

First experimental evidence for active farming in ambrosia beetles and strong heredity of garden microbiomes - Fungal Analysis

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Data Preparation

load required packages

```
library(lme4)
library(permute)
library(lattice)
library(vegan)
library(phyloseq)
library(ggplot2)
library(dplyr)
library(scales)
library(grid)
library(DHARMa)
library(ggeffects)
library(glmmTMB)
library(lmerTest)
library(emmeans)
library(sjPlot)
library(fitdistrplus)
library(GLMMadaptive)
library(microbiome)
library(microbiomeutilities)
library(knitr)
library(ggpubr)
library(doBy)
library(performance)
library(see)
library(patchwork)
library(pairwiseAdonis)
library(cowplot)
library(multcomp)
library(car)
library(forcats)
library(ggrepel)
library(tidyverse)
```

loading the data files for fungi

```
dataLSU <- otu_table(read.table("28S_Rem_zotu_table.txt", sep="\t", header=T, row.names=1, check.names =  
taxLSU <- tax_table(as.matrix(read.table("28S_Rem_zotus97.tax.txt", sep="\t", header=T, row.names=1, fi  
datasampleLSU <- sample_data(read.table("28S_map_removal.txt", sep="\t", header=T, row.names=1)))
```

merge data into phyloseq object

```
(allLSU <- merge_phyloseq(dataLSU, taxLSU, datasampleLSU))
```

```
## phyloseq-class experiment-level object  
## otu_table() OTU Table: [ 212 taxa and 57 samples ]  
## sample_data() Sample Data: [ 57 samples by 16 sample variables ]  
## tax_table() Taxonomy Table: [ 212 taxa by 8 taxonomic ranks ]
```

copy taxonomic classification in tax_table columns with gaps and add "__spc"

```
dataset.LSU = subset_taxa(allLSU, (Kingdom == "k:Fungi"))  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Phylum"]=="", "Phylum"] <- paste(tax_table(dataset.LSU)[tax_  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Class"]=="", "Class"] <- paste(tax_table(dataset.LSU)[tax_  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Order"]=="", "Order"] <- paste(tax_table(dataset.LSU)[tax_  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Family"]=="", "Family"] <- paste(tax_table(dataset.LSU)[tax_  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Genus"]=="", "Genus"] <- paste(tax_table(dataset.LSU)[tax_  
tax_table(dataset.LSU)[tax_table(dataset.LSU)[,"Species"]=="", "Species"] <- paste(tax_table(dataset.LSU)[
```

start filtering out all ZOTUs that were only assigned to Kingdom (Fungi)

excluding all taxa assigned to Fungi_spc

```
dataset.LSU.ordi = subset_taxa(dataset.LSU, (Kingdom == "k:Fungi"))  
dataset.LSU.ordi = subset_taxa(dataset.LSU.ordi, Phylum != "k:Fungi_spc")
```

check all the columns for patterns ranging from [a-z] joined by ____ like this [a-z]____ and substitute it with "" i.e. nothing.

```
tax_table(dataset.LSU.ordi)[, colnames(tax_table(dataset.LSU.ordi))] <- gsub(tax_table(dataset.LSU.ordi)  
tax_table(dataset.LSU.ordi)[, colnames(tax_table(dataset.LSU.ordi))] <- gsub(tax_table(dataset.LSU.ordi)  
tax_table(dataset.LSU.ordi)[, colnames(tax_table(dataset.LSU.ordi))] <- gsub(tax_table(dataset.LSU.ordi)  
tax_table(dataset.LSU.ordi)[, colnames(tax_table(dataset.LSU.ordi))] <- gsub(tax_table(dataset.LSU.ordi)  
tax_table(dataset.LSU.ordi) <- tax_table(dataset.LSU.ordi)[,2:8]
```

Format the phyloseq object to add the best taxonomy in phyloseq object (tax_table and otu_table).

```
dataset.LSU.ordi <- format_to_besthit(dataset.LSU.ordi)
taxa_names(dataset.LSU.ordi)[1:5]
```

```
## [1] "LSUzotu2:Raffaelea_sulphurea" "LSUzotu7:Raffaelea_sulphurea"
## [3] "LSUzotu1:Chaetomium_globosum" "LSUzotu12:Raffaelea_sulphurea"
## [5] "LSUzotu4:Raffaelea_canadensis"
```

check the table for total reads per sample

```
colSums(otu_table(dataset.LSU.ordi))
```

```
##      B0-19a      B0-19b      B0-22a      B0-22b      B0-24      B0-27      B10-01      B10-06
##      8655      19999      19671      37621      27652      16442      24646      26723
##      B10-09a      B10-09b      B10-18      B10-24      B10-25a      B10-25b      B10-26a      B15-01a
##      26663      39932      6770      10235      24348      14123      26861      12155
##      B15-01b      B15-06      B15-07a      B15-07b      B15-11      B15-20      B15-24      B15-25a
##      24509      2378      19916      9950      19307      14350      21346      8108
##      B15-25b      B16-01a      B16-04      B16-31a      B16-31b      B20-04a      B20-08a      B20-08b
##      27304      20358      7521      15823      23816      34274      27572      19430
##      B20-11      B23-21      B23-23a      B24-02a      B24-26a      B24-26b      B24-28a      B24-29
##      8395      27931      26875      1087      16017      10464      19882      7041
##      B36-14a      B36-14b      B36-30      B36-31a      B36-32      B39-15      B39-16      B39-17a
##      8615      25211      8240      17279      1092      12342      11854      12233
##      B46-03      B7-07      B7-08a      medium1      medium2      negative2      negative1      mock
##      21081      5815      4694      11      98      41      259      23868
##      standard
##      26
```

no losses

check controls from dataset: only ten most abundant ZOTUs are picked for visualisation prae decontam

```
neg.controls<-subset_samples(dataset.LSU.ordi, Treatment=="medium" | Treatment=="negative")
sample_names(neg.controls)
```

```
## [1] "medium1" "medium2" "negative2" "negative1"
```

visualisation of negative controls

```
filtaxa <- names (sort(rowSums(otu_table(neg.controls)),decreasing=T))[1:10]
tax_table(dataset.LSU.ordi)[filtaxa]
```

```
## Taxonomy Table:      [10 taxa by 8 taxonomic ranks]:
##                                Domain  Phylum      Class
## LSUzotu1:Chaetomium_globosum      "Fungi" "Ascomycota" "Sordariomycetes"
```

## LSUzotu2:Raffaelea_sulphurea	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu87:Cladosporium_sp._CHTAE11	"Fungi"	"Ascomycota"	"Dothideomycetes"
## LSUzotu26:Chaetomium_spc	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu25:Raffaelea_sulphurea	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu159:Raffaelea_sulphurea	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu4:Raffaelea_canadensis	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu72:Raffaelea_sulphurea	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu6:Sporothrix_stenoceras	"Fungi"	"Ascomycota"	"Sordariomycetes"
## LSUzotu130:Chaetomiaceae_spc	"Fungi"	"Ascomycota"	"Sordariomycetes"
##	Order	Family	
## LSUzotu1:Chaetomium_globosum	"Sordariales"	"Chaetomiaceae"	
## LSUzotu2:Raffaelea_sulphurea	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu87:Cladosporium_sp._CHTAE11	"Capnodiales"	"Cladosporiaceae"	
## LSUzotu26:Chaetomium_spc	"Sordariales"	"Chaetomiaceae"	
## LSUzotu25:Raffaelea_sulphurea	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu159:Raffaelea_sulphurea	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu4:Raffaelea_canadensis	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu72:Raffaelea_sulphurea	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu6:Sporothrix_stenoceras	"Ophiostomatales"	"Ophiostomataceae"	
## LSUzotu130:Chaetomiaceae_spc	"Sordariales"	"Chaetomiaceae"	
##	Genus		
## LSUzotu1:Chaetomium_globosum	"Chaetomium"		
## LSUzotu2:Raffaelea_sulphurea	"Raffaelea"		
## LSUzotu87:Cladosporium_sp._CHTAE11	"Cladosporium"		
## LSUzotu26:Chaetomium_spc	"Chaetomium"		
## LSUzotu25:Raffaelea_sulphurea	"Raffaelea"		
## LSUzotu159:Raffaelea_sulphurea	"Raffaelea"		
## LSUzotu4:Raffaelea_canadensis	"Raffaelea"		
## LSUzotu72:Raffaelea_sulphurea	"Raffaelea"		
## LSUzotu6:Sporothrix_stenoceras	"Sporothrix"		
## LSUzotu130:Chaetomiaceae_spc	"Chaetomiaceae_spc"		
##	Species		
## LSUzotu1:Chaetomium_globosum	"Chaetomium_globosum"		
## LSUzotu2:Raffaelea_sulphurea	"Raffaelea_sulphurea"		
## LSUzotu87:Cladosporium_sp._CHTAE11	"Cladosporium_sp._CHTAE11"		
## LSUzotu26:Chaetomium_spc	"Chaetomium_spc"		
## LSUzotu25:Raffaelea_sulphurea	"Raffaelea_sulphurea"		
## LSUzotu159:Raffaelea_sulphurea	"Raffaelea_sulphurea"		
## LSUzotu4:Raffaelea_canadensis	"Raffaelea_canadensis"		
## LSUzotu72:Raffaelea_sulphurea	"Raffaelea_sulphurea"		
## LSUzotu6:Sporothrix_stenoceras	"Sporothrix_stenoceras"		
## LSUzotu130:Chaetomiaceae_spc	"Chaetomiaceae_spc"		
##	best_hit		
## LSUzotu1:Chaetomium_globosum	"LSUzotu1:Chaetomium_globosum"		
## LSUzotu2:Raffaelea_sulphurea	"LSUzotu2:Raffaelea_sulphurea"		
## LSUzotu87:Cladosporium_sp._CHTAE11	"LSUzotu87:Cladosporium_sp._CHTAE11"		
## LSUzotu26:Chaetomium_spc	"LSUzotu26:Chaetomium_spc"		
## LSUzotu25:Raffaelea_sulphurea	"LSUzotu25:Raffaelea_sulphurea"		
## LSUzotu159:Raffaelea_sulphurea	"LSUzotu159:Raffaelea_sulphurea"		
## LSUzotu4:Raffaelea_canadensis	"LSUzotu4:Raffaelea_canadensis"		
## LSUzotu72:Raffaelea_sulphurea	"LSUzotu72:Raffaelea_sulphurea"		
## LSUzotu6:Sporothrix_stenoceras	"LSUzotu6:Sporothrix_stenoceras"		
## LSUzotu130:Chaetomiaceae_spc	"LSUzotu130:Chaetomiaceae_spc"		

```
round(otu_table(dataset.LSU.ordi)[filtaxa], digits = 4)
```

```
## OTU Table:          [10 taxa and 57 samples]
##                      taxa are rows
##
##                      B0-19a B0-19b B0-22a B0-22b B0-24 B0-27
## LSUzotu1:Chaetomium_globosum      258 19888 19631 34239 7239 1340
## LSUzotu2:Raffaelea_sulphurea     7705      9    22      6 15410 10421
## LSUzotu87:Cladosporium_sp._CHTAE11      0      0      0      0      0      0
## LSUzotu26:Chaetomium_spc            0      0      0      0     86     22
## LSUzotu25:Raffaelea_sulphurea        0      0      0      0     97     15
## LSUzotu159:Raffaelea_sulphurea        0      0      0      0      0      0
## LSUzotu4:Raffaelea_canadensis       251      1     11   2284  2764  3024
## LSUzotu72:Raffaelea_sulphurea         1      0      0      0     27     13
## LSUzotu6:Sporothrix_stenoceras        0      0      2      0      0      0
## LSUzotu130:Chaetomiaceae_spc          0      0      0    179     22      3
##
##                      B10-01 B10-06 B10-09a B10-09b B10-18 B10-24
## LSUzotu1:Chaetomium_globosum        170 11309 18613 35695 2094 1159
## LSUzotu2:Raffaelea_sulphurea     16220 13569 5195      7 3573 7565
## LSUzotu87:Cladosporium_sp._CHTAE11      0      0      0      0      0      0
## LSUzotu26:Chaetomium_spc            2    165    184      0     48     25
## LSUzotu25:Raffaelea_sulphurea         4    179    213      0     43     19
## LSUzotu159:Raffaelea_sulphurea       148      0      0      0     10     12
## LSUzotu4:Raffaelea_canadensis         13      0      1      0      2      0
## LSUzotu72:Raffaelea_sulphurea         3     52     54      0      8      7
## LSUzotu6:Sporothrix_stenoceras         0      0      1      0      0      0
## LSUzotu130:Chaetomiaceae_spc          0      0      0      0      0      0
##
##                      B10-25a B10-25b B10-26a B15-01a B15-01b
## LSUzotu1:Chaetomium_globosum       6733      95  4421      33 20163
## LSUzotu2:Raffaelea_sulphurea     13414    7353 18965    9796 2454
## LSUzotu87:Cladosporium_sp._CHTAE11      0      0      0      0      0
## LSUzotu26:Chaetomium_spc           287      0    255      0    125
## LSUzotu25:Raffaelea_sulphurea       305      3    413      0    169
## LSUzotu159:Raffaelea_sulphurea        40    111     20      0      0
## LSUzotu4:Raffaelea_canadensis          2      0      1   1260    257
## LSUzotu72:Raffaelea_sulphurea         72      1     98      0     51
## LSUzotu6:Sporothrix_stenoceras         3      0      0     28      0
## LSUzotu130:Chaetomiaceae_spc          0      0      0      0     20
##
##                      B15-06 B15-07a B15-07b B15-11 B15-20 B15-24
## LSUzotu1:Chaetomium_globosum         4   15782   3284  4560  7674  3569
## LSUzotu2:Raffaelea_sulphurea       1589   2382   4285  8639  3977  8446
## LSUzotu87:Cladosporium_sp._CHTAE11      0      0      0      0      0      0
## LSUzotu26:Chaetomium_spc              0    137     34     58     65     42
## LSUzotu25:Raffaelea_sulphurea          0     97     27     60     73     39
## LSUzotu159:Raffaelea_sulphurea          0      0      0      0      0      0
## LSUzotu4:Raffaelea_canadensis         524    178   1572  4372  1616  7115
## LSUzotu72:Raffaelea_sulphurea          0     40     17     14     11      8
## LSUzotu6:Sporothrix_stenoceras          0      0      0      0      0      0
## LSUzotu130:Chaetomiaceae_spc          0      9      8     27     20     21
##
##                      B15-25a B15-25b B16-01a B16-04 B16-31a
## LSUzotu1:Chaetomium_globosum         466   3571  20350     33 10485
## LSUzotu2:Raffaelea_sulphurea       5434  14262      3  7443    793
## LSUzotu87:Cladosporium_sp._CHTAE11      0      0      0      0      0
## LSUzotu26:Chaetomium_spc              6    189      0      0      3
```

## LSUzotu25:Raffaelea_sulphurea	5	280	0	0	0	
## LSUzotu159:Raffaelea_sulphurea	0	0	0	0	0	
## LSUzotu4:Raffaelea_canadensis	1663	3320	1	1	29	
## LSUzotu72:Raffaelea_sulphurea	1	59	0	0	1	
## LSUzotu6:Sporothrix_stenoceras	1	0	3	0	23	
## LSUzotu130:Chaetomiaceae_spc	0	35	0	0	3	
##	B16-31b	B20-04a	B20-08a	B20-08b	B20-11	
## LSUzotu1:Chaetomium_globosum	10239	34162	21	3445	6388	
## LSUzotu2:Raffaelea_sulphurea	33	30	49	7	3	
## LSUzotu87:Cladosporium_sp._CHTAE11	0	0	0	0	0	
## LSUzotu26:Chaetomium_spc	0	0	0	0	0	
## LSUzotu25:Raffaelea_sulphurea	0	0	5	0	0	
## LSUzotu159:Raffaelea_sulphurea	0	0	0	0	0	
## LSUzotu4:Raffaelea_canadensis	4	1	3	0	2	
## LSUzotu72:Raffaelea_sulphurea	0	0	1	0	0	
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu130:Chaetomiaceae_spc	0	0	0	3	0	
##	B23-21	B23-23a	B24-02a	B24-26a	B24-26b	
## LSUzotu1:Chaetomium_globosum	22924	8567	1031	11771	5163	
## LSUzotu2:Raffaelea_sulphurea	4734	14764	43	2542	3448	
## LSUzotu87:Cladosporium_sp._CHTAE11	0	0	0	0	0	
## LSUzotu26:Chaetomium_spc	11	291	1	117	117	
## LSUzotu25:Raffaelea_sulphurea	23	491	0	160	160	
## LSUzotu159:Raffaelea_sulphurea	0	13	0	1	10	
## LSUzotu4:Raffaelea_canadensis	2	1	1	0	0	
## LSUzotu72:Raffaelea_sulphurea	8	147	0	56	25	
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu130:Chaetomiaceae_spc	0	0	0	0	0	
##	B24-28a	B24-29	B36-14a	B36-14b	B36-30	
## LSUzotu1:Chaetomium_globosum	6763	4311	3226	6297	6760	
## LSUzotu2:Raffaelea_sulphurea	8373	2555	4297	15265	1367	
## LSUzotu87:Cladosporium_sp._CHTAE11	0	0	0	0	0	
## LSUzotu26:Chaetomium_spc	144	17	35	198	4	
## LSUzotu25:Raffaelea_sulphurea	201	11	48	348	5	
## LSUzotu159:Raffaelea_sulphurea	36	0	4	35	0	
## LSUzotu4:Raffaelea_canadensis	29	1	3	0	3	
## LSUzotu72:Raffaelea_sulphurea	61	0	10	64	2	
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu130:Chaetomiaceae_spc	0	0	0	0	0	
##	B36-31a	B36-32	B39-15	B39-16	B39-17a	B46-03
## LSUzotu1:Chaetomium_globosum	16058	367	5922	2153	11890	20657
## LSUzotu2:Raffaelea_sulphurea	499	699	6225	7681	130	15
## LSUzotu87:Cladosporium_sp._CHTAE11	0	0	0	0	0	0
## LSUzotu26:Chaetomium_spc	40	0	9	0	9	0
## LSUzotu25:Raffaelea_sulphurea	37	0	3	0	4	0
## LSUzotu159:Raffaelea_sulphurea	0	0	0	2	0	0
## LSUzotu4:Raffaelea_canadensis	3	0	2	0	5	1
## LSUzotu72:Raffaelea_sulphurea	11	0	0	1	3	0
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu130:Chaetomiaceae_spc	0	0	0	0	0	0
##	B7-07	B7-08a	medium1	medium2	negative2	
## LSUzotu1:Chaetomium_globosum	2230	4660	2	12	11	
## LSUzotu2:Raffaelea_sulphurea	3522	5	7	23	21	
## LSUzotu87:Cladosporium sp. CHTAE11	0	0	0	56	0	

## LSUzotu26:Chaetomium_spc	5	0	0	1	2
## LSUzotu25:Raffaelea_sulphurea	5	0	0	2	0
## LSUzotu159:Raffaelea_sulphurea	0	0	0	3	0
## LSUzotu4:Raffaelea_canadensis	0	14	0	0	2
## LSUzotu72:Raffaelea_sulphurea	0	0	0	0	0
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	1
## LSUzotu130:Chaetomiaceae_spc	0	2	0	0	2
##	negative1	mock	standard		
## LSUzotu1:Chaetomium_globosum	219	4546	6		
## LSUzotu2:Raffaelea_sulphurea	24	747	16		
## LSUzotu87:Cladosporium_sp._CHTAE11	6	0	0		
## LSUzotu26:Chaetomium_spc	0	4	0		
## LSUzotu25:Raffaelea_sulphurea	1	2	0		
## LSUzotu159:Raffaelea_sulphurea	0	0	0		
## LSUzotu4:Raffaelea_canadensis	0	1822	0		
## LSUzotu72:Raffaelea_sulphurea	2	3	0		
## LSUzotu6:Sporothrix_stenoceras	1	15551	0		
## LSUzotu130:Chaetomiaceae_spc	0	22	0		

negative2 seems to have cross contaminations from samples → consider cross-contamination within samples of this PCR plate? → switched primers, mock and neg2 and corrected this now in tax_table Weirdly Sporothrix is not abundant in samples (highest contaminant here), C.globosum (Zotu 1) highly abundant in all samples, R.canadensis (Zotu4) only half of the samples higher abundant → Line B15 + B0, R. sulphurea (Zotu 2) most samples higher abundant with some lower ones, rest show now abundances in samples

check for ten most abundant ZOTUs in pos.controls

```
pos.controls<-subset_samples(dataset.LSU.ordi, Treatment=="standard" | Treatment=="mock")
sample_names(pos.controls)
```

```
## [1] "mock"      "standard"
```

```
filtaxa <- names (sort(rowSums(otu_table(pos.controls)) ,decreasing=T))[1:10]
tax_table(dataset.LSU.ordi)[filtaxa]
```

```
## Taxonomy Table:      [10 taxa by 8 taxonomic ranks]:
##
##      Domain  Phylum  Class
## LSUzotu6:Sporothrix_stenoceras "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu1:Chaetomium_globosum   "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu4:Raffaelea_canadensis  "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu2:Raffaelea_sulphurea   "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu66:Sordariomycetes_spc  "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu54:Sporothrix_stenoceras "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu138:Sporothrix_stenoceras "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu158:Sporothrix_stenoceras "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu128:Sordariomycetes_spc  "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu198:Sordariomycetes_spc  "Fungi" "Ascomycota" "Sordariomycetes"
##
##      Order  Family
## LSUzotu6:Sporothrix_stenoceras "Ophiostomatales" "Ophiostomataceae"
## LSUzotu1:Chaetomium_globosum   "Sordariales"     "Chaetomiaceae"
## LSUzotu4:Raffaelea_canadensis  "Ophiostomatales" "Ophiostomataceae"
## LSUzotu2:Raffaelea_sulphurea   "Ophiostomatales" "Ophiostomataceae"
## LSUzotu66:Sordariomycetes_spc  "Sordariomycetes_spc" "Sordariomycetes_spc"
```

```

## LSUzotu54:Sporothrix_stenoceras "Ophiostomatales" "Ophiostomataceae"
## LSUzotu138:Sporothrix_stenoceras "Ophiostomatales" "Ophiostomataceae"
## LSUzotu158:Sporothrix_stenoceras "Ophiostomatales" "Ophiostomataceae"
## LSUzotu128:Sordariomycetes_spc "Sordariomycetes_spc" "Sordariomycetes_spc"
## LSUzotu198:Sordariomycetes_spc "Sordariomycetes_spc" "Sordariomycetes_spc"
##
## Genus Species
## LSUzotu6:Sporothrix_stenoceras "Sporothrix" "Sporothrix_stenoceras"
## LSUzotu1:Chaetomium_globosum "Chaetomium" "Chaetomium_globosum"
## LSUzotu4:Raffaelea_canadensis "Raffaelea" "Raffaelea_canadensis"
## LSUzotu2:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu66:Sordariomycetes_spc "Sordariomycetes_spc" "Sordariomycetes_spc"
## LSUzotu54:Sporothrix_stenoceras "Sporothrix" "Sporothrix_stenoceras"
## LSUzotu138:Sporothrix_stenoceras "Sporothrix" "Sporothrix_stenoceras"
## LSUzotu158:Sporothrix_stenoceras "Sporothrix" "Sporothrix_stenoceras"
## LSUzotu128:Sordariomycetes_spc "Sordariomycetes_spc" "Sordariomycetes_spc"
## LSUzotu198:Sordariomycetes_spc "Sordariomycetes_spc" "Sordariomycetes_spc"
##
## best_hit
## LSUzotu6:Sporothrix_stenoceras "LSUzotu6:Sporothrix_stenoceras"
## LSUzotu1:Chaetomium_globosum "LSUzotu1:Chaetomium_globosum"
## LSUzotu4:Raffaelea_canadensis "LSUzotu4:Raffaelea_canadensis"
## LSUzotu2:Raffaelea_sulphurea "LSUzotu2:Raffaelea_sulphurea"
## LSUzotu66:Sordariomycetes_spc "LSUzotu66:Sordariomycetes_spc"
## LSUzotu54:Sporothrix_stenoceras "LSUzotu54:Sporothrix_stenoceras"
## LSUzotu138:Sporothrix_stenoceras "LSUzotu138:Sporothrix_stenoceras"
## LSUzotu158:Sporothrix_stenoceras "LSUzotu158:Sporothrix_stenoceras"
## LSUzotu128:Sordariomycetes_spc "LSUzotu128:Sordariomycetes_spc"
## LSUzotu198:Sordariomycetes_spc "LSUzotu198:Sordariomycetes_spc"

```

```
round(otu_table(dataset.LSU.ordi)[filtaxa], digits = 4)
```

```

## OTU Table:          [10 taxa and 57 samples]
##                      taxa are rows
##
## B0-19a B0-19b B0-22a B0-22b B0-24 B0-27 B10-01
## LSUzotu6:Sporothrix_stenoceras      0      0      2      0      0      0      0
## LSUzotu1:Chaetomium_globosum    258  19888  19631  34239  7239  1340   170
## LSUzotu4:Raffaelea_canadensis    251      1     11   2284  2764  3024    13
## LSUzotu2:Raffaelea_sulphurea   7705      9     22      6 15410 10421 16220
## LSUzotu66:Sordariomycetes_spc      0      0      0      0      0      0      0
## LSUzotu54:Sporothrix_stenoceras      0      0      0      0      0      0      0
## LSUzotu138:Sporothrix_stenoceras      0      0      0      0      0      0      0
## LSUzotu158:Sporothrix_stenoceras      0      0      0      0      0      0      0
## LSUzotu128:Sordariomycetes_spc      0      0      0      0      0      0      0
## LSUzotu198:Sordariomycetes_spc      0      0      0      0      0      0      0
##
## B10-06 B10-09a B10-09b B10-18 B10-24 B10-25a
## LSUzotu6:Sporothrix_stenoceras      0      1      0      0      0      3
## LSUzotu1:Chaetomium_globosum   11309  18613  35695  2094  1159  6733
## LSUzotu4:Raffaelea_canadensis      0      1      0      2      0      2
## LSUzotu2:Raffaelea_sulphurea   13569  5195      7  3573  7565 13414
## LSUzotu66:Sordariomycetes_spc      0      0      0      0      0      0
## LSUzotu54:Sporothrix_stenoceras      0      0      0      0      0      0
## LSUzotu138:Sporothrix_stenoceras      0      0      0      0      0      0
## LSUzotu158:Sporothrix_stenoceras      0      0      0      0      0      0
## LSUzotu128:Sordariomycetes_spc      0      0      0      0      0      0
## LSUzotu198:Sordariomycetes_spc      0      0      0      0      0      0

```


##	B10-25b	B10-26a	B15-01a	B15-01b	B15-06	B15-07a
## LSUzotu6:Sporothrix_stenoceras	0	0	28	0	0	0
## LSUzotu1:Chaetomium_globosum	95	4421	33	20163	4	15782
## LSUzotu4:Raffaelea_canadensis	0	1	1260	257	524	178
## LSUzotu2:Raffaelea_sulphurea	7353	18965	9796	2454	1589	2382
## LSUzotu66:Sordariomycetes_spc	0	0	1	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	1	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B15-07b	B15-11	B15-20	B15-24	B15-25a	B15-25b
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	1	0
## LSUzotu1:Chaetomium_globosum	3284	4560	7674	3569	466	3571
## LSUzotu4:Raffaelea_canadensis	1572	4372	1616	7115	1663	3320
## LSUzotu2:Raffaelea_sulphurea	4285	8639	3977	8446	5434	14262
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B16-01a	B16-04	B16-31a	B16-31b	B20-04a	B20-08a
## LSUzotu6:Sporothrix_stenoceras	3	0	23	0	0	0
## LSUzotu1:Chaetomium_globosum	20350	33	10485	10239	34162	21
## LSUzotu4:Raffaelea_canadensis	1	1	29	4	1	3
## LSUzotu2:Raffaelea_sulphurea	3	7443	793	33	30	49
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	2	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B20-08b	B20-11	B23-21	B23-23a	B24-02a	B24-26a
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu1:Chaetomium_globosum	3445	6388	22924	8567	1031	11771
## LSUzotu4:Raffaelea_canadensis	0	2	2	1	1	0
## LSUzotu2:Raffaelea_sulphurea	7	3	4734	14764	43	2542
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B24-26b	B24-28a	B24-29	B36-14a	B36-14b	B36-30
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu1:Chaetomium_globosum	5163	6763	4311	3226	6297	6760
## LSUzotu4:Raffaelea_canadensis	0	29	1	3	0	3
## LSUzotu2:Raffaelea_sulphurea	3448	8373	2555	4297	15265	1367
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0

## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B36-31a	B36-32	B39-15	B39-16	B39-17a	B46-03
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu1:Chaetomium_globosum	16058	367	5922	2153	11890	20657
## LSUzotu4:Raffaelea_canadensis	3	0	2	0	5	1
## LSUzotu2:Raffaelea_sulphurea	499	699	6225	7681	130	15
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	0
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	0
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	0
##	B7-07	B7-08a	medium1	medium2	negative2	
## LSUzotu6:Sporothrix_stenoceras	0	0	0	0	1	
## LSUzotu1:Chaetomium_globosum	2230	4660	2	12	11	
## LSUzotu4:Raffaelea_canadensis	0	14	0	0	2	
## LSUzotu2:Raffaelea_sulphurea	3522	5	7	23	21	
## LSUzotu66:Sordariomycetes_spc	0	0	0	0	0	
## LSUzotu54:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu138:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu158:Sporothrix_stenoceras	0	0	0	0	0	
## LSUzotu128:Sordariomycetes_spc	0	0	0	0	0	
## LSUzotu198:Sordariomycetes_spc	0	0	0	0	0	
##	negative1	mock	standard			
## LSUzotu6:Sporothrix_stenoceras	1	15551	0			
## LSUzotu1:Chaetomium_globosum	219	4546	6			
## LSUzotu4:Raffaelea_canadensis	0	1822	0			
## LSUzotu2:Raffaelea_sulphurea	24	747	16			
## LSUzotu66:Sordariomycetes_spc	0	412	0			
## LSUzotu54:Sporothrix_stenoceras	0	163	0			
## LSUzotu138:Sporothrix_stenoceras	0	136	0			
## LSUzotu158:Sporothrix_stenoceras	0	112	0			
## LSUzotu128:Sordariomycetes_spc	0	56	0			
## LSUzotu198:Sordariomycetes_spc	0	38	0			

=> fungi from Zymo communitiy standard not sequenced, mock-community (R. sulphurea, R. canadensis, C. globosum, O. stenoceras, yeast (C. pichia)) represented in mock sample

plot controls

prepare data for plotting

```
mock <- subset_samples(dataset.LSU.ordi, Sample == "mock")
std <- subset_samples(dataset.LSU.ordi, Sample == "standard")

negs <- subset_samples(dataset.LSU.ordi, Treatment == "negative" | Treatment == "medium")

Fungi_Species.mock <- mock %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)
```

```
Fungi_Species.mock$Species<-as.character(Fungi_Species.mock$Species)
Fungi_Species.mock$Species[Fungi_Species.mock$Abundance<0.01]<-"Others"
Fungi_Species.mock$Class<-as.character(Fungi_Species.mock$Class)
Fungi_Species.mock$Class[Fungi_Species.mock$Abundance<0.01]<-"Others"
```

```
Fungi_Species.std <- std %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)
```

```
Fungi_Species.std$Species<-as.character(Fungi_Species.std$Species)
Fungi_Species.std$Species[Fungi_Species.std$Abundance<0.01]<-"Others"
Fungi_Species.std$Class<-as.character(Fungi_Species.std$Class)
Fungi_Species.std$Class[Fungi_Species.std$Abundance<0.01]<-"Others"
```

```
Fungi_Species.neg <- negs %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)
```

```
Fungi_Species.neg$Species<-as.character(Fungi_Species.neg$Species)
Fungi_Species.neg$Species[Fungi_Species.neg$Abundance<0.01]<-"Others"
Fungi_Species.neg$Class<-as.character(Fungi_Species.neg$Class)
Fungi_Species.neg$Class[Fungi_Species.neg$Abundance<0.01]<-"Others"
```

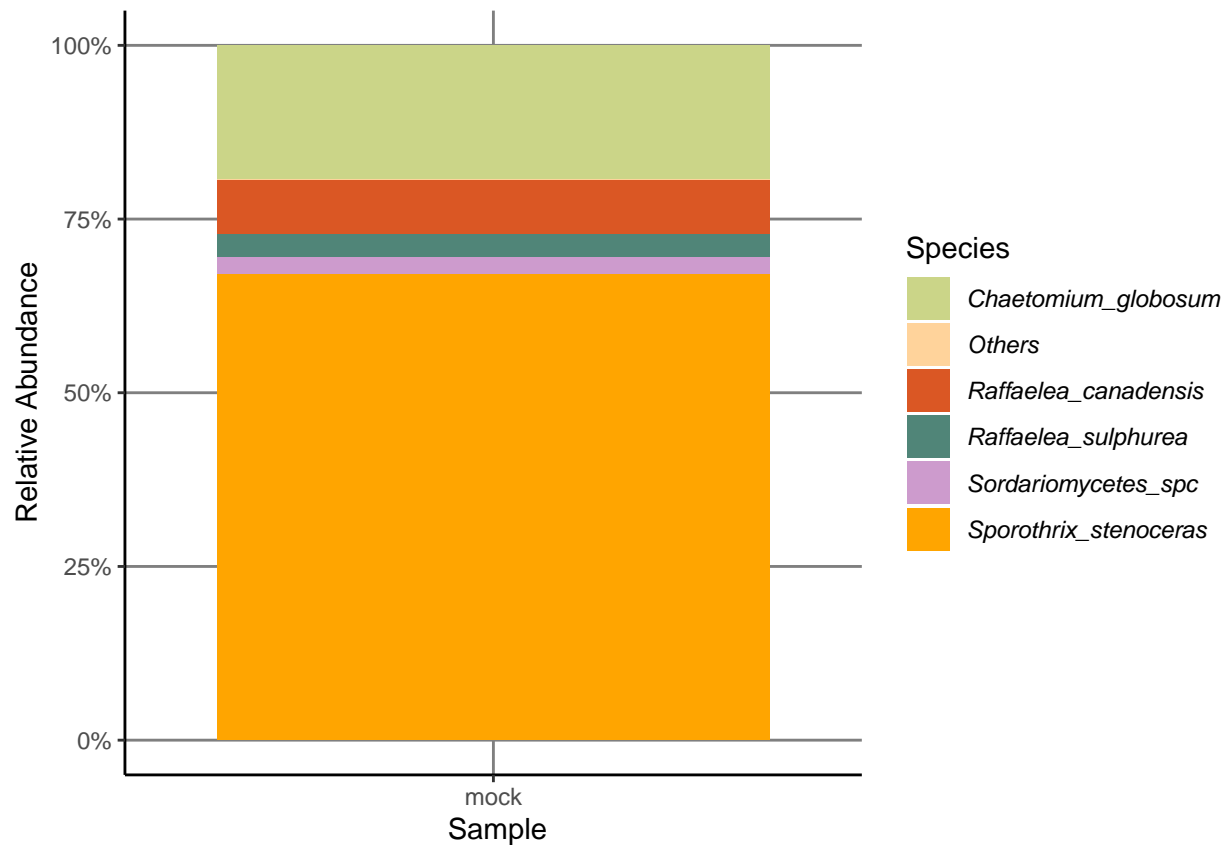
plot positive controls prae decontam

```
Family_colors <- c("#CBD588", "burlywood1", "#DA5724", "#508578", "#CD9BCD", "orange" , "#AD6F3B", "#6A5ACD")
```

```
Fungi_Species.mock_plot <-ggplot(Fungi_Species.mock, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")
```

```
g1<-Fungi_Species.mock_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))
```

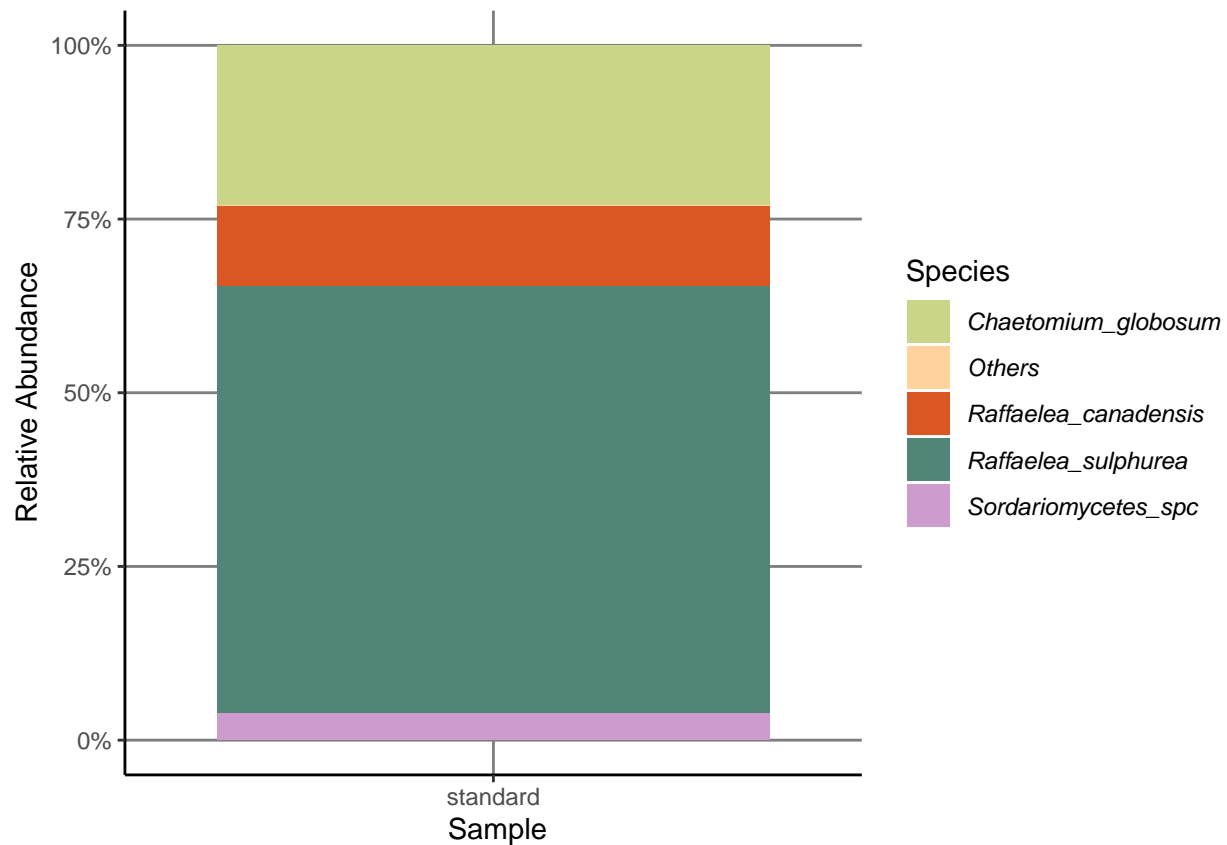
g1



```
Fungi_Species.std_plot <-ggplot(Fungi_Species.std, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")
```

```
g2<-Fungi_Species.std_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))
```

g2

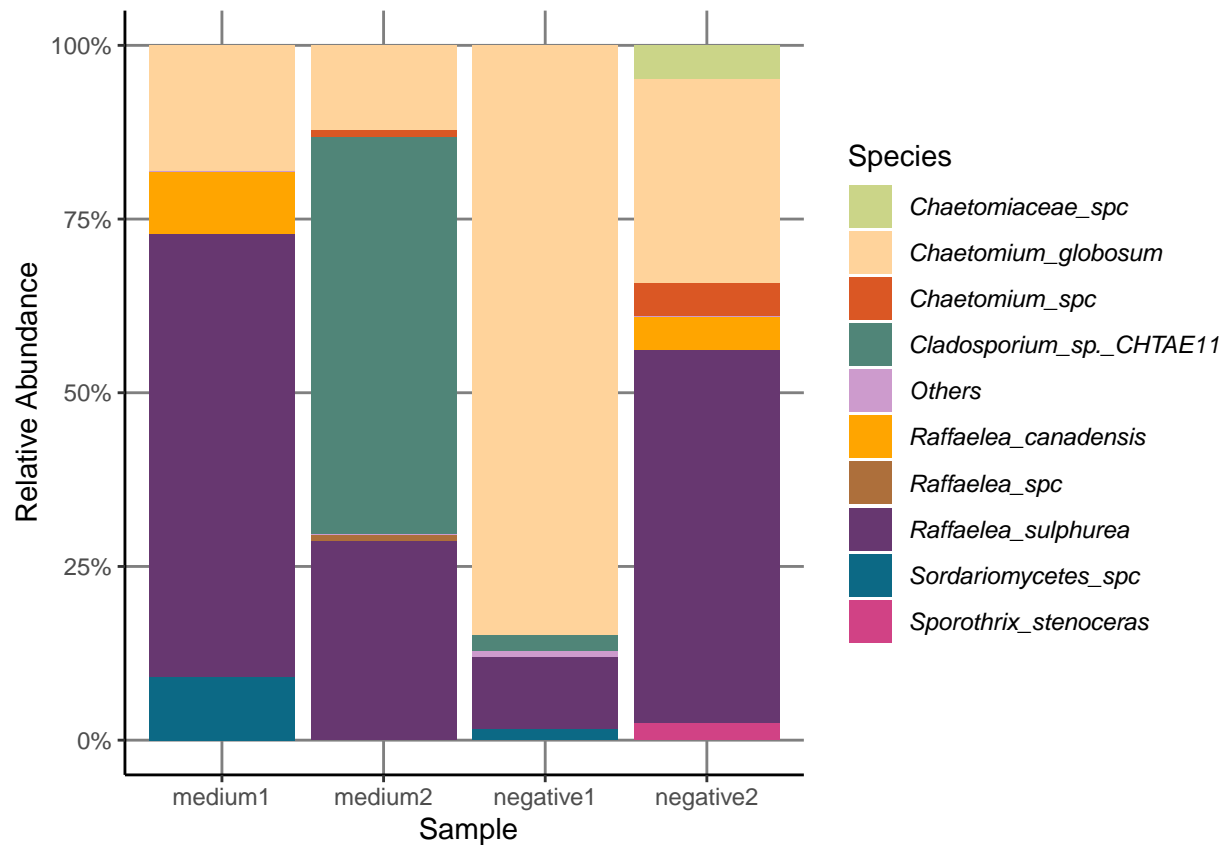


negative controls prae decontam

```
Fungi_Species.neg_plot <-ggplot(Fungi_Species.neg, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")

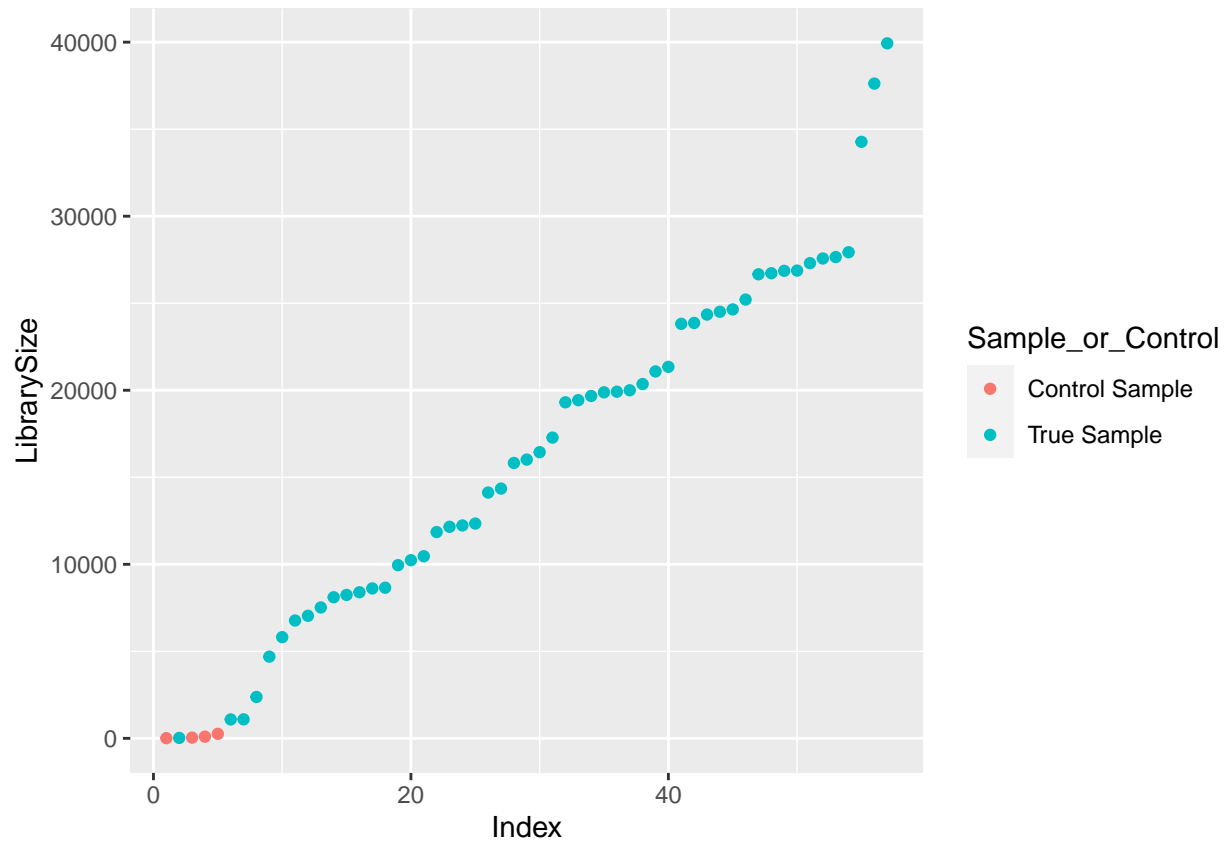
g3<-Fungi_Species.neg_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))

g3
```



run decontam

```
library(decontam)
df <- as.data.frame(sample_data(dataset.LSU.ordi)) # Put sample_data into a ggplot-friendly data.frame
df$LibrarySize <- sample_sums(dataset.LSU.ordi)
df <- df[order(df$LibrarySize),]
df$Index <- seq(nrow(df))
ggplot(data=df, aes(x=Index, y=LibrarySize, color=Sample_or_Control)) + geom_point()
```



