

# 03\_fungi

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## 0. Data importation & functions loading

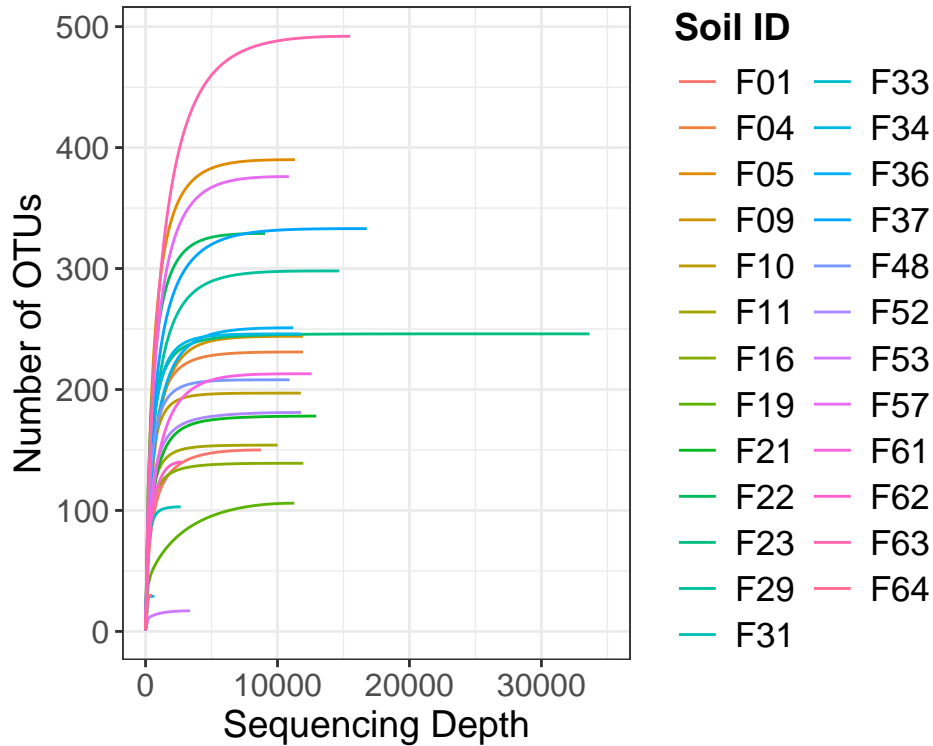
In this step we create the phyloseq object that contains the following data: 1. OTU counts 2. OTU Taxonomy 3. Design file

### 0.1 OTU filtering and TSS scaling

All (operational taxonomic units) OTUs that were not verified as fungi by the ITSx software (Bengtsson-Palme et al., 2013) as well as those with a count of less than five across all samples were excluded from further analysis.

## 1. Rarefaction analysis

Rarefaction analysis confirmed that a sufficient sequencing depth had been reached to capture fungal diversity in most samples



## 2. Mapping the inoculum with native soil fungi.

First, we asked if we could find the inoculated *C. tenuissimum* or closely related sequences in the tested soils.

