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

```
data16S <- otu_table(read.table("16S_Rem_zotu_table.txt",sep="\t", header=T, row.names=1, check.names =
tax16S <- tax_table(as.matrix(read.table("16S_Rem_zotus.tax.txt", sep="\t", header=T, row.names=1, fill=
datasample16S  <- sample_data(read.table("16S_map_removal.txt", sep="\t", header=T, row.names=1))
```

merge data into phyloseq object

```
(all16S <- merge_phyloseq(data16S, tax16S, datasample16S))
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 365 taxa and 57 samples ]
## sample_data() Sample Data:       [ 57 samples by 16 sample variables ]
## tax_table()   Taxonomy Table:    [ 365 taxa by 8 taxonomic ranks ]
```

copy taxonomic classification in tax_table collumns with gaps and add "_spc"

```
dataset.16S =  subset_taxa(all16S, (Domain == "d:Bacteria"))
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Phylum"]=="","Phylum"]<-paste(tax_table(dataset.16S)[ta
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Class"]=="","Class"]<-paste(tax_table(dataset.16S)[tax_
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Order"]=="","Order"]<-paste(tax_table(dataset.16S)[tax_
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Family"]=="","Family"]<-paste(tax_table(dataset.16S)[ta
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Genus"]=="","Genus"]<-paste(tax_table(dataset.16S)[tax_
tax_table(dataset.16S)[tax_table(dataset.16S)[,"Species"]=="","Species"]<-paste(tax_table(dataset.16S)[
```

start filtering out all ZOTUs that were only assigned to Domain (Bacteria and Archaea), as well as Chloroplasts

```
dataset.16S.ordi =  subset_taxa(dataset.16S, (Domain == "d:Bacteria" | Domain =="d:Archaea"))
dataset.16S.ordi =  subset_taxa(dataset.16S.ordi, Phylum != "d:Bacteria_spc")
dataset.16S.ordi =  subset_taxa(dataset.16S.ordi, Phylum != "d:Archaea_spc")
dataset.16S.ordi =  subset_taxa(dataset.16S.ordi, Class != "c:Chloroplast")
```

controlled for most abundant ZOTUS in the dataset with Bacteria sp. and Archaea sp. still included in https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome ==> mitochondrial DNA

Zotu1 –> Leptographium (Ophiostomataceae) max/total score: 340, Query Cover: 100%, e-value: 2e-89, Per. Ident 93.81%

Zotu3 –> Leptographium (Ophiostomataceae) max/total score: 274, Query Cover: 100%, e-value: 2e-69, Per. Ident 88.60%

Zotu4 –> Ophiostoma (Query Cover : 100%, Per. Ident: 89.96%) or Grosmannia (Query Cover: 93%, Per. Ident: 91.51%)

Zotu6 –> Hydropisphaera (Trichoderma, Fusarium close) max/total score: 316, Query Cover: 100%, e-value: 3e-82, Per. Ident 92.44%

Zotu7 –> Wolbachia rRNA max/total score: 468, Query Cover: 100%, e-value: 8e-128, Per. Ident 100%

Zotu11 –> Clematis sp. or Ranunculus sp. max/total score: 464, Query Cover: 100%, e-value: 1e-126, Per. Ident 100%

Zotu12 –> Cannabis max/total score: 464, Query Cover: 100%, e-value: 1e-126, Per. Ident 100%

note: we need to exclude these Zotus since they are not bacterial species (except Wolbachia), keep this in mind for the fungal analysis

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.16S.ordi)[, colnames(tax_table(dataset.16S.ordi))] <- gsub(tax_table(dataset.16S.ordi
tax_table(dataset.16S.ordi)[, colnames(tax_table(dataset.16S.ordi))] <- gsub(tax_table(dataset.16S.ordi
tax_table(dataset.16S.ordi)[, colnames(tax_table(dataset.16S.ordi))] <- gsub(tax_table(dataset.16S.ordi
tax_table(dataset.16S.ordi)[, colnames(tax_table(dataset.16S.ordi))] <- gsub(tax_table(dataset.16S.ordi
tax_table(dataset.16S.ordi) <- tax_table(dataset.16S.ordi)[,2:8]
```

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

```
dataset.16S.ordi <- format_to_besthit(dataset.16S.ordi)
taxa_names(dataset.16S.ordi)[1:5]
```