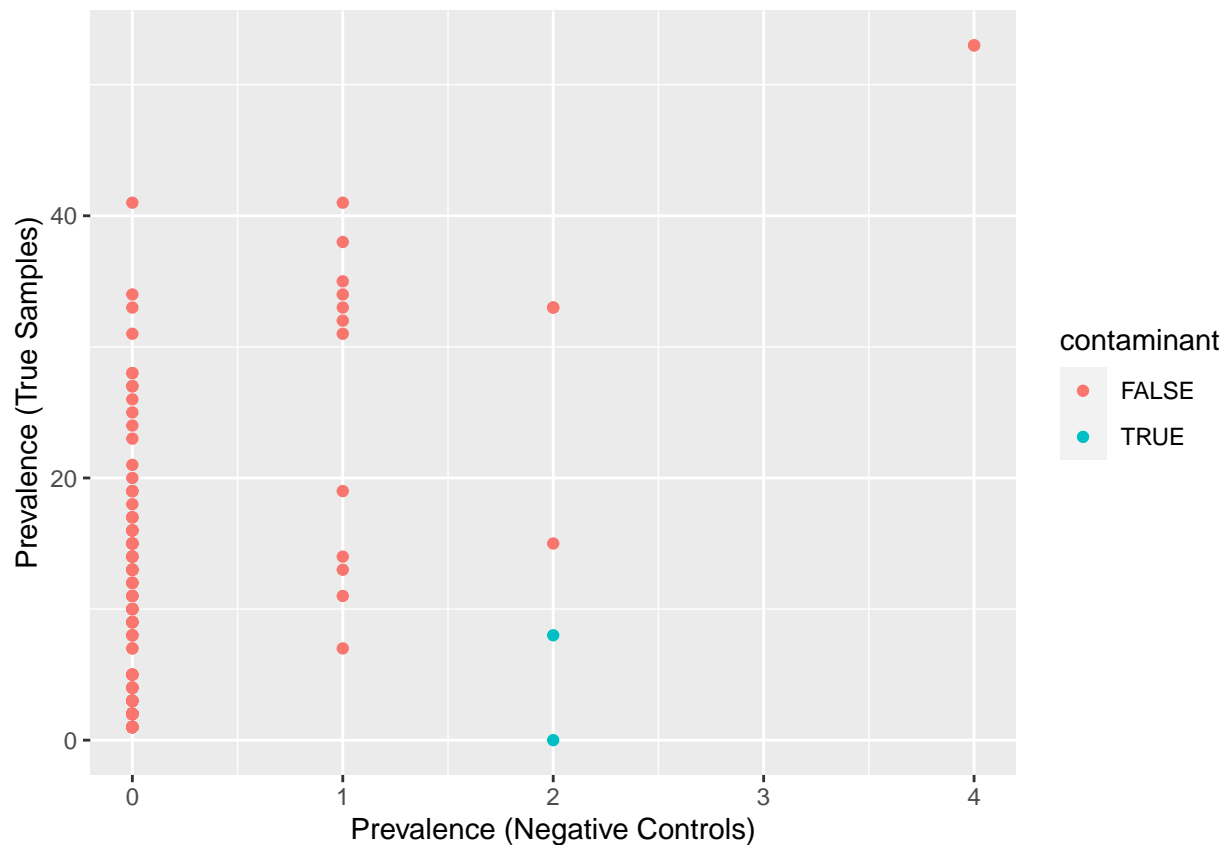
```
sample_data(dataset.LSU.ordi)$is.neg <- sample_data(dataset.LSU.ordi)$Sample_or_Control == "Control Sample"
contamdf.prev <- isContaminant(dataset.LSU.ordi, method="prevalence", neg="is.neg")
table(contamdf.prev$contaminant)
```

```
##
## FALSE TRUE
##    209    2
```

```
ps.pa <- transform_sample_counts(dataset.LSU.ordi, function(abund) 1*(abund>0))
ps.pa.neg <- prune_samples(sample_data(ps.pa)$Sample_or_Control == "Control Sample", ps.pa)
ps.pa.pos <- prune_samples(sample_data(ps.pa)$Sample_or_Control == "True Sample", ps.pa)
```

Make data.frame of prevalence in positive and negative samples

```
df.pa <- data.frame(pa.pos=taxa_sums(ps.pa.pos), pa.neg=taxa_sums(ps.pa.neg), contaminant=contamdf.prev$contaminant)
ggplot(data=df.pa, aes(x=pa.neg, y=pa.pos, color=contaminant)) + geom_point() + xlab("Prevalence (Negative Samples)") + ylab("Prevalence (Positive Samples)")
```



```
ps.noncontam_dataset.LSU.ordi <- prune_taxa(!contamdf.prev$contaminant, dataset.LSU.ordi)
ps.noncontam_dataset.LSU.ordi
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 209 taxa and 57 samples ]
## sample_data() Sample Data: [ 57 samples by 17 sample variables ]
## tax_table() Taxonomy Table: [ 209 taxa by 8 taxonomic ranks ]
```

```
smin <- min(sample_sums(ps.noncontam_dataset.LSU.ordi))
smean <- mean(sample_sums(ps.noncontam_dataset.LSU.ordi))
smax <- max(sample_sums(ps.noncontam_dataset.LSU.ordi))
cat("The minimum sample read count is:",smin) #11
```

```
## The minimum sample read count is: 11
```

```
cat("The average sample read count is:",smean) #15670.75
```

```
## The average sample read count is: 15670.75
```

```
cat("The maximum sample read count is:",smax) #39932
```

```
## The maximum sample read count is: 39932
```



```
contaminants <- subset(contamdf.prev, contaminant == "TRUE")
```

create a list of all excluded contaminants

check controls post decontam

prepare data for plotting

```
mock2 <- subset_samples(ps.noncontam_dataset.LSU.ordi, Sample == "mock")
std2 <- subset_samples(ps.noncontam_dataset.LSU.ordi, Sample == "standard")

negs2 <- subset_samples(ps.noncontam_dataset.LSU.ordi, Treatment == "negative" | Treatment == "medium")

Fungi_Species.mock2 <- mock2 %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)

Fungi_Species.mock2$Species<-as.character(Fungi_Species.mock2$Species)
Fungi_Species.mock2$Species[Fungi_Species.mock2$Abundance<0.01]<- "Others"
Fungi_Species.mock2$Class<-as.character(Fungi_Species.mock2$Class)
Fungi_Species.mock2$Class[Fungi_Species.mock2$Abundance<0.01]<- "Others"

Fungi_Species.std2 <- std2 %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)

Fungi_Species.std2$Species<-as.character(Fungi_Species.std2$Species)
Fungi_Species.std2$Species[Fungi_Species.std2$Abundance<0.01]<- "Others"
Fungi_Species.std2$Class<-as.character(Fungi_Species.std2$Class)
Fungi_Species.std2$Class[Fungi_Species.std2$Abundance<0.01]<- "Others"

Fungi_Species.neg2 <- negs2 %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)

Fungi_Species.neg2$Species<-as.character(Fungi_Species.neg2$Species)
Fungi_Species.neg2$Species[Fungi_Species.neg2$Abundance<0.01]<- "Others"
Fungi_Species.neg2$Class<-as.character(Fungi_Species.neg2$Class)
Fungi_Species.neg2$Class[Fungi_Species.neg2$Abundance<0.01]<- "Others"
```

positive controls post decontam

```
Fungi_Species.mock2_plot <-ggplot(Fungi_Species.mock2, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
```

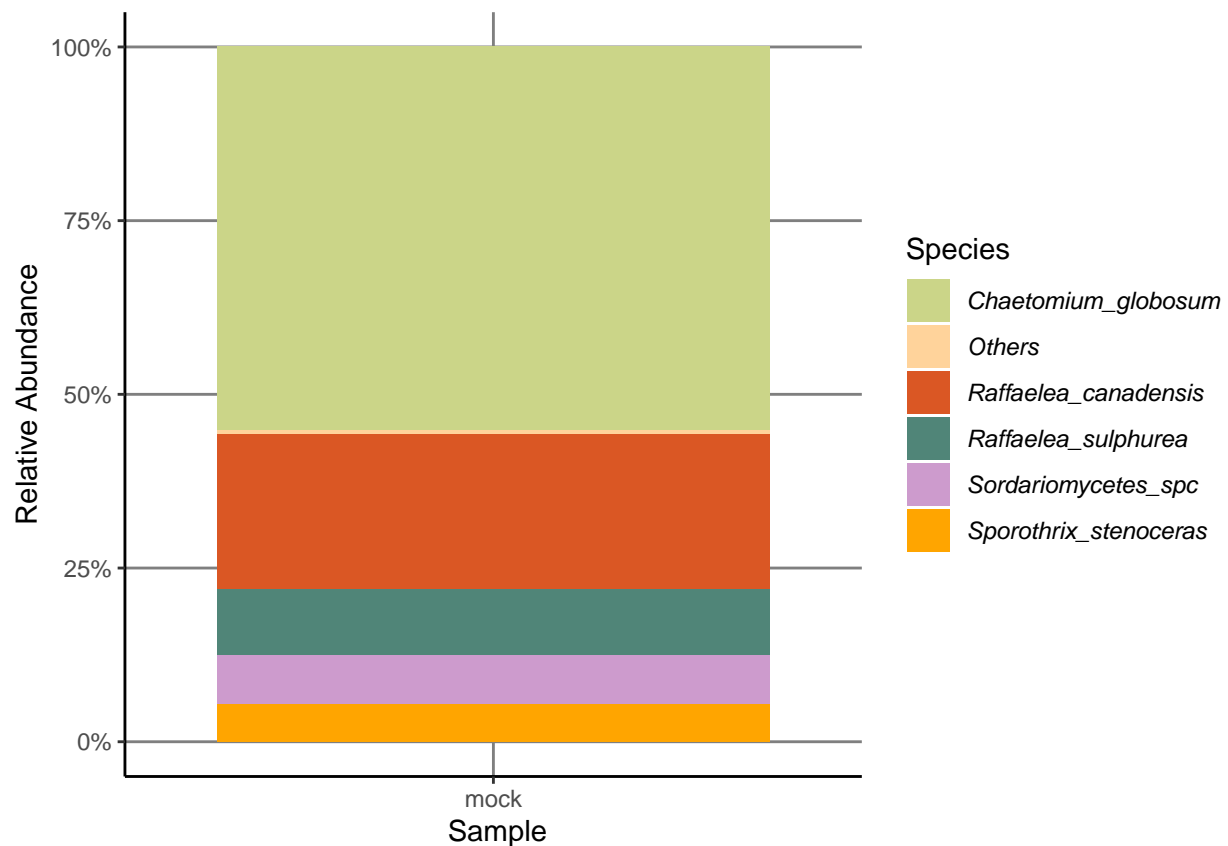
```

scale_fill_manual(values = Family_colors, name = "Species")

g4<-Fungi_Species.mock2_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))

```

g4



standard controls post decontam

```

Fungi_Species.std2_plot <-ggplot(Fungi_Species.std2, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")

g5<-Fungi_Species.std2_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+

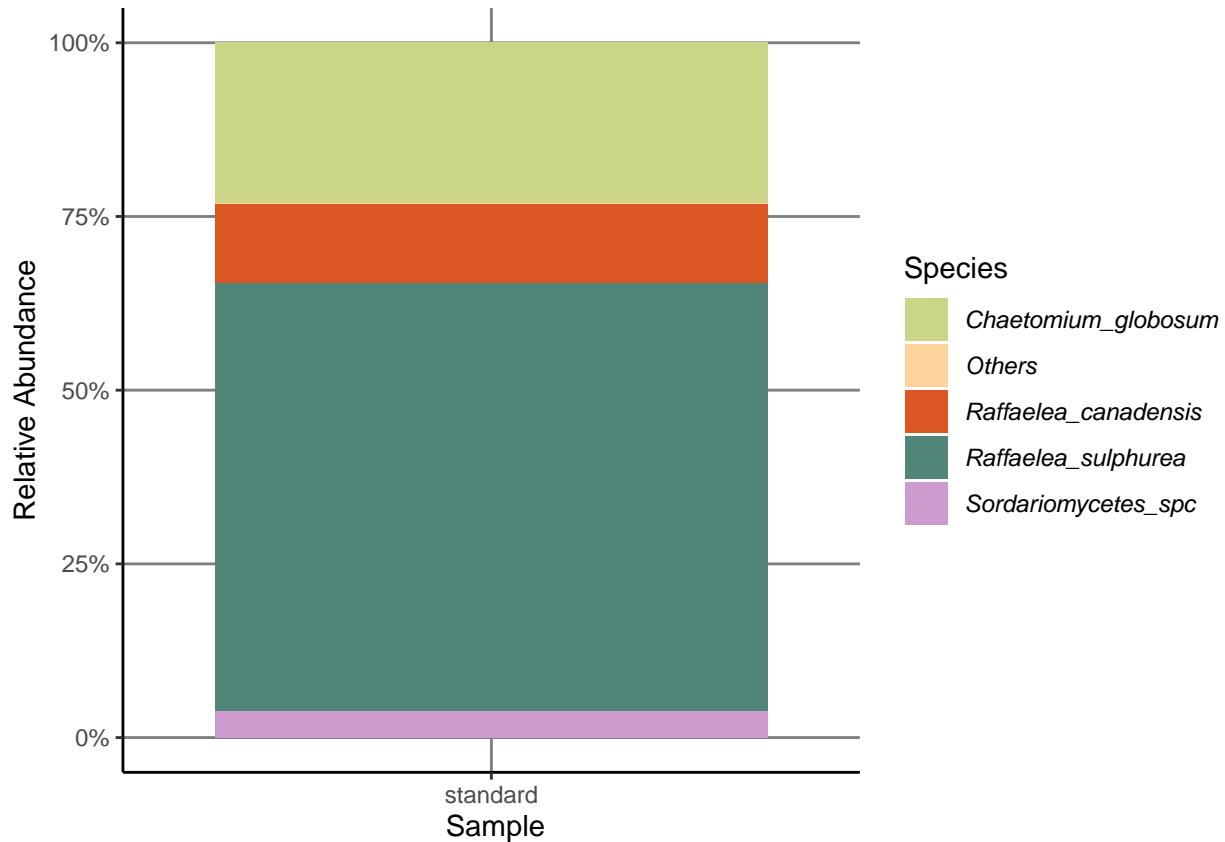
```

```

theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))

```

g5



negative controls post decontam

```

Fungi_Species.neg2_plot <-ggplot(Fungi_Species.neg2, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")

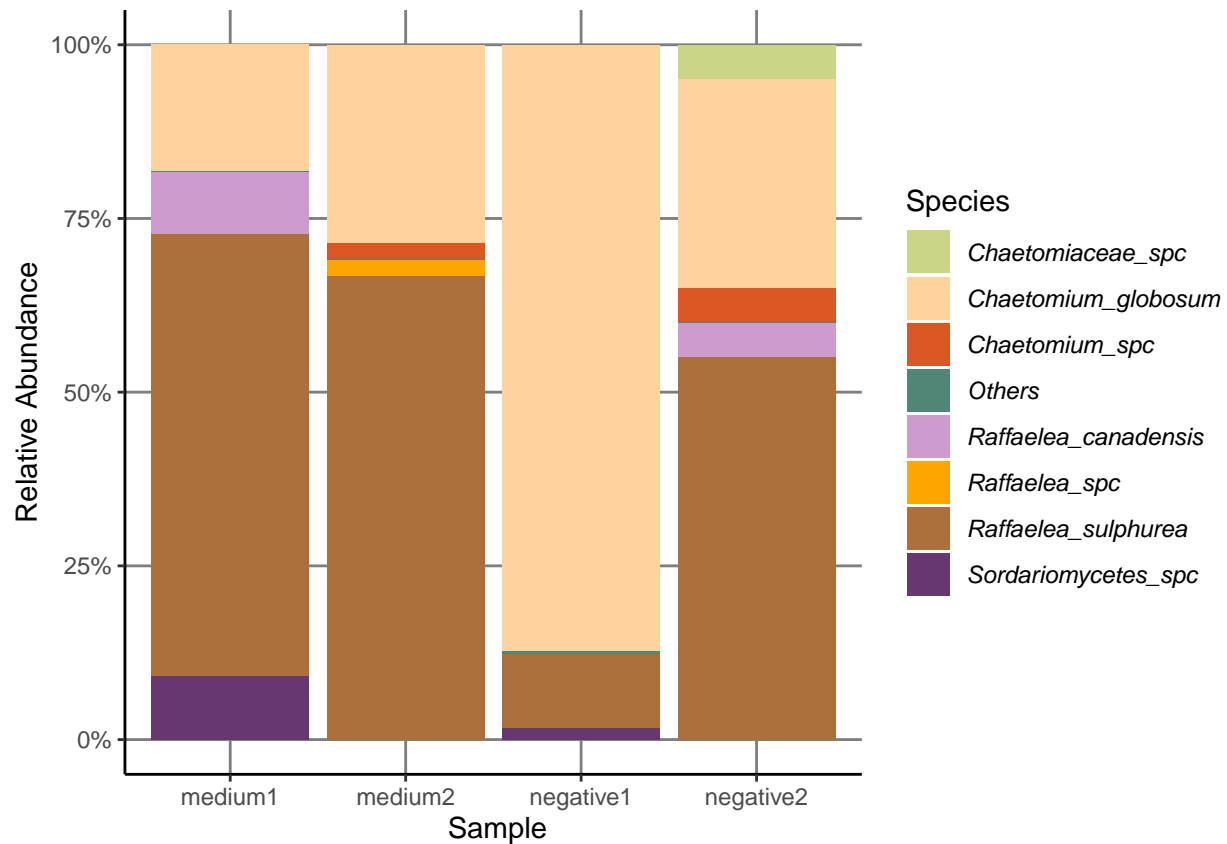
```

```

g6<-Fungi_Species.neg2_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme(text = element_text(size=20, face = "bold"))+
  theme(axis.text.x=element_text(size = rel(0.5)))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x=" Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(legend.text = element_text(face = "italic"))

```

g6



exclude negative and positive samples for a look of the ten most abundant ZOTUS in our samples

```
fun.without.controls <- subset_samples(ps.noncontam_dataset.LSU.ordi, Treatment!="medium")
fun.without.controls2 <- subset_samples(fun.without.controls, Treatment!="negative")
fun.without.controls3 <- subset_samples(fun.without.controls2, Treatment!="mock")
fwc <- subset_samples(fun.without.controls3, Treatment!="standard")
```

get rid of no read taxa and unimportant metadata in dataset

```
fwc <- prune_taxa(taxa_sums(fwc) > 0, fwc)

sample_data(fwc) <- sample_data(fwc)[,c("Sample", "Nest", "Lineage", "Treatment", "age_sampling_d.", "Group")]
```

now have a look at the 15 most abundant ZOTUS in our samples

```
most.abundant <- subset_samples(fwc, Treatment=="control" | Treatment=="removal" | Treatment=="2nd-founda")
```

```
filtaxa <- names (sort(rowSums(otu_table(most.abundant)) ,decreasing=T))[1:15]
tax_table(fwc)[filtaxa]
```

```
## Taxonomy Table:      [15 taxa by 8 taxonomic ranks]:
##
##      Domain  Phylum      Class
## LSUzotu1:Chaetomium_globosum "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu2:Raffaelea_sulphurea "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu3:Acremonium_biseptum "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu4:Raffaelea_canadensis "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu5:Raffaelea_canadensis "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu8:Penicillium_commune "Fungi" "Ascomycota" "Eurotiomycetes"
## LSUzotu19:Chaetomium_globosum "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu23:Raffaelea_sulphurea "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu25:Raffaelea_sulphurea "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu7:Raffaelea_sulphurea "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu26:Chaetomium_spc "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu9:Raffaelea_spc "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu36:Raffaelea_sulphurea "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu11:Raffaelea_canadensis "Fungi" "Ascomycota" "Sordariomycetes"
## LSUzotu37:Raffaelea_canadensis "Fungi" "Ascomycota" "Sordariomycetes"
##
##      Order      Family
## LSUzotu1:Chaetomium_globosum "Sordariales" "Chaetomiaceae"
## LSUzotu2:Raffaelea_sulphurea "Ophiostomatales" "Ophiostomataceae"
## LSUzotu3:Acremonium_biseptum "Hypocreales" "Bionectriaceae"
## LSUzotu4:Raffaelea_canadensis "Ophiostomatales" "Ophiostomataceae"
## LSUzotu5:Raffaelea_canadensis "Ophiostomatales" "Ophiostomataceae"
## LSUzotu8:Penicillium_commune "Eurotiales" "Trichocomaceae"
## LSUzotu19:Chaetomium_globosum "Sordariales" "Chaetomiaceae"
## LSUzotu23:Raffaelea_sulphurea "Ophiostomatales" "Ophiostomataceae"
## LSUzotu25:Raffaelea_sulphurea "Ophiostomatales" "Ophiostomataceae"
## LSUzotu7:Raffaelea_sulphurea "Ophiostomatales" "Ophiostomataceae"
## LSUzotu26:Chaetomium_spc "Sordariales" "Chaetomiaceae"
## LSUzotu9:Raffaelea_spc "Ophiostomatales" "Ophiostomataceae"
## LSUzotu36:Raffaelea_sulphurea "Ophiostomatales" "Ophiostomataceae"
## LSUzotu11:Raffaelea_canadensis "Ophiostomatales" "Ophiostomataceae"
## LSUzotu37:Raffaelea_canadensis "Ophiostomatales" "Ophiostomataceae"
##
##      Genus      Species
## LSUzotu1:Chaetomium_globosum "Chaetomium" "Chaetomium_globosum"
## LSUzotu2:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu3:Acremonium_biseptum "Acremonium" "Acremonium_biseptum"
## LSUzotu4:Raffaelea_canadensis "Raffaelea" "Raffaelea_canadensis"
## LSUzotu5:Raffaelea_canadensis "Raffaelea" "Raffaelea_canadensis"
## LSUzotu8:Penicillium_commune "Penicillium" "Penicillium_commune"
## LSUzotu19:Chaetomium_globosum "Chaetomium" "Chaetomium_globosum"
## LSUzotu23:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu25:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu7:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu26:Chaetomium_spc "Chaetomium" "Chaetomium_spc"
## LSUzotu9:Raffaelea_spc "Raffaelea" "Raffaelea_spc"
## LSUzotu36:Raffaelea_sulphurea "Raffaelea" "Raffaelea_sulphurea"
## LSUzotu11:Raffaelea_canadensis "Raffaelea" "Raffaelea_canadensis"
## LSUzotu37:Raffaelea_canadensis "Raffaelea" "Raffaelea_canadensis"
##
##      best_hit
```

```
## LSUzotu1:Chaetomium_globosum "LSUzotu1:Chaetomium_globosum"
## LSUzotu2:Raffaelea_sulphurea "LSUzotu2:Raffaelea_sulphurea"
## LSUzotu3:Acremonium_biseptum "LSUzotu3:Acremonium_biseptum"
## LSUzotu4:Raffaelea_canadensis "LSUzotu4:Raffaelea_canadensis"
## LSUzotu5:Raffaelea_canadensis "LSUzotu5:Raffaelea_canadensis"
## LSUzotu8:Penicillium_commune "LSUzotu8:Penicillium_commune"
## LSUzotu19:Chaetomium_globosum "LSUzotu19:Chaetomium_globosum"
## LSUzotu23:Raffaelea_sulphurea "LSUzotu23:Raffaelea_sulphurea"
## LSUzotu25:Raffaelea_sulphurea "LSUzotu25:Raffaelea_sulphurea"
## LSUzotu7:Raffaelea_sulphurea "LSUzotu7:Raffaelea_sulphurea"
## LSUzotu26:Chaetomium_spc "LSUzotu26:Chaetomium_spc"
## LSUzotu9:Raffaelea_spc "LSUzotu9:Raffaelea_spc"
## LSUzotu36:Raffaelea_sulphurea "LSUzotu36:Raffaelea_sulphurea"
## LSUzotu11:Raffaelea_canadensis "LSUzotu11:Raffaelea_canadensis"
## LSUzotu37:Raffaelea_canadensis "LSUzotu37:Raffaelea_canadensis"
```

```
round(otu_table(fwc)[filtaxa], digits = 4)
```

```
## OTU Table:          [15 taxa and 51 samples]
##                      taxa are rows
##
##                      B0-19a B0-19b B0-22a B0-22b B0-24 B0-27 B10-01
## LSUzotu1:Chaetomium_globosum      258  19888  19631  34239  7239  1340   170
## LSUzotu2:Raffaelea_sulphurea    7705      9      22      6 15410 10421 16220
## LSUzotu3:Acremonium_biseptum        0      0      0      0      0      0      0
## LSUzotu4:Raffaelea_canadensis     251      1     11   2284  2764  3024    13
## LSUzotu5:Raffaelea_canadensis        0     45      4      0      1      1  3221
## LSUzotu8:Penicillium_commune        0      0      0      0      0      0      0
## LSUzotu19:Chaetomium_globosum        0      0      0      2     99     20      3
## LSUzotu23:Raffaelea_sulphurea        0      0      0      0    172     30      6
## LSUzotu25:Raffaelea_sulphurea        0      0      0      0     97     15      4
## LSUzotu7:Raffaelea_sulphurea     386      0      0      0    548    380   109
## LSUzotu26:Chaetomium_spc            0      0      0      0     86     22      2
## LSUzotu9:Raffaelea_spc              2      0      0      0    170    192      2
## LSUzotu36:Raffaelea_sulphurea        0      0      0      0     63     19      2
## LSUzotu11:Raffaelea_canadensis       0      0      0      0      0      0   535
## LSUzotu37:Raffaelea_canadensis       3      0      0      0    123    187      0
##
##                      B10-06 B10-09a B10-09b B10-18 B10-24 B10-25a
## LSUzotu1:Chaetomium_globosum    11309  18613  35695  2094   1159   6733
## LSUzotu2:Raffaelea_sulphurea    13569   5195      7   3573   7565  13414
## LSUzotu3:Acremonium_biseptum        0      0      0      0      0      0
## LSUzotu4:Raffaelea_canadensis       0      1      0      2      0      2
## LSUzotu5:Raffaelea_canadensis     161      7   2030   540   885   861
## LSUzotu8:Penicillium_commune        0      0      0      0      0      0
## LSUzotu19:Chaetomium_globosum     192     491      0     48     24   452
## LSUzotu23:Raffaelea_sulphurea     312     673      0     39     35   255
## LSUzotu25:Raffaelea_sulphurea     179     213      0     43     19   305
## LSUzotu7:Raffaelea_sulphurea     125     115      0     40     64   116
## LSUzotu26:Chaetomium_spc          165     184      0     48     25   287
## LSUzotu9:Raffaelea_spc              4      0      0      1      1    14
## LSUzotu36:Raffaelea_sulphurea     139     150      0     41     20   267
## LSUzotu11:Raffaelea_canadensis      11      0      0     38    102   132
## LSUzotu37:Raffaelea_canadensis       0      0      1      0      0      0
##
##                      B10-25b B10-26a B15-01a B15-01b B15-06 B15-07a
## LSUzotu1:Chaetomium_globosum       95   4421      33  20163      4  15782
```

## LSUzotu2:Raffaelea_sulphurea	7353	18965	9796	2454	1589	2382
## LSUzotu3:Acremonium_biseptum	0	0	102	0	96	29
## LSUzotu4:Raffaelea_canadensis	0	1	1260	257	524	178
## LSUzotu5:Raffaelea_canadensis	3875	413	0	0	0	0
## LSUzotu8:Penicillium_commune	0	0	0	0	0	0
## LSUzotu19:Chaetomium_globosum	1	354	0	355	0	278
## LSUzotu23:Raffaelea_sulphurea	6	247	0	202	0	240
## LSUzotu25:Raffaelea_sulphurea	3	413	0	169	0	97
## LSUzotu7:Raffaelea_sulphurea	58	93	63	47	20	47
## LSUzotu26:Chaetomium_spc	0	255	0	125	0	137
## LSUzotu9:Raffaelea_spc	0	5	187	34	14	7
## LSUzotu36:Raffaelea_sulphurea	2	269	0	114	0	80
## LSUzotu11:Raffaelea_canadensis	754	59	0	0	0	0
## LSUzotu37:Raffaelea_canadensis	0	0	197	6	52	6
##	B15-07b	B15-11	B15-20	B15-24	B15-25a	B15-25b
## LSUzotu1:Chaetomium_globosum	3284	4560	7674	3569	466	3571
## LSUzotu2:Raffaelea_sulphurea	4285	8639	3977	8446	5434	14262
## LSUzotu3:Acremonium_biseptum	0	0	0	0	0	0
## LSUzotu4:Raffaelea_canadensis	1572	4372	1616	7115	1663	3320
## LSUzotu5:Raffaelea_canadensis	0	0	0	0	0	0
## LSUzotu8:Penicillium_commune	0	0	1	0	0	0
## LSUzotu19:Chaetomium_globosum	50	79	71	62	12	292
## LSUzotu23:Raffaelea_sulphurea	23	95	137	67	1	146
## LSUzotu25:Raffaelea_sulphurea	27	60	73	39	5	280
## LSUzotu7:Raffaelea_sulphurea	49	91	79	115	50	73
## LSUzotu26:Chaetomium_spc	34	58	65	42	6	189
## LSUzotu9:Raffaelea_spc	105	223	58	345	76	1081
## LSUzotu36:Raffaelea_sulphurea	25	39	43	58	4	177
## LSUzotu11:Raffaelea_canadensis	0	0	0	0	0	0
## LSUzotu37:Raffaelea_canadensis	123	144	24	257	124	412
##	B16-01a	B16-04	B16-31a	B16-31b	B20-04a	B20-08a
## LSUzotu1:Chaetomium_globosum	20350	33	10485	10239	34162	21
## LSUzotu2:Raffaelea_sulphurea	3	7443	793	33	30	49
## LSUzotu3:Acremonium_biseptum	0	0	0	0	0	26272
## LSUzotu4:Raffaelea_canadensis	1	1	29	4	1	3
## LSUzotu5:Raffaelea_canadensis	1	10	113	376	52	573
## LSUzotu8:Penicillium_commune	0	0	1520	6660	0	1
## LSUzotu19:Chaetomium_globosum	0	0	2	0	0	0
## LSUzotu23:Raffaelea_sulphurea	0	0	0	0	0	0
## LSUzotu25:Raffaelea_sulphurea	0	0	0	0	0	5
## LSUzotu7:Raffaelea_sulphurea	0	34	7	0	0	1
## LSUzotu26:Chaetomium_spc	0	0	3	0	0	0
## LSUzotu9:Raffaelea_spc	0	0	2	0	0	0
## LSUzotu36:Raffaelea_sulphurea	0	0	1	0	0	3
## LSUzotu11:Raffaelea_canadensis	0	0	1	2	3	0
## LSUzotu37:Raffaelea_canadensis	0	0	2	0	0	1
##	B20-08b	B20-11	B23-21	B23-23a	B24-02a	B24-26a
## LSUzotu1:Chaetomium_globosum	3445	6388	22924	8567	1031	11771
## LSUzotu2:Raffaelea_sulphurea	7	3	4734	14764	43	2542
## LSUzotu3:Acremonium_biseptum	5057	0	0	1	1	0
## LSUzotu4:Raffaelea_canadensis	0	2	2	1	1	0
## LSUzotu5:Raffaelea_canadensis	5452	1957	19	297	0	280
## LSUzotu8:Penicillium_commune	0	0	0	0	0	0
## LSUzotu19:Chaetomium_globosum	0	0	22	530	0	218

## LSUzotu23:Raffaelea_sulphurea	0	0	48	253	1	149
## LSUzotu25:Raffaelea_sulphurea	0	0	23	491	0	160
## LSUzotu7:Raffaelea_sulphurea	0	1	46	67	0	47
## LSUzotu26:Chaetomium_spc	0	0	11	291	1	117
## LSUzotu9:Raffaelea_spc	0	0	0	2	1	0
## LSUzotu36:Raffaelea_sulphurea	0	0	16	308	0	67
## LSUzotu11:Raffaelea_canadensis	1	0	0	54	0	21
## LSUzotu37:Raffaelea_canadensis	0	0	0	0	0	0
##	B24-26b	B24-28a	B24-29	B36-14a	B36-14b	B36-30
## LSUzotu1:Chaetomium_globosum	5163	6763	4311	3226	6297	6760
## LSUzotu2:Raffaelea_sulphurea	3448	8373	2555	4297	15265	1367
## LSUzotu3:Acremonium_biseptum	0	8	0	0	0	0
## LSUzotu4:Raffaelea_canadensis	0	29	1	3	0	3
## LSUzotu5:Raffaelea_canadensis	323	1842	62	407	743	23
## LSUzotu8:Penicillium_commune	0	0	0	0	0	0
## LSUzotu19:Chaetomium_globosum	240	300	10	42	379	7
## LSUzotu23:Raffaelea_sulphurea	140	163	22	19	140	6
## LSUzotu25:Raffaelea_sulphurea	160	201	11	48	348	5
## LSUzotu7:Raffaelea_sulphurea	33	87	21	50	109	24
## LSUzotu26:Chaetomium_spc	117	144	17	35	198	4
## LSUzotu9:Raffaelea_spc	5	17	0	0	8	0
## LSUzotu36:Raffaelea_sulphurea	92	126	4	21	205	1
## LSUzotu11:Raffaelea_canadensis	56	232	0	53	145	0
## LSUzotu37:Raffaelea_canadensis	0	0	0	0	0	0
##	B36-31a	B36-32	B39-15	B39-16	B39-17a	B46-03
## LSUzotu1:Chaetomium_globosum	16058	367	5922	2153	11890	20657
## LSUzotu2:Raffaelea_sulphurea	499	699	6225	7681	130	15
## LSUzotu3:Acremonium_biseptum	0	0	0	0	1	0
## LSUzotu4:Raffaelea_canadensis	3	0	2	0	5	1
## LSUzotu5:Raffaelea_canadensis	19	19	98	1914	32	385
## LSUzotu8:Penicillium_commune	0	0	0	0	1	0
## LSUzotu19:Chaetomium_globosum	95	2	3	0	25	0
## LSUzotu23:Raffaelea_sulphurea	50	0	1	0	6	0
## LSUzotu25:Raffaelea_sulphurea	37	0	3	0	4	0
## LSUzotu7:Raffaelea_sulphurea	15	4	73	83	1	0
## LSUzotu26:Chaetomium_spc	40	0	9	0	9	0
## LSUzotu9:Raffaelea_spc	0	0	0	0	0	0
## LSUzotu36:Raffaelea_sulphurea	32	0	0	1	9	0
## LSUzotu11:Raffaelea_canadensis	0	1	0	10	0	0
## LSUzotu37:Raffaelea_canadensis	0	0	0	0	0	0
##	B7-07	B7-08a				
## LSUzotu1:Chaetomium_globosum	2230	4660				
## LSUzotu2:Raffaelea_sulphurea	3522	5				
## LSUzotu3:Acremonium_biseptum	0	2				
## LSUzotu4:Raffaelea_canadensis	0	14				
## LSUzotu5:Raffaelea_canadensis	0	0				
## LSUzotu8:Penicillium_commune	0	0				
## LSUzotu19:Chaetomium_globosum	11	0				
## LSUzotu23:Raffaelea_sulphurea	3	2				
## LSUzotu25:Raffaelea_sulphurea	5	0				
## LSUzotu7:Raffaelea_sulphurea	23	0				
## LSUzotu26:Chaetomium_spc	5	0				
## LSUzotu9:Raffaelea_spc	0	1				
## LSUzotu36:Raffaelea_sulphurea	5	0				

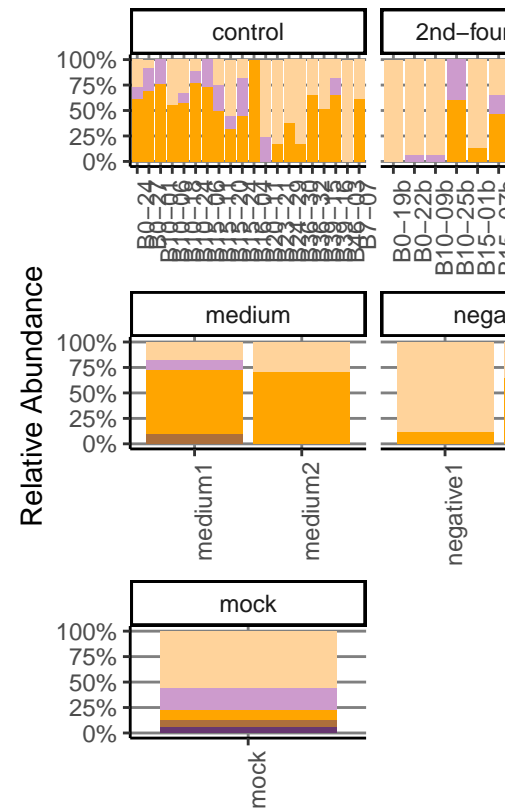

```
## LSUzotu11:Raffaelea_canadensis      0      0
## LSUzotu37:Raffaelea_canadensis      0      0
```

```
Fungi_Species.all. <- ps.noncontam_dataset.LSU.ordi %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  filter(Abundance > 0.05) %>%
  arrange(Species)

Fungi_Species.all.$Treatment <- factor(Fungi_Species.all.$Treatment, levels = c("control", "2nd-foundat.

Fungi_Species_plot <-ggplot(Fungi_Species.all., aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", position="fill") +
  scale_fill_manual(values = Family_colors, name = "Species")

Fungi_Species_plot +
  facet_wrap("Treatment", scales = "free_x", drop = TRUE)+
  theme(plot.title = element_text(size = 16, face = "bold")) +
  theme(text = element_text(size=16, face = "bold"))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 14))+
  theme_classic()+      theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x="Sample", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(axis.text.x=element_text(angle = 90, hjust = 1, size = rel(1)))+
  theme(legend.text = element_text(face = "italic"))
```



plot rel. abundance of present dataset to check abundances in sampels

Most abundant taxa are *Chaetomium*, *Raffaelea*, *Acremonium* & *Penicillium*. Last two appear only in sample B16-31a/b and B20-08a/b.

extract general information on data

```
microbiome::summarize_phyloseq(fwc)
```

```
## Compositional = N02
```

```
## 1] Min. number of reads = 10872] Max. number of reads = 399323] Total number of reads = 8845454] Average number of reads = 17344.0196078431] Number of sample variants = 19801980198019810] (i.e. exactly one read detected across all samples)
```

```
## [[1]]
## [1] "1] Min. number of reads = 1087"
##
## [[2]]
## [1] "2] Max. number of reads = 39932"
##
## [[3]]
## [1] "3] Total number of reads = 884545"
##
## [[4]]
## [1] "4] Average number of reads = 17344.0196078431"
##
## [[5]]
## [1] "5] Median number of reads = 17279"
```

```
##
## [[6]]
## [1] "7] Sparsity = 0.822267520869734"
##
## [[7]]
## [1] "6] Any OTU sum to 1 or less? YES"
##
## [[8]]
## [1] "8] Number of singletons = 4"
##
## [[9]]
## [1] "9] Percent of OTUs that are singletons \n          (i.e. exactly one read detected across all sampl
##
## [[10]]
## [1] "10] Number of sample variables are: 6"
##
## [[11]]
## [1] "Sample"          "Nest"          "Linage"          "Treatment"
## [5] "age_sampling_d." "Group"
```

```
replicates <- subset.data.frame(sample_data(fwc))
table(replicates$Treatment)
```

```
##
## 2nd-foundation      control      removal
##           11           20           20
```

```
sample_data(fwc)$ColSums <- colSums(otu_table(fwc))
```

Analysis

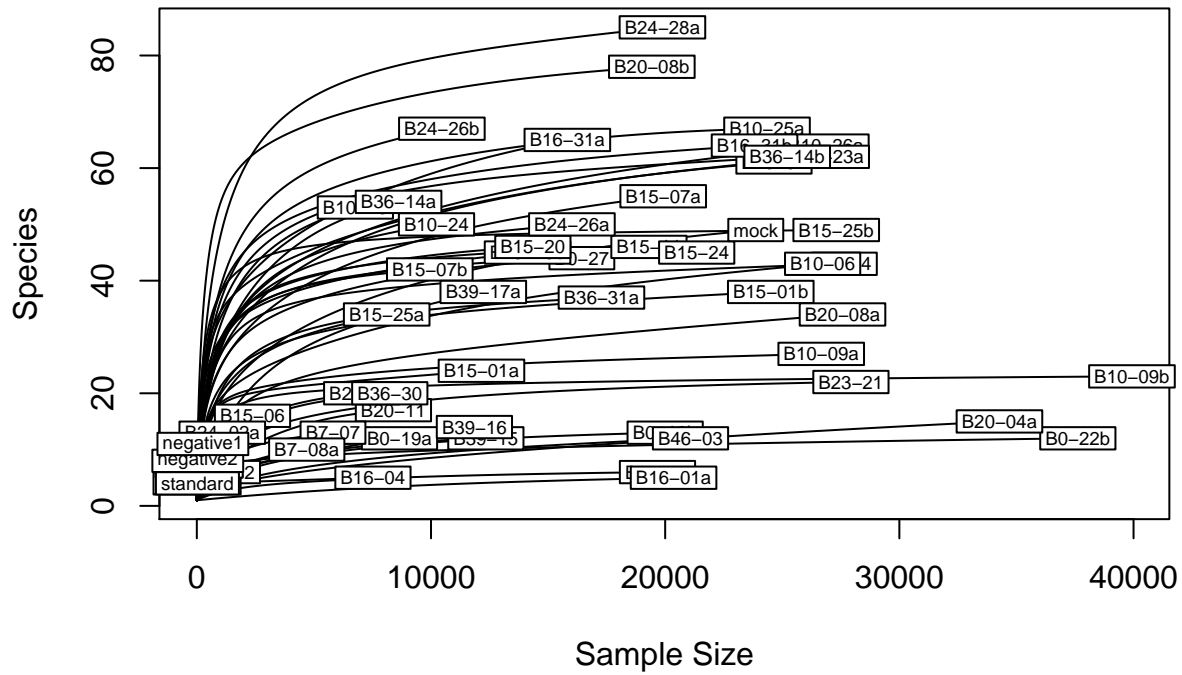
Rarefaction curves

1. all samples with controls
2. all samples with controls after decontam
3. all samples without controls

Rarefaction is used to simulate even number of reads per sample.