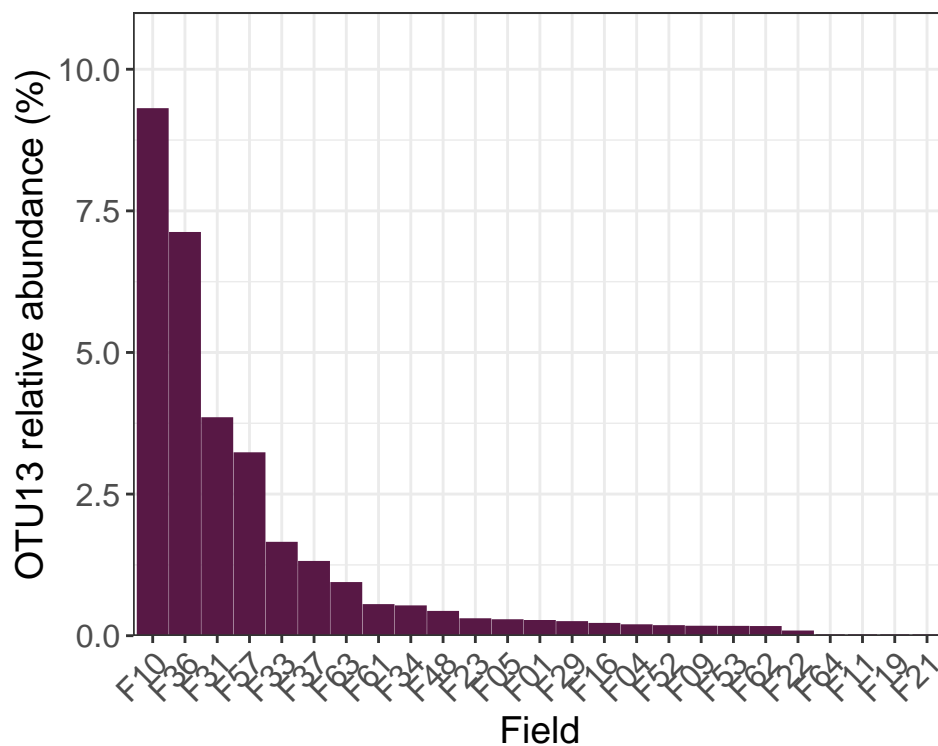
### 2.1 *C. tenuissimum* sanger sequence

### 2.2 Sequence mapping with soil fungal OTUs present in the soils before inoculation.

```
## [1] "Cladosporium mapped to: OTU13 with a similarity of: 99.62%"
```

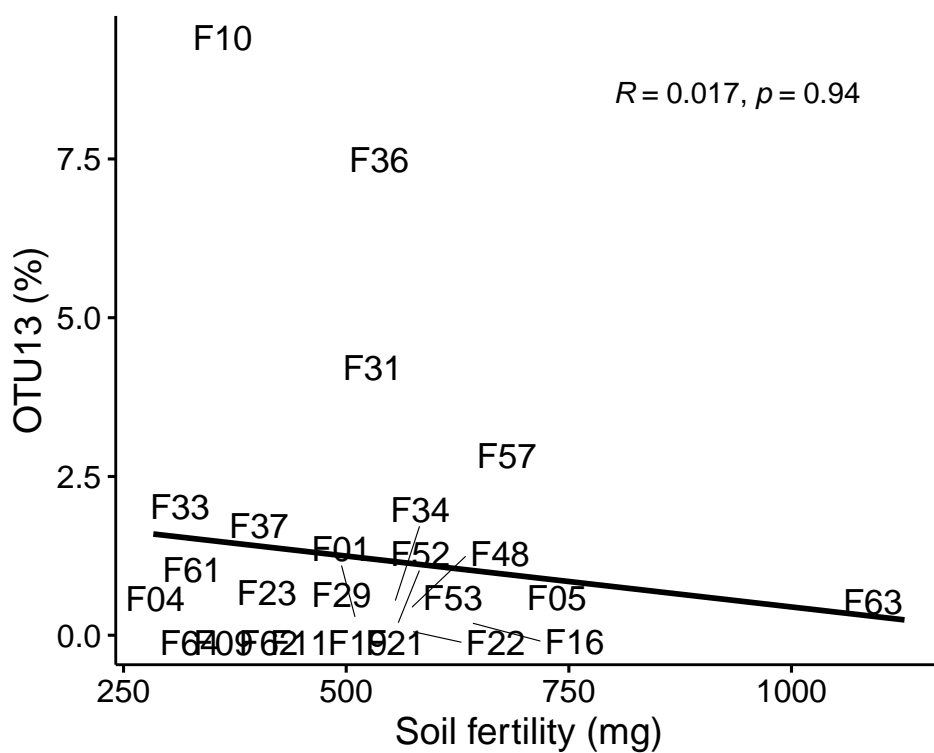
### 2.3 Figure S6A

The abundance of OTU13 ranged from absent to 9.3% relative abundance with presences  $>1\%$  in ca. 1/4 and  $<1\%$  in ca. 3/4 of the soils.