```
## [1] "Zotu2:Pseudoxanthomonas_spadix" "Zotu48:Microbacterium_spc"
## [3] "Zotu21:Microbacterium_pygmaeum" "Zotu66:Sphingobacterium_spc"
## [5] "Zotu260:Gp3_spc"
```

```
colSums(otu_table(dataset.16S.ordi))
```

check the table without Bacteria and Archaea sp. for total reads per sample

```
##    B0-19a    B0-19b    B0-22a    B0-22b     B0-24     B0-27    B10-01    B10-06
##     17492     17718      1365      2348      1424       803       659       226
##   B10-09a   B10-09b   B10-18    B10-24   B10-25a   B10-25b   B10-26a   B15-01a
##       133       132       175       221        98       202       261       359
##   B15-01b    B15-06   B15-07a   B15-07b    B15-11    B15-20    B15-24   B15-25a
##       306      3589      3152      1184      3184      4624      1181       281
##   B15-25b   B16-01a    B16-04   B16-31a   B16-31b   B20-04a   B20-08a   B20-08b
##      1156     24582     24435     36064     20380     28703      7581     14344
##    B20-11    B23-21   B23-23a   B24-02a   B24-26a   B24-26b   B24-28a    B24-29
##     22250       312       472     21494     44940      9135     14354       174
##   B36-14a   B36-14b    B36-30   B36-31a    B36-32    B39-15    B39-16   B39-17a
##     12948     10866     11903     24530     24335     26401     26823     29121
##    B46-03     B7-07    B7-08a   medium1   medium2 negative1 negative2 standard1
##     15551     19370     12785      3672      6445      2584      3926     10263
## standard2
##      9062
```

there are now samples that lost a lot of reads due to this filtering step –> not good for later analysis, some samples might get excluded ahead to secure robustness

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

```
neg.controls<-subset_samples(dataset.16S.ordi, Treatment=="medium" | Treatment=="negative")
sample_names(neg.controls)
```

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

```
neg <- prune_taxa(taxa_sums(neg.controls) > 0, neg.controls)
```

visualisation of negative controls

```
filtaxa <- names (sort(rowSums(otu_table(neg)) ,decreasing=T))[1:10]
tax_table(dataset.16S.ordi)[filtaxa]
```

```
## Taxonomy Table:     [10 taxa by 8 taxonomic ranks]:
##                                         Domain     Phylum
## Zotu19:Streptomyces_xanthophaeus        "Bacteria" "Actinobacteria"
## Zotu20:Pseudomonas_brenneri             "Bacteria" "Proteobacteria"
## Zotu28:Halomonadaceae_spc               "Bacteria" "Proteobacteria"
## Zotu36:Pelomonas_aquatica               "Bacteria" "Proteobacteria"
## Zotu17:Staphylococcus_aureus            "Bacteria" "Firmicutes"
## Zotu33:Sediminibacterium_spc            "Bacteria" "Bacteroidetes"
## Zotu42:Halomonas_desiderata             "Bacteria" "Proteobacteria"
## Zotu39:Acinetobacter_indicus            "Bacteria" "Proteobacteria"
## Zotu41:Lactobacillus_spc                "Bacteria" "Firmicutes"
## Zotu40:Undibacterium_oligocarboniphilum "Bacteria" "Proteobacteria"
##                                         Class
## Zotu19:Streptomyces_xanthophaeus        "Actinobacteria"
## Zotu20:Pseudomonas_brenneri             "Gammaproteobacteria"
## Zotu28:Halomonadaceae_spc               "Gammaproteobacteria"
## Zotu36:Pelomonas_aquatica               "Betaproteobacteria"
## Zotu17:Staphylococcus_aureus            "Bacilli"
## Zotu33:Sediminibacterium_spc            "Sphingobacteriia"
## Zotu42:Halomonas_desiderata             "Gammaproteobacteria"
## Zotu39:Acinetobacter_indicus            "Gammaproteobacteria"
## Zotu41:Lactobacillus_spc                "Bacilli"
## Zotu40:Undibacterium_oligocarboniphilum "Betaproteobacteria"
##                                         Order
## Zotu19:Streptomyces_xanthophaeus        "Actinomycetales"
## Zotu20:Pseudomonas_brenneri             "Pseudomonadales"
## Zotu28:Halomonadaceae_spc               "Oceanospirillales"
## Zotu36:Pelomonas_aquatica               "Burkholderiales"
## Zotu17:Staphylococcus_aureus            "Bacillales"
## Zotu33:Sediminibacterium_spc            "Sphingobacteriales"
## Zotu42:Halomonas_desiderata             "Oceanospirillales"
## Zotu39:Acinetobacter_indicus            "Pseudomonadales"
```