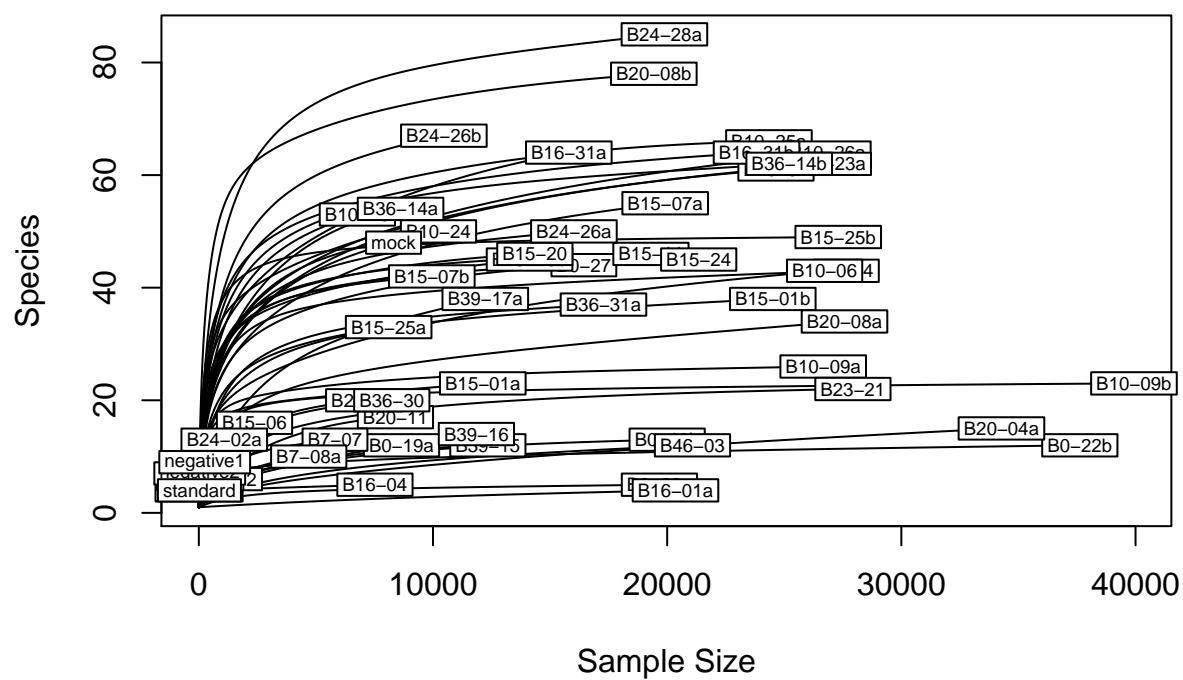
```
rarecurve(t(otu_table(dataset.LSU.ordi)), cex=0.6, step = 20, main = "Rarefaction curve of all samples")
```

Rarefaction curve of all samples (incl. controls)



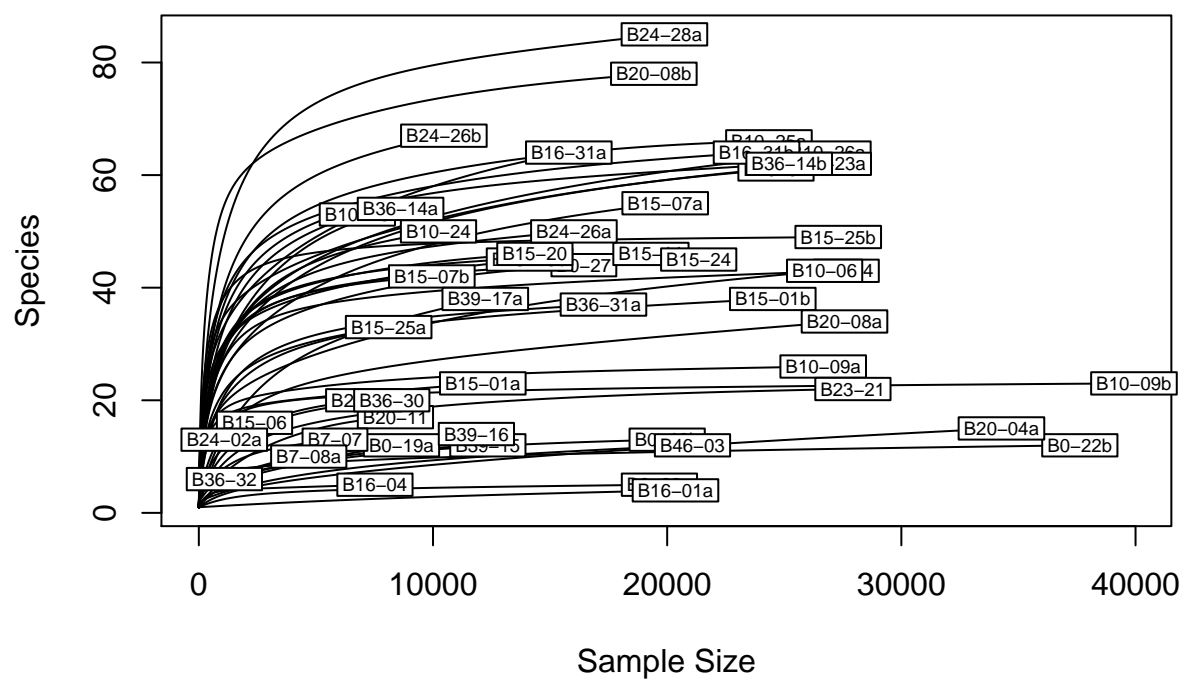
```
rarecurve(t(otu_table(ps.noncontam_dataset.LSU.ordi)), cex=0.6, step = 20, main = "Rarefaction curve of
```

Rarefaction curve of all samples (incl. controls) after decontaminatic



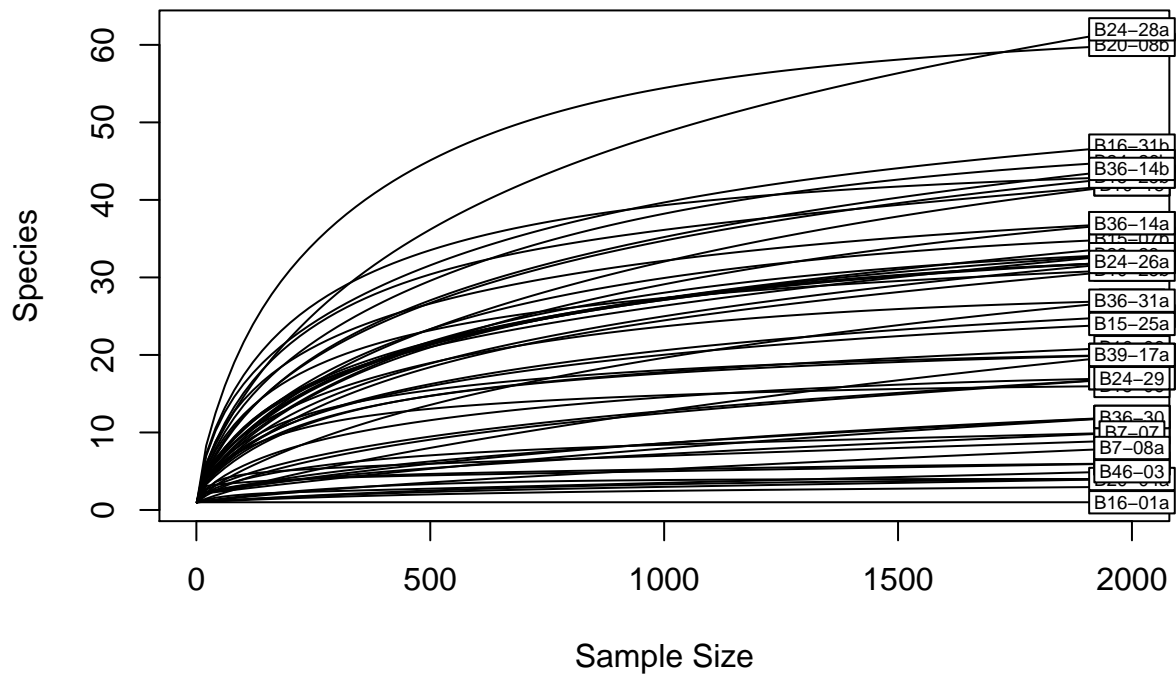
```
rarecurve(t(otu_table(fwc)), cex=0.6, step = 20, main = "Rarefaction curve of samples (excl. controls)");
```

Rarefaction curve of samples (excl. controls)



rarefy to 2000 reads

```
ps2 <-fwc
set.seed(1)
ps2 <- rarefy_even_depth(ps2,sample.size=2000, replace=FALSE, rngseed = 1)
rarecurve(t(otu_table(ps2)), cex=0.6, step = 20)
```



```
replicates3 <- subset.data.frame(sample_data(ps2))
table(replicates3$Treatment)
```

```
##
## 2nd-foundation      control      removal
##           11           19           19
```

subset data to two comparison groups

```
#control vs. removal
CR <- subset_samples(fwc, Treatment!="2nd-foundation")
CRrf <- subset_samples(ps2, Treatment!="2nd-foundation")

#removal vs. 2nd attempt
R2nd <- subset_samples(fwc, Treatment!="control")
R2ndrf <- subset_samples(ps2, Treatment!="control")
```

Alpha diversity

Diversity plots

This returns a table with selected diversity indicators (Shannon diversity & Observed richness).

```
tabCR <- microbiome::alpha(CRrf, index = c("diversity_shannon","observed"))
tabR2nd <- microbiome::alpha(R2ndrf, index = c("diversity_shannon","observed"))
```

Prepare data for vizualisation Now, get the metadata (sample_data) from the phyloseq object

```
ps1.meta.CR <- meta(CRrf)
ps1.meta.R2nd <- meta(R2ndrf)
```

Add the diversity table to metadata

```
ps1.meta.CR$Shannon <- tabCR$diversity_shannon
ps1.meta.CR$Observed <- tabCR$observed

ps1.meta.R2nd$Shannon <- tabR2nd$diversity_shannon
ps1.meta.R2nd$Observed <- tabR2nd$observed
```

plot control vs. removal (rarefied, 2000 reads)

```
ps1.meta.CR$Treatment <- as.factor(ps1.meta.CR$Treatment)
ps1.meta.CR$Treatment <- factor(ps1.meta.CR$Treatment, levels = c("control", "removal"))
```

#Shannon diversity index

```
(a <- summaryBy(Shannon ~ Treatment, ps1.meta.CR, FUN = c(mean, sd)))
```

```
## Treatment Shannon.mean Shannon.sd
## 1 control 0.9571066 0.4540992
## 2 removal 0.7803962 0.5793697
```

```
p1 <- ggplot(ps1.meta.CR, aes(x = Treatment, y = Shannon))
shan_fun <- p1 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
  geom_pointrange(data = a, mapping = aes(x = Treatment, y = Shannon.mean, ymin = Shannon.mean-Shannon.sd, ymax = Shannon.mean+Shannon.sd),
  theme_bw()+
  ylim(NA, 1.95)+
  labs(x = "Treatment group", y = "Shannon Diversity Index")+
  theme(axis.title = element_text(size = 18, face = "bold"), axis.text = element_text(size = 16))
```

#Observed richness

```
(b <- summaryBy(Observed ~ Treatment, ps1.meta.CR, FUN = c(mean, sd)))
```

```
## Treatment Observed.mean Observed.sd
## 1 control 20.68421 12.79346
## 2 removal 24.36842 15.64612
```

```
p2 <- ggplot(ps1.meta.CR, aes(x = Treatment, y = Observed))
obs_fun <- p2 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
  geom_pointrange(data = b, mapping = aes(x = Treatment, y = Observed.mean, ymin = Observed.mean-Observed.sd, ymax = Observed.mean+Observed.sd),
  theme_bw()+
```



```

ylim(NA, 70)
labs(x = "Treatment group", y = "Observed richness")+
theme(axis.title = element_text(size = 18, face = "bold"), axis.text = element_text(size = 16))

## NULL

removal vs. 2nd attempt (rarefied, 2000 reads)

ps1.meta.R2nd$Treatment <- as.factor(ps1.meta.R2nd$Treatment)
ps1.meta.R2nd$Treatment <- factor(ps1.meta.R2nd$Treatment, levels = c("removal", "2nd-foundation"), lab

#Shannon diversity index
(c <- summaryBy(Shannon ~ Treatment, ps1.meta.R2nd, FUN = c(mean, sd)))

##      Treatment Shannon.mean Shannon.sd
## 1      removal      0.7803962  0.5793697
## 2 2nd-attempt      1.2684611  0.7431823

p3 <- ggplot(ps1.meta.R2nd, aes(x = Treatment, y = Shannon))
shan_fun2 <- p3 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
  geom_pointrange(data = c, mapping = aes(x = Treatment, y = Shannon.mean, ymin = Shannon.mean-Shannon
  theme_bw()+
  ylim(NA, 2.7))+
  labs(x = "Treatment group", y = "Shannon Diversity Index")+
  theme(axis.title = element_text(size = 18, face = "bold"), axis.text = element_text(size = 16))

#Observed richness
(d <- summaryBy(Observed ~ Treatment, ps1.meta.R2nd, FUN = c(mean, sd)))

##      Treatment Observed.mean Observed.sd
## 1      removal      24.36842  15.64612
## 2 2nd-attempt      32.81818  17.28478

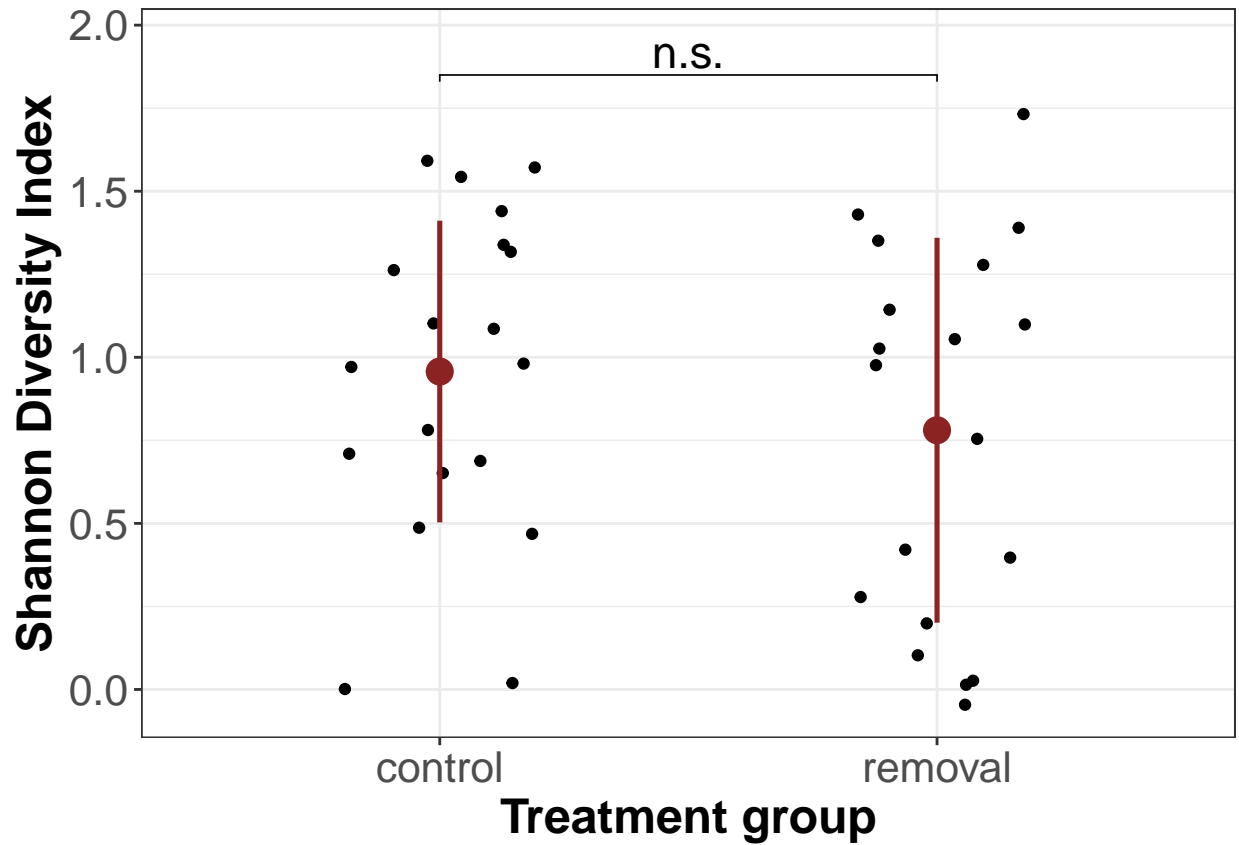
p4 <- ggplot(ps1.meta.R2nd, aes(x = Treatment, y = Observed))
obs_fun2 <- p4 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
  geom_pointrange(data = d, mapping = aes(x = Treatment, y = Observed.mean, ymin = Observed.mean-Obse
  theme_bw()+
  ylim(NA, 70))+
  labs(x = "Treatment group", y = "Observed richness")+
  theme(axis.title = element_text(size = 18, face = "bold"), axis.text = element_text(size = 16))

add statistical data output to plot

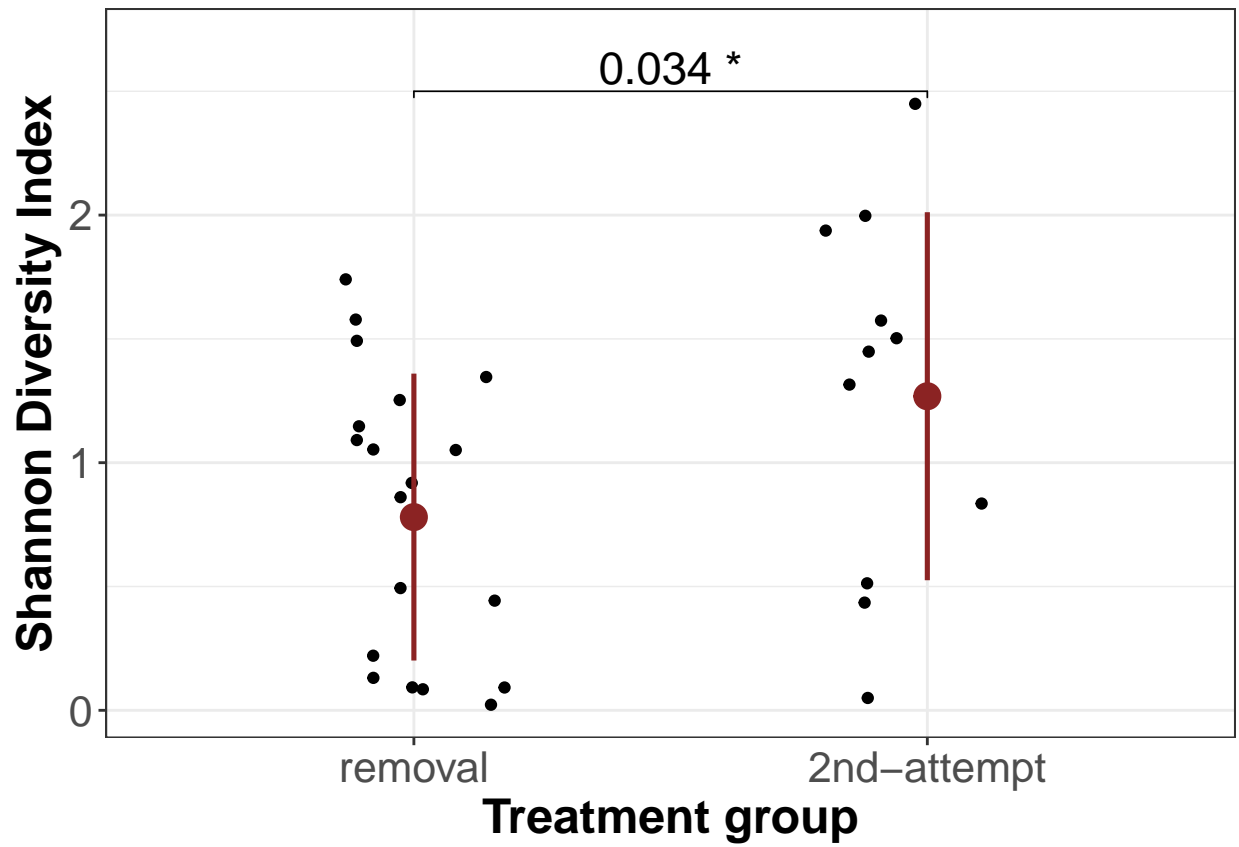
shan_fun <- shan_fun + font("axis.title", size = 18)+
  font("xylab", size = 18)+
  geom_bracket(
    xmin = "control", xmax = "removal", y.position = 1.85, label = "n.s.", label.size = 6,tip.l

shan_fun

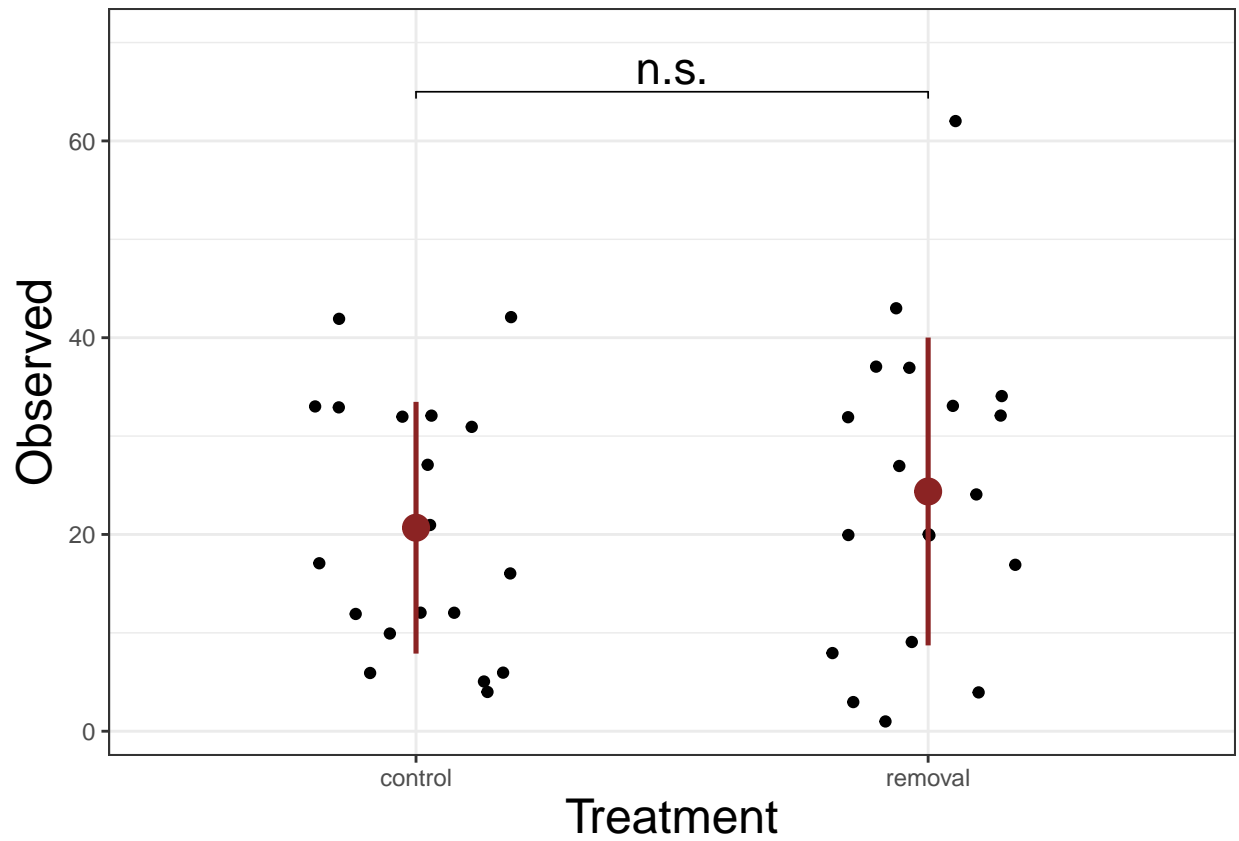
```



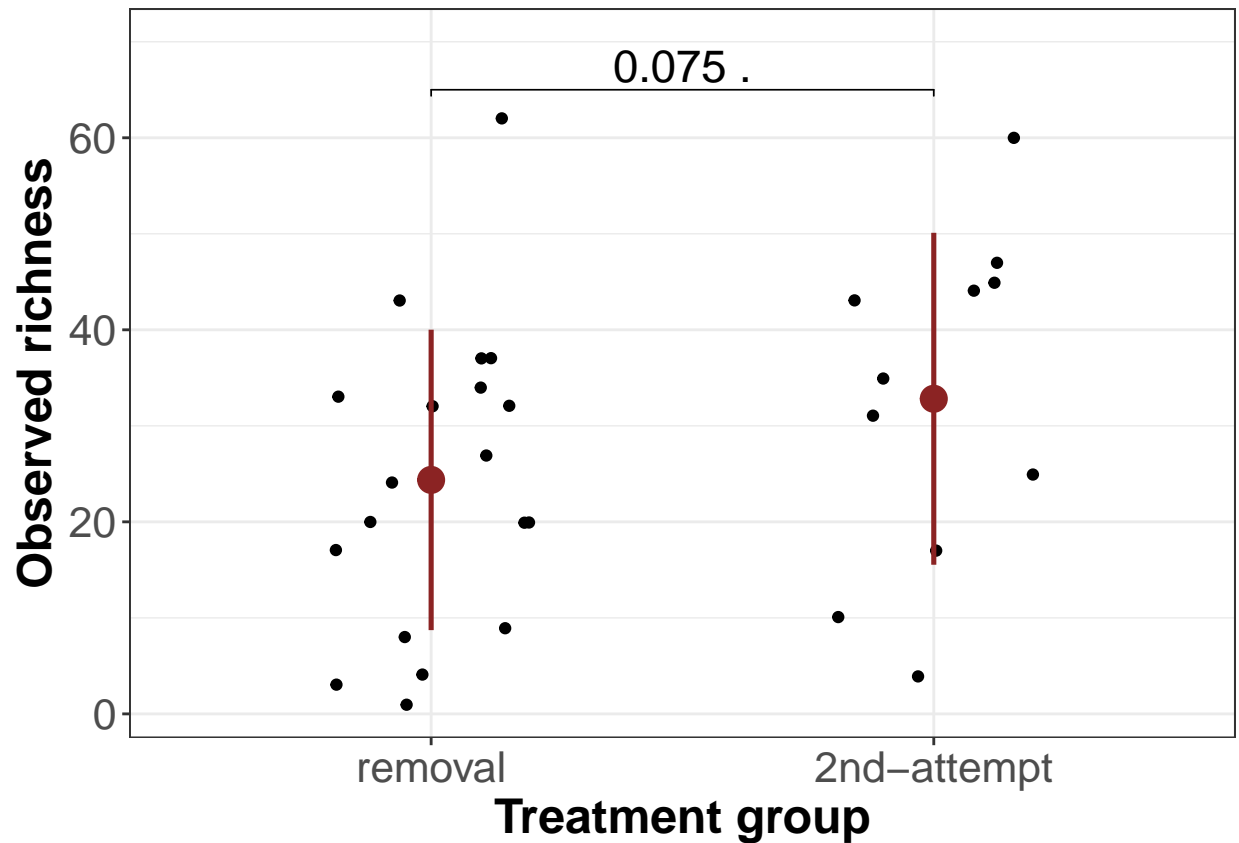
```
shan_fun2 <- shan_fun2 + font("axis.title", size = 18)+
  font("xylab", size = 18)+
  geom_bracket(
    xmin = "removal", xmax = "2nd-attempt", y.position = 2.5, label = "0.034 *", label.size = 6
  )
shan_fun2
```



```
obs_fun <- obs_fun + font("axis.title", size = 18)+
  font("xylab", size = 18)+
  geom_bracket(
    xmin = "control", xmax = "removal", y.position = 65, label = "n.s.", label.size = 6,tip.len=
obs_fun
```



```
obs_fun2 <- obs_fun2 + font("axis.title", size = 18)+
  font("xylab", size = 18)+
  geom_bracket(
    xmin = "removal", xmax = "2nd-attempt", y.position = 65, label = "0.075 .", label.size = 6,
obs_fun2
```



Statistics

Testing differences in alpha diversity

create dataframe for analysis

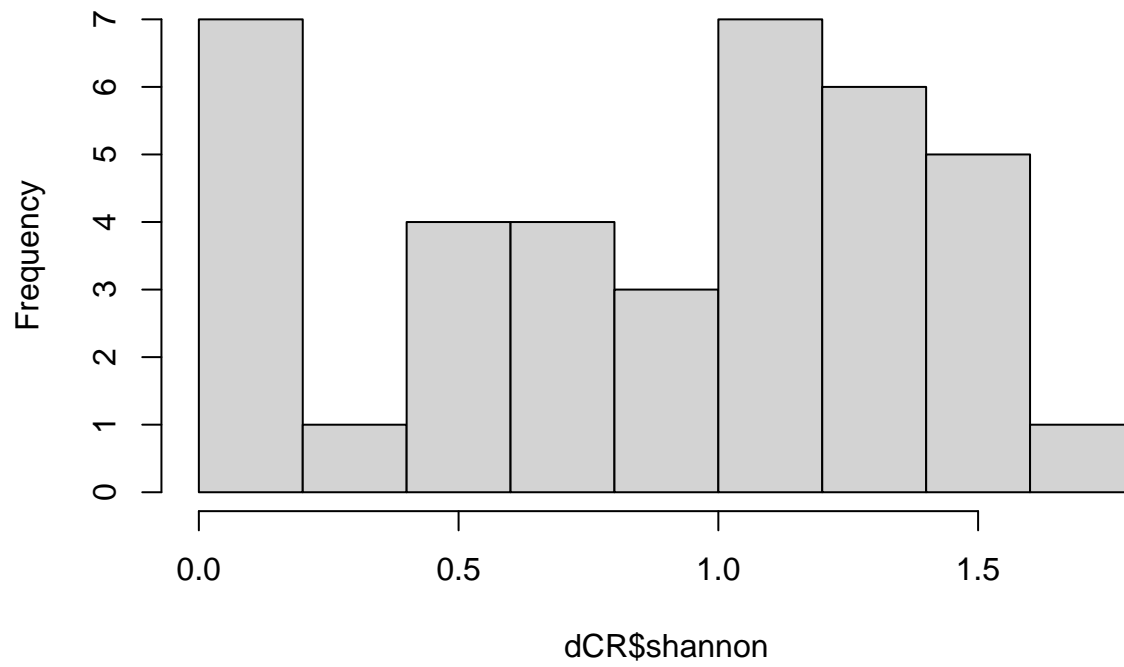
```
#control vs. removal
dCR <- meta(CRrf)
shannon <- diversity(CRrf, "shannon")
dCR$shannon <- shannon$shannon
observed <- alpha(CRrf, index = "observed", zeroes = TRUE)
dCR$observed <- observed$observed

#removal vs. 2nd attempt
dR2nd <- meta(R2ndrf)
shannon <- diversity(R2ndrf, "shannon")
dR2nd$shannon <- shannon$shannon
observed <- alpha(R2ndrf, index = "observed", zeroes = TRUE)
dR2nd$observed <- observed$observed
```

Shannon diversity test distribution

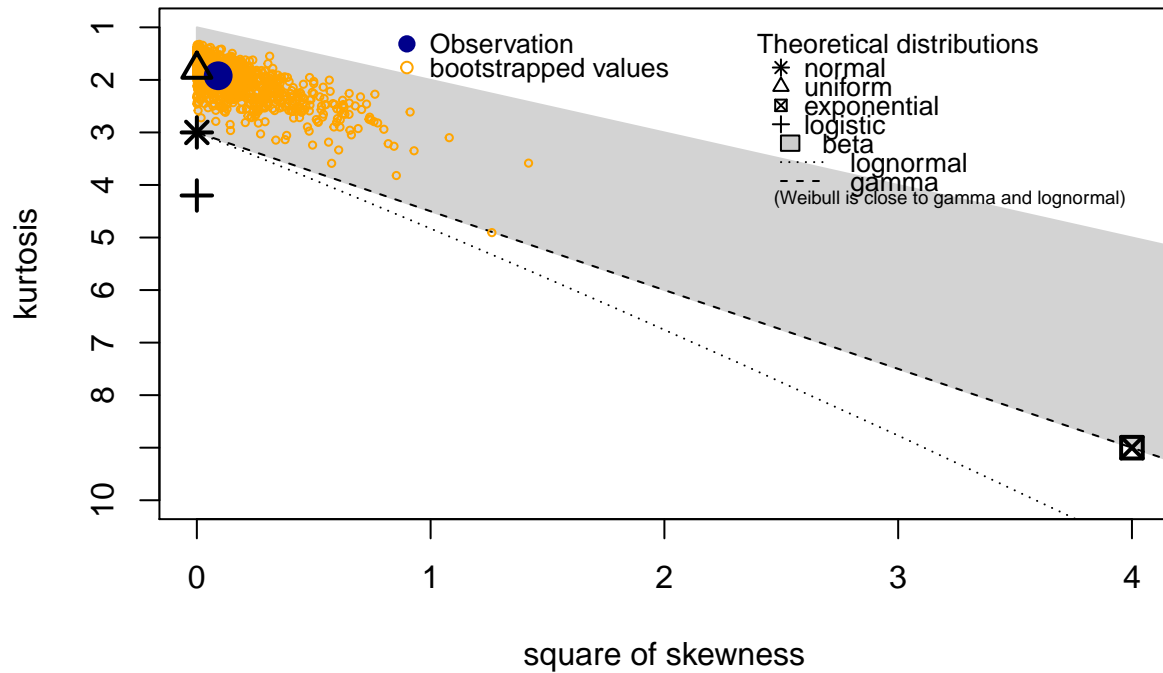
```
hist(dCR$shannon)
```

Histogram of dCR\$shannon



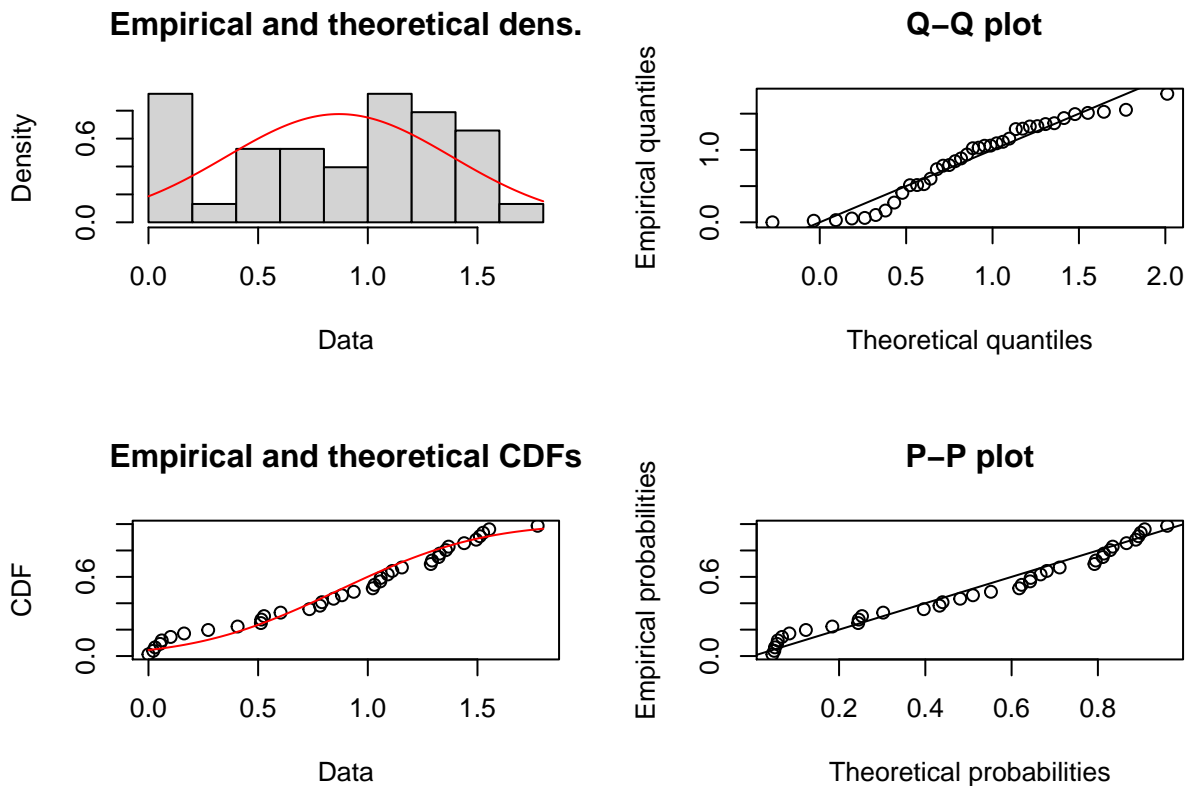
```
descdist(dCR$shannon, boot = 1000)
```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 0    max: 1.774394
## median: 0.9793887
## mean: 0.8687514
## estimated sd: 0.5211839
## estimated skewness: -0.3021322
## estimated kurtosis: 1.921262
```

```
fit.norm <- fitdist(dCR$shannon, distr = "norm", method = "mme") # looks good
plot(fit.norm)
```



→ gamma

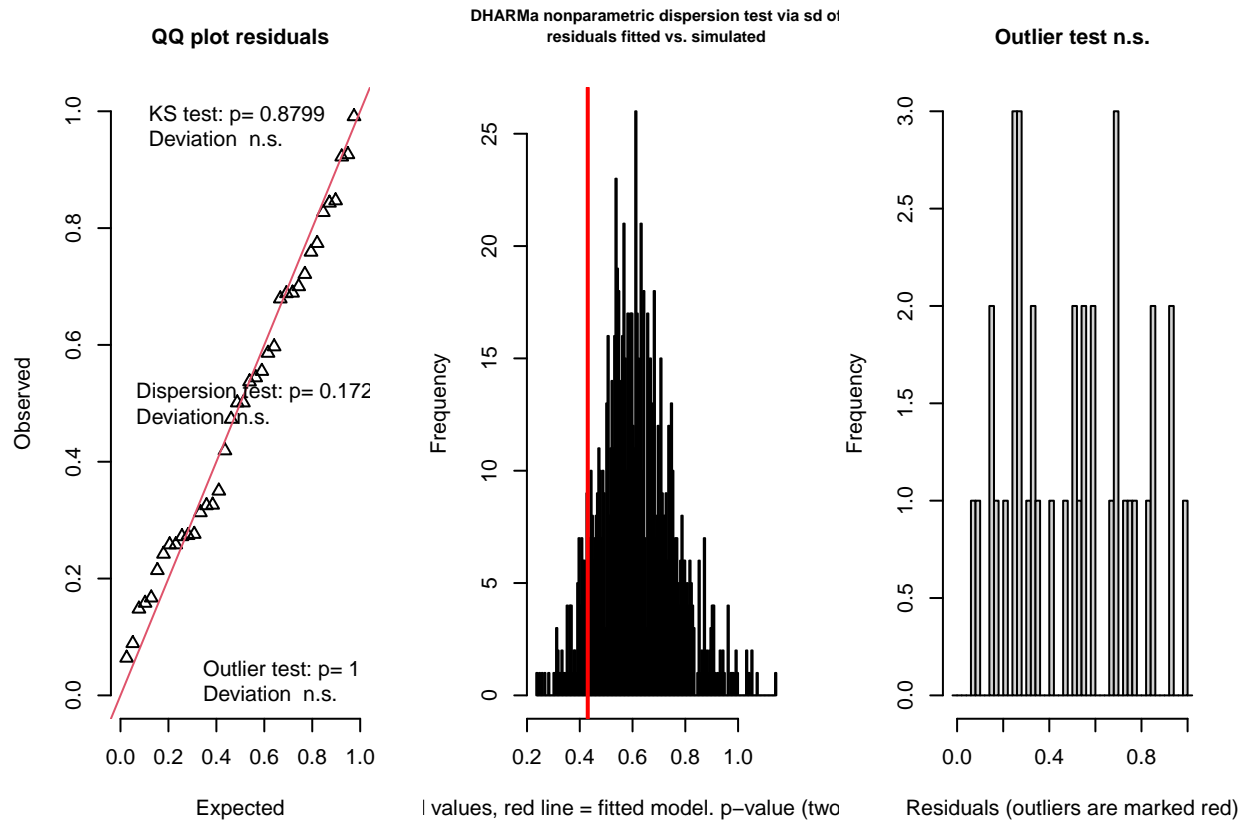
test with subset 1