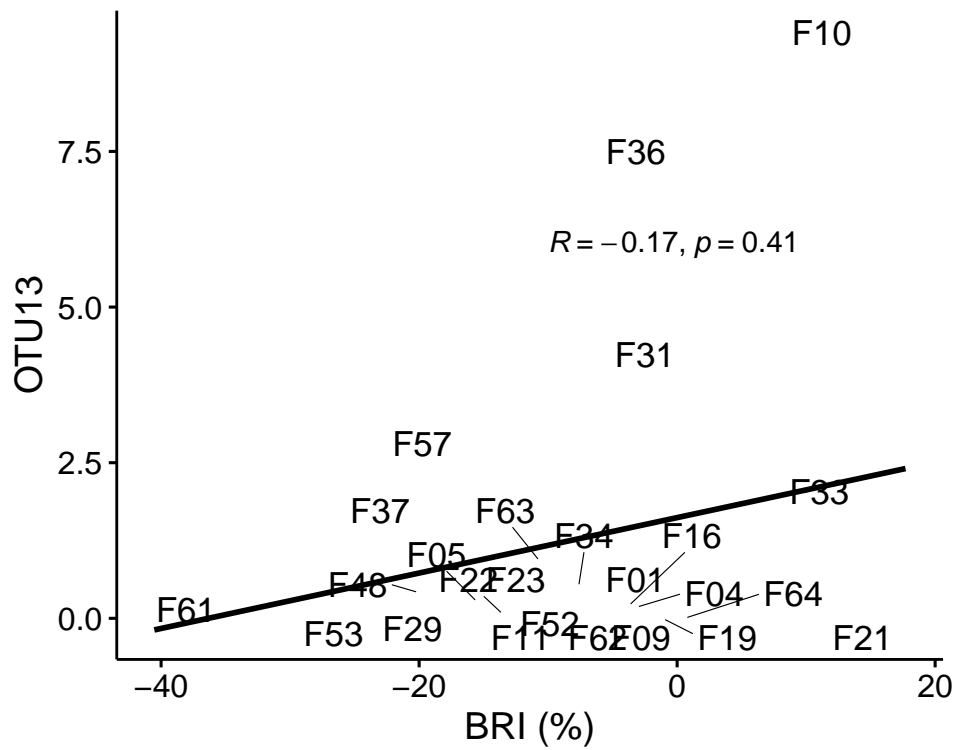
## 2.4 Figure S6B

OTU13 abundance did not correlate with soil fertility



## 2.5 Figure S6C

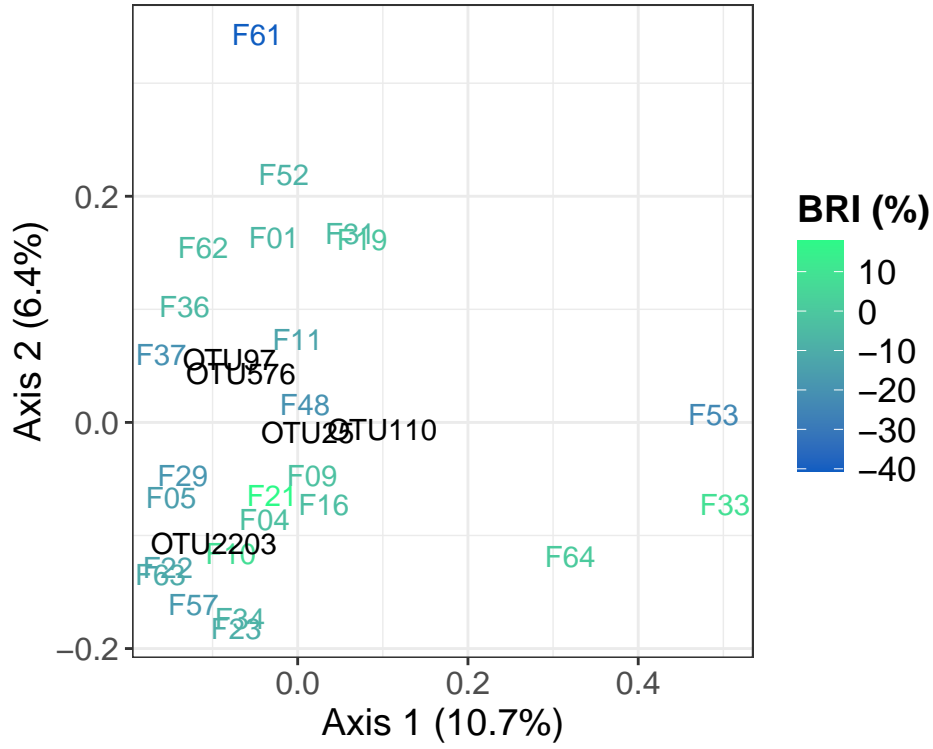
OTU13 abundance did not correlate with BRI



## 3. PCoA with all soil fungal OTUs

### 3.1 Figure 4A

We examined the relationship between soil fungal community composition and BRI using Principal Coordinate Analysis (PCoA), which revealed no clustering of fungal communities with the degree of BRI



### 3.2 PERMANOVA

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = distance_matrix ~ BRI$'BRI (%)')
##          Df SumOfSqs      R2      F Pr(>F)
## BRI$'BRI (%)'  1  0.3459 0.04696 1.1334 0.142
## Residual      23  7.0184 0.95304
## Total         24  7.3643 1.00000
```

### 3.3 Table S7

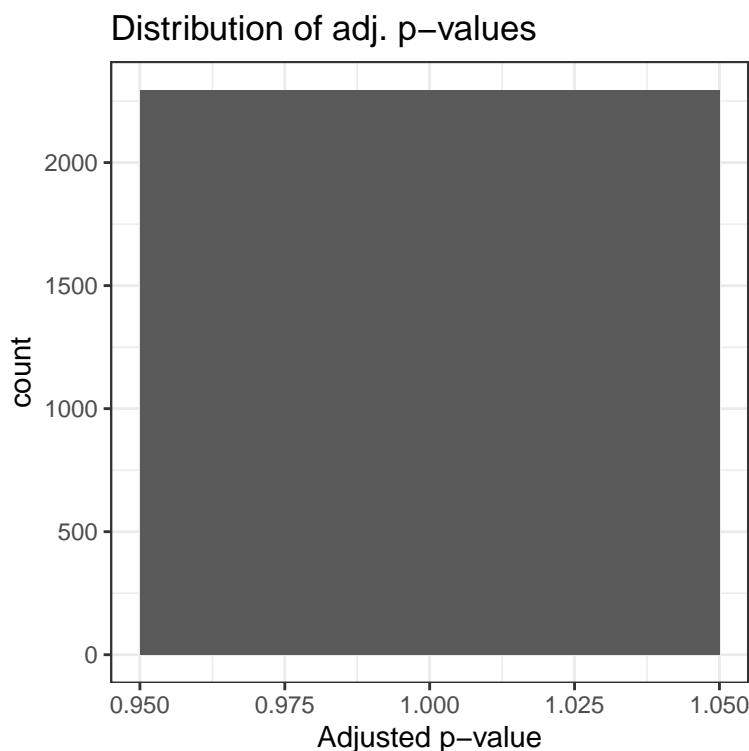
This was supported by permutational multivariate analysis of variance (PERMANOVA), which confirmed no significant relationship between BRI and soil fungal community composition

Table S7

	Df	SumOfSqs	R2	F	Pr(>F)
BRI\$BRI (%)	1	0.3458634	0.0469648	1.133421	0.142
Residual	23	7.0184480	0.9530352	NA	NA
Total	24	7.3643114	1.0000000	NA	NA

## 4. Pairwise correlation BRI ~ soil fungi

After p-value adjustment, none of the Spearman pairwise correlation is significant.



## 5. Random Forest feature selection

As with the analysis of physicochemical soil parameters, we reduced the number of soil fungal taxa for further analysis (identification of OTUs predictive for BRI using Random Forest).

### 5.1 Table S8

Table S8

