraceae
f__Phanerochaetaceae
f__Bulleribasidiaceae
k__Fungi
f__Didymosphaeriaceae
f__Filobasidiaceae
f__Malasseziaceae
f__Polyporaceae
f__Didymellaceae
f__Pleosporaceae
f__Cladosporiaceae
f__Mycosphaerellaceae
f__Aspergillaceae


Fungi: Top 35 Genus per country (averaged)
Abundance
0
20
40
60
80
FI
NG
NL
US
Sample
Genus
g__Exidia
g__Fusarium
g__Cyberlindnera
g__Neoascochyta
g__Candida
g__Knufia
g__Didymella
g__Rhodotorula
g__Filobasidium
g__Nigrospora
g__Wallemia
g__Septoria
g__Gibberella
g__Aureobasidium
g__Funalia
g__Curvularia
f__Malasseziaceae
g__Schizophyllum
f__Sclerotiniaceae
g__Bjerkandera
g__Heterobasidion
p__Ascomycota
g__Peniophora
g__Vishniacozyma
k__Fungi
g__Pseudopithomyces
g__Naganishia
g__Malassezia
g__Trametes
g__Epicoccum
g__Alternaria
g__Penicillium
g__Cladosporium
g__Mycosphaerella
g__Aspergillus

Beta diversity

PCoA plot will be generated with the following code:

```
source("scripts/ordination_plots_fungi.R")
```

Process takes too long and I have not been able to create ordination figures. Thus, I will keep the analysis up to here.

Package References

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Study References

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