```
dCR$Treatment <- as.factor(dCR$Treatment)
dCR <- within(dCR, Treatment <- relevel(Treatment, ref = "control"))

shan1 <- glm(shannon ~ Treatment + Linage, data = dCR)
Anova(shan1, type = "II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: shannon
##          LR Chisq Df Pr(>Chisq)
## Treatment  1.2779  1  0.25830
## Linage     22.5454 10  0.01255 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_shan1 <- simulateResiduals(shan1, n = 1000)
testResiduals(res_shan1)
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.095368, p-value = 0.8799
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.70198, p-value = 0.172
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 38, p-value = 1
```

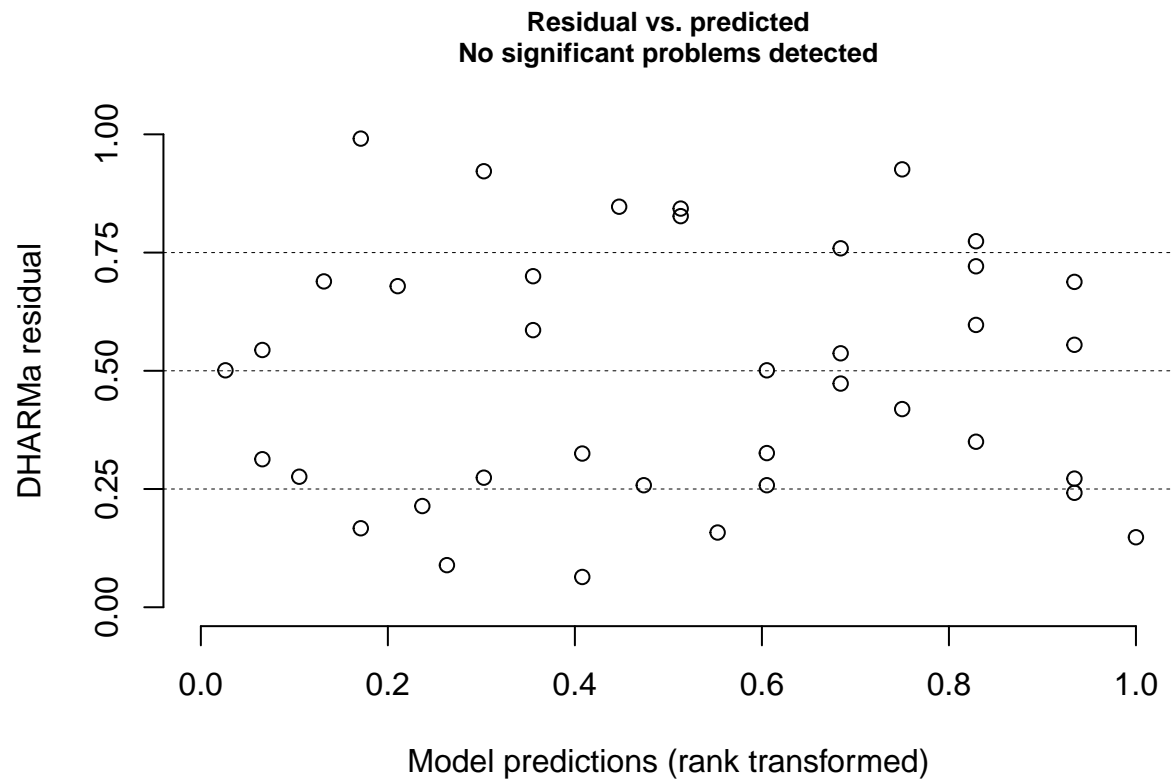
```

## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.00000000 0.09251276
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.095368, p-value = 0.8799
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.70198, p-value = 0.172
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 38, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.00000000 0.09251276
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

```

```
plotResiduals(res_shan1)
```

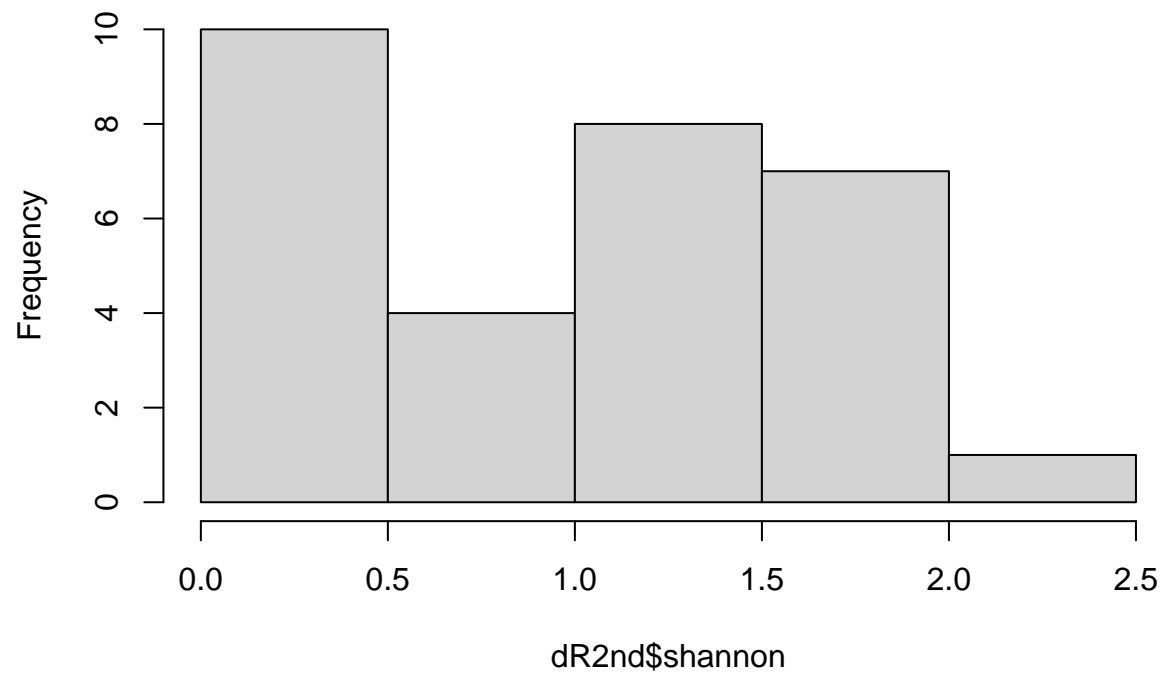


-> strong Lineage effect in B24, Lineage with high abundant *Erwinia*, *Ochrobactrum* and *Microbacterium* inside

test with subset 2

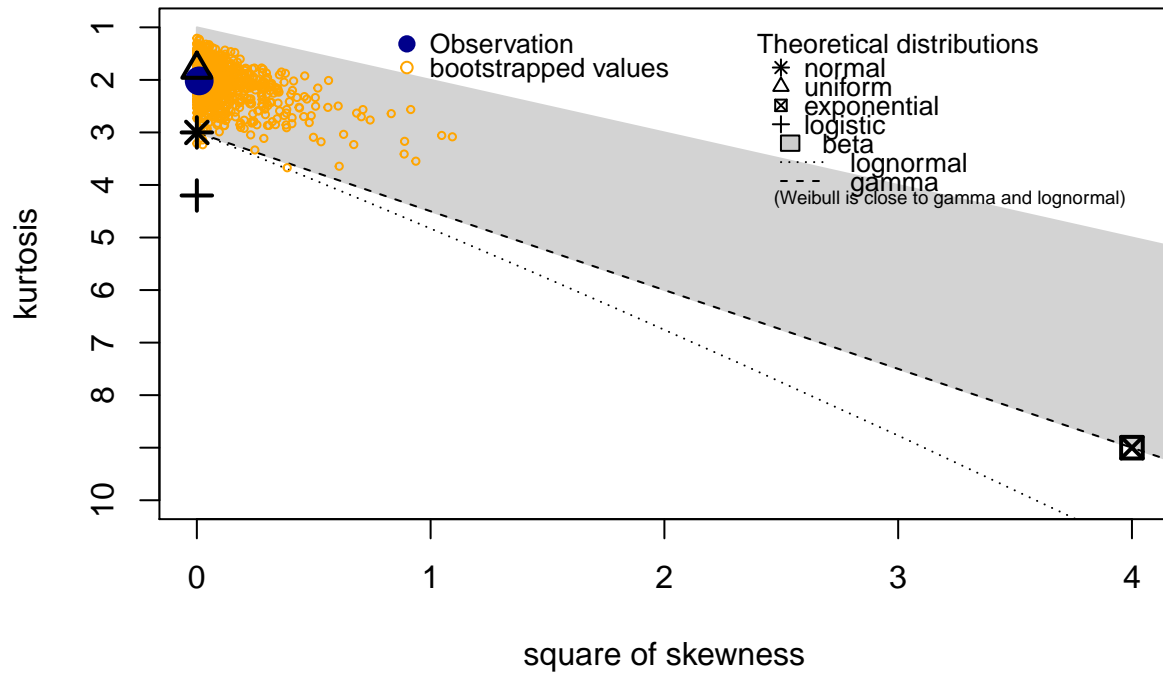
```
hist(dR2nd$shannon)
```

Histogram of dR2nd\$shannon



```
descdist(dR2nd$shannon, boot = 1000)
```

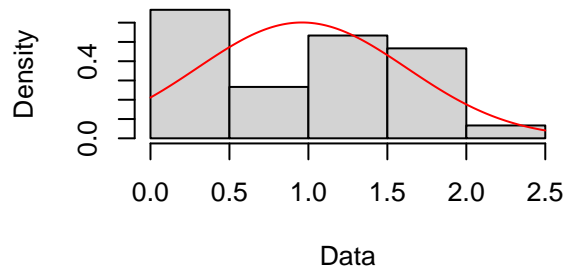
Cullen and Frey graph



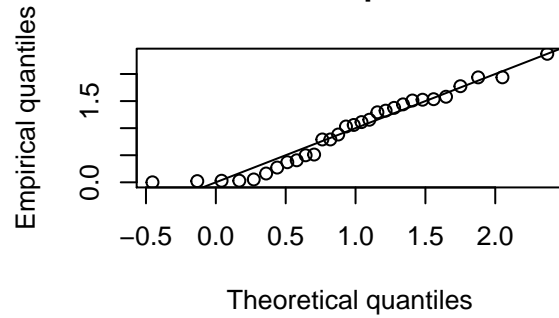
```
## summary statistics
## -----
## min: 0    max: 2.372804
## median: 1.044649
## mean: 0.9593534
## estimated sd: 0.675297
## estimated skewness: 0.1004296
## estimated kurtosis: 2.019042
```

```
fit.norm <- fitdist(dR2nd$shannon, distr = "norm", method = "mme") # looks good
plot(fit.norm)
```

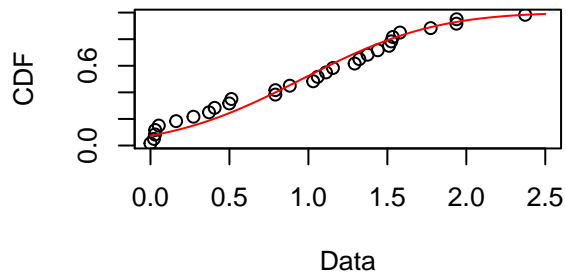
Empirical and theoretical dens.



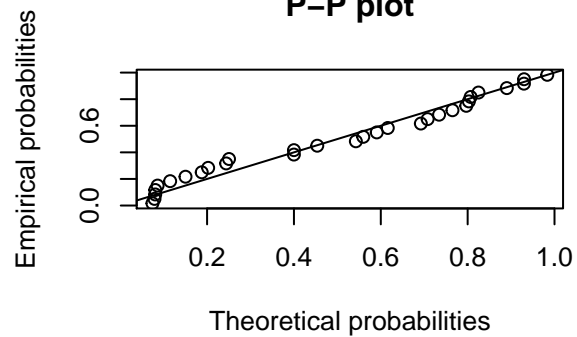
Q-Q plot



Empirical and theoretical CDFs



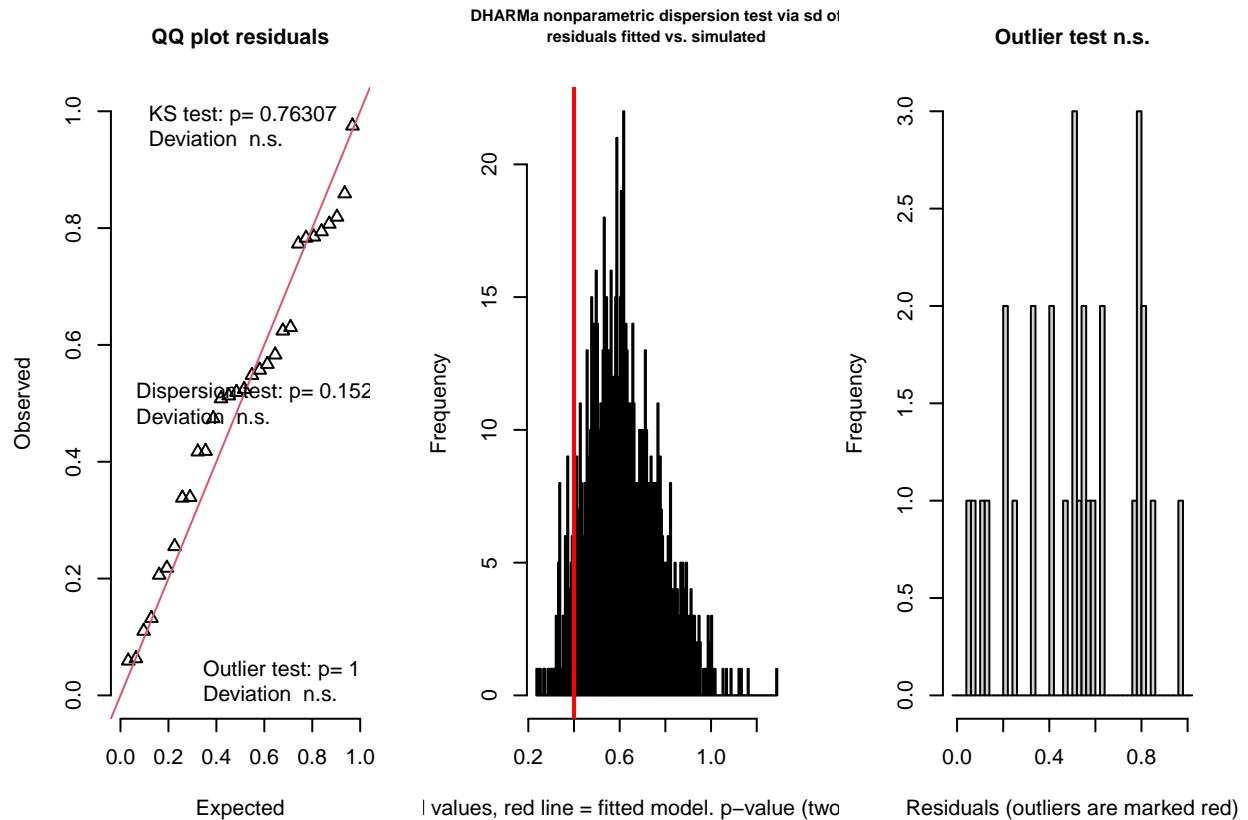
P-P plot



```
shan <- glm(shannon ~ Treatment + Linage, data = dR2nd)
Anova(shan, type = "II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: shannon
##      LR Chisq Df Pr(>Chisq)
## Treatment  4.5059 1  0.03378 *
## Linage     14.1368 9  0.11754
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_shan <- simulateResiduals(shan, n = 1000)
testResiduals(res_shan)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.117, p-value = 0.7631
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.65369, p-value = 0.152
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 30, p-value = 1
```

```

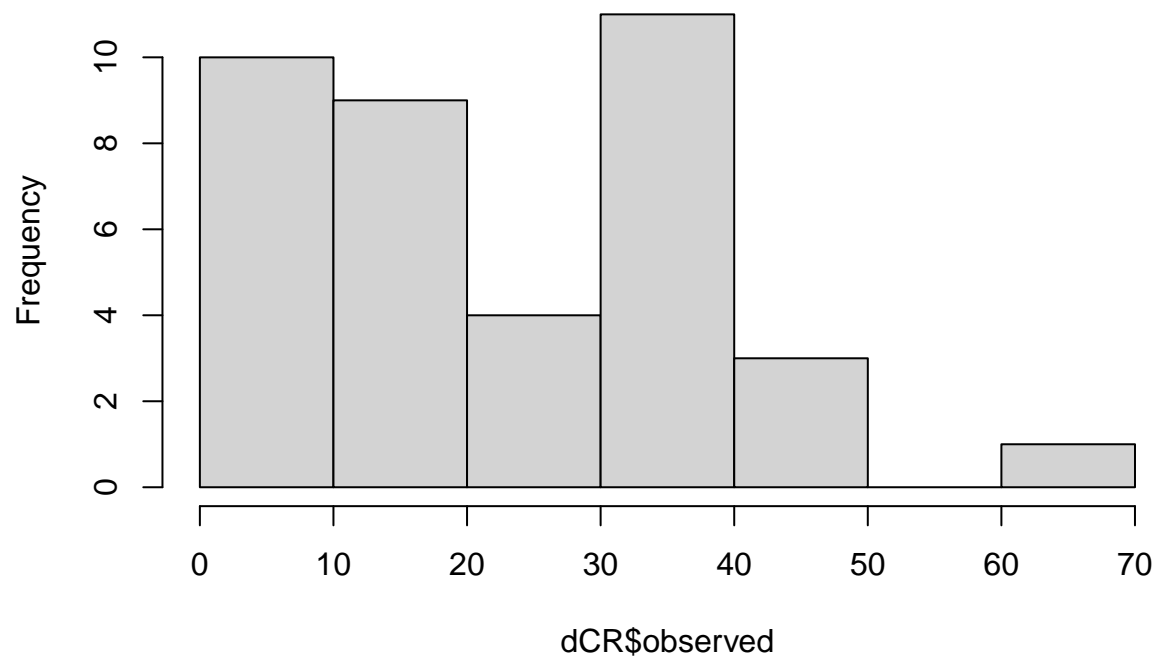
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1157033
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.117, p-value = 0.7631
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.65369, p-value = 0.152
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 30, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1157033
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

```

```
hist(dCR$observed)
```

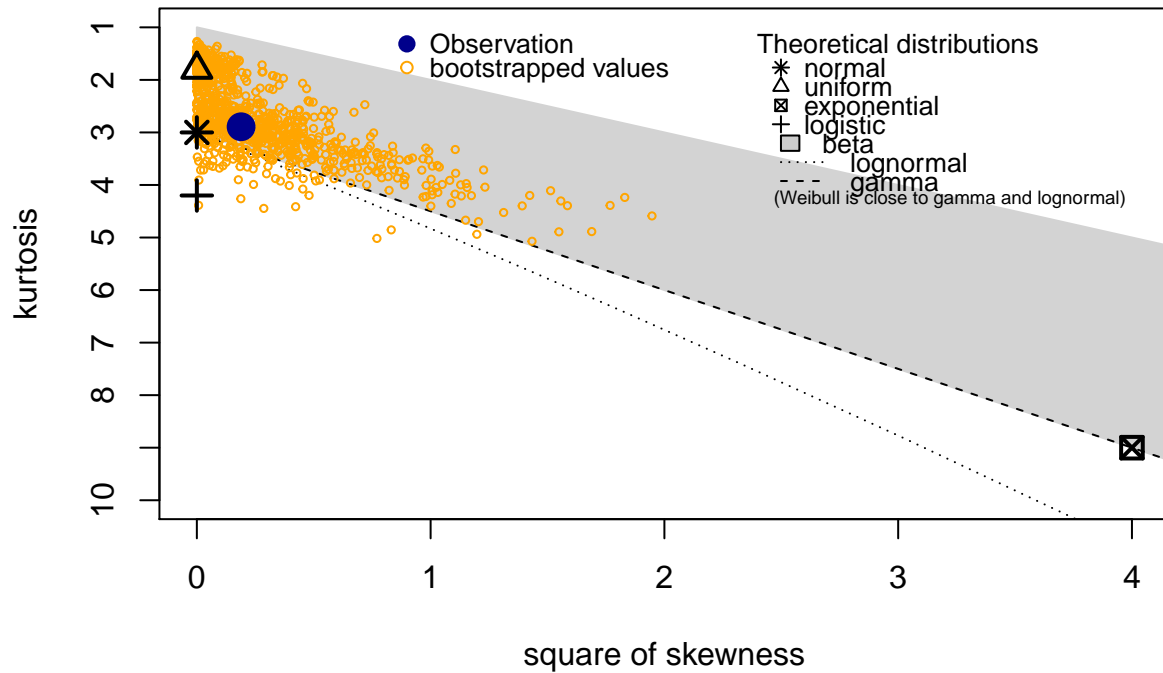

Histogram of dCR\$observed



Observed richness

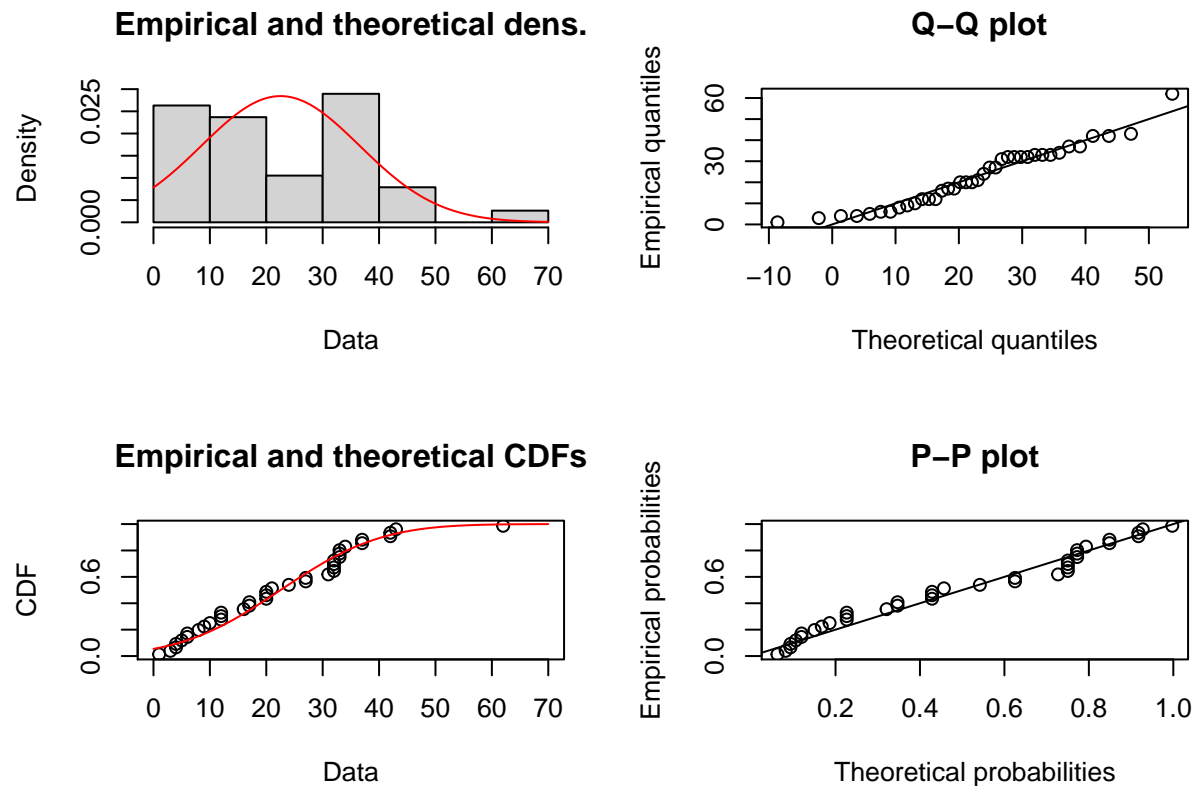
```
descdist(dCR$observed, boot = 1000)
```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 1   max: 62
## median: 20.5
## mean: 22.52632
## estimated sd: 14.21977
## estimated skewness: 0.4350977
## estimated kurtosis: 2.891807
```

```
fit.norm <- fitdist(dCR$observed, distr = "norm", method = "mme") # looks good
plot(fit.norm)
```



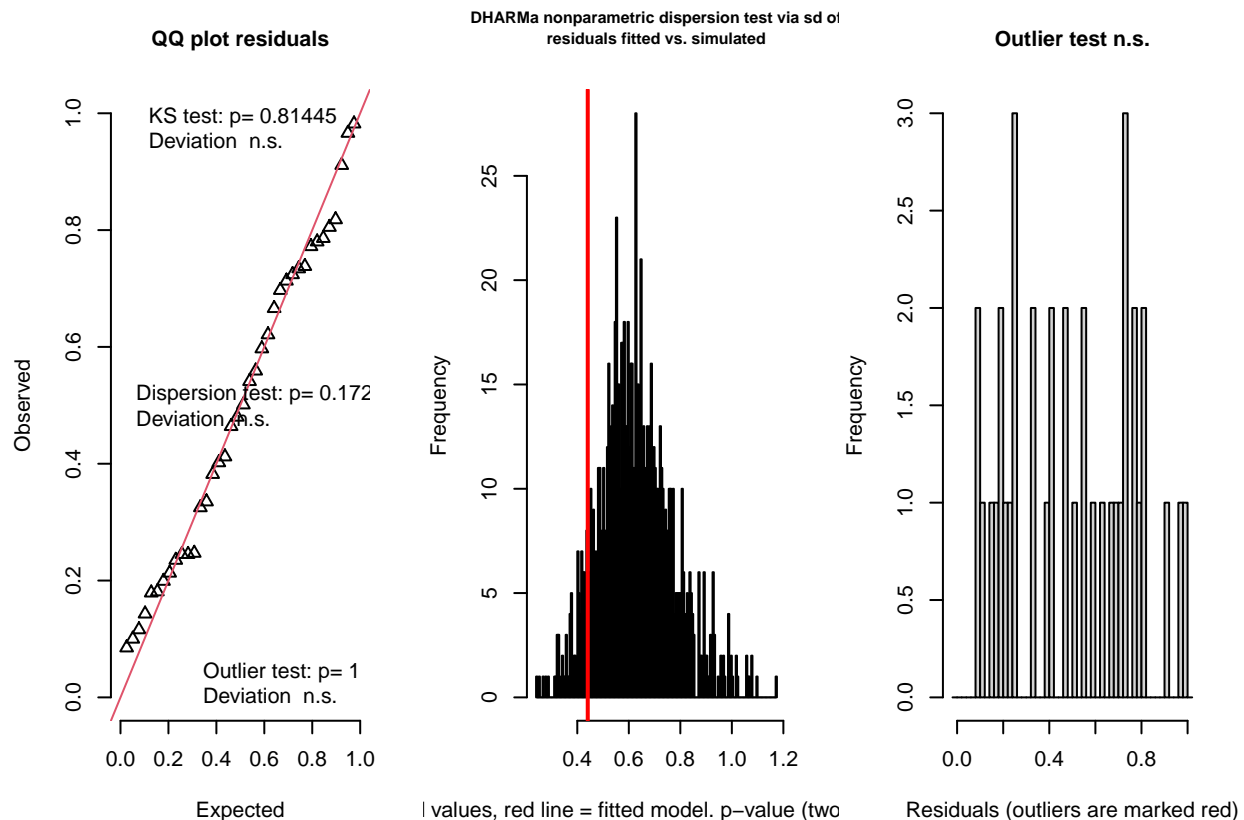
run model

```
dCR$Treatment <- as.factor(dCR$Treatment)
dCR <- within(dCR, Treatment <- relevel(Treatment, ref = "control"))

ob1 <- glm(observed ~ Treatment + Linage, data = dCR)
Anova(ob1, type = "II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: observed
##          LR Chisq Df Pr(>Chisq)
## Treatment   0.6393  1   0.42395
## Linage    21.9446 10   0.01539 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_ob1 <- simulateResiduals(ob1, n = 1000)
testResiduals(res_ob1)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.10305, p-value = 0.8144
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.70198, p-value = 0.172
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 38, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.00000000 0.09251276
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

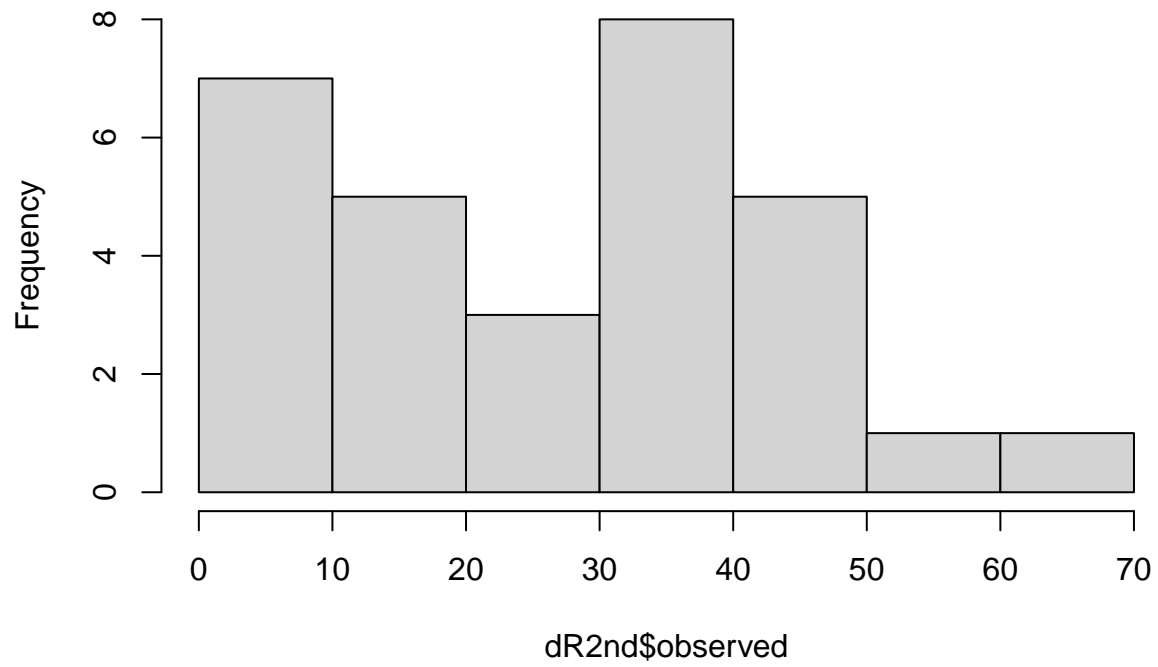
```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.10305, p-value = 0.8144
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.70198, p-value = 0.172
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 38, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.00000000 0.09251276
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

choose normal distribution

test with subset 2

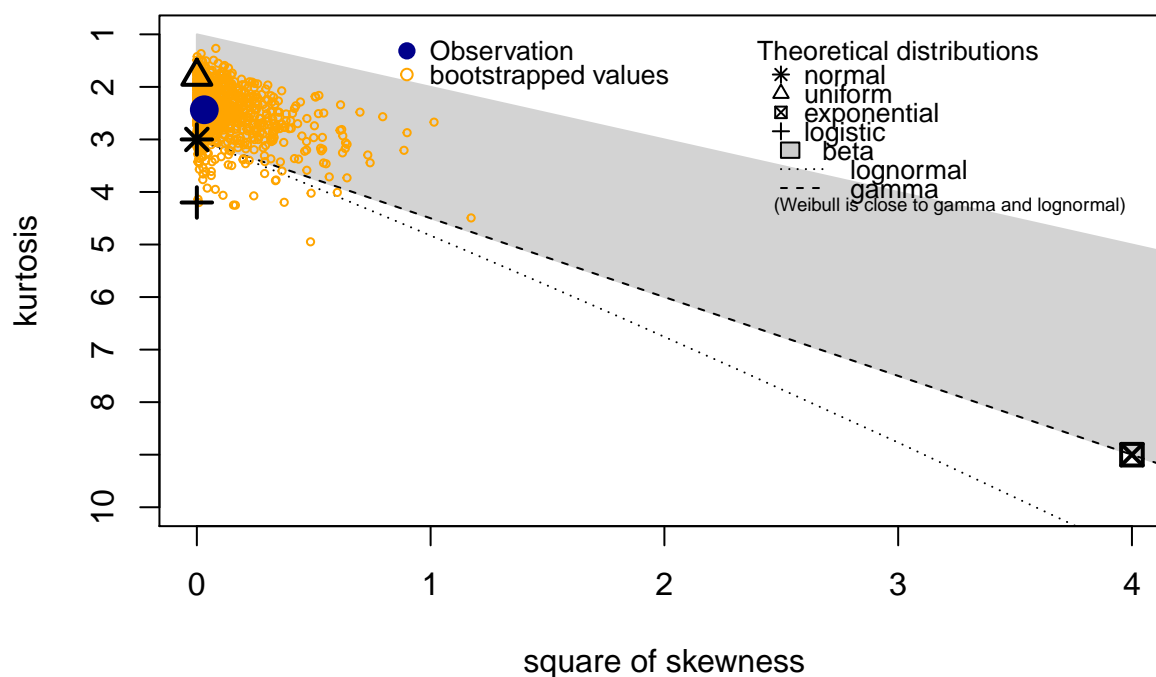
```
hist(dR2nd$observed)
```

Histogram of dR2nd\$observed



```
descdist(dR2nd$observed, boot = 1000)
```

Cullen and Frey graph

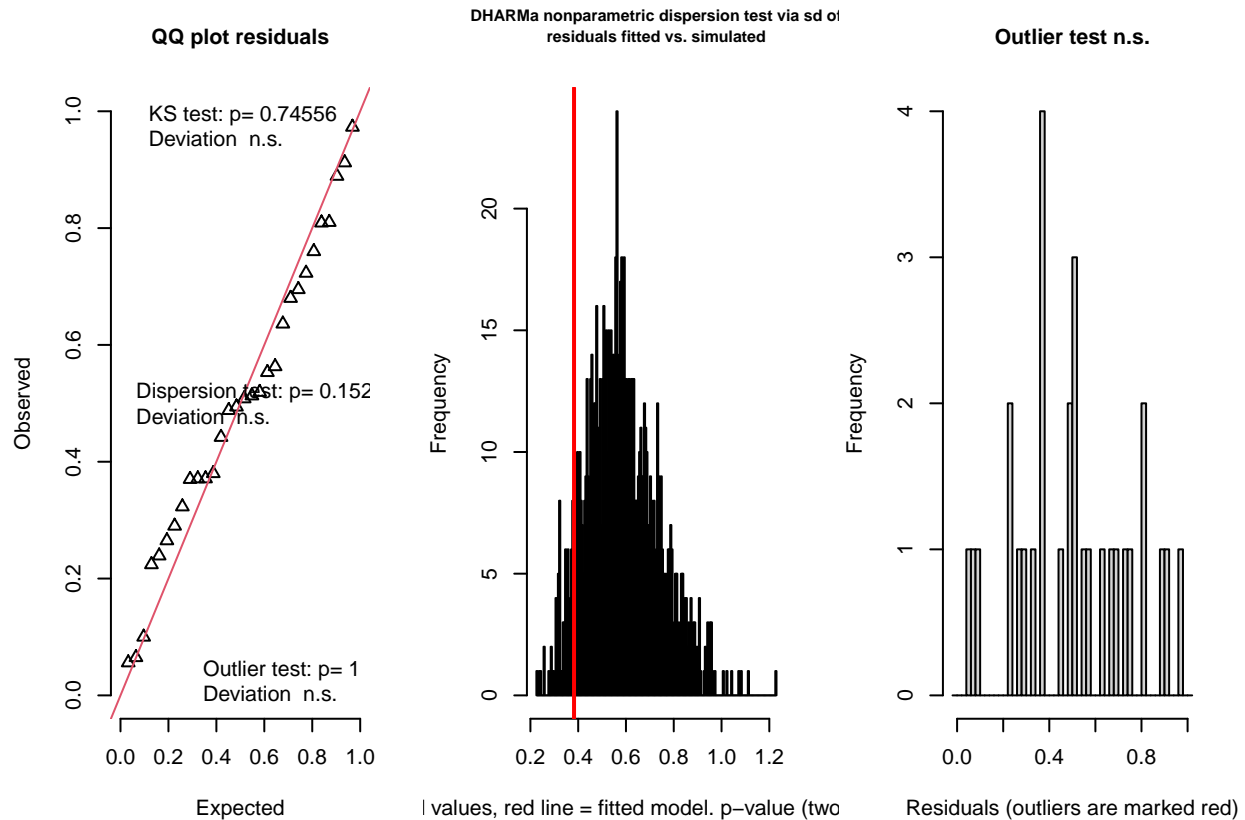


```
## summary statistics
## -----
## min: 1    max: 62
## median: 29
## mean: 27.46667
## estimated sd: 16.49605
## estimated skewness: 0.1784444
## estimated kurtosis: 2.434842
```

```
ob2 <- glm(observed ~ Treatment + Linage, data = dR2nd)
Anova(ob2, type = "II")
```

```
## Analysis of Deviance Table (Type II tests)
##
## Response: observed
##          LR Chisq Df Pr(>Chisq)
## Treatment   3.1731  1   0.07486 .
## Linage     18.8360  9   0.02662 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_ob2 <- simulateResiduals(ob2, n = 1000)
testResiduals(res_ob2)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.124, p-value = 0.7456
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.65369, p-value = 0.152
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 30, p-value = 1
```



```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1157033
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.124, p-value = 0.7456
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.65369, p-value = 0.152
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 30, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1157033
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

Beta Diversity

Beta diversity and microbiome divergence

Beta diversity quantifies dissimilarity in community composition between samples. Dissimilarity can be also quantified by distance or divergence. These measures have a broad use in statistical data analysis.