Kingdom	Phylum	Class	Order	Family	Genus
OTU25 Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusicolla
OTU576 Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria
OTU543 NA	NA	NA	NA	NA	NA
OTU241 NA	NA	NA	NA	NA	NA
OTU14 Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	Pyronemataceae_gen_Incertae_sedis
OTU5 Fungi	Zoopagomycota	Zoopagomycetes	Zoopagales	Piptocephalidaceae	Syncephalis
OTU2 Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium
OTU3 Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	NA
OTU220 Fungi	Ascomycota	Sordariomycetes	Glomerellales	Plectosphaerellaceae	Acrestalagmus
OTU287 NA	NA	NA	NA	NA	NA
OTU17 Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella

	Kingdom	Phylum	Class	Order	Family	Genus
OTU305	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Lycoperdaceae	Lycoperdon
OTU4	Fungi	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	Pseudeurotium
OTU97	Fungi	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	NA
OTU15	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Talaromyces
OTU163	Fungi	Chytridiomycota	Rhizophyidiomycetes	Rhizophydiales	Rhizophydiaceae	Rhizophydiaceae_gen_Incertae_sedis
OTU110	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Cladosporiaceae	Cladosporium
OTU47	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae	Talaromyces
OTU11	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium
OTU10	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella

## 6. Glmulti for feature selection

Again, we screened for the best OLS model based on reduced sets of soil fungi