```
rel.CR <- CR %>%
  transform_sample_counts(function(x) {x/sum(x)} )

rel.R2nd <- R2nd %>%
  transform_sample_counts(function(x) {x/sum(x)} )
```

relative Abundance plots agglomerate data to Species level, transform to rel. abundance, melt long format and sort data frame alph. by Species

```
Fungi_Species1 <- CR %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)

Fungi_Species2 <- R2nd %>%
  tax_glom(taxrank = "Species") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Species)
```

find the mean and standard deviation by Species

```
rem <- subset(Fungi_Species1, Treatment == "removal")
mean.rem <- tapply(rem$Abundance, rem$Species, mean)
mean.rem
```

##	Acremonium_biseptum	Acremonium_spc
##	4.822926e-02	1.825963e-04
##	Ascomycota_spc_spc	Aspergillus_costiformis
##	1.921889e-03	2.792152e-05
##	Chaetomiaceae_spc	Chaetomium_globosum
##	1.527656e-03	5.751256e-01
##	Chaetomium_spc	Diatrypella_vulgaris
##	5.217036e-03	7.370756e-05
##	Eurotiales_spc	Eurotiomycetes_spc
##	1.585447e-04	6.946107e-05
##	Hypocreales_spc	Ophiostomataceae_spc
##	3.086386e-04	1.346598e-04
##	Ophiostomatales_spc	Paecilomyces_spc
##	1.862119e-04	4.264759e-05
##	Penicillium_commune	Penicillium_spc
##	7.113496e-03	2.412351e-03
##	Raffaelea_canadensis	Raffaelea_spc
##	3.755341e-02	1.845842e-03
##	Raffaelea_sulphurea	Sordariales_spc
##	3.076030e-01	3.200399e-04
##	Sordariomycetes_spc	Sporothrix_stenoceras
##	8.133021e-03	1.045215e-05
##	Talaromyces_rugulosus	Thermoascaceae_spc
##	1.273286e-03	8.691522e-05
##	Xenopolyscytalum_sp._I12F_02273	Xylariales_spc
##	3.227091e-04	1.196866e-04

```
SD.rem <- tapply(rem$Abundance, rem$Species, sd)
SD.rem
```

##	Acremonium_biseptum	Acremonium_spc
##	2.129341e-01	7.380513e-04

##	Ascomycota_spc_spc	Aspergillus_costiformis
##	7.016012e-03	1.248688e-04
##	Chaetomiaceae_spc	Chaetomium_globosum
##	2.777907e-03	3.863477e-01
##	Chaetomium_spc	Diatrypella_vulgaris
##	5.606403e-03	3.109255e-04
##	Eurotiales_spc	Eurotiomycetes_spc
##	5.080569e-04	1.680653e-04
##	Hypocreales_spc	Ophiostomataceae_spc
##	1.303065e-03	3.373166e-04
##	Ophiostomatales_spc	Paecilomyces_spc
##	3.095223e-04	1.685520e-04
##	Penicillium_commune	Penicillium_spc
##	3.178475e-02	1.076897e-02
##	Raffaelea_canadensis	Raffaelea_spc
##	5.985818e-02	4.362601e-03
##	Raffaelea_sulphurea	Sordariales_spc
##	3.306086e-01	4.252054e-04
##	Sordariomycetes_spc	Sporothrix_stenoceras
##	8.708500e-03	3.295761e-05
##	Talaromyces_rugulosus	Thermoascaceae_spc
##	4.413233e-03	2.700954e-04
##	Xenopolyscytalum_sp._I12F_02273	Xylariales_spc
##	7.445484e-04	5.352549e-04

```
sec <- subset(Fungi_Species2, Treatment == "2nd-foundation")
mean.sec <- tapply(sec$Abundance, sec$Species, mean)
mean.sec
```

##	Acremonium_biseptum	Acremonium_spc
##	0.0236606934	0.0039535863
##	Ascomycota_spc_spc	Aspergillus_costiformis
##	0.0032163024	0.0000000000
##	Chaetomiaceae_spc	Chaetomium_globosum
##	0.0073566780	0.5073275038
##	Chaetomium_spc	Diatrypella_vulgaris
##	0.0085913959	0.0000000000
##	Eurotiales_spc	Eurotiomycetes_spc
##	0.0007557944	0.0000877943
##	Hypocreales_spc	Ophiostomataceae_spc
##	0.0032611238	0.0005139207
##	Ophiostomatales_spc	Paecilomyces_spc
##	0.0007235568	0.0000000000
##	Penicillium_commune	Penicillium_spc
##	0.0316822915	0.0072273551
##	Raffaelea_canadensis	Raffaelea_spc
##	0.1175589366	0.0072412047
##	Raffaelea_sulphurea	Sordariales_spc
##	0.2531221210	0.0016430442
##	Sordariomycetes_spc	Sporothrix_stenoceras
##	0.0207058085	0.0000000000
##	Talaromyces_rugulosus	Thermoascaceae_spc
##	0.0000000000	0.0000000000
##	Xenopolyscytalum_sp._I12F_02273	Xylariales_spc

```
##                0.0000000000                0.0013708885
```

```
SD.sec <- tapply(sec$Abundance, sec$Species, sd)
SD.sec
```

```
##                Acremonium_biseptum                Acremonium_spc
##                0.0784736423                0.0131125623
##                Ascomycota_spc_spc                Aspergillus_costiformis
##                0.0089808210                0.0000000000
##                Chaetomiaceae_spc                Chaetomium_globosum
##                0.0091540276                0.3533535207
##                Chaetomium_spc                Diatrypella_vulgaris
##                0.0099731689                0.0000000000
##                Eurotiales_spc                Eurotiomycetes_spc
##                0.0025066865                0.0002911808
##                Hypocreales_spc                Ophiostomataceae_spc
##                0.0108159242                0.0013543344
##                Ophiostomatales_spc                Paecilomyces_spc
##                0.0011593941                0.0000000000
##                Penicillium_commune                Penicillium_spc
##                0.1050782734                0.0239371110
##                Raffaelea_canadensis                Raffaelea_spc
##                0.1306026315                0.0142054109
##                Raffaelea_sulphurea                Sordariales_spc
##                0.2771804794                0.0029798185
##                Sordariomycetes_spc                Sporothrix_stenoceras
##                0.0243502551                0.0000000000
##                Talaromyces_rugulosus                Thermoascaceae_spc
##                0.0000000000                0.0000000000
##                Xenopolyscytalum_sp._I12F_02273                Xylariales_spc
##                0.0000000000                0.0045467228
```

```
control <- subset(Fungi_Species1, Treatment == "control")
mean.control <- tapply(control$Abundance, control$Species, mean)
mean.control
```

```
##                Acremonium_biseptum                Acremonium_spc
##                2.018503e-03                6.968641e-06
##                Ascomycota_spc_spc                Aspergillus_costiformis
##                6.965760e-05                8.988316e-05
##                Chaetomiaceae_spc                Chaetomium_globosum
##                4.590560e-04                3.796378e-01
##                Chaetomium_spc                Diatrypella_vulgaris
##                2.400139e-03                0.000000e+00
##                Eurotiales_spc                Eurotiomycetes_spc
##                0.000000e+00                0.000000e+00
##                Hypocreales_spc                Ophiostomataceae_spc
##                0.000000e+00                2.804989e-04
##                Ophiostomatales_spc                Paecilomyces_spc
##                2.988574e-04                0.000000e+00
##                Penicillium_commune                Penicillium_spc
##                1.328225e-05                0.000000e+00
##                Raffaelea_canadensis                Raffaelea_spc
```

```
##          1.104248e-01          3.631699e-03
##          Raffaelea_sulphurea          Sordariales_spc
##          4.948844e-01          1.094521e-04
##          Sordariomycetes_spc          Sporothrix_stenoceras
##          5.383031e-03          0.000000e+00
##          Talaromyces_rugulosus          Thermoascaceae_spc
##          2.899075e-04          0.000000e+00
## Xenopolyscytalum_sp._I12F_02273          Xylariales_spc
##          2.028727e-06          0.000000e+00
```

```
SD.control <- tapply(control$Abundance, control$Species, sd)
SD.control
```

```
##          Acremonium_biseptum          Acremonium_spc
##          9.027020e-03          3.116471e-05
##          Ascomycota_spc_spc          Aspergillus_costiformis
##          1.752356e-04          2.666390e-04
##          Chaetomiaceae_spc          Chaetomium_globosum
##          7.011706e-04          2.980927e-01
##          Chaetomium_spc          Diatrypella_vulgaris
##          2.929313e-03          0.000000e+00
##          Eurotiales_spc          Eurotiomycetes_spc
##          0.000000e+00          0.000000e+00
##          Hypocreales_spc          Ophiostomataceae_spc
##          0.000000e+00          1.103085e-03
##          Ophiostomatales_spc          Paecilomyces_spc
##          8.186757e-04          0.000000e+00
##          Penicillium_commune          Penicillium_spc
##          3.777937e-05          0.000000e+00
##          Raffaelea_canadensis          Raffaelea_spc
##          1.134036e-01          5.389495e-03
##          Raffaelea_sulphurea          Sordariales_spc
##          2.584721e-01          2.370017e-04
##          Sordariomycetes_spc          Sporothrix_stenoceras
##          9.740509e-03          0.000000e+00
##          Talaromyces_rugulosus          Thermoascaceae_spc
##          1.026525e-03          0.000000e+00
## Xenopolyscytalum_sp._I12F_02273          Xylariales_spc
##          9.072742e-06          0.000000e+00
```

edit object for plotting

```
#control vs. removal
Fungi_Species1$Species<-as.character(Fungi_Species1$Species)
Fungi_Species1$Species[Fungi_Species1$Abundance<0.05]<-"Others"
Fungi_Species1$Class<-as.character(Fungi_Species1$Class)
Fungi_Species1$Class[Fungi_Species1$Abundance<0.05]<-"Others"
Fungi_Species1$Species<-factor(Fungi_Species1$Species)
Fungi_Species1<-droplevels(Fungi_Species1)
Fungi_Species1$Treatment<-factor(Fungi_Species1$Treatment,levels=c("control", "removal"))
Fungi_Species1$Species <- factor(Fungi_Species1$Species, levels = c("Others", "Sordariomycetes_spc", "P

#removal vs. 2nd attempt
```

```

Fungi_Species2$Species<-as.character(Fungi_Species2$Species)
Fungi_Species2$Species[Fungi_Species2$Abundance<0.05]<-"Others"
Fungi_Species2$Class<-as.character(Fungi_Species2$Class)
Fungi_Species2$Class[Fungi_Species2$Abundance<0.05]<-"Others"
Fungi_Species2$Species<-factor(Fungi_Species2$Species)
Fungi_Species2<-droplevels(Fungi_Species2)
Fungi_Species2$Treatment<-factor(Fungi_Species2$Treatment,levels=c("removal", "2nd-foundation"))
Fungi_Species2$Species<-factor(Fungi_Species2$Species,levels=levels(Fungi_Species2$Species))
Fungi_Species2$Species <- factor(Fungi_Species2$Species, levels = c("Others", "Sordariomycetes_spc", "P

```

define plot colors

```

Plot_colors_s <- c("grey87", "forestgreen", "peachpuff3", "lightskyblue", "goldenrod3", "gold")

Plot_colors_s2 <- c("grey87", "bisque", "darkolivegreen3", "darkgreen", "forestgreen", "peachpuff3", "g

Plot_colors_s3 <- c("grey87", "bisque", "darkgreen", "forestgreen", "peachpuff3", "lightskyblue", "gold

```

```

Fun2 <- Fungi_Species1
Fun2$Sample <- factor(Fun2$Sample, levels = c("B0-24", "B0-27", "B0-19a", "B0-22a", "B15-06", "B15-11",
Fun2$Linage <- factor(Fun2$Linage, levels = c("B0", "B15", "B16", "B20", "B24", "B36", "B39", "B46", "B

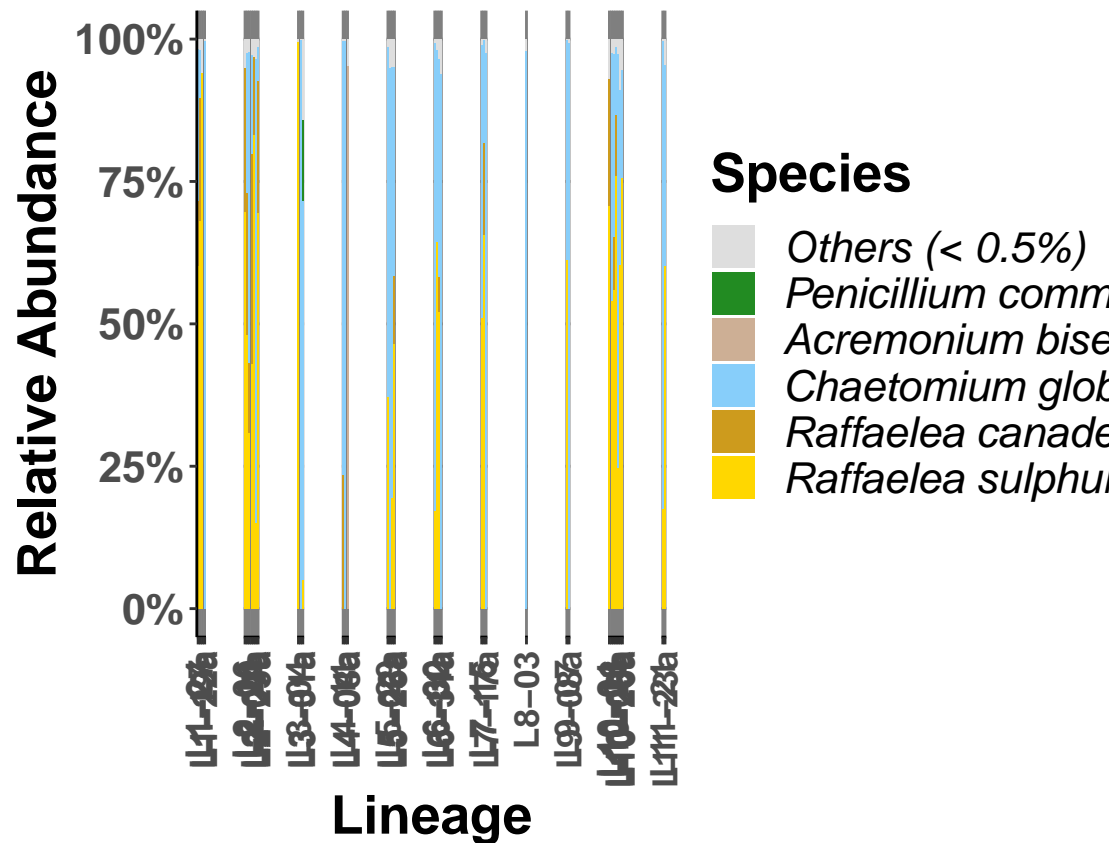
Fungi_Species_plot2 <- ggplot(Fun2, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Plot_colors_s, name = "Species")

relAb_fun<-Fungi_Species_plot2 +
  facet_grid(~ Linage, scales = "free_x", space = "free_x")+
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme_classic()+ #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x="Linage", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(axis.text.x=element_text(size = 13, angle = 90, vjust = 0.5))+
  theme(text = element_text(size=20, face = "bold"))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 16))+
  theme(legend.text = element_text(face = "italic"))+
  theme(strip.text.x = element_blank())

relAb_fun <- relAb_fun + theme(panel.spacing.x = unit(0.5, "cm"))

relAb_fun

```



plot control vs. removal

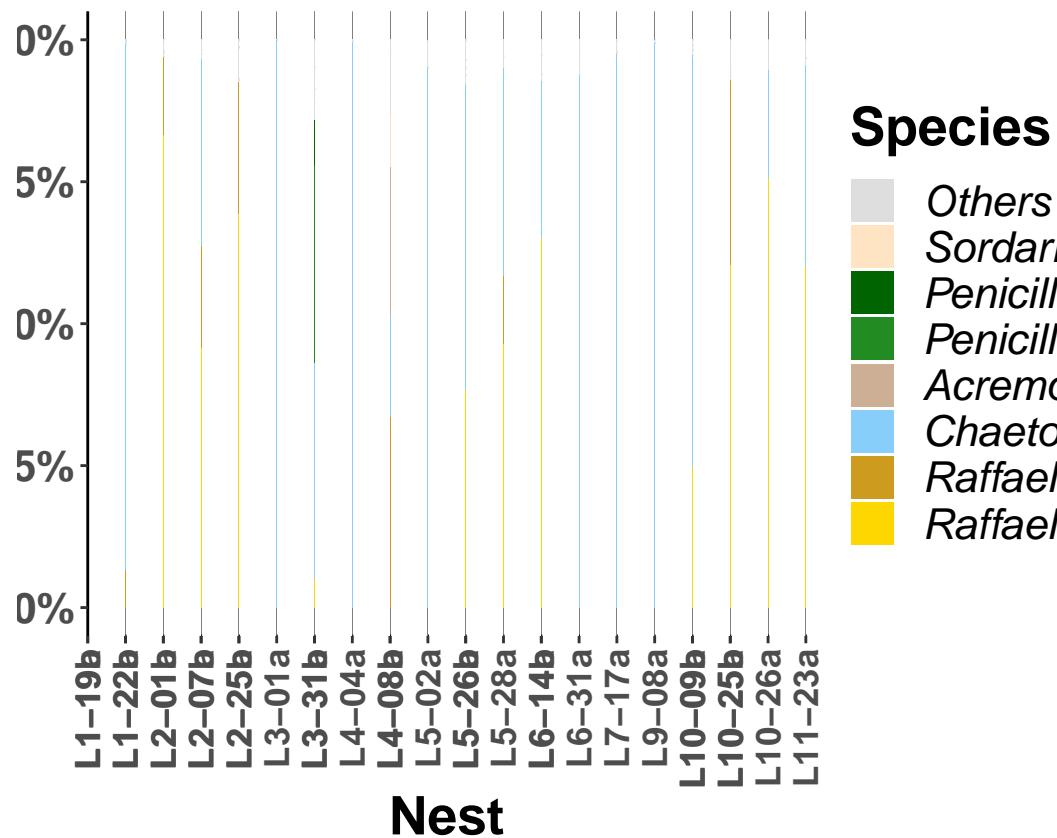
```
Fun3 <- Fungi_Species2
Fun3$Nest <- factor(Fun3$Nest, levels = c("B0-19", "B0-22", "B15-01", "B15-07", "B15-25", "B16-01", "B16-02", "B16-03", "B16-04", "B16-05", "B16-06", "B16-07", "B16-08", "B16-09", "B16-10", "B16-11", "B16-12", "B16-13", "B16-14", "B16-15", "B16-16", "B16-17", "B16-18", "B16-19", "B16-20", "B16-21", "B16-22", "B16-23", "B16-24", "B16-25", "B16-26", "B16-27", "B16-28", "B16-29", "B16-30", "B16-31", "B16-32", "B16-33", "B16-34", "B16-35", "B16-36", "B16-37", "B16-38", "B16-39", "B16-40", "B16-41", "B16-42", "B16-43", "B16-44", "B16-45", "B16-46", "B16-47", "B16-48", "B16-49", "B16-50", "B16-51", "B16-52", "B16-53", "B16-54", "B16-55", "B16-56", "B16-57", "B16-58", "B16-59", "B16-60", "B16-61", "B16-62", "B16-63", "B16-64", "B16-65", "B16-66", "B16-67", "B16-68", "B16-69", "B16-70", "B16-71", "B16-72", "B16-73", "B16-74", "B16-75", "B16-76", "B16-77", "B16-78", "B16-79", "B16-80", "B16-81", "B16-82", "B16-83", "B16-84", "B16-85", "B16-86", "B16-87", "B16-88", "B16-89", "B16-90", "B16-91", "B16-92", "B16-93", "B16-94", "B16-95", "B16-96", "B16-97", "B16-98", "B16-99", "B16-100"))
Fun3$Sample <- factor(Fun3$Sample, levels = c("B0-19a", "B0-19b", "B0-22a", "B0-22b", "B15-01a", "B15-01b", "B15-07a", "B15-07b", "B15-25a", "B15-25b", "B16-01a", "B16-01b", "B16-02a", "B16-02b", "B16-03a", "B16-03b", "B16-04a", "B16-04b", "B16-05a", "B16-05b", "B16-06a", "B16-06b", "B16-07a", "B16-07b", "B16-08a", "B16-08b", "B16-09a", "B16-09b", "B16-10a", "B16-10b", "B16-11a", "B16-11b", "B16-12a", "B16-12b", "B16-13a", "B16-13b", "B16-14a", "B16-14b", "B16-15a", "B16-15b", "B16-16a", "B16-16b", "B16-17a", "B16-17b", "B16-18a", "B16-18b", "B16-19a", "B16-19b", "B16-20a", "B16-20b", "B16-21a", "B16-21b", "B16-22a", "B16-22b", "B16-23a", "B16-23b", "B16-24a", "B16-24b", "B16-25a", "B16-25b", "B16-26a", "B16-26b", "B16-27a", "B16-27b", "B16-28a", "B16-28b", "B16-29a", "B16-29b", "B16-30a", "B16-30b", "B16-31a", "B16-31b", "B16-32a", "B16-32b", "B16-33a", "B16-33b", "B16-34a", "B16-34b", "B16-35a", "B16-35b", "B16-36a", "B16-36b", "B16-37a", "B16-37b", "B16-38a", "B16-38b", "B16-39a", "B16-39b", "B16-40a", "B16-40b", "B16-41a", "B16-41b", "B16-42a", "B16-42b", "B16-43a", "B16-43b", "B16-44a", "B16-44b", "B16-45a", "B16-45b", "B16-46a", "B16-46b", "B16-47a", "B16-47b", "B16-48a", "B16-48b", "B16-49a", "B16-49b", "B16-50a", "B16-50b", "B16-51a", "B16-51b", "B16-52a", "B16-52b", "B16-53a", "B16-53b", "B16-54a", "B16-54b", "B16-55a", "B16-55b", "B16-56a", "B16-56b", "B16-57a", "B16-57b", "B16-58a", "B16-58b", "B16-59a", "B16-59b", "B16-60a", "B16-60b", "B16-61a", "B16-61b", "B16-62a", "B16-62b", "B16-63a", "B16-63b", "B16-64a", "B16-64b", "B16-65a", "B16-65b", "B16-66a", "B16-66b", "B16-67a", "B16-67b", "B16-68a", "B16-68b", "B16-69a", "B16-69b", "B16-70a", "B16-70b", "B16-71a", "B16-71b", "B16-72a", "B16-72b", "B16-73a", "B16-73b", "B16-74a", "B16-74b", "B16-75a", "B16-75b", "B16-76a", "B16-76b", "B16-77a", "B16-77b", "B16-78a", "B16-78b", "B16-79a", "B16-79b", "B16-80a", "B16-80b", "B16-81a", "B16-81b", "B16-82a", "B16-82b", "B16-83a", "B16-83b", "B16-84a", "B16-84b", "B16-85a", "B16-85b", "B16-86a", "B16-86b", "B16-87a", "B16-87b", "B16-88a", "B16-88b", "B16-89a", "B16-89b", "B16-90a", "B16-90b", "B16-91a", "B16-91b", "B16-92a", "B16-92b", "B16-93a", "B16-93b", "B16-94a", "B16-94b", "B16-95a", "B16-95b", "B16-96a", "B16-96b", "B16-97a", "B16-97b", "B16-98a", "B16-98b", "B16-99a", "B16-99b", "B16-100a", "B16-100b"))

Fungi_Species2_plot2 <- ggplot(Fun3, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position="fill") +
  scale_fill_manual(values = Plot_colors_s3, name = "Species")

g4<-Fungi_Species2_plot2 +
  facet_grid(~ Nest, scales = "free_x", space = "free_x")+
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme_classic()+
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50"))+
  labs(x="Nest", y="Relative Abundance")+
  scale_y_continuous(labels=percent_format())+
  theme(axis.text.x=element_text(size = 13, angle = 90, vjust = 0.5))+
  theme(text = element_text(size=20, face = "bold"))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 16))+
  theme(legend.text = element_text(face = "italic"))+
  theme(strip.text.x = element_blank())

relAb_fun2 <- g4 + theme(panel.spacing.x = unit(0.5, "cm"))
```

```
relAb_fun2
```



plot removal vs. 2nd-attempt

Microbiome composition

Composition heatmaps plot control vs. removal (>5% abundance)

```
pseq <- aggregate_rare(rel.CR, level = "Species", detection = 5/100, prevalence = 10/100)

pseqh <- pseq %>%
  psmelt()

pseqh$Treatment<-factor(pseqh$Treatment,levels=c("control", "removal"))
pseqh$Species<-factor(pseqh$Species,levels=c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_c..."

p.heat <- ggplot(pseqh, aes(x = Sample, y = Species)) + geom_tile(aes(fill = Abundance))

# Change color
p.heat <- p.heat + scale_fill_distiller("Abundance", palette = "RdYlBu", labels = scales::percent) + th...

# Make bacterial names italics
p.heat <- p.heat + theme(axis.text.y = element_text(colour = 'black',
  size = 10,
  face = 'italic'))

# Make separate samples based on main variable
```

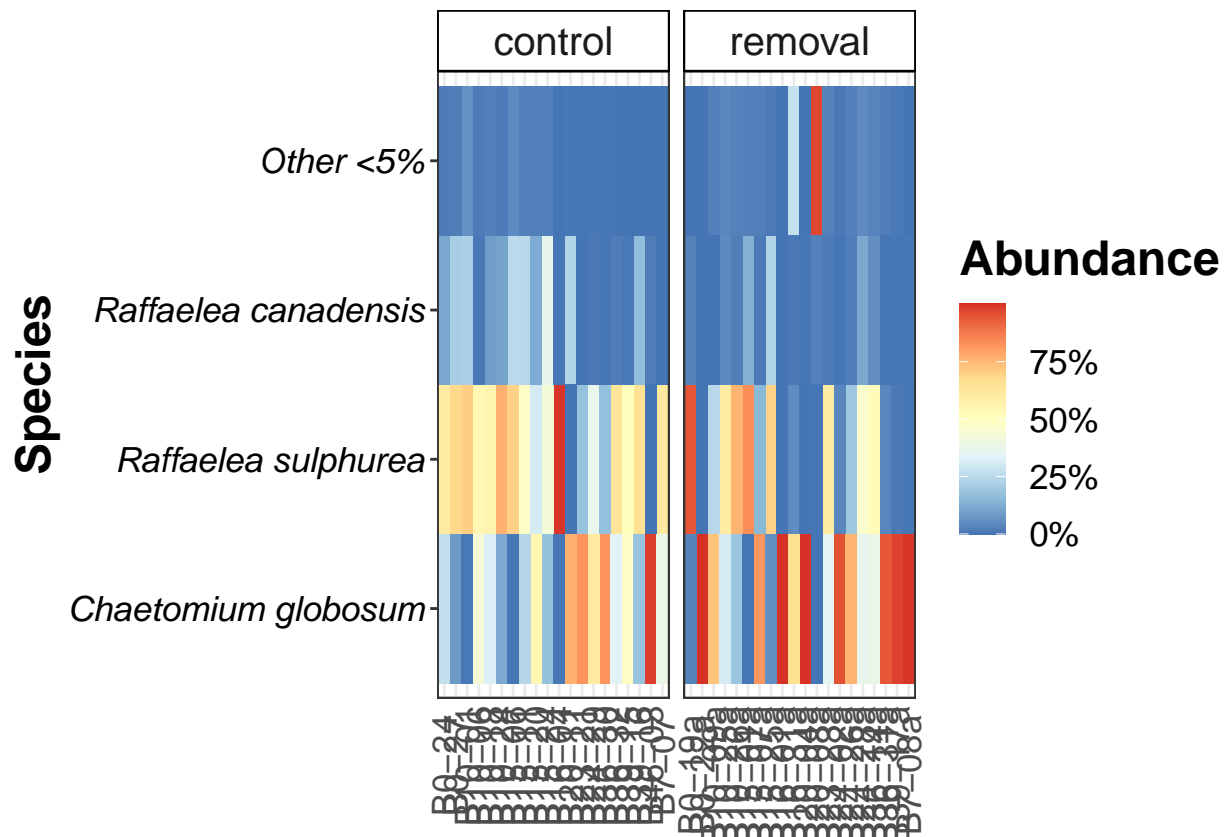


```
p.heat <- p.heat + facet_grid(~Treatment,
                             scales = "free") + rremove("x.text")

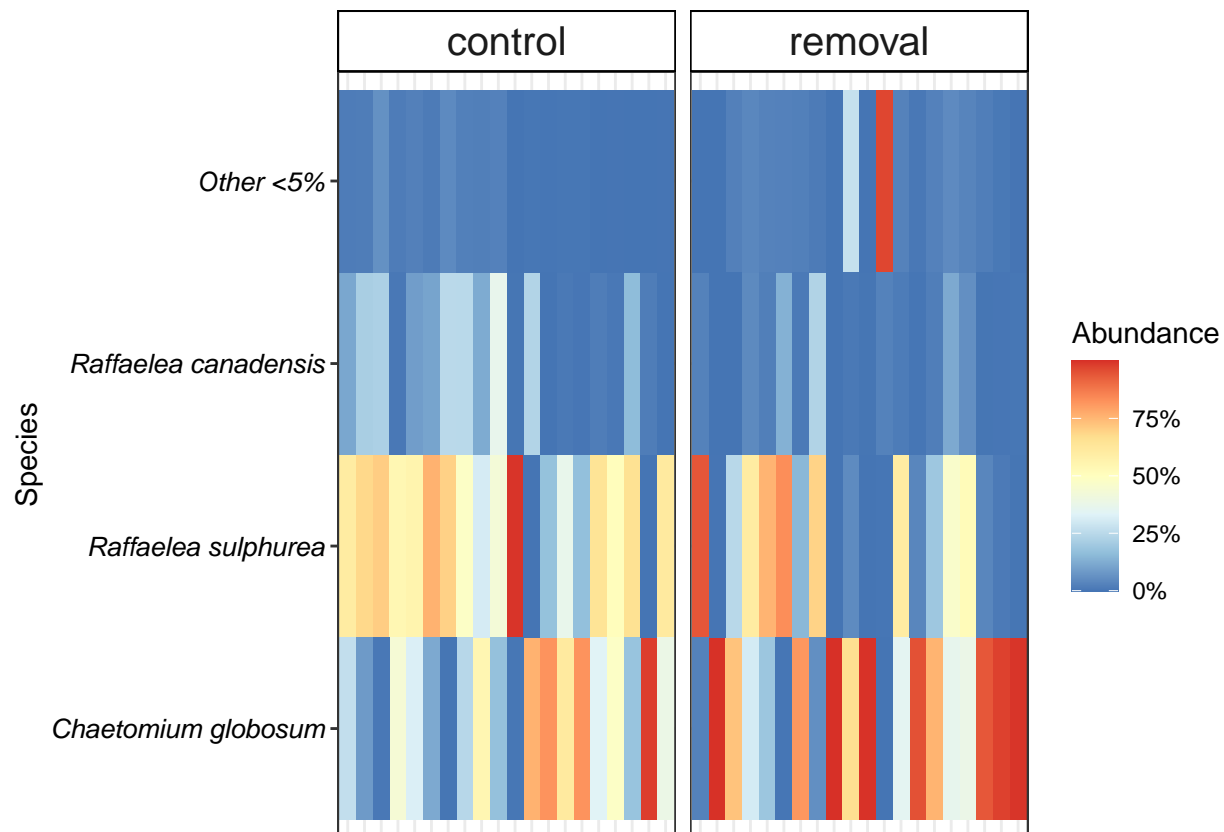
#Clean the x-axis
p.heat <- p.heat + theme(axis.title.x=element_blank(),
                        axis.text.x=element_blank(),
                        axis.ticks.x=element_blank())

# Clean the facet label box
p.heat <- p.heat + theme(legend.key = element_blank(),
                        strip.background = element_rect(colour="black", fill="white"))+
                        theme(strip.text = element_text(size = 15))

p.heat + theme(axis.text.x=element_text(angle = 90, size = 13))+
theme(axis.title.y = element_text(size = 18, face = "bold"), axis.title.x = element_blank())+
theme(axis.text.y = element_text(size = 13, face = "italic"))+
theme(legend.text = element_text(size = 13))+
theme(legend.title = element_text(size = 18, face = "bold"))
```



p.heat



plot control vs. 2nd attempt (>5% abundance)

```
pseq2 <- aggregate_rare(rel.R2nd, level = "Species", detection = 5/100, prevalence = 10/100)

pseqh2 <- pseq2 %>%
  psmelt()

pseqh2$Treatment<-factor(pseqh2$Treatment,levels=c("removal", "2nd-foundation"))
pseqh2$Species<-factor(pseqh2$Species,levels=c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_

p.heat <- ggplot(pseqh, aes(x = Sample, y = Species)) + geom_tile(aes(fill = Abundance))

# Change color
p.heat <- p.heat + scale_fill_distiller("Abundance", palette = "RdYlBu", labels = scales::percent) + th

# Make bacterial names italics
p.heat <- p.heat + theme(axis.text.y = element_text(colour = 'black',
                                                    size = 10,
                                                    face = 'italic'))

# Make separate samples based on main variable
p.heat <- p.heat + facet_grid(~Treatment,
                             scales = "free") + rremove("x.text")

#Clean the x-axis
p.heat <- p.heat + theme(axis.title.x=element_blank(),
                        axis.text.x=element_blank(),
```

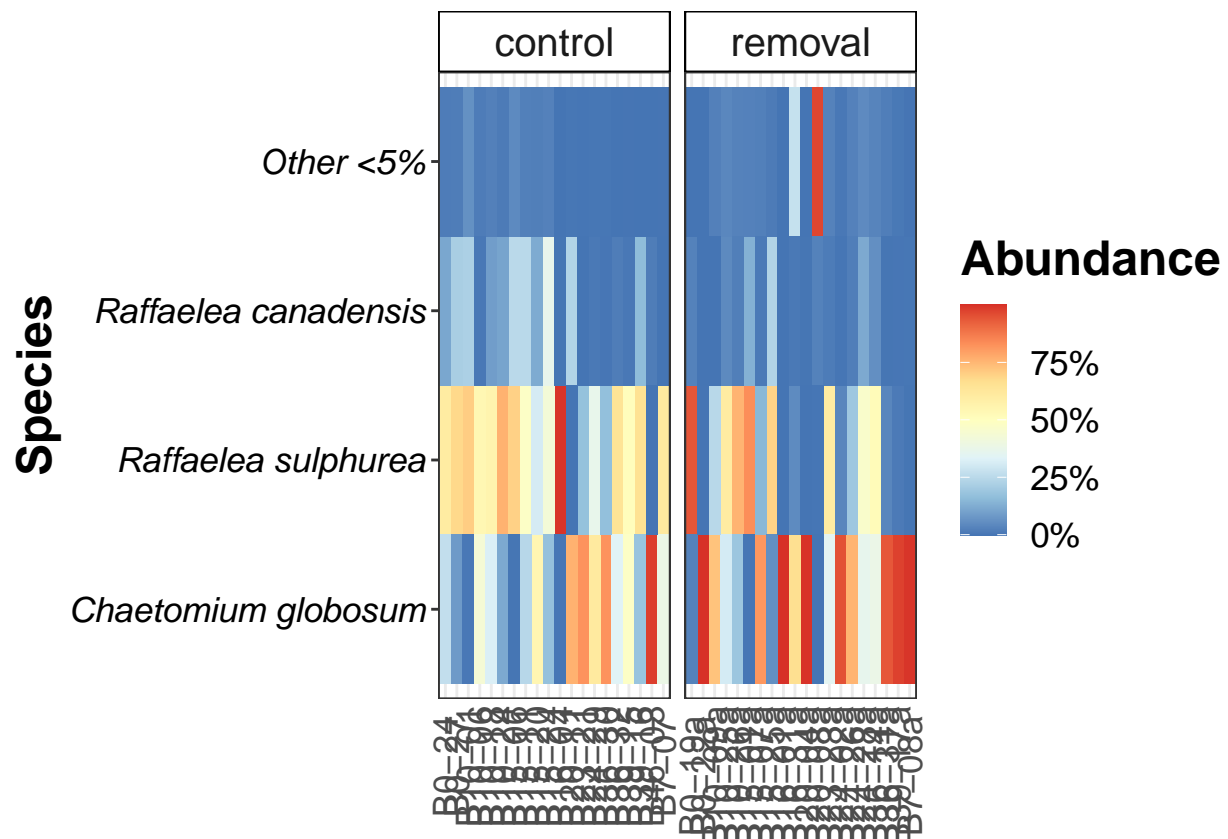
```

axis.ticks.x=element_blank())

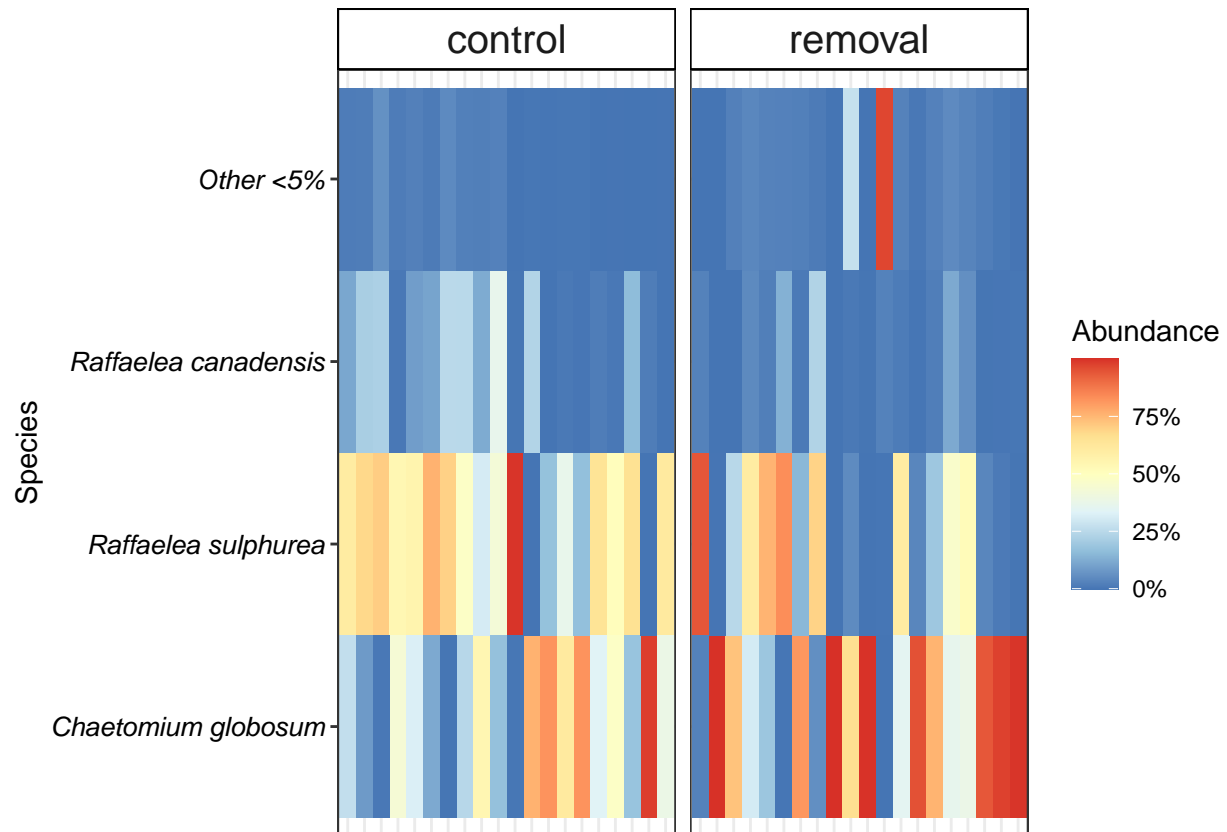
# Clean the facet label box
p.heat <- p.heat + theme(legend.key = element_blank(),
  strip.background = element_rect(colour="black", fill="white"))+
  theme(strip.text = element_text(size = 15))

p.heat + theme(axis.text.x=element_text(angle = 90, size = 13))+
  theme(axis.title.y = element_text(size = 18, face = "bold"), axis.title.x = element_blank())+
  theme(axis.text.y = element_text(size = 13, face = "italic"))+
  theme(legend.text = element_text(size = 13))+
  theme(legend.title = element_text(size = 18, face = "bold"))

```



p.heat



Core taxa abundance plot define color bar

```
colpal_fun <- c("lightskyblue", "gold", "goldenrod3", "forestgreen", "peachpuff3", "darkolivegreen3")
```

plot core taxa of control vs. removal with relative abundance

relative Abundance

```
ps4 <- prune_taxa(taxa_sums(rel.CR) > 0, rel.CR)
ps4 <- tax_glom(ps4, taxrank = 'Species')
psOrd4 = subset_taxa(ps4, Species == "Chaetomium_globosum" | Species == "Raffaelea_sulphurea" | Species == "Raffaelea_canadensis")
psctr = subset_samples(psOrd4, Treatment=="control")
#Melt and plot
melt3<-psmelt(psOrd4)
melt4<-psmelt(psctr)
melt3$Treament <- factor(melt3$Treatment, levels = c("control","removal"))
melt3$Species <- factor(melt3$Species, levels = c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_canadensis"))
melt4$Treament <- factor(melt4$Treatment, levels = c("control","removal"))
melt4$Species <- factor(melt4$Species, levels = c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_canadensis"))
a_mean <- melt4 %>%
  group_by(Species) %>%
  summarize(mean_val = mean(Abundance))
print(a_mean)
```

```
## # A tibble: 3 x 2
```

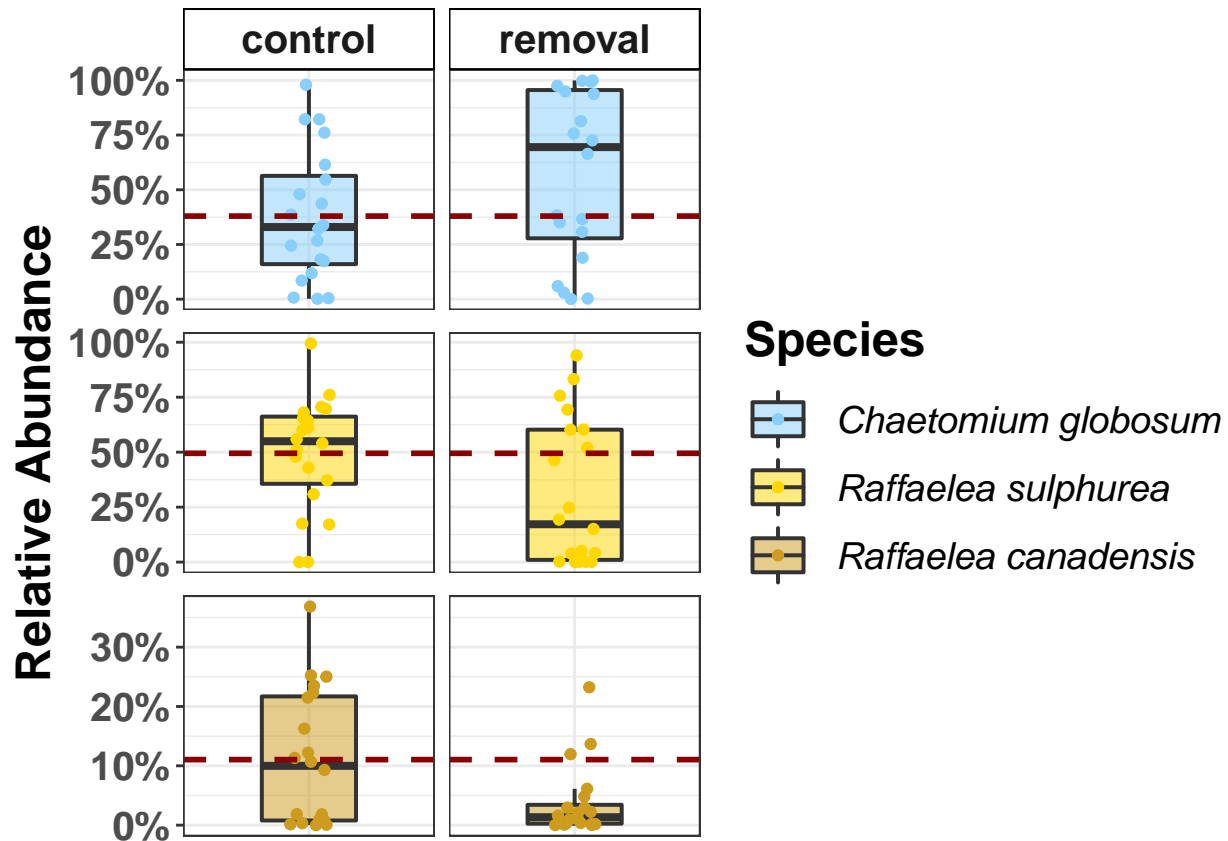
##	Species	mean_val
##	<fct>	<dbl>
## 1	Chaetomium globosum	0.380
## 2	Raffaelea sulphurea	0.495
## 3	Raffaelea canadensis	0.110

```

p2<-ggplot(data = melt3, aes(x = Treatment, y = Abundance)) +
  geom_boxplot(aes(fill=Species),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,
  scale_fill_manual(values = colpal_fun, name = "Species")+
  scale_color_manual(values = colpal_fun, name = "Species")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Species~fct_relevel(Treatment, "control", "removal"), scales = "free")+theme_bw()
p2<-p2+ theme(legend.position="right")+ylab("Relative Abundance")
p2<-p2+ theme(legend.text=element_text(size=14, face = "italic"))+
  theme(legend.key = element_rect(color = NA, fill = NA),legend.key.size = unit(0.9, "cm"))+
  theme(legend.title = element_text(size = 18, face = "bold"))+
  scale_y_continuous(labels=percent_format())
abu2<-p2 + theme(strip.background =element_rect(fill="white", color="black"))+
  theme(strip.text.x = element_text(size = 15, face = "bold"))
Treatment2<-abu2+theme(axis.title.y = element_text(size=18, face="bold"))+theme(axis.text.y = element_t
  theme(axis.text.x = element_text(size=18, angle=90,face="bold"))+
  theme(axis.title.x = element_blank(), axis.text.x=element_blank(),
    axis.ticks.x=element_blank())+
  theme(strip.text.y = element_blank())
relAbcoreCR <- Treatment2 + theme(panel.spacing.y = unit(0.3, "cm"))

relAbcoreCR

```



plot core taxa of removal vs. 2nd attempt with relative abundance

```
Treats <- c('removal' = "removal", '2nd-foundation' = "2nd-att.")
ps3 <- prune_taxa(taxa_sums(rel.R2nd) > 0, rel.R2nd)
ps3 <- tax_glom(ps3, taxrank = 'Species')
psOrd3 = subset_taxa(ps3, Species == "Chaetomium_globosum" | Species == "Raffaelea_sulphurea" | Species == "Raffaelea_canadensis")
psctr = subset_samples(psOrd3, Treatment == "removal")
#Melt and plot
melt<-psmelt(psOrd3)
melt2<-psmelt(psctr)
melt$Treatment <- factor(melt$Treatment, levels = c("removal", "2nd-foundation"))
melt$Species <- factor(melt$Species, levels = c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_canadensis"))
melt2$Treatment <- factor(melt2$Treatment, levels = c("removal", "2nd-foundation"))
melt2$Species <- factor(melt2$Species, levels = c("Chaetomium_globosum", "Raffaelea_sulphurea", "Raffaelea_canadensis"))
a_mean <- melt2 %>%
  group_by(Species) %>%
  summarize(mean_val = mean(Abundance))
print(a_mean)
```

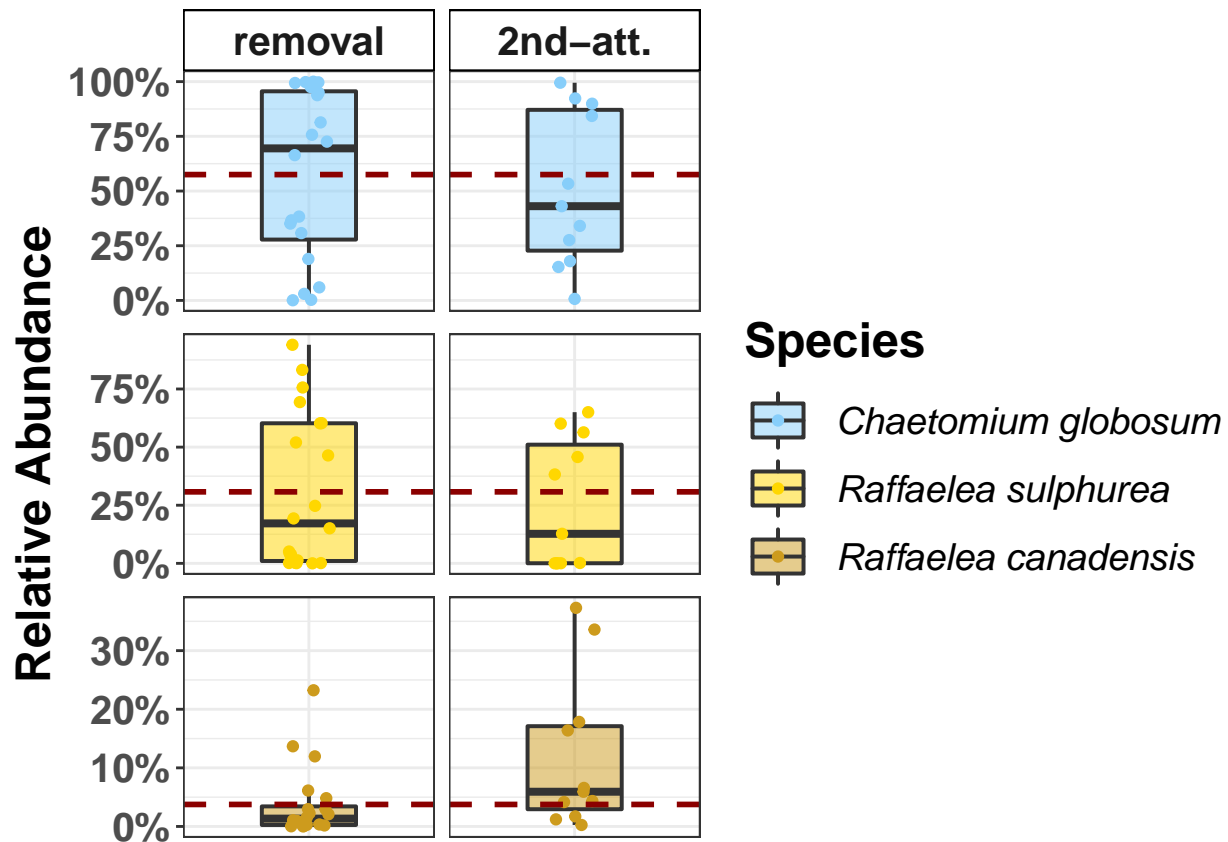
```
## # A tibble: 3 x 2
##   Species          mean_val
##   <fct>          <dbl>
## 1 Chaetomium globosum 0.575
## 2 Raffaelea sulphurea 0.308
## 3 Raffaelea canadensis 0.0376
```

```

p<-ggplot(data = melt, aes(x = Treatment, y = Abundance)) +
  geom_boxplot(aes(fill=Species),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,
  scale_fill_manual(values = colpal_fun, name = "Species")+
  scale_color_manual(values = colpal_fun, name = "Species")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Species~fct_relevel(Treatment, "removal", "2nd-foundation"), labeller = as_labeller(Treats
  theme_bw()
p<-p+ theme(legend.position="right")+
  ylab("Relative Abundance")
p<-p+ theme(legend.text=element_text(size=14, face = "italic"))+theme(legend.key = element_rect(color =
  scale_y_continuous(labels=percent_format())
abu<-p + theme(strip.background =element_rect(fill="white", color="black"))+
  theme(strip.text.x = element_text(size = 15, face = "bold"))
TreatmentCR<-abu+theme(axis.title.y = element_text(size=18, face="bold"))+
  theme(axis.text.y = element_text(size=15, face="bold")) +
  theme(axis.text.x = element_text(size=18, angle=90,face="bold"))+
  theme(axis.title.x = element_blank(), axis.text.x=element_blank(),
  axis.ticks.x=element_blank())+
  theme(strip.text.y = element_blank())
relAbcoreR2nd_fun <- TreatmentCR + theme(panel.spacing.y = unit(0.3, "cm"))

relAbcoreR2nd_fun

```



Ordination analysis

Sample ordination

```
set.seed(1)
ordi.CR = ordinate(rel.CR, "NMDS", "bray", k=3, trymax=100)
```

NMDS

```
## Run 0 stress 0.0346926
## Run 1 stress 0.03892109
## Run 2 stress 0.04011849
## Run 3 stress 0.03469555
## ... Procrustes: rmse 0.0006566098  max resid 0.00193732
## ... Similar to previous best
## Run 4 stress 0.03911702
## Run 5 stress 0.03469257
## ... New best solution
## ... Procrustes: rmse 4.163446e-05  max resid 0.0001359446
## ... Similar to previous best
## Run 6 stress 0.03418742
## ... New best solution
## ... Procrustes: rmse 0.03183313  max resid 0.1870848
## Run 7 stress 0.03418735
## ... New best solution
## ... Procrustes: rmse 0.0002360602  max resid 0.0008162302
## ... Similar to previous best
## Run 8 stress 0.03419229
## ... Procrustes: rmse 0.0008910375  max resid 0.004884492
## ... Similar to previous best
## Run 9 stress 0.03907424
## Run 10 stress 0.03902262
## Run 11 stress 0.03469247
## Run 12 stress 0.03849743
## Run 13 stress 0.0346925
## Run 14 stress 0.0388932
## Run 15 stress 0.03469337
## Run 16 stress 0.03418733
## ... New best solution
## ... Procrustes: rmse 0.0001105944  max resid 0.000336442
## ... Similar to previous best
## Run 17 stress 0.03418721
## ... New best solution
## ... Procrustes: rmse 9.364042e-05  max resid 0.0004726928
## ... Similar to previous best
## Run 18 stress 0.03418759
## ... Procrustes: rmse 0.0005333684  max resid 0.002974389
## ... Similar to previous best
## Run 19 stress 0.03469241
## Run 20 stress 0.03469295
## *** Solution reached
```



```
ordi.CR$stress #0.03418721 --> good fit
```

```
## [1] 0.03418721
```

```
set.seed(1)
```

```
ordi.R2nd = ordinate(rel.R2nd, "NMDS", "bray", k=3, trymax=100)
```

```
## Run 0 stress 0.03165789
## Run 1 stress 0.03305765
## Run 2 stress 0.03345132
## Run 3 stress 0.03165796
## ... Procrustes: rmse 2.985695e-05 max resid 0.000119893
## ... Similar to previous best
## Run 4 stress 0.03512227
## Run 5 stress 0.03165843
## ... Procrustes: rmse 0.0001765007 max resid 0.0007290808
## ... Similar to previous best
## Run 6 stress 0.03305756
## Run 7 stress 0.03305762
## Run 8 stress 0.03165782
## ... New best solution
## ... Procrustes: rmse 4.148317e-05 max resid 0.0001625033
## ... Similar to previous best
## Run 9 stress 0.03165798
## ... Procrustes: rmse 7.899082e-05 max resid 0.0003264773
## ... Similar to previous best
## Run 10 stress 0.03345129
## Run 11 stress 0.03305763
## Run 12 stress 0.03512172
## Run 13 stress 0.03512318
## Run 14 stress 0.03165778
## ... New best solution
## ... Procrustes: rmse 2.469497e-05 max resid 0.000106505
## ... Similar to previous best
## Run 15 stress 0.03305745
## Run 16 stress 0.03512266
## Run 17 stress 0.03306002
## Run 18 stress 0.03165773
## ... New best solution
## ... Procrustes: rmse 0.0001553175 max resid 0.0004708727
## ... Similar to previous best
## Run 19 stress 0.03305738
## Run 20 stress 0.0334517
## *** Solution reached
```

```
ordi.R2nd$stress #0.03165773
```

```
## [1] 0.03165773
```

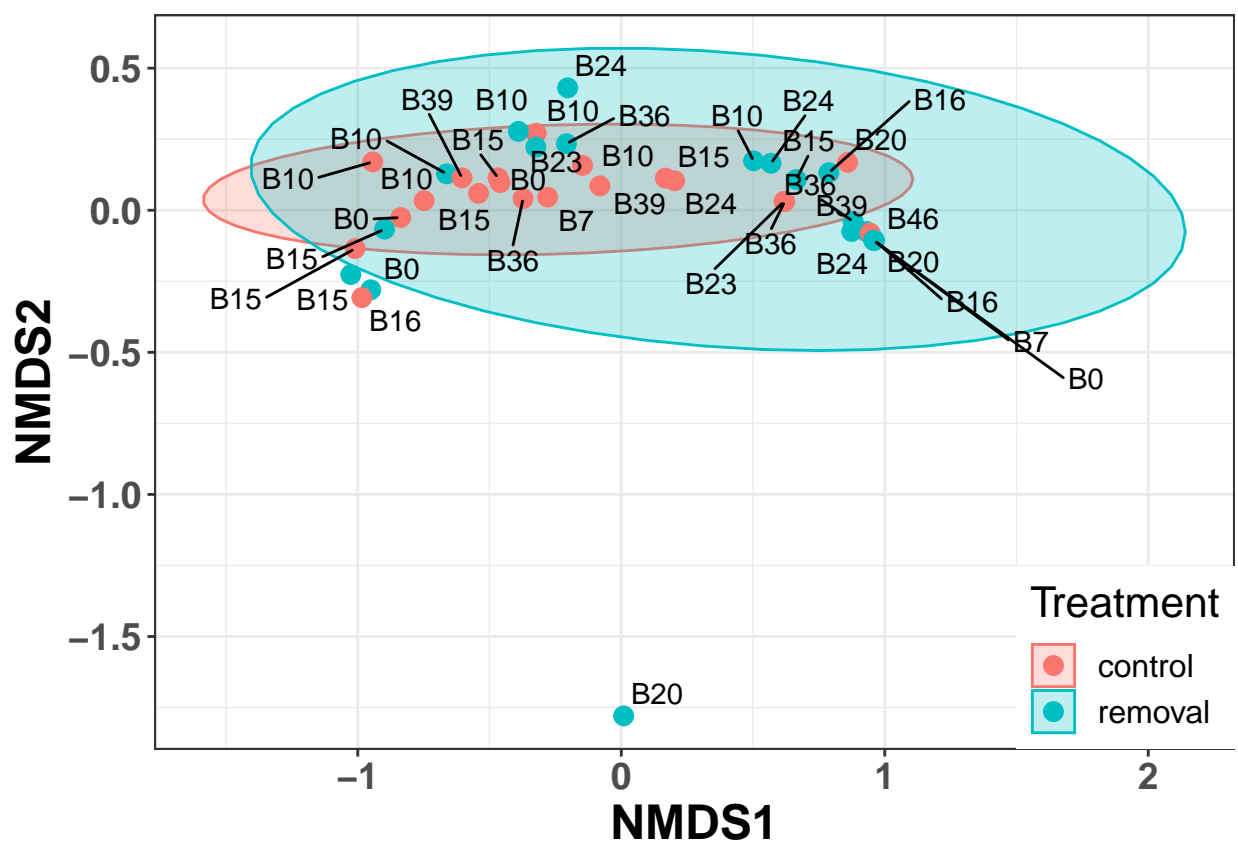
```
plot NMDS
```

```

NMDS_CR_fun <- plot_ordination(rel.CR, ordi.CR, color = "Treatment", axes=c(1,2))+
  stat_ellipse(level = 0.95, geom = "polygon", aes(fill = Treatment), alpha = 0.25)+
  geom_point(size=3, inherit.aes=T)+
  geom_text_repel(aes(label=Linage), color = "black", size = 4, max.overlaps = Inf)+
  theme_bw()+
  theme(legend.justification=c(1,0), legend.position=c(1,0))+
  theme(axis.text = element_text(size = 14, face = "bold"))+
  theme(axis.title = element_text(size = 18, face = "bold"))+
  theme(legend.text = element_text(size = 12))+
  theme(legend.title = element_text(size = 16))+
  theme(title = element_text(size = 18))

```

NMDS_CR_fun



```

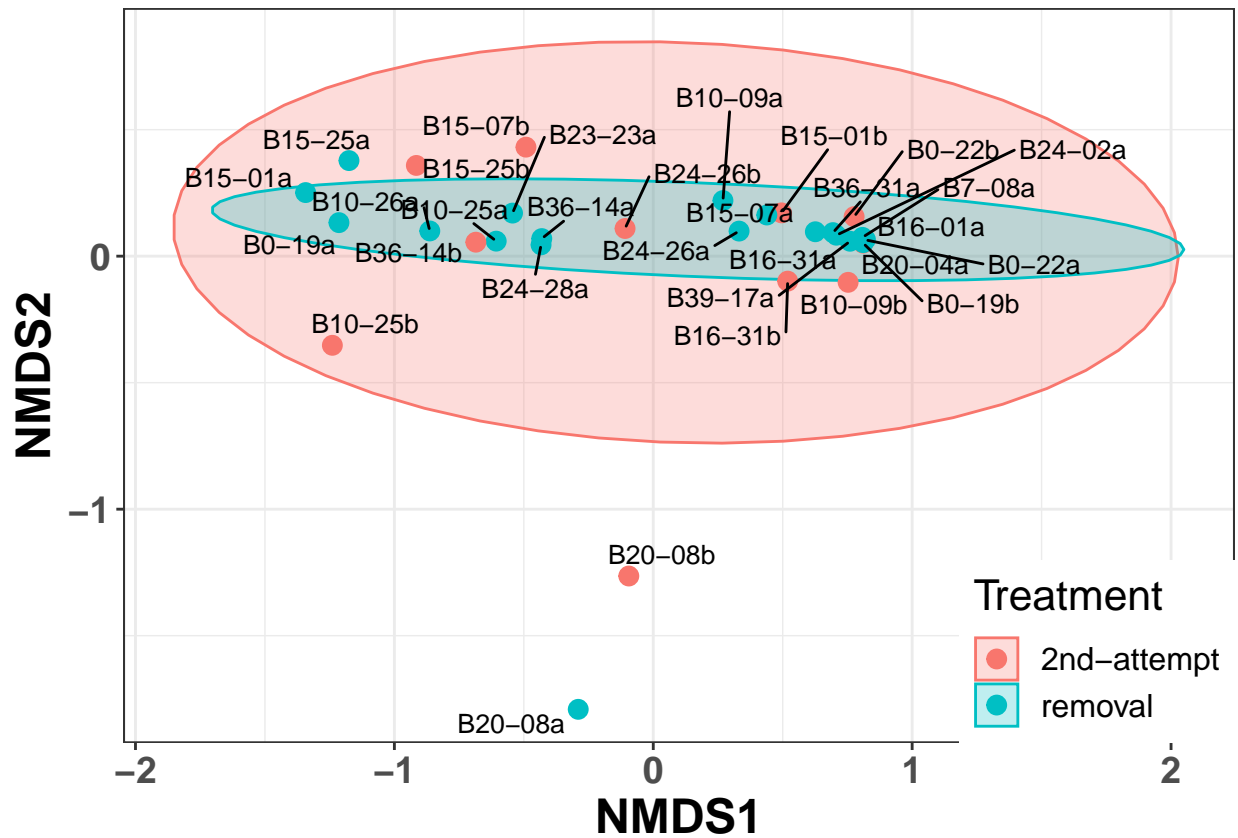
sample_data(rel.R2nd)$Treatment <- as.factor(sample_data(rel.R2nd)$Treatment)
levels(sample_data(rel.R2nd)$Treatment) = c("2nd-attempt", "removal")

NMDS_R2nd_fun <- plot_ordination(rel.R2nd, ordi.R2nd, color = "Treatment", axes=c(1,2))+
  stat_ellipse(level = 0.95, geom = "polygon", aes(fill = Treatment), alpha = 0.25)+
  geom_point(size=3, inherit.aes=T)+
  geom_text_repel(aes(label=Sample), color = "black", size = 3.5, max.overlaps = Inf)+
  theme_bw()+
  theme(legend.justification=c(1,0), legend.position=c(1,0))+
  theme(axis.text = element_text(size = 14, face = "bold"))+
  theme(axis.title = element_text(size = 18, face = "bold"))+

```

```
theme(legend.text = element_text(size = 12))+
theme(legend.title = element_text(size = 16))+
theme(title = element_text(size = 18))
```

NMDS_R2nd_fun



Permanova for community-level multivariate comparisons

PERMANOVA quantifies multivariate community-level differences between groups.

```
# Pick relative abundances (compositional) and sample metadata
otu.CR <- abundances(rel.CR)
meta.CR <- meta(rel.CR)

otu.R2nd <- abundances(rel.R2nd)
meta.R2nd <- meta(rel.R2nd)
```

PERMANOVA significance test for group-level differences Now let us evaluate whether the Treatment has a significant effect on overall garden microbiota composition. Perform PERMANOVA:

```
# control vs. removal
set.seed(1)
adonis2(distance(rel.CR, method = "bray") ~ Treatment + Linage, data = meta.CR)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = distance(rel.CR, method = "bray") ~ Treatment + Linage, data = meta.CR)
##           Df SumOfSqs      R2      F Pr(>F)
## Treatment  1   0.5422 0.08329 3.9420  0.032 *
## Linage     10   2.1167 0.32514 1.5389  0.107
## Residual   28   3.8513 0.59157
## Total      39   6.5102 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#removal vs. 2nd attempt
set.seed(1)
adonis2(distance(rel.R2nd, method = "bray") ~ Treatment + Linage, data = meta.R2nd)

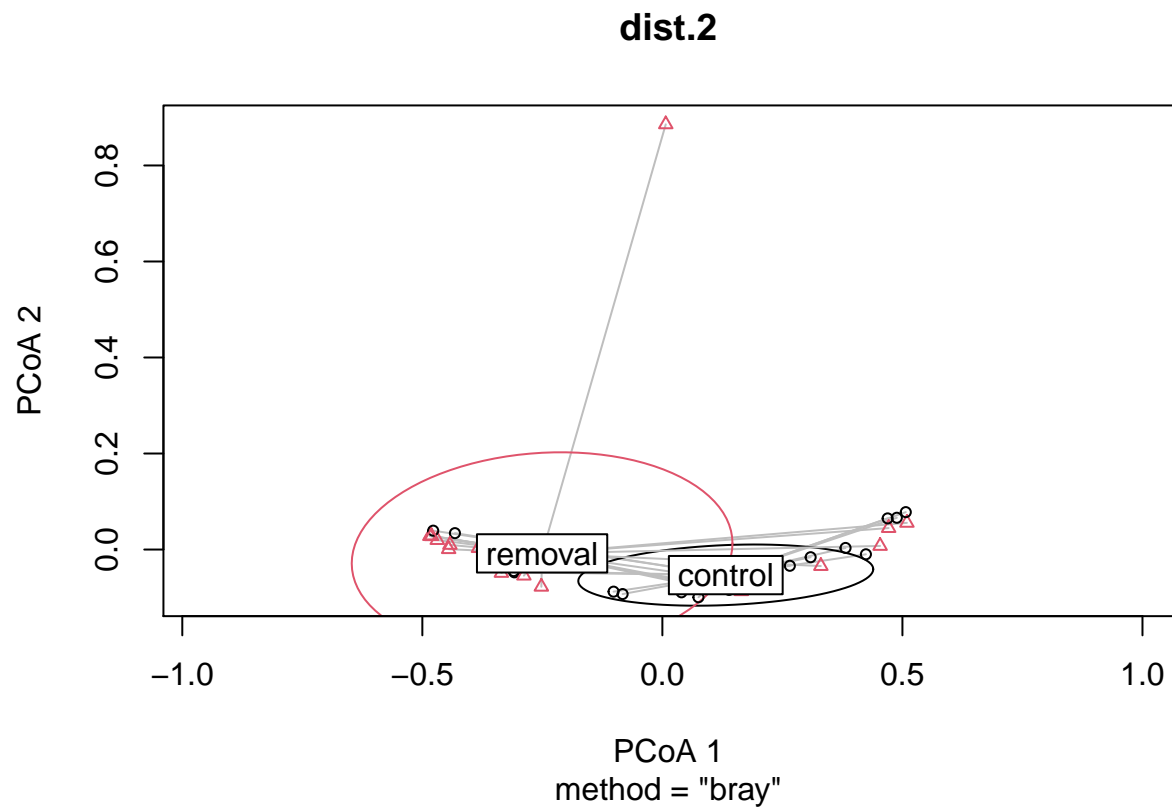
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = distance(rel.R2nd, method = "bray") ~ Treatment + Linage, data = meta.R2nd)
##           Df SumOfSqs      R2      F Pr(>F)
## Treatment  1   0.0951 0.01614 0.5438  0.641
## Linage      9   2.2989 0.39029 1.4612  0.132
## Residual   20   3.4962 0.59357
## Total      30   5.8902 1.00000
```

Checking the homogeneity condition Check that variance homogeneity assumptions hold (to ensure the reliability of the results):

```
# control vs. removal
dist <- vegdist(t(otu.CR))
dist.2 <- betadisper(dist, meta.CR$Treatment)
anova(dist.2)

## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      1 0.08264 0.082640  1.7441 0.1945
## Residuals   38 1.80055 0.047383

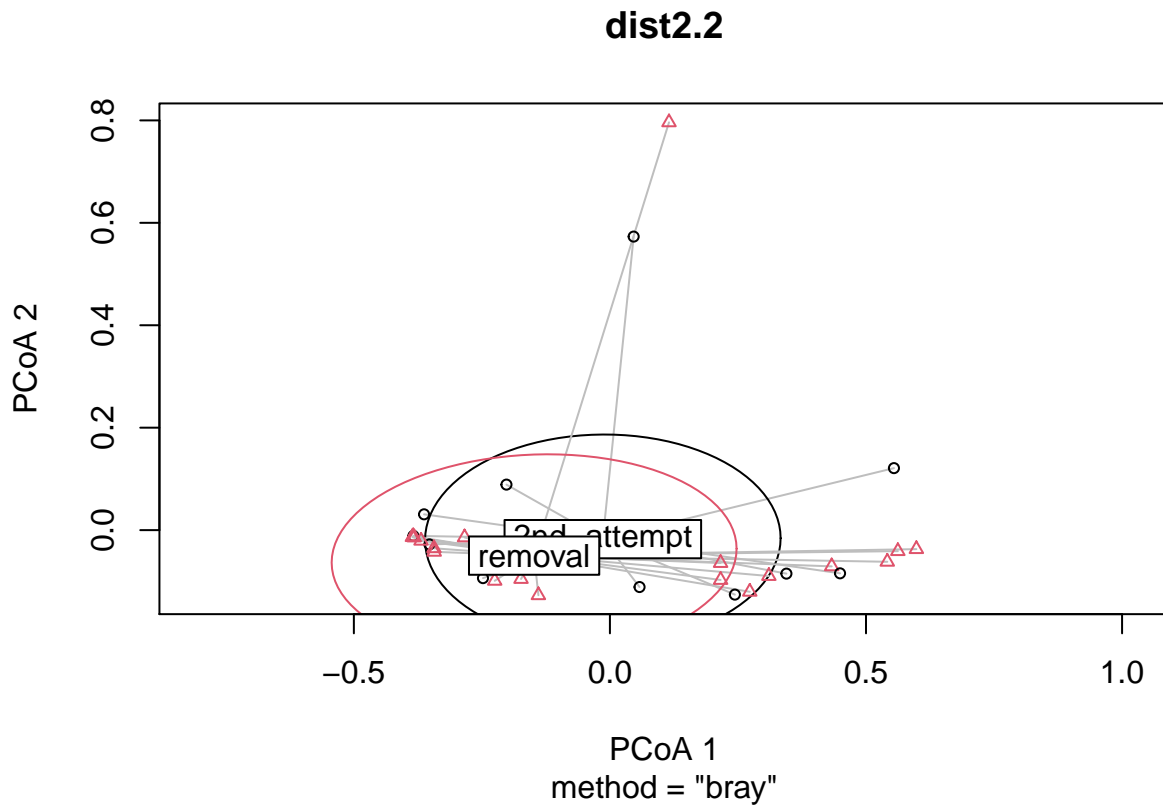
plot(dist.2, hull = FALSE, ellipse = TRUE)
```



```
# removal vs. 2nd attempt
dist2 <- vegdist(t(otu.R2nd))
dist2.2 <- betadisper(dist2, meta.R2nd$Treatment)
anova(dist2.2)
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups    1 0.00751 0.007511  0.1497 0.7017
## Residuals 29 1.45538 0.050185
```

```
plot(dist2.2, hull = FALSE, ellipse = TRUE)
```



closer look at most abundant taxa

subset datasets of both combinations to most abundant Geni (each Chaetomiaceae or *Raffaelea*) (rel. abundances)

```
CG_CR <- subset_taxa(rel.CR, Species == "Chaetomium_globosum")
CG_CR <- CG_CR %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()

CG_R2nd <- subset_taxa(rel.R2nd, Species == "Chaetomium_globosum")
CG_R2nd <- CG_R2nd %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()

RS_CR <- subset_taxa(rel.CR, Species == "Raffaelea_sulphurea")
RS_CR <- RS_CR %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()

RS_R2nd <- subset_taxa(rel.R2nd, Species == "Raffaelea_sulphurea")
RS_R2nd <- RS_R2nd %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()
```

```

RC_CR <- subset_taxa(rel.CR, Species == "Raffaelea_canadensis")
RC_CR <- RC_CR %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()

RC_R2nd <- subset_taxa(rel.R2nd, Species == "Raffaelea_canadensis")
RC_R2nd <- RC_R2nd %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()

```

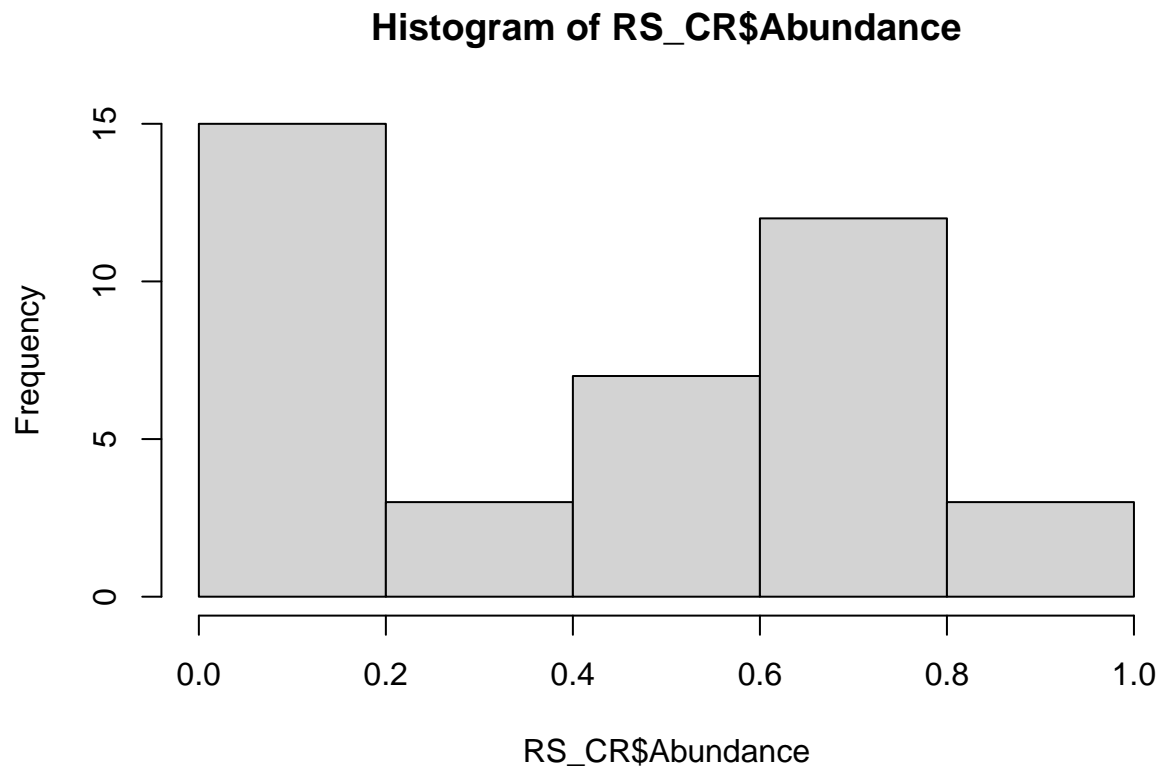
dataset *Raffaelea sulphurea*

```

RS_CR$Treatment <- as.factor(RS_CR$Treatment)
RS_CR <- within(RS_CR, Treatment <- relevel(Treatment, ref = "control"))

#control vs. removal
hist(RS_CR$Abundance)

```

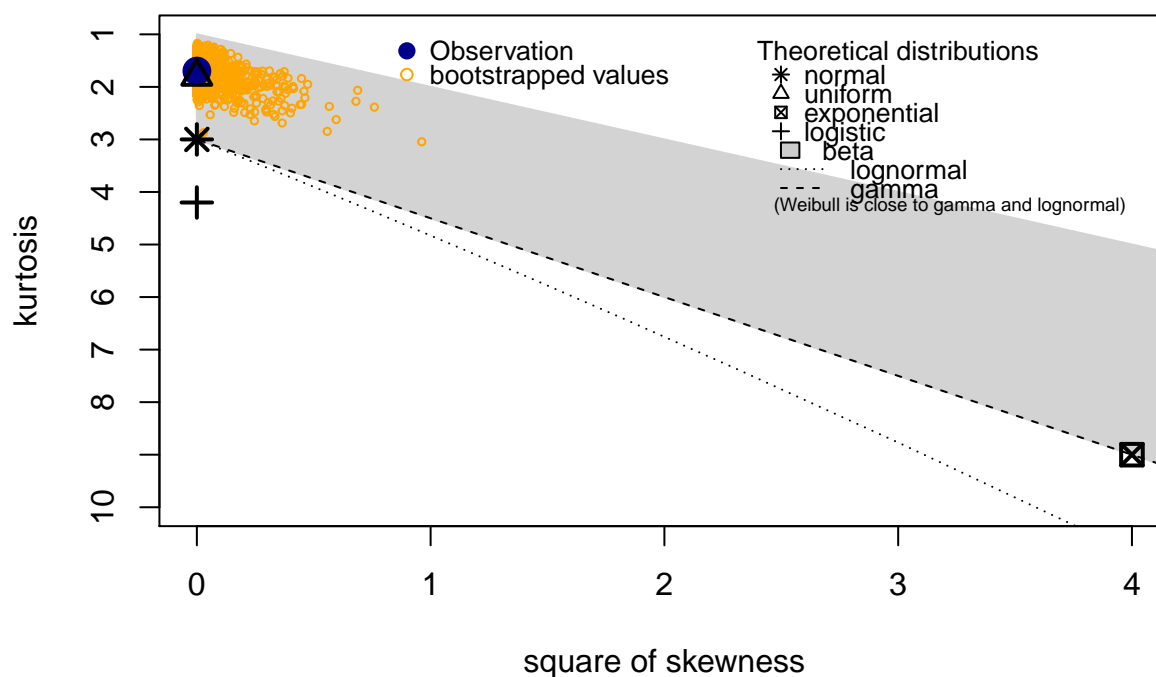


```

descdist(RS_CR$Abundance, boot = 1000)

```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 0.0001473839   max: 0.9941497
## median: 0.4719555
## mean: 0.4012437
## estimated sd: 0.3078807
## estimated skewness: 0.01282538
## estimated kurtosis: 1.698684
```

```
logistic <- function(p) log(p / (1-p) +0.01)
```

```
RS.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = RS_CR)
summary(RS.mod)
```

```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = RS_CR)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -3.6243 -1.1048 -0.0834  0.9287  4.8945
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.5922     1.0502   0.564   0.5773
```

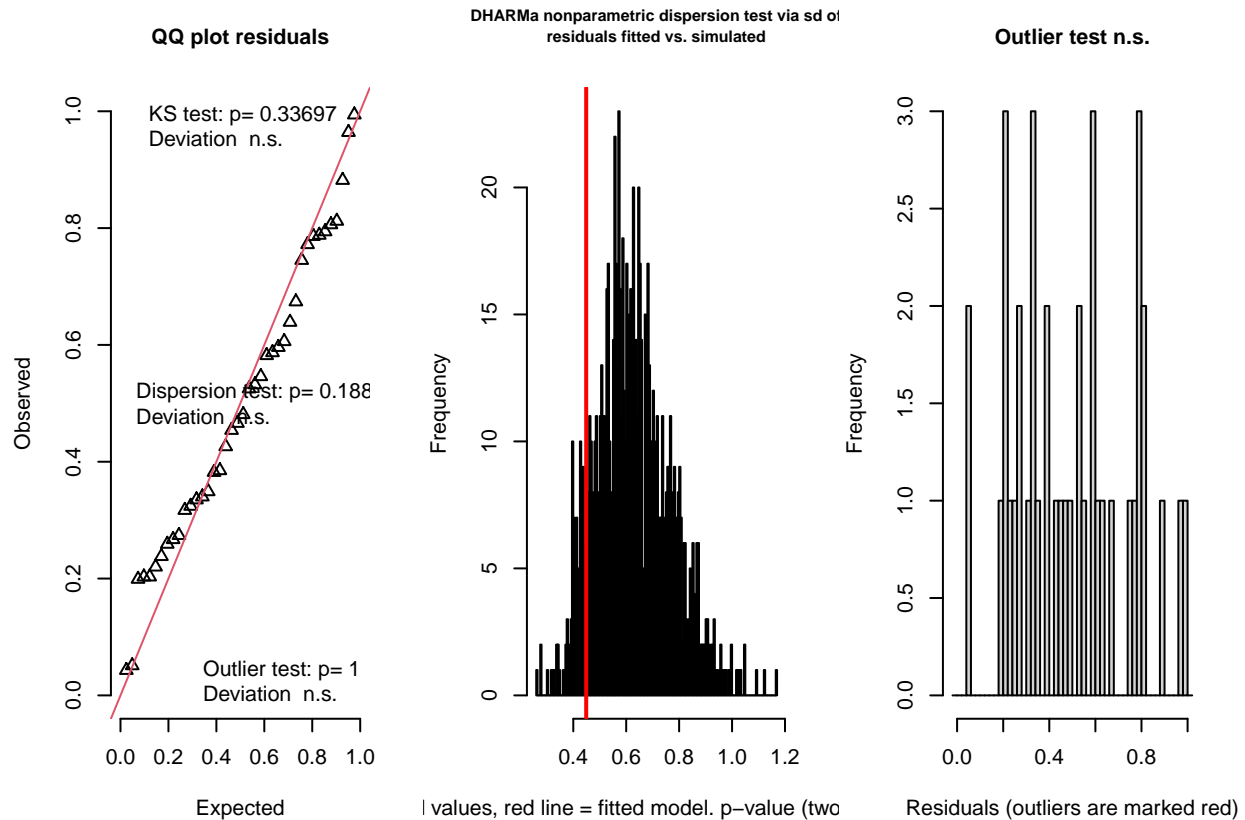


```
## Treatmentremoval -1.4670      0.6584 -2.228  0.0341 *
## LinageB10      0.4553      1.2510  0.364  0.7186
## LinageB15      0.1023      1.2510  0.082  0.9354
## LinageB16     -0.3513      1.5273 -0.230  0.8198
## LinageB20     -4.1102      1.5273 -2.691  0.0119 *
## LinageB23     -0.4007      1.7273 -0.232  0.8182
## LinageB24     -0.7373      1.4199 -0.519  0.6077
## LinageB36     -0.8169      1.4104 -0.579  0.5671
## LinageB39     -1.1260      1.5273 -0.737  0.4671
## LinageB46     -5.1286      2.2541 -2.275  0.0307 *
## LinageB7      -1.8596      1.7273 -1.077  0.2909
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.995 on 28 degrees of freedom
## Multiple R-squared:  0.4629, Adjusted R-squared:  0.2519
## F-statistic: 2.194 on 11 and 28 DF, p-value: 0.04585
```

```
Anova(RS.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##      Sum Sq Df F value    Pr(>F)
## Treatment  19.752  1  4.9650 0.03407 *
## Linage    74.132 10  1.8634 0.09476 .
## Residuals 111.391 28
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_RS <- simulateResiduals(fittedModel=RS.mod, n = 1000)
testResiduals(res_RS)
```



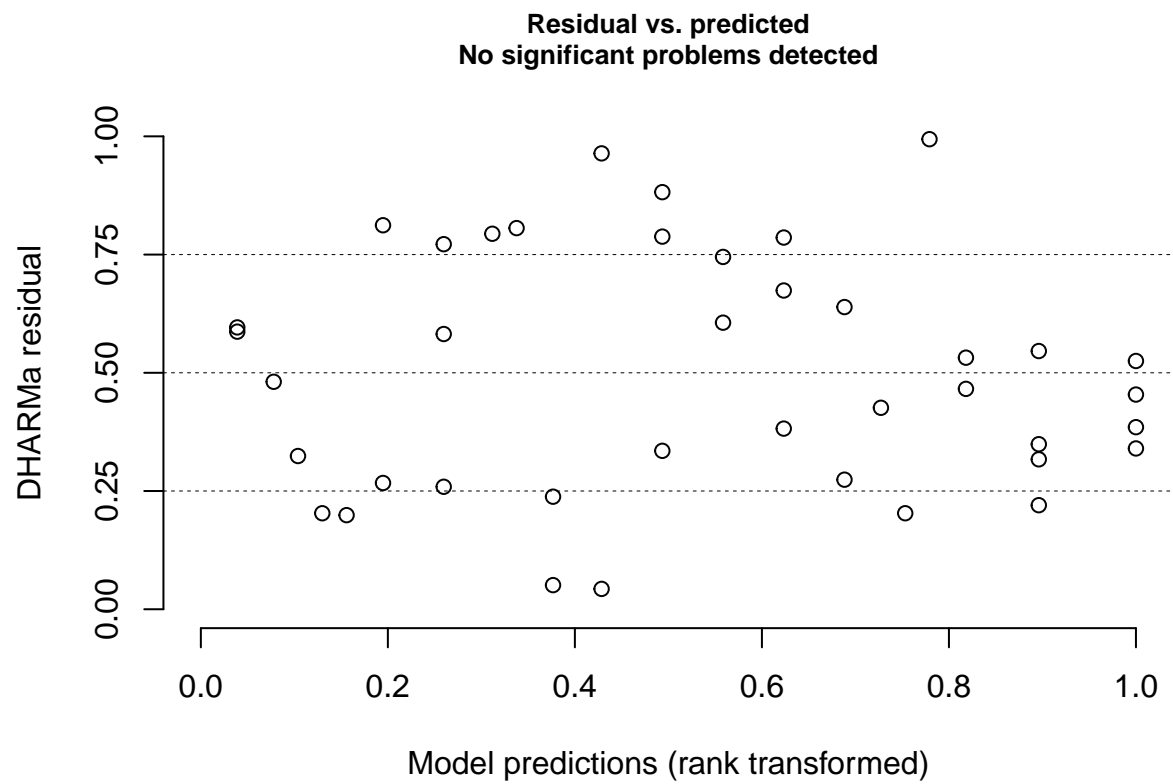
```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.149, p-value = 0.337
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.149, p-value = 0.337
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

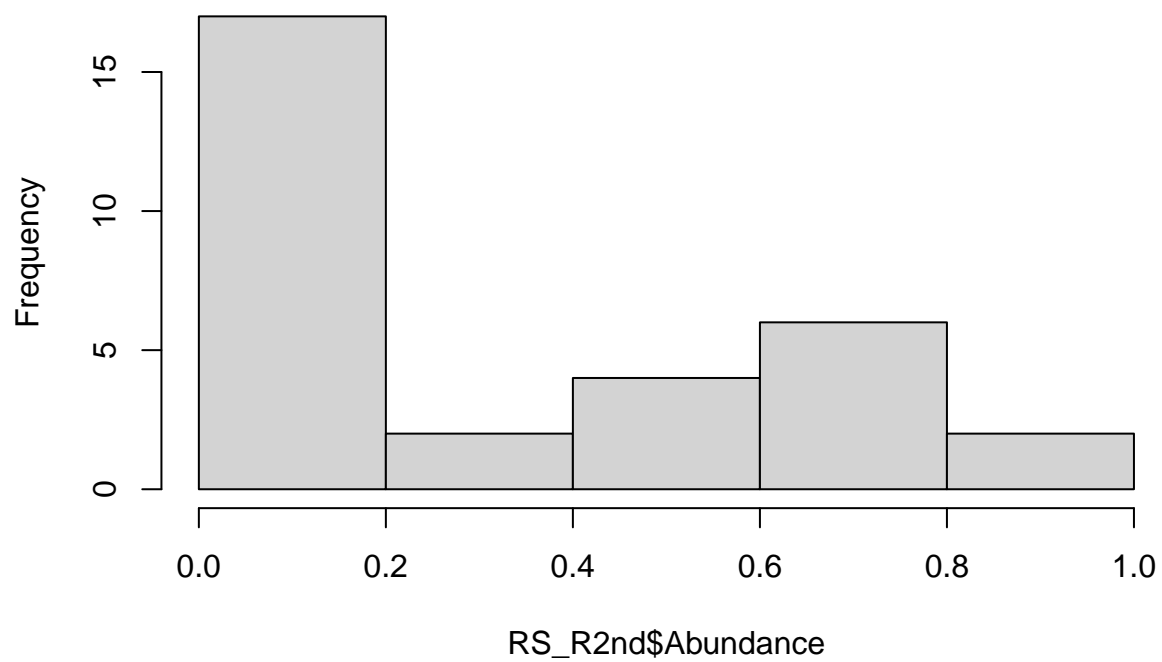
```
plotResiduals(res_RS)
```

```
## Unable to calculate quantile regression for quantile 0.25. Possibly to few (unique) data points / pr
## Unable to calculate quantile regression for quantile 0.5. Possibly to few (unique) data points / pre
## Unable to calculate quantile regression for quantile 0.75. Possibly to few (unique) data points / pr
```



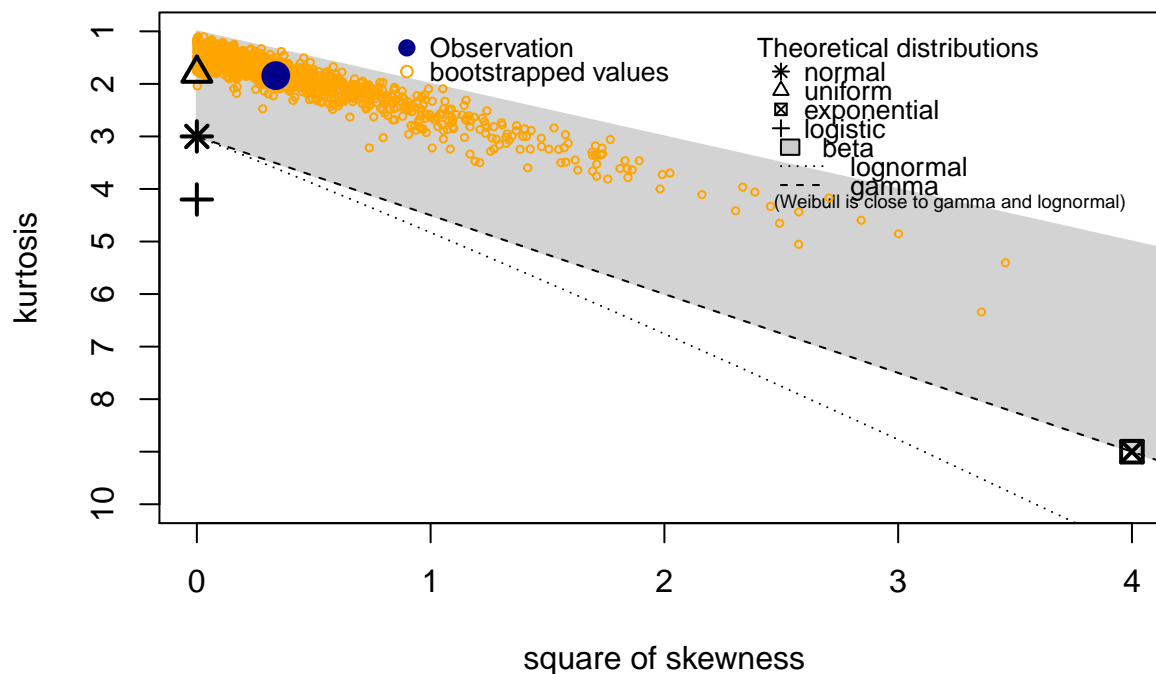
```
#removal vs. 2nd attempt  
hist(RS_R2nd$Abundance)
```

Histogram of RS_R2nd\$Abundance