```
##
## Call:
## fitfunc(formula = as.formula(x), data = data, trace = ..1)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -14.788  -2.715   1.584   3.547  11.667
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -10.641      2.290  -4.646 0.000176 ***
## OTU25         2.465      1.003   2.457 0.023782 *
## OTU576       -71.760     26.474  -2.711 0.013869 *
## OTU2203      346.930    106.735   3.250 0.004210 **
## OTU97        -13.635      6.426  -2.122 0.047226 *
## OTU110        8.770      3.015   2.908 0.009015 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 6.524 on 19 degrees of freedom
## Multiple R-squared:  0.757, Adjusted R-squared:  0.6931
## F-statistic: 11.84 on 5 and 19 DF, p-value: 2.658e-05
```

### 6.1 Normality assumptions

Assessing the residuals normality assumptions the produced model. The best model is not fully respecting the residuals normality assumptions. These values are aggregated into the Table S5 for each model.

```
## -----
##      Test           Statistic      pvalue
## -----
## Shapiro-Wilk         0.9001        0.0185
## Kolmogorov-Smirnov    0.1605        0.4906
## Cramer-von Mises      2.0168        0.0000
## Anderson-Darling      1.0325        0.0084
## -----
```

## 6.2 Predictor relative importance

The relative importance of each predictor in the previously selected model is computed in this step. These values are then used to complete the figure 4B in illustrator.

## 6.3 Table S9

Summary of the model coefficients and relative importance.

Table S9

Parameter	Coefficient	Relative importance
OTU25	2.465479	0.0675290
OTU110	8.769654	0.1364049
OTU2203	346.929572	0.1538133
OTU97	-13.635160	0.1711882
OTU576	-71.760013	0.2280798
(Intercept)	-10.640738	NA

## 6.4 Figure 4B



## 7. Similarity between OTU110 (Cladosporium selected by the model) and OTU13 C. tenuissimum

```
## [1] "Cladosporium (OTU110) and Cladosporium (OTU13) sequences share a similarity of: 97.62%"
```