```
descdist(RS_R2nd$Abundance, boot = 1000)
```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 0.0001473839 max: 0.9401502
## median: 0.1507833
## mean: 0.2882711
## estimated sd: 0.3090897
## estimated skewness: 0.5810069
## estimated kurtosis: 1.842227
```

```
RS.mod2 <- lm(logistic(Abundance) ~ Treatment + Linage, data = RS_R2nd)
summary(RS.mod2)
```

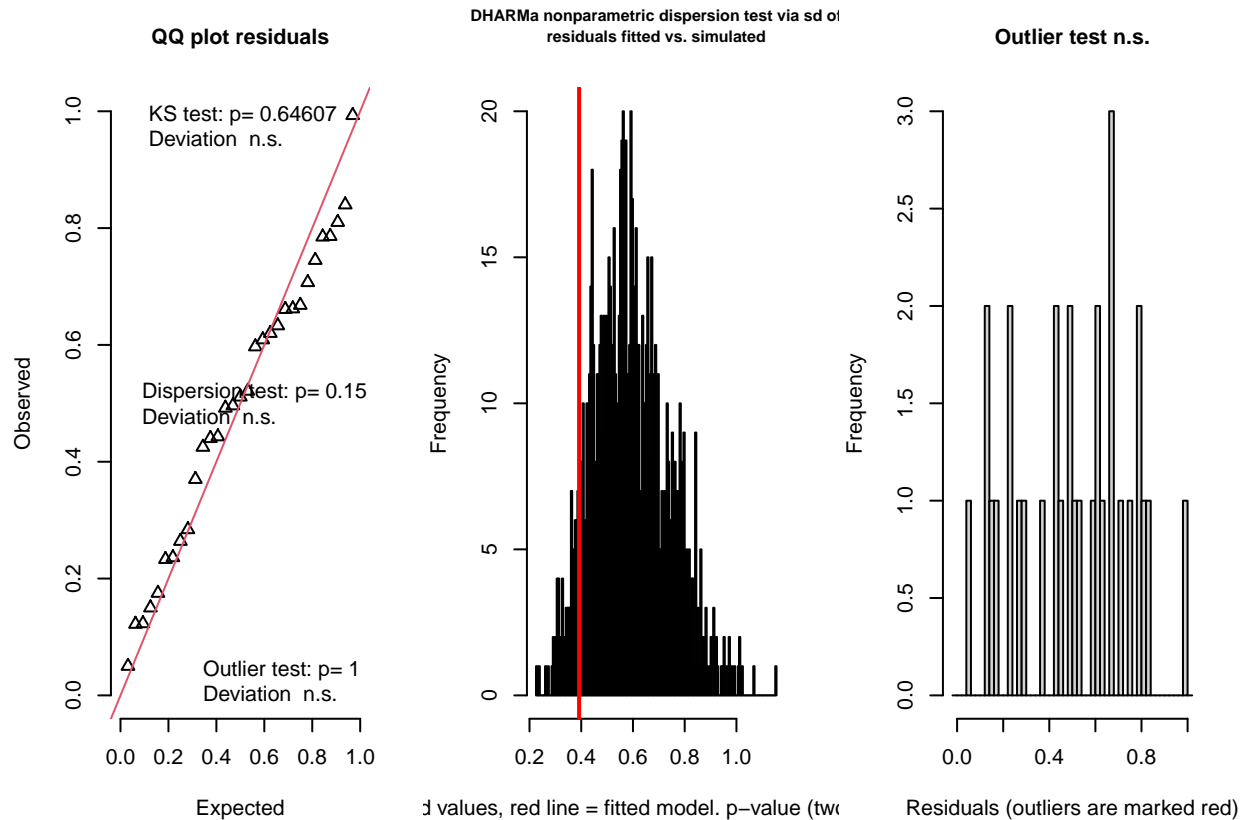
```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = RS_R2nd)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -3.2509 -1.1071  0.0000  0.8656  5.0054
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    -3.1968     1.0690  -2.991  0.00723 **
## Treatmentremoval  0.9462     0.7833   1.208  0.24112
## LinageB10       1.8599     1.3367   1.391  0.17939
```

```
## LinageB15      2.5816      1.2841      2.011      0.05806 .
## LinageB16     -1.3710      1.5249     -0.899      0.37933
## LinageB20     -1.9304      1.5249     -1.266      0.22010
## LinageB23      2.6719      2.2583      1.183      0.25062
## LinageB24      1.2528      1.4202      0.882      0.38817
## LinageB36      1.8078      1.5249      1.185      0.24972
## LinageB39     -1.5180      2.2583     -0.672      0.50914
## LinageB7      -2.2154      2.2583     -0.981      0.33830
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.989 on 20 degrees of freedom
## Multiple R-squared:  0.5235, Adjusted R-squared:  0.2853
## F-statistic: 2.197 on 10 and 20 DF, p-value: 0.06429
```

```
Anova(RS.mod2, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##      Sum Sq Df F value  Pr(>F)
## Treatment  5.775  1  1.4594 0.24112
## Linage    84.589  9  2.3751 0.05143 .
## Residuals 79.142 20
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_RS2 <- simulateResiduals(fittedModel=RS.mod2, n = 1000)
testResiduals(res_RS2)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12774, p-value = 0.6461
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
```

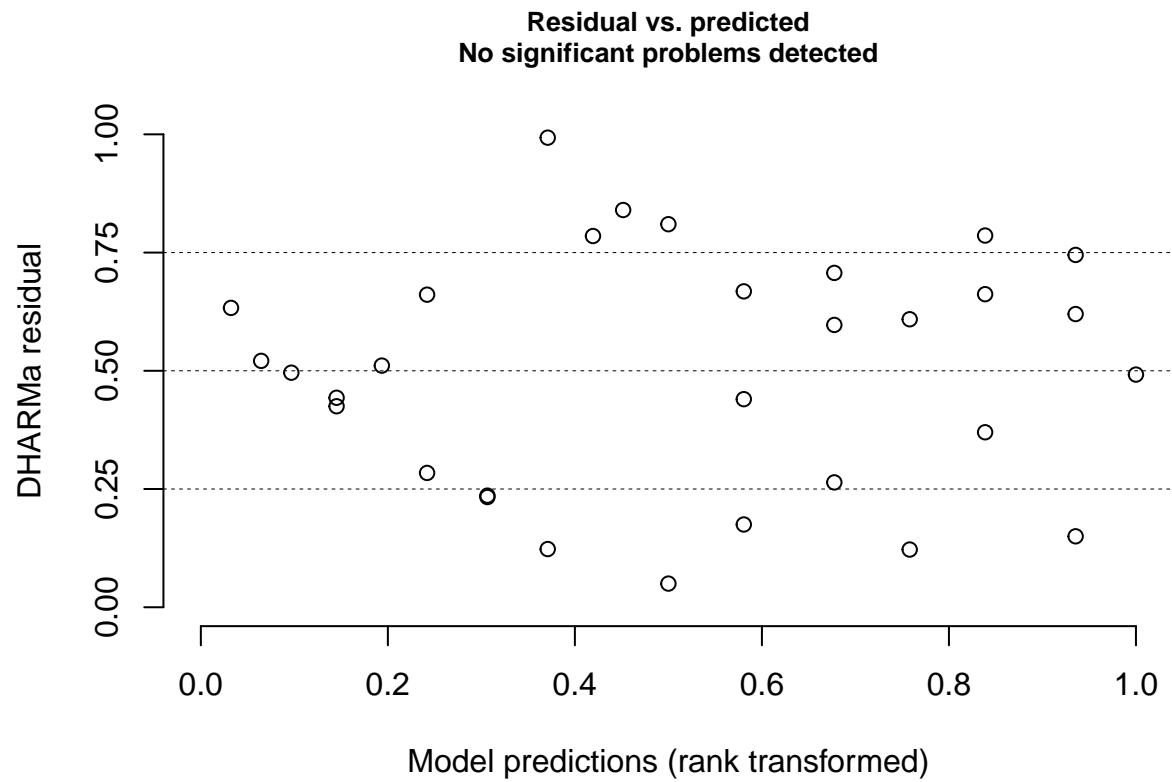


```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12774, p-value = 0.6461
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
plotResiduals(res_RS2)
```

```
## Unable to calculate quantile regression for quantile 0.25. Possibly to few (unique) data points / pr
## Unable to calculate quantile regression for quantile 0.5. Possibly to few (unique) data points / pre
## Unable to calculate quantile regression for quantile 0.75. Possibly to few (unique) data points / pr
```

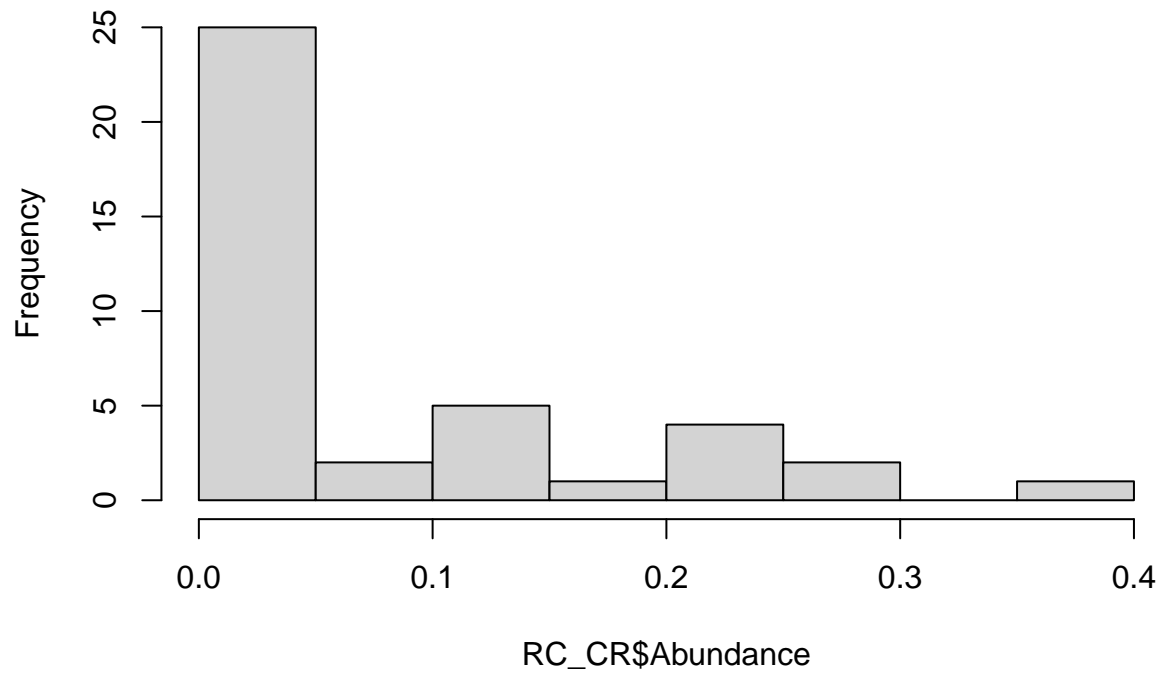


dataset *Raffaelea canadensis*

```
RC_CR$Treatment <- as.factor(RC_CR$Treatment)
RC_CR <- within(RC_CR, Treatment <- relevel(Treatment, ref = "control"))

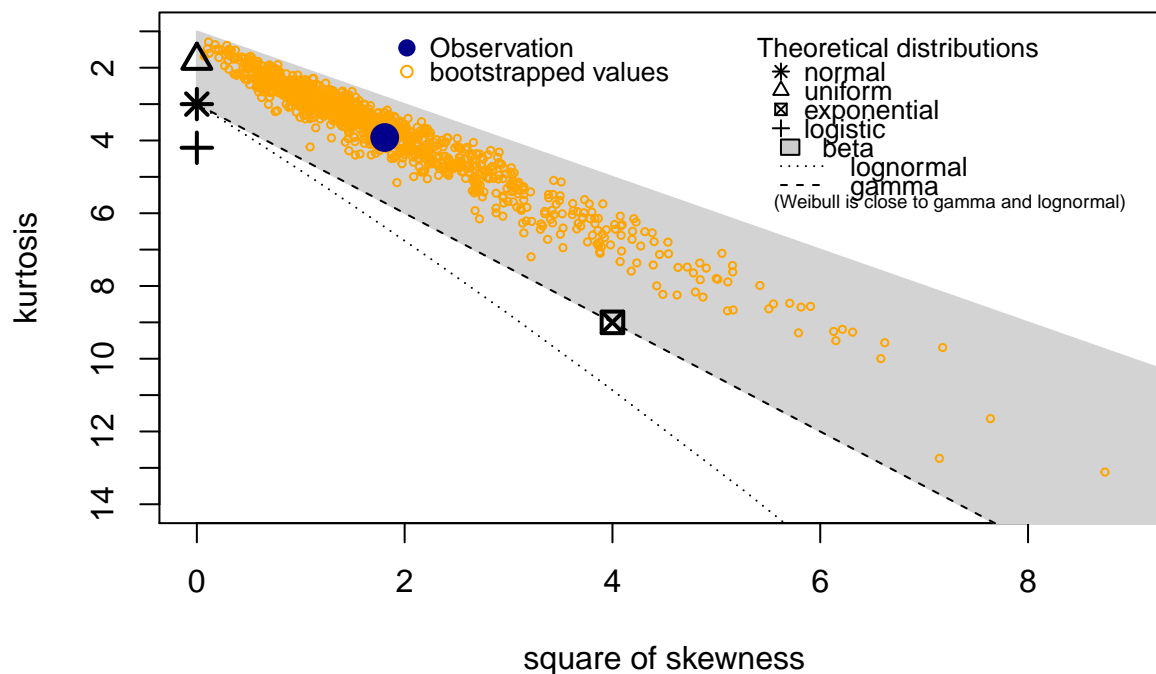
#control vs. removal
hist(RC_CR$Abundance)
```

Histogram of RC_CR\$Abundance



```
descdist(RC_CR$Abundance, boot = 1000)
```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 0    max: 0.3683126
## median: 0.01966896
## mean: 0.0739891
## estimated sd: 0.09681155
## estimated skewness: 1.344583
## estimated kurtosis: 3.919206
```

```
RC_CR.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = RC_CR)
summary(RC_CR.mod)
```

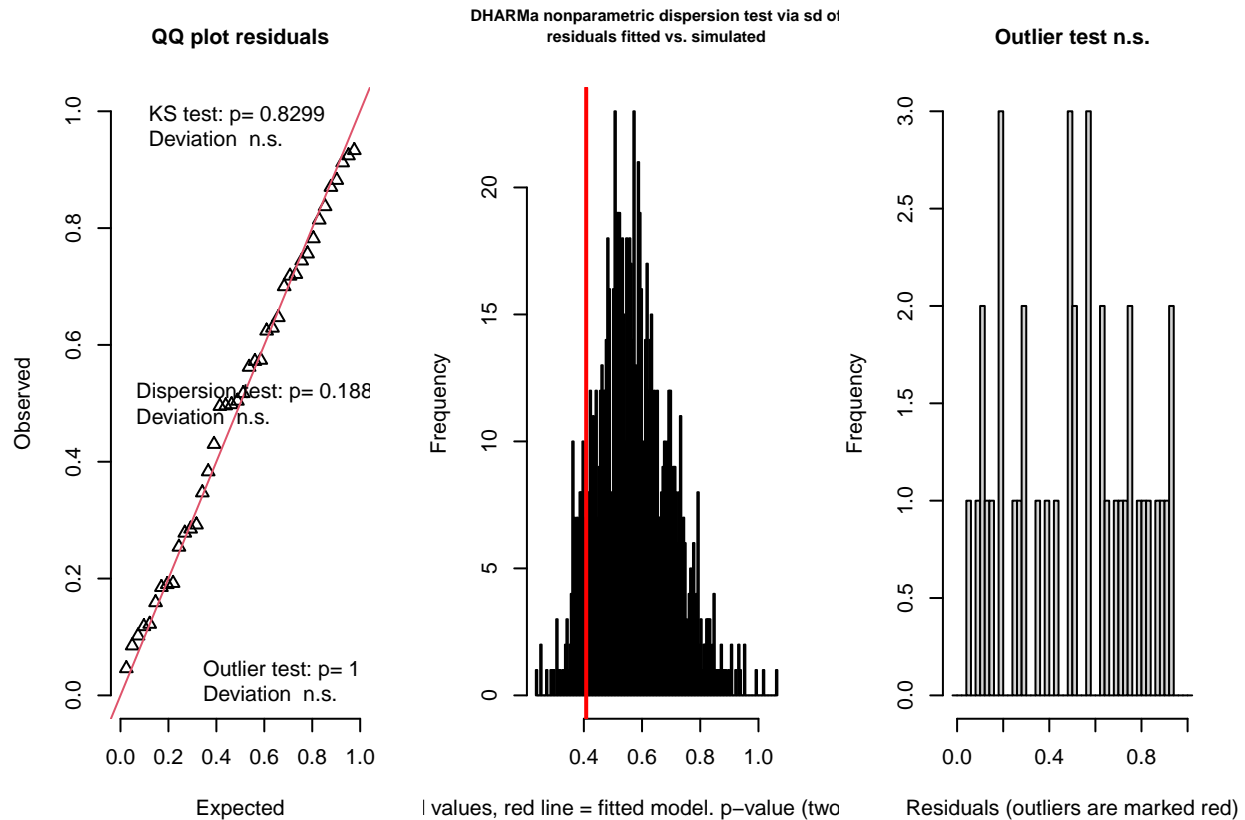
```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = RC_CR)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.78787 -0.68776  0.00395  0.58529  1.69934
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -2.34061    0.56519  -4.141 0.000287 ***
## Treatmentremoval -0.80637    0.35431  -2.276 0.030700 *
## LinageB10      -0.21455    0.67328  -0.319 0.752341
```

```
## LinageB15      1.06255    0.67328    1.578 0.125759
## LinageB16     -1.45631    0.82196   -1.772 0.087320 .
## LinageB20     -0.05887    0.82196   -0.072 0.943417
## LinageB23     -1.34091    0.92961   -1.442 0.160269
## LinageB24     -0.47751    0.76417   -0.625 0.537111
## LinageB36     -0.98234    0.75902   -1.294 0.206162
## LinageB39     -0.69249    0.82196   -0.842 0.406652
## LinageB46     -1.20338    1.21312   -0.992 0.329710
## LinageB7      -1.70636    0.92961   -1.836 0.077064 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.073 on 28 degrees of freedom
## Multiple R-squared:  0.5171, Adjusted R-squared:  0.3274
## F-statistic: 2.726 on 11 and 28 DF,  p-value: 0.01576
```

```
Anova(RC_CR.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##      Sum Sq Df F value    Pr(>F)
## Treatment  5.968  1  5.1797 0.03070 *
## Linage    26.848 10  2.3301 0.03805 *
## Residuals 32.262 28
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_RC_CR <- simulateResiduals(fittedModel=RC_CR.mod, n = 1000)
testResiduals(res_RC_CR)
```



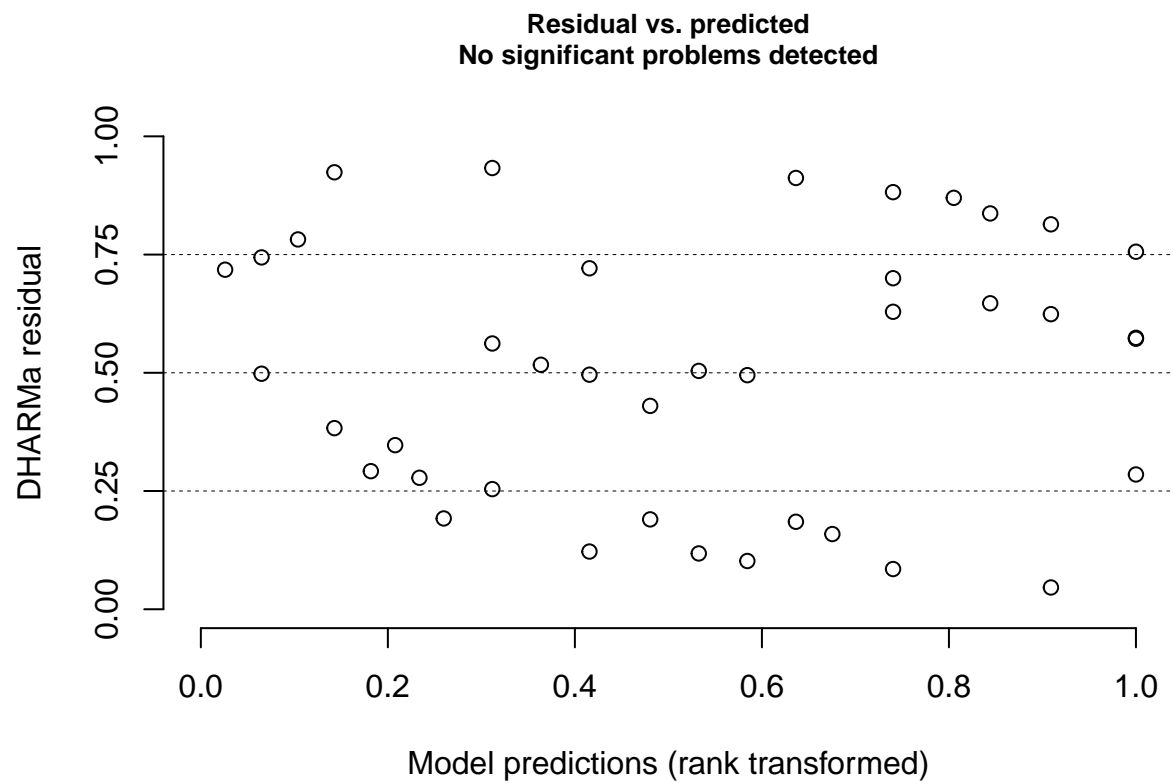
```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.095, p-value = 0.8299
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.095, p-value = 0.8299
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

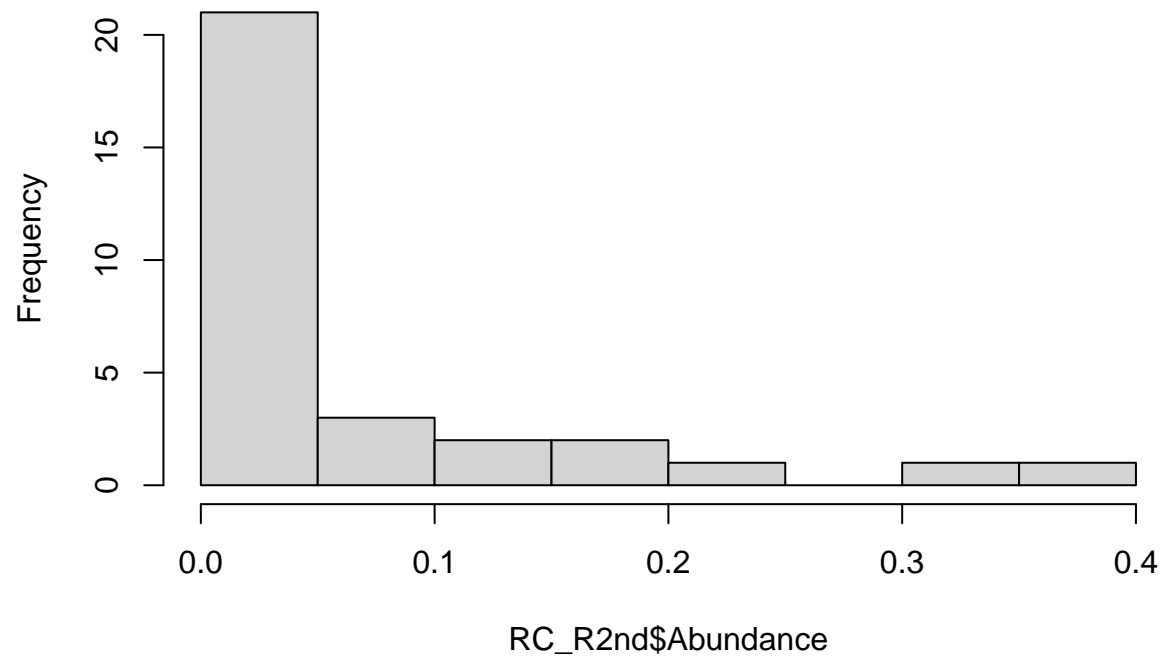
```
plotResiduals(res_RC_CR)
```

```
## Unable to calculate quantile regression for quantile 0.25. Possibly to few (unique) data points / pr
## Unable to calculate quantile regression for quantile 0.5. Possibly to few (unique) data points / pre
## Unable to calculate quantile regression for quantile 0.75. Possibly to few (unique) data points / pr
```



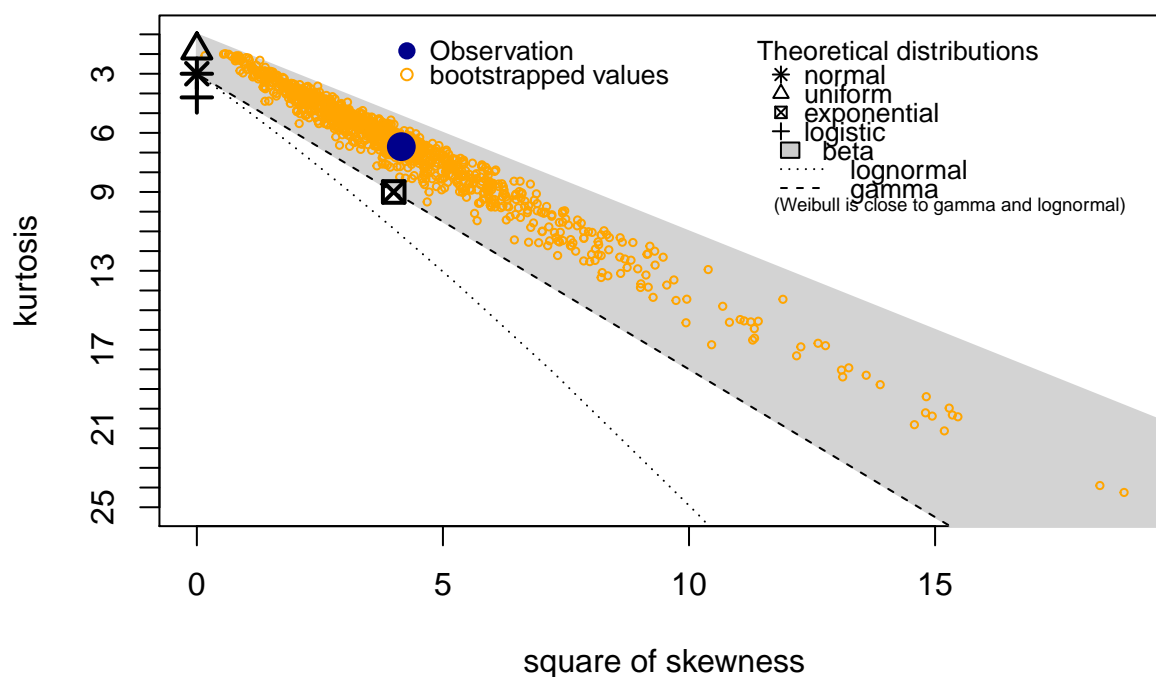
```
#removal vs. 2nd attempt  
hist(RC_R2nd$Abundance)
```


Histogram of RC_R2nd\$Abundance



```
descdist(RC_R2nd$Abundance, boot = 1000)
```

Cullen and Frey graph



```
## summary statistics
## -----
## min: 9.825596e-05 max: 0.3730086
## median: 0.02241167
## mean: 0.06594247
## estimated sd: 0.09730933
## estimated skewness: 2.038278
## estimated kurtosis: 6.698162
```

```
RC_R2nd.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = RC_R2nd)
summary(RC_R2nd.mod)
```

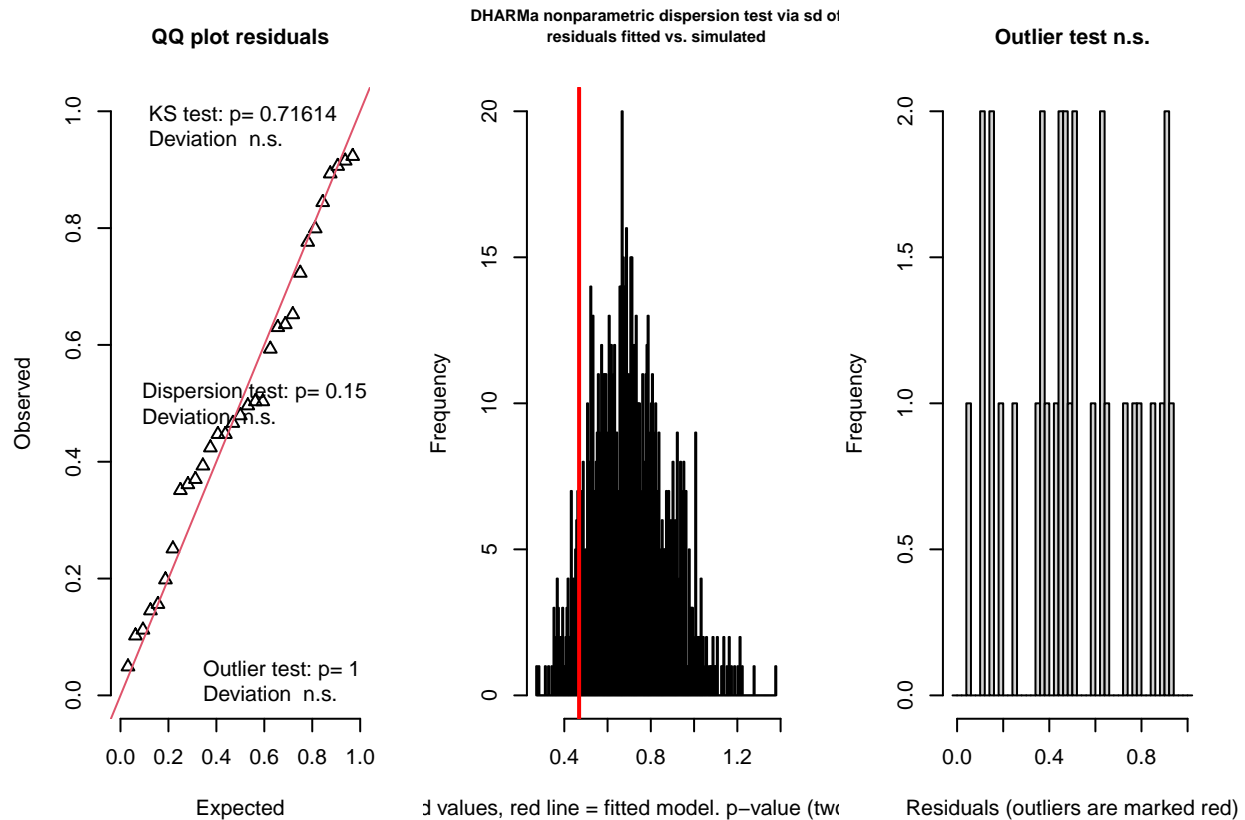
```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = RC_R2nd)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.99379 -0.41802 -0.06997  0.54212  1.71614
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -3.1885     0.6183  -5.157 4.79e-05 ***
## Treatmentremoval -0.9391     0.4530  -2.073  0.0513 .
## LinageB10       0.9697     0.7731   1.254  0.2242
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## LinageB15      1.3776      0.7427      1.855      0.0784 .
## LinageB16     -0.2286      0.8820     -0.259      0.7981
## LinageB20      1.0399      0.8820      1.179      0.2522
## LinageB23      0.4908      1.3061      0.376      0.7110
## LinageB24      0.7313      0.8214      0.890      0.3839
## LinageB36      0.4945      0.8820      0.561      0.5812
## LinageB39     -0.1815      1.3061     -0.139      0.8909
## LinageB7       -0.1675      1.3061     -0.128      0.8992
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.151 on 20 degrees of freedom
## Multiple R-squared:  0.4011, Adjusted R-squared:  0.1016
## F-statistic: 1.339 on 10 and 20 DF,  p-value: 0.2765
```

```
Anova(RC_R2nd.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##      Sum Sq Df F value    Pr(>F)
## Treatment  5.6882  1  4.2971 0.05131 .
## Linage     9.4990  9  0.7973 0.62301
## Residuals 26.4747 20
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_RC_R2nd <- simulateResiduals(fittedModel=RC_R2nd.mod, n = 1000)
testResiduals(res_RC_R2nd)
```



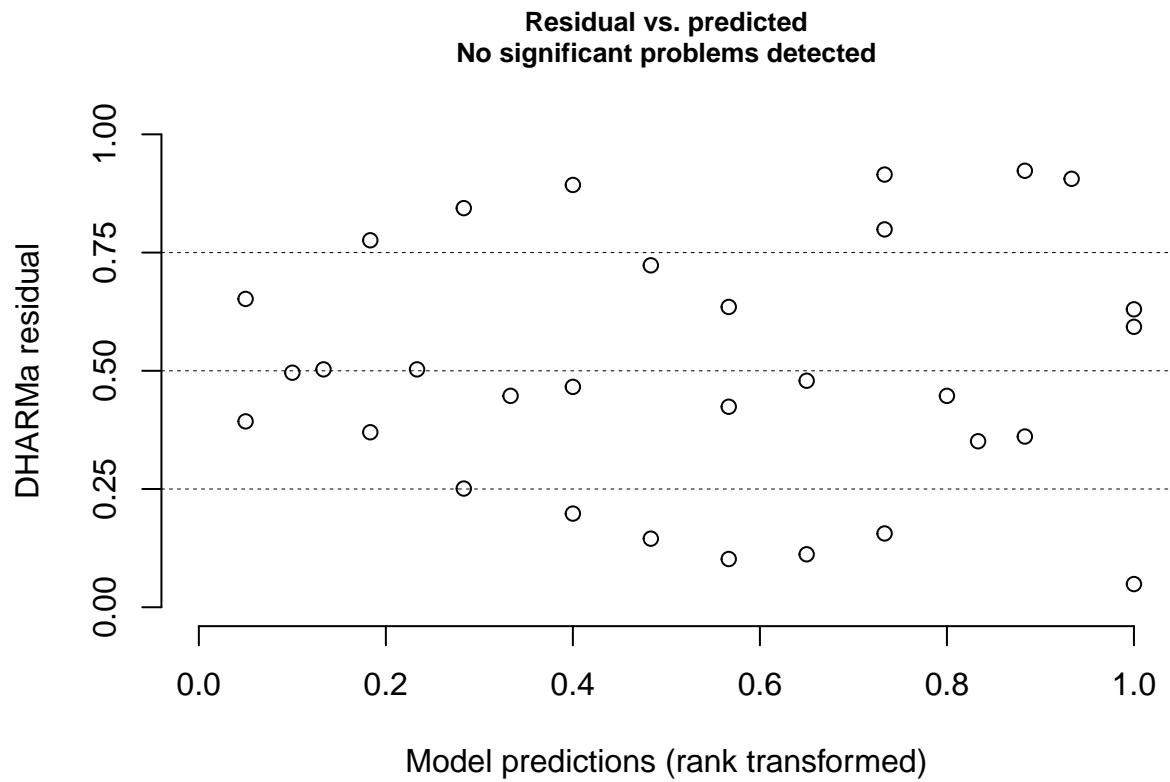
```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12519, p-value = 0.7161
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12519, p-value = 0.7161
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
plotResiduals(res_RC_R2nd)
```

```
## Unable to calculate quantile regression for quantile 0.25. Possibly to few (unique) data points / pr
## Unable to calculate quantile regression for quantile 0.5. Possibly to few (unique) data points / pre
## Unable to calculate quantile regression for quantile 0.75. Possibly to few (unique) data points / pr
```

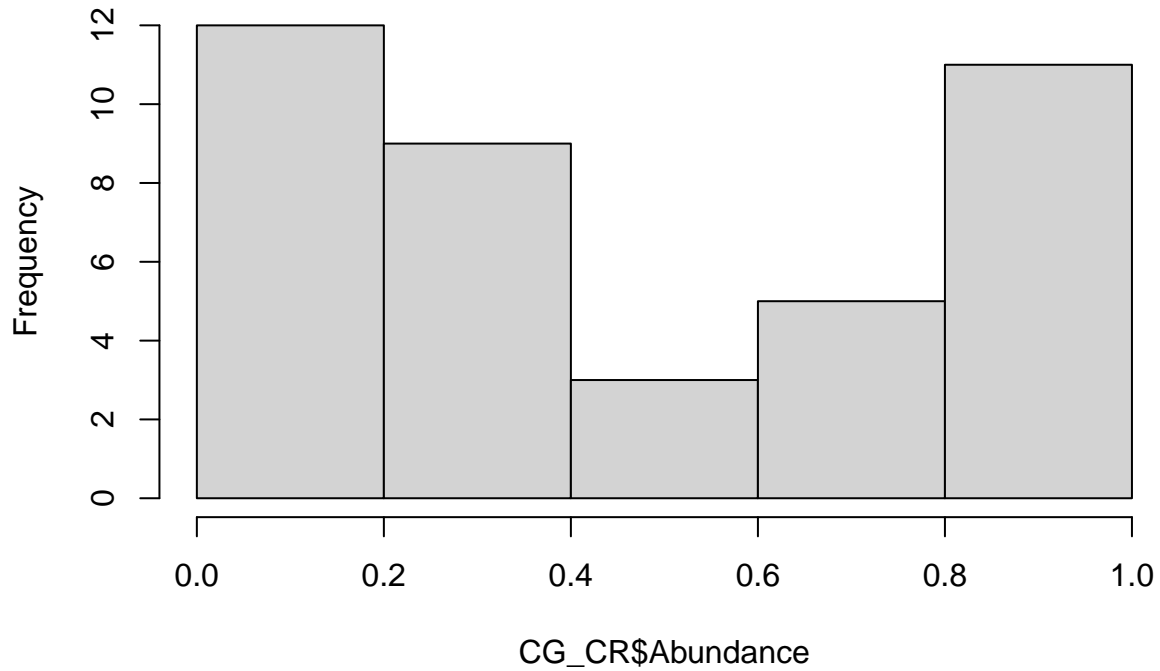


dataset *Chaetomium globosum*

```
CG_CR$Treatment <- as.factor(CG_CR$Treatment)
CG_CR <- within(CG_CR, Treatment <- relevel(Treatment, ref = "control"))

#control vs. removal
hist(CG_CR$Abundance)
```

Histogram of CG_CR\$Abundance



```
CG_CR.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = CG_CR)
summary(CG_CR.mod)
```

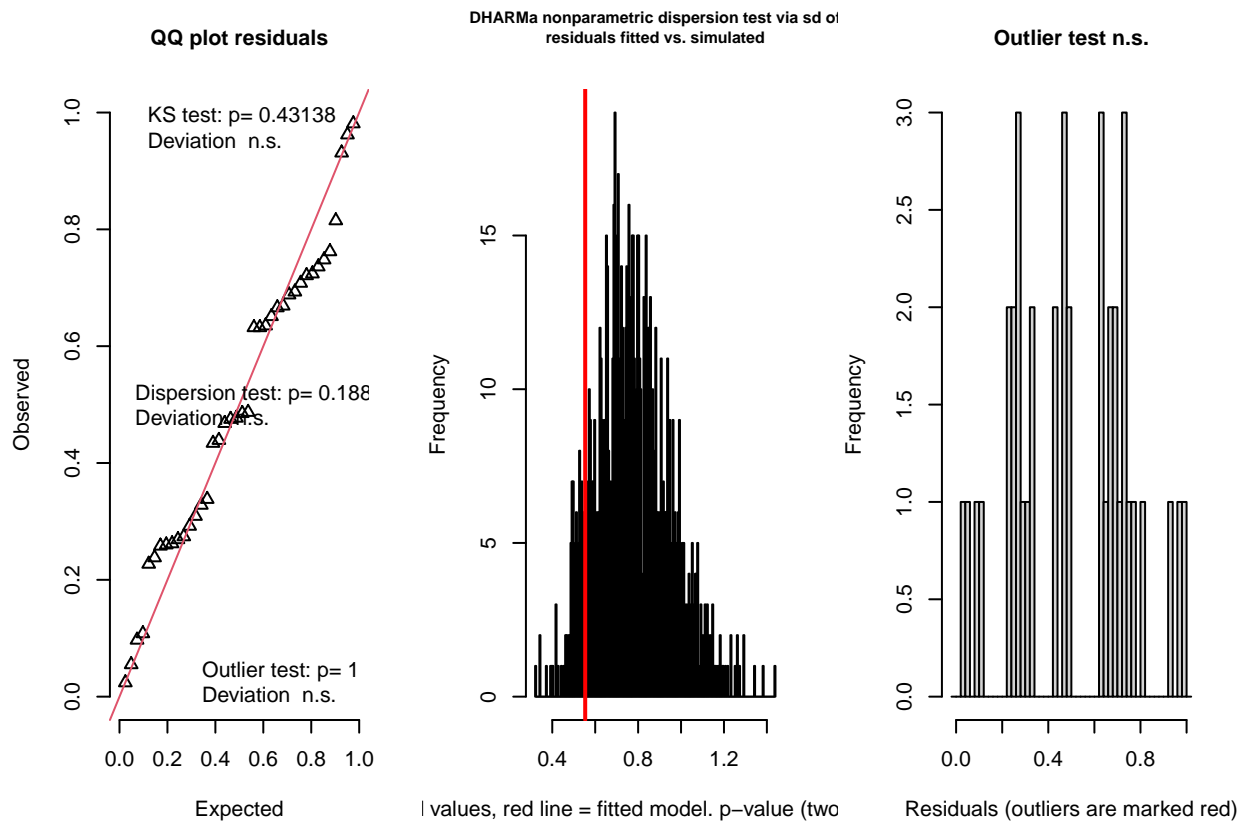
```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = CG_CR)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -5.9174 -1.8652 -0.0126  1.5953  6.1238
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)   -0.9525     1.5687  -0.607   0.5486
## Treatmentremoval  1.8051     0.9834   1.836   0.0771 .
## LinageB10      -0.9916     1.8687  -0.531   0.5999
## LinageB15     -1.5876     1.8687  -0.850   0.4027
## LinageB16       1.3350     2.2814   0.585   0.5631
## LinageB20       0.5364     2.2814   0.235   0.8158
## LinageB23       0.5182     2.5801   0.201   0.8423
## LinageB24       0.6024     2.1210   0.284   0.7785
## LinageB36       0.8342     2.1067   0.396   0.6951
## LinageB39       1.0642     2.2814   0.466   0.6445
## LinageB46       4.8388     3.3670   1.437   0.1618
## LinageB7        2.3669     2.5801   0.917   0.3668
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.979 on 28 degrees of freedom
## Multiple R-squared:  0.2958, Adjusted R-squared:  0.01916
## F-statistic: 1.069 on 11 and 28 DF,  p-value: 0.4186
```

```
Anova(CG_CR.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##           Sum Sq Df F value    Pr(>F)
## Treatment  29.908  1   3.3695 0.07706 .
## Linage     72.260 10   0.8141 0.61777
## Residuals 248.530 28
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res.CG_CR.mod <- simulateResiduals(CG_CR.mod, n = 1000)
testResiduals(res.CG_CR.mod)
```



```
## $uniformity
##
```



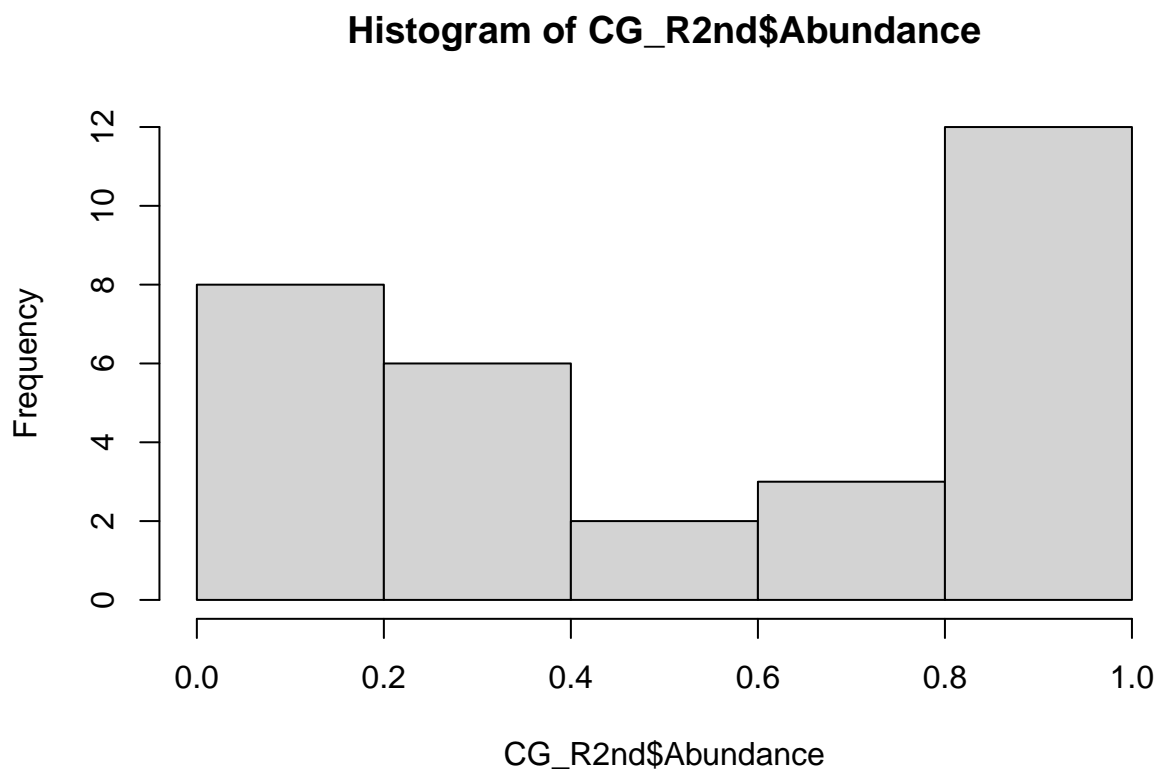
```

## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.138, p-value = 0.4314
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##
##
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.138, p-value = 0.4314
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.71597, p-value = 0.188
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations

```

```
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 40, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.0880973
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0
```

```
#removal vs. 2nd attempt
hist(CG_R2nd$Abundance)
```



```
CG_R2nd.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = CG_R2nd)
summary(CG_R2nd.mod)
```

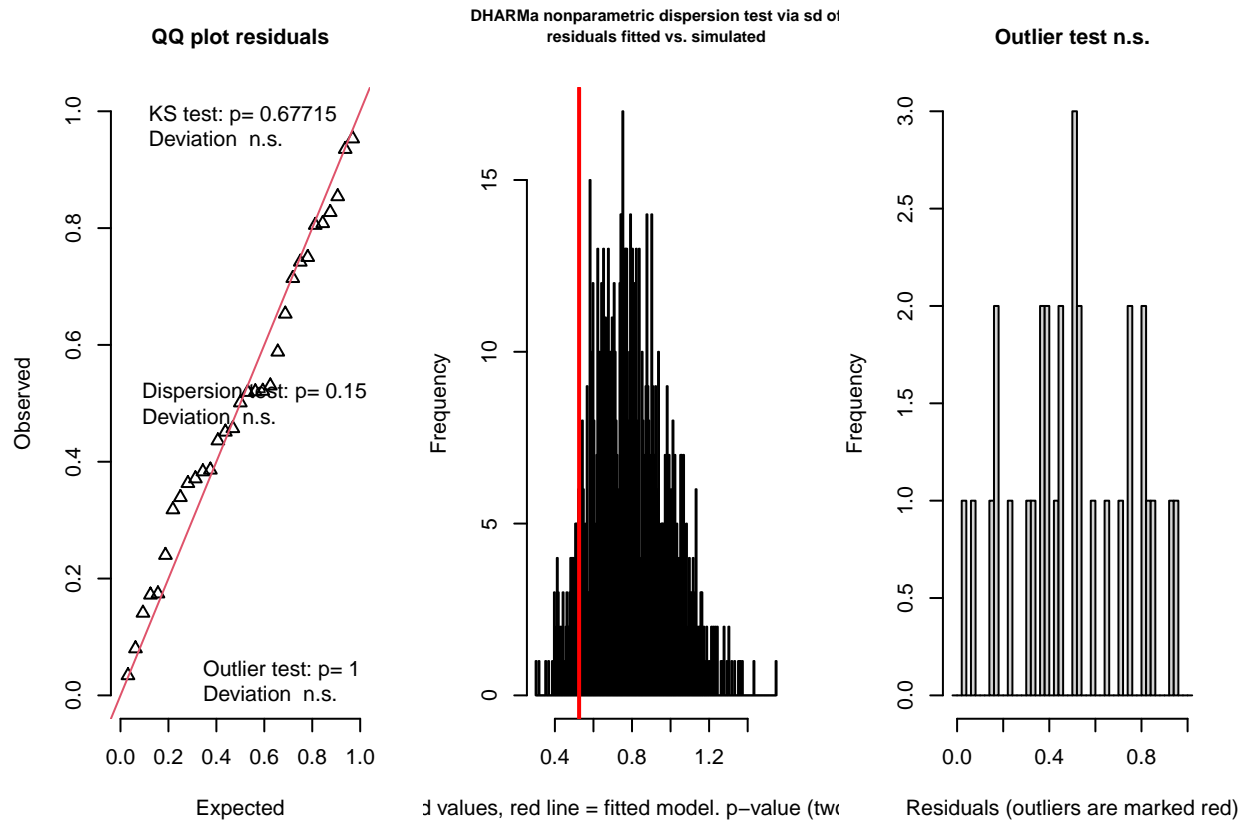
```
##
## Call:
## lm(formula = logistic(Abundance) ~ Treatment + Linage, data = CG_R2nd)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -6.1054 -1.3363 -0.0285  2.0304  5.6895
##
## Coefficients:
```

```
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)      2.5165      1.7758   1.417  0.1719
## Treatmentremoval  0.3940      1.3012   0.303  0.7652
## LinageB10        -3.3741      2.2207  -1.519  0.1443
## LinageB15        -3.7326      2.1332  -1.750  0.0955 .
## LinageB16         0.1315      2.5333   0.052  0.9591
## LinageB20        -2.8706      2.5333  -1.133  0.2706
## LinageB23        -3.5060      3.7516  -0.935  0.3612
## LinageB24        -1.8909      2.3593  -0.801  0.4323
## LinageB36        -2.3398      2.5333  -0.924  0.3667
## LinageB39         0.7595      3.7516   0.202  0.8416
## LinageB7          2.1701      3.7516   0.578  0.5694
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.305 on 20 degrees of freedom
## Multiple R-squared:  0.2923, Adjusted R-squared:  -0.06151
## F-statistic: 0.8262 on 10 and 20 DF,  p-value: 0.6092
```

```
Anova(CG_R2nd.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##           Sum Sq Df F value Pr(>F)
## Treatment   1.001  1  0.0917 0.7652
## Linage     85.990  9  0.8748 0.5623
## Residuals 218.424 20
```

```
res.CG_R2nd.mod <- simulateResiduals(CG_R2nd.mod, n = 1000)
testResiduals(res.CG_R2nd.mod)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12445, p-value = 0.6771
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
```

```

## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.12445, p-value = 0.6771
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.66599, p-value = 0.15
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 31, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1121887
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

```

testing difference of ‘successfull’ vs. ‘failed’ nests

create subset of samples from ‘removal’ group

```

REM <- subset_samples(rel.R2nd, Treatment == "removal")
REM <- prune_taxa(taxa_sums(REM) > 0, REM)

sample_data(REM)

```

```

##           Sample   Nest Linage Treatment age_sampling_d. Group ColSums
## B0-19a    B0-19a  B0-19    B0    removal           31 Fungi    8655
## B0-22a    B0-22a  B0-22    B0    removal           31 Fungi   19669

```

```
## B10-09a B10-09a B10-09      B10      removal      30 Fungi      26662
## B10-25a B10-25a B10-25      B10      removal      30 Fungi      24345
## B10-26a B10-26a B10-26      B10      removal      30 Fungi      26861
## B15-01a B15-01a B15-01      B15      removal      33 Fungi      12127
## B15-07a B15-07a B15-07      B15      removal      29 Fungi      19916
## B15-25a B15-25a B15-25      B15      removal      28 Fungi       8107
## B16-01a B16-01a B16-01      B16      removal      32 Fungi      20355
## B16-31a B16-31a B16-31      B16      removal      30 Fungi      15800
## B20-04a B20-04a B20-04      B20      removal      35 Fungi      34274
## B20-08a B20-08a B20-08      B20      removal      31 Fungi      27572
## B23-23a B23-23a B23-23      B23      removal      32 Fungi      26875
## B24-02a B24-02a B24-02      B24      removal      32 Fungi       1087
## B24-26a B24-26a B24-26      B24      removal      32 Fungi      16017
## B24-28a B24-28a B24-28      B24      removal      32 Fungi      19882
## B36-14a B36-14a B36-14      B36      removal      19 Fungi       8615
## B36-31a B36-31a B36-31      B36      removal      32 Fungi      17279
## B39-17a B39-17a B39-17      B39      removal      31 Fungi      12233
## B7-08a   B7-08a   B7-08       B7       removal      33 Fungi       4694
```

```
suc = c("successfull", "successfull", "successfull", "successfull", "failed", "successfull", "successfull")
sample_data(REM)$success <- suc

dfREM <- REM %>%
  tax_glom(taxrank = "Species") %>%
  psmelt()
```

```
otu.REM <- abundances(REM)
meta.REM <- meta(REM)

set.seed(1)
adonis2(distance(REM, method = "bray") ~ success, data = meta.REM)
```

Permanova on data REM

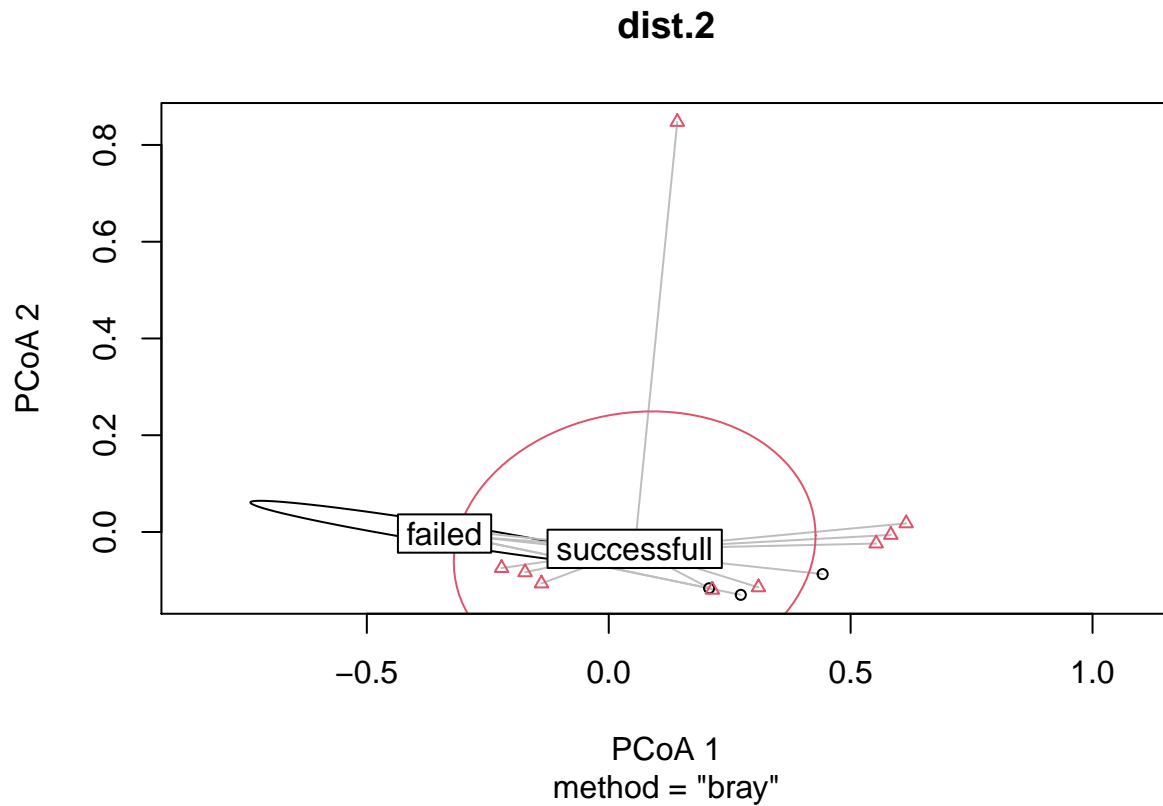
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = distance(REM, method = "bray") ~ success, data = meta.REM)
##           Df SumOfSqs      R2      F Pr(>F)
## success    1   0.3363 0.09082 1.7981  0.164
## Residual  18   3.3662 0.90918
## Total     19   3.7024 1.00000
```

```
dist <- vegdist(t(otu.REM))
dist.2 <- betadisper(dist, meta.REM$success)
anova(dist.2)
```

```
## Analysis of Variance Table
```

```
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups    1 0.15332 0.153318   2.109 0.1636
## Residuals 18 1.30858 0.072699
```

```
plot(dist.2, hull = FALSE, ellipse = TRUE)
```



prepare data and plot

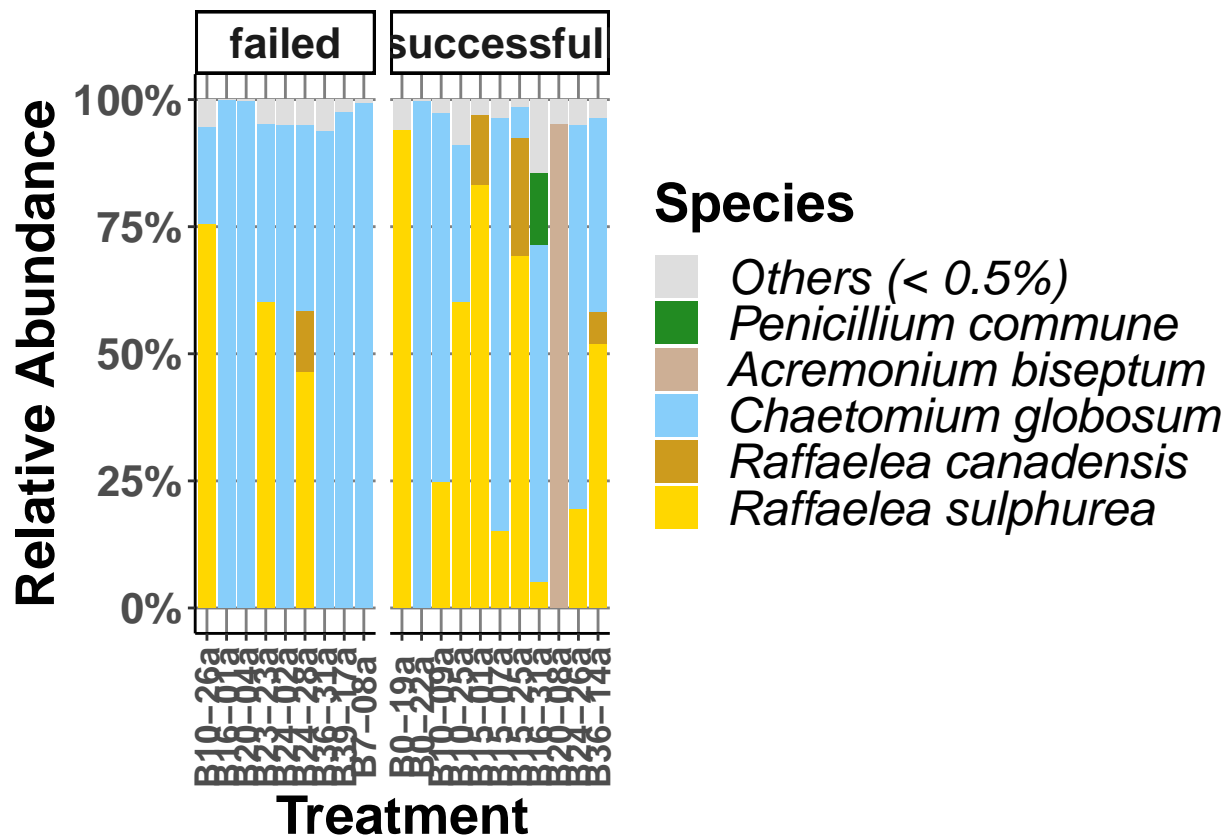
```
dfREM.plot <- dfREM
dfREM.plot$Species <- as.character(dfREM.plot$Species)
dfREM.plot$Species[dfREM.plot$Abundance < 0.05] <- "Others"
dfREM.plot$Species <- factor(dfREM.plot$Species, levels = c("Others", "Penicillium_commune", "Acremonium_l"))

Fungi_Success_plot <- ggplot(dfREM.plot, aes(x = Sample, y = Abundance, fill = Species)) +
  geom_bar(stat = "identity", color = NA, position = "fill") +
  scale_fill_manual(values = Plot_colors_s, name = "Species") +
  facet_grid(~success, scales = "free_x", space = "free_x")

FS <- Fungi_Success_plot +
  theme(plot.title = element_text(size = 20, face = "bold")) +
  theme_classic() +
  #gets rid of background
  theme(panel.grid.major = element_line(colour = "grey50")) +
  labs(x = "Treatment", y = "Relative Abundance") +
```

```
scale_y_continuous(labels=percent_format())+
theme(axis.text.x=element_text(size = 13, angle = 90, vjust = 0.5))+
theme(text = element_text(size=20, face = "bold"))+
theme(legend.title = element_text(size = 20), legend.text = element_text(size = 18))+
theme(legend.text = element_text(face = "italic"))
```

FS



```
dfREM.sub <- subset(dfREM, Species == "Chaetomium_globosum" | Species == "Raffaelea_canadensis" | Species == "Acremonium_bisseptum")
ptax_col <- c("lightskyblue", "goldenrod3", "gold")
dfREM.sub$Species <- factor(dfREM.sub$Species, labels = c("Chaetomium globosum", "Raffaelea canadensis", "Acremonium bisseptum"))
ptax<-ggplot(data = dfREM.sub, aes(x = success, y = Abundance)) +
  geom_boxplot(aes(fill=Species),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,
  scale_fill_manual(values = ptax_col, name = "Species")+
  scale_color_manual(values = ptax_col, name = "Species")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Species~fct_relevel(success, "successfull", "failed"), scales = "free")+theme_bw()
ptax<-ptax+ theme(legend.position="right")+ylab("Relative Abundance")
ptax<-ptax+ theme(legend.text=element_text(size=15, face = "italic"))+
  theme(legend.key = element_rect(color = NA, fill = NA),legend.key.size = unit(0.9, "cm"))+
  theme(legend.title = element_text(size = 20, face = "bold"))+
  scale_y_continuous(labels=percent_format())
```

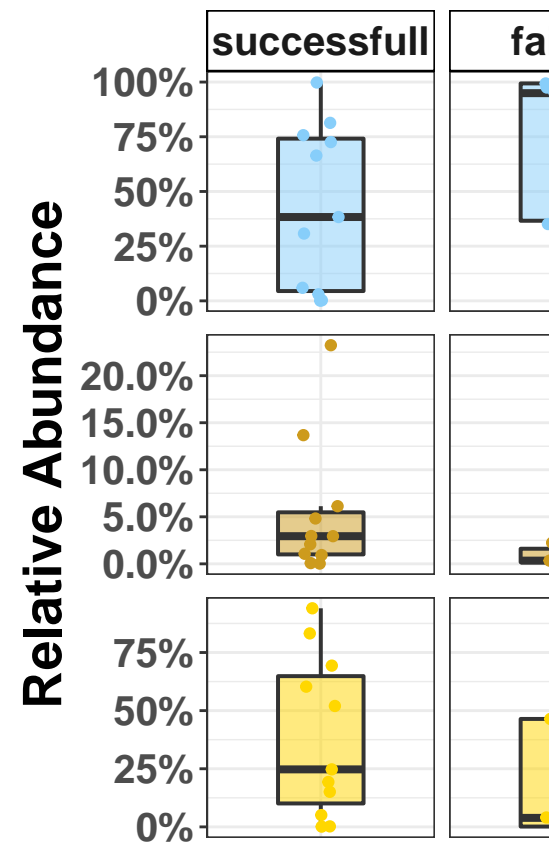


```

abu2<-ptax + theme(strip.background =element_rect(fill="white", color="black"))+
  theme(strip.text.x = element_text(size = 15, face = "bold"))
Success.plot<-abu2+theme(axis.title.y = element_text(size=20, face="bold"))+theme(axis.text.y = element.
  theme(axis.text.x = element_text(size=20, angle=90,face="bold"))+
  theme(axis.title.x = element_blank(), axis.text.x=element_blank(),
    axis.ticks.x=element_blank())+
  theme(strip.text.y = element_blank())
Success.plot_fun <- Success.plot + theme(panel.spacing.y = unit(0.3, "cm"))

Success.plot_fun

```



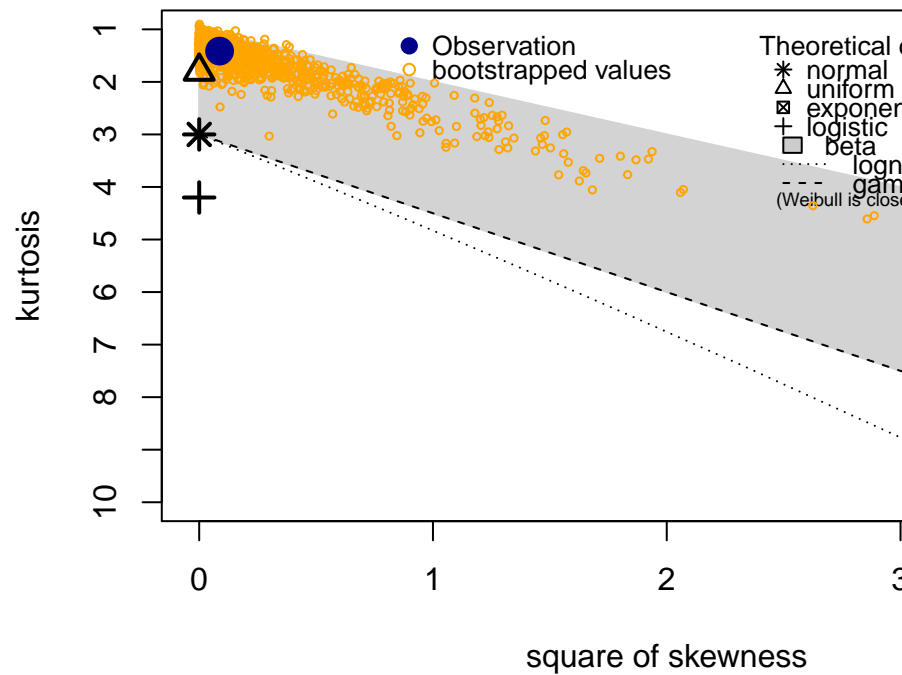
plot core taxa of successfull vs. failed nests with relative abundance

```

CGrem <- subset(dfREM, Species == "Chaetomium_globosum")
descdist(CGrem$Abundance, boot = 1000)

```

Cullen and Frey graph



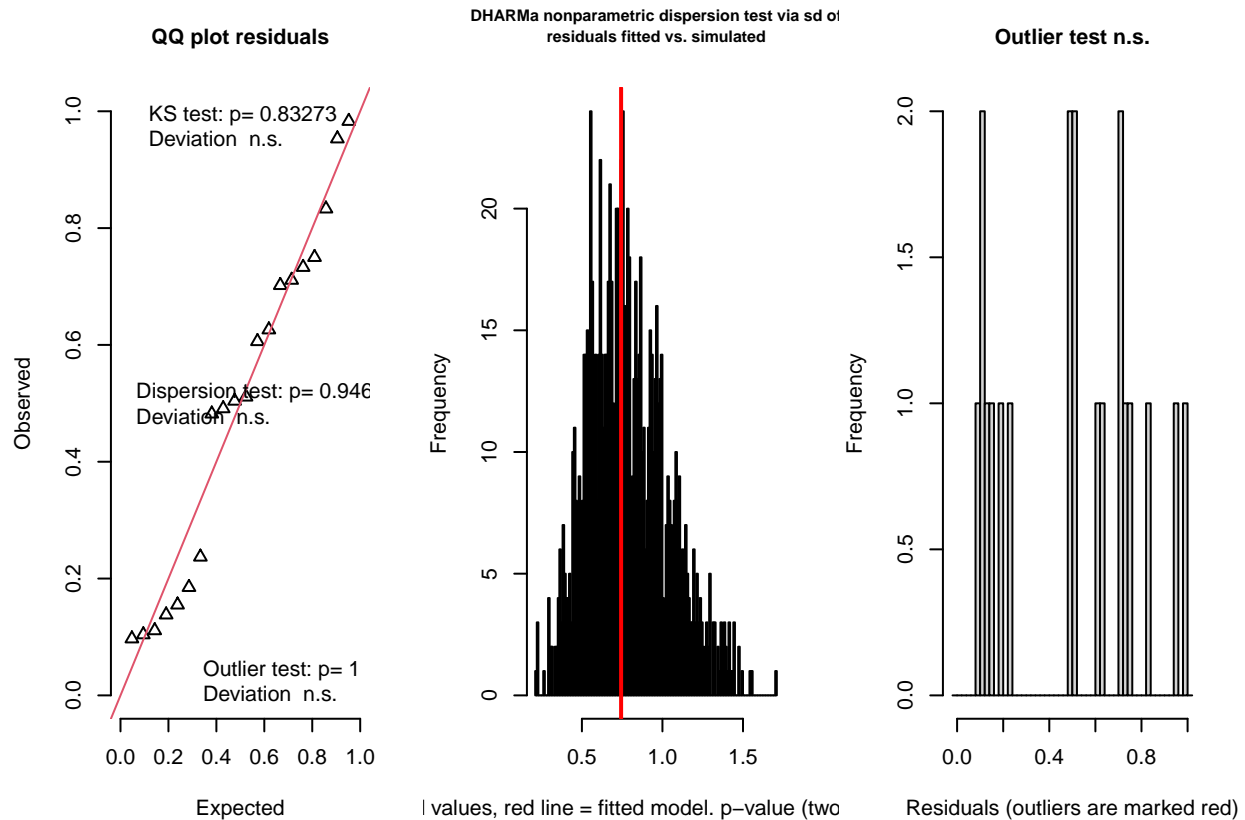
test if taxa different in these two groups

```
## summary statistics
## -----
## min: 0.0007979109 max: 0.9997544
## median: 0.6949201
## mean: 0.5751256
## estimated sd: 0.3863477
## estimated skewness: -0.2953997
## estimated kurtosis: 1.414034
```

```
CGrem.mod <- lm(logistic(Abundance) ~ success, data = CGrem)
Anova(CGrem.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##          Sum Sq Df F value    Pr(>F)
## success    56.252  1  5.5052 0.03061 *
## Residuals 183.925 18
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_CGrem.mod <- simulateResiduals(CGrem.mod, n = 1000)
testResiduals(res_CGrem.mod)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.132, p-value = 0.8327
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
```

```

## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.132, p-value = 0.8327
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

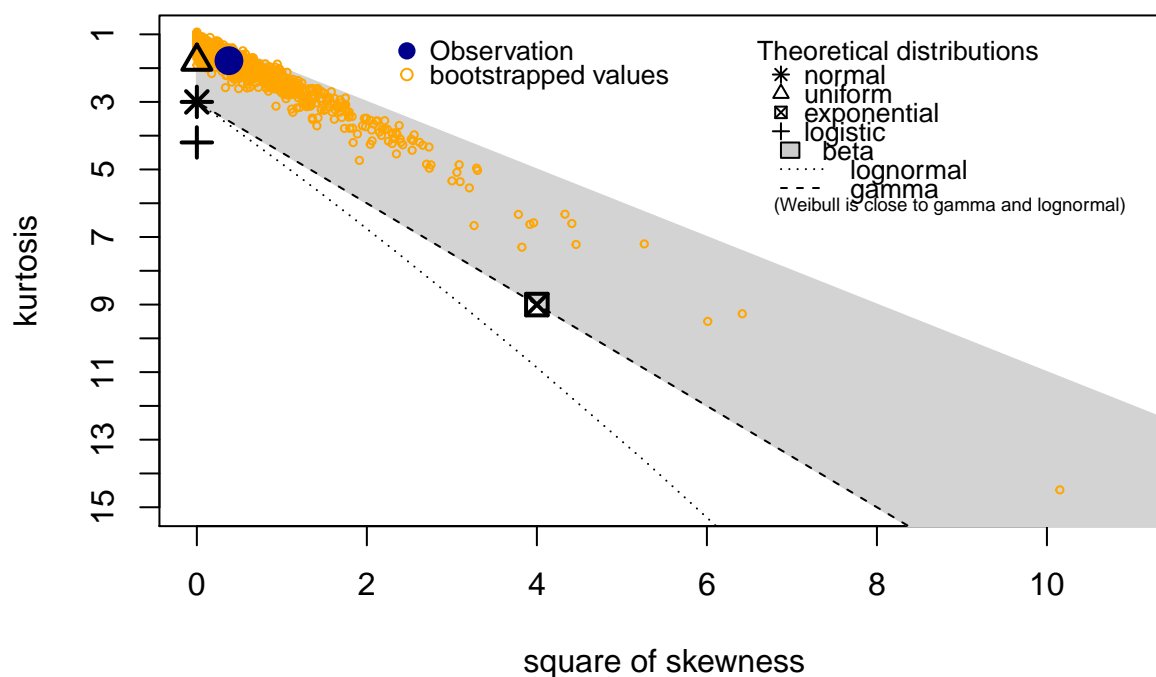
```

```

RSrem <- subset(dfREM, Species == "Raffaelea_sulphurea")
descdist(RSrem$Abundance, boot = 1000)

```

Cullen and Frey graph

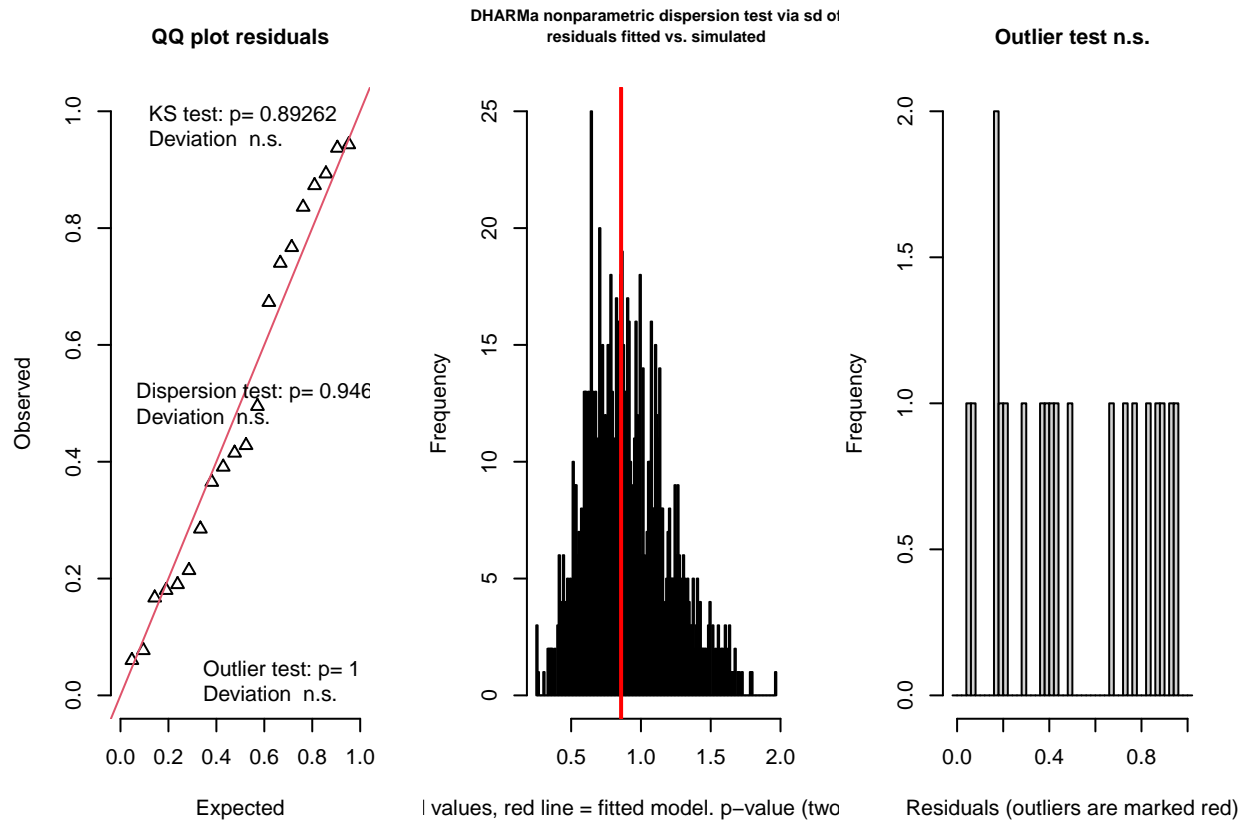


```
## summary statistics
## -----
## min: 0.0001473839   max: 0.9401502
## median: 0.1719765
## mean: 0.307603
## estimated sd: 0.3306086
## estimated skewness: 0.6144493
## estimated kurtosis: 1.77946
```

```
RSrem.mod <- lm(logistic(Abundance) ~ success, data = RSrem)
Anova(RSrem.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##          Sum Sq Df F value Pr(>F)
## success   11.281  1  2.1023 0.1643
## Residuals  96.585 18
```

```
res_RSrem.mod <- simulateResiduals(RSrem.mod, n = 1000)
testResiduals(res_RSrem.mod)
```



```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.122, p-value = 0.8926
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
```

```

## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.122, p-value = 0.8926
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

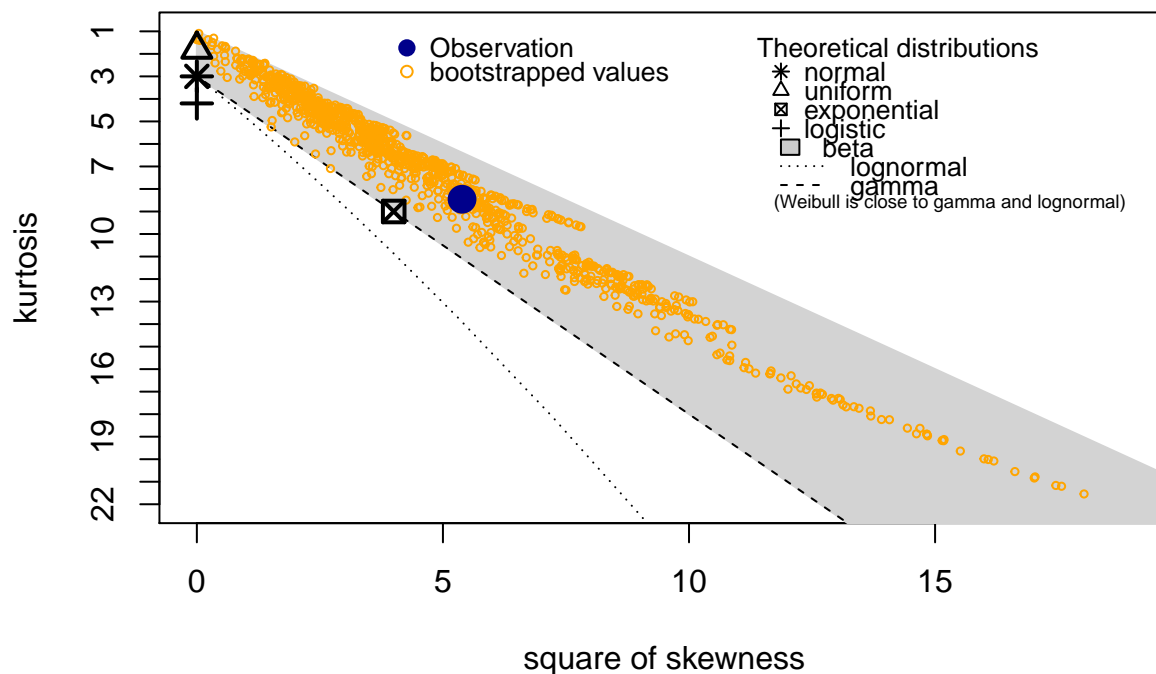
```

```

RCrem <- subset(dfREM, Species == "Raffaelea_canadensis")
descdist(RCrem$Abundance, boot = 1000)

```

Cullen and Frey graph

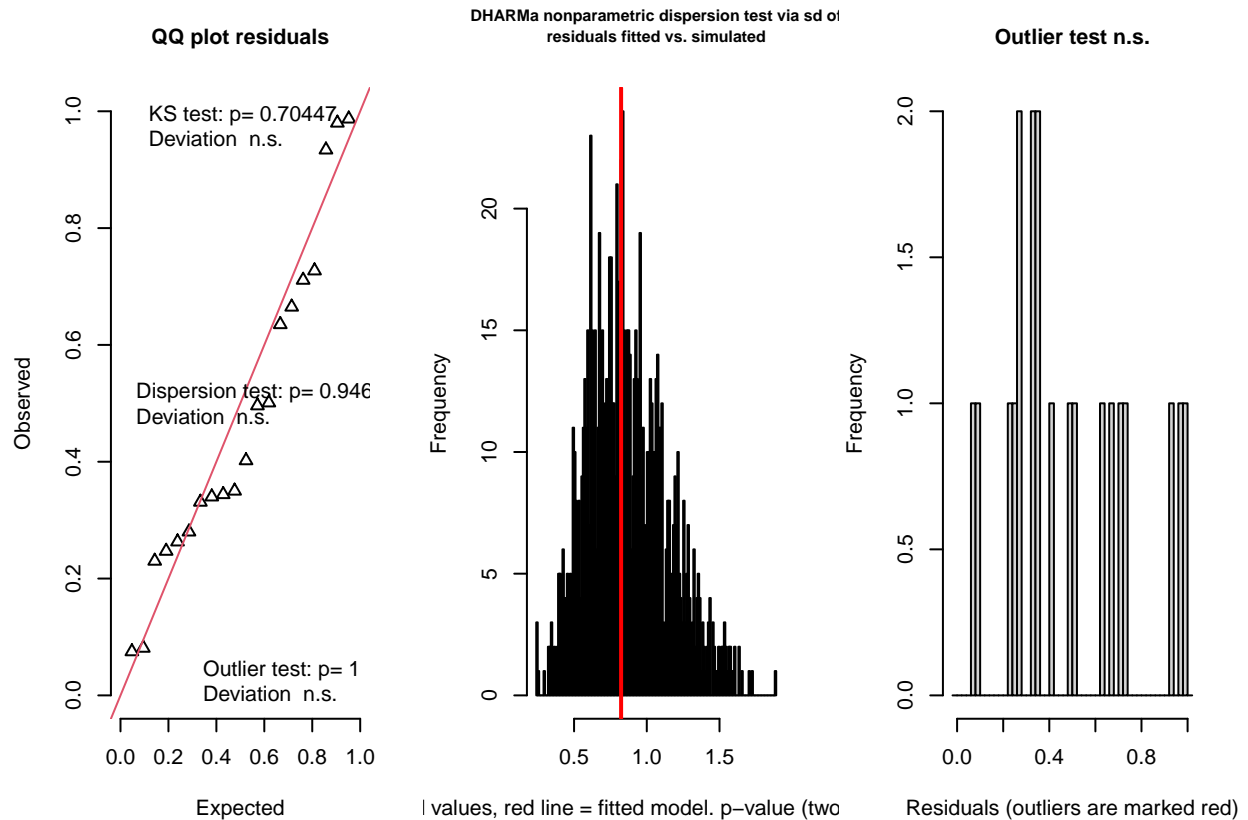


```
## summary statistics
## -----
## min: 9.825596e-05 max: 0.2323918
## median: 0.01338467
## mean: 0.03755341
## estimated sd: 0.05985818
## estimated skewness: 2.321288
## estimated kurtosis: 8.455446
```

```
RCrem.mod <- lm(logistic(Abundance) ~ success, data = RCrem)
Anova(RCrem.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##          Sum Sq Df F value Pr(>F)
## success    2.7337  1  2.8548 0.1083
## Residuals 17.2368 18
```

```
res_RCrem.mod <- simulateResiduals(RCrem.mod, n = 1000)
testResiduals(res_RCrem.mod)
```

```
## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.15, p-value = 0.7045
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
```

```

## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

## $uniformity
##
## One-sample Kolmogorov-Smirnov test
##
## data: simulationOutput$scaledResiduals
## D = 0.15, p-value = 0.7045
## alternative hypothesis: two-sided
##
##
## $dispersion
##
## DHARMA nonparametric dispersion test via sd of residuals fitted vs.
## simulated
##
## data: simulationOutput
## dispersion = 0.94547, p-value = 0.946
## alternative hypothesis: two.sided
##
##
## $outliers
##
## DHARMA outlier test based on exact binomial test with approximate
## expectations
##
## data: simulationOutput
## outliers at both margin(s) = 0, observations = 20, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
## 0.0000000 0.1684335
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
## 0

```

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