```
## Zotu41:Lactobacillus_spc                "Lactobacillales"
## Zotu40:Undibacterium_oligocarboniphilum "Burkholderiales"
##                                         Family
## Zotu19:Streptomyces_xanthophaeus        "Streptomycetaceae"
## Zotu20:Pseudomonas_brenneri             "Pseudomonadaceae"
## Zotu28:Halomonadaceae_spc               "Halomonadaceae"
## Zotu36:Pelomonas_aquatica               "Comamonadaceae"
## Zotu17:Staphylococcus_aureus            "Staphylococcaceae"
## Zotu33:Sediminibacterium_spc            "Chitinophagaceae"
## Zotu42:Halomonas_desiderata             "Halomonadaceae"
## Zotu39:Acinetobacter_indicus            "Moraxellaceae"
## Zotu41:Lactobacillus_spc                "Lactobacillaceae"
## Zotu40:Undibacterium_oligocarboniphilum "Oxalobacteraceae"
##                                         Genus
## Zotu19:Streptomyces_xanthophaeus        "Streptomyces"
## Zotu20:Pseudomonas_brenneri             "Pseudomonas"
## Zotu28:Halomonadaceae_spc               "Halomonadaceae_spc"
## Zotu36:Pelomonas_aquatica               "Pelomonas"
## Zotu17:Staphylococcus_aureus            "Staphylococcus"
## Zotu33:Sediminibacterium_spc            "Sediminibacterium"
## Zotu42:Halomonas_desiderata             "Halomonas"
## Zotu39:Acinetobacter_indicus            "Acinetobacter"
## Zotu41:Lactobacillus_spc                "Lactobacillus"
## Zotu40:Undibacterium_oligocarboniphilum "Undibacterium"
##                                         Species
## Zotu19:Streptomyces_xanthophaeus        "Streptomyces_xanthophaeus"
## Zotu20:Pseudomonas_brenneri             "Pseudomonas_brenneri"
## Zotu28:Halomonadaceae_spc               "Halomonadaceae_spc"
## Zotu36:Pelomonas_aquatica               "Pelomonas_aquatica"
## Zotu17:Staphylococcus_aureus            "Staphylococcus_aureus"
## Zotu33:Sediminibacterium_spc            "Sediminibacterium_spc"
## Zotu42:Halomonas_desiderata             "Halomonas_desiderata"
## Zotu39:Acinetobacter_indicus            "Acinetobacter_indicus"
## Zotu41:Lactobacillus_spc                "Lactobacillus_spc"
## Zotu40:Undibacterium_oligocarboniphilum "Undibacterium_oligocarboniphilum"
##                                         best_hit
## Zotu19:Streptomyces_xanthophaeus        "Zotu19:Streptomyces_xanthophaeus"
## Zotu20:Pseudomonas_brenneri             "Zotu20:Pseudomonas_brenneri"
## Zotu28:Halomonadaceae_spc               "Zotu28:Halomonadaceae_spc"
## Zotu36:Pelomonas_aquatica               "Zotu36:Pelomonas_aquatica"
## Zotu17:Staphylococcus_aureus            "Zotu17:Staphylococcus_aureus"
## Zotu33:Sediminibacterium_spc            "Zotu33:Sediminibacterium_spc"
## Zotu42:Halomonas_desiderata             "Zotu42:Halomonas_desiderata"
## Zotu39:Acinetobacter_indicus            "Zotu39:Acinetobacter_indicus"
## Zotu41:Lactobacillus_spc                "Zotu41:Lactobacillus_spc"
## Zotu40:Undibacterium_oligocarboniphilum "Zotu40:Undibacterium_oligocarboniphilum"
```

```
round(otu_table(dataset.16S.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
## Zotu19:Streptomyces_xanthophaeus            29      0      7      0     2     0
## Zotu20:Pseudomonas_brenneri                  8      0     46      0     0     1
```

```
## Zotu28:Halomonadaceae_spc                  10      1     23      0     0     0
## Zotu36:Pelomonas_aquatica                  10      0     18      2     4     1
## Zotu17:Staphylococcus_aureus                7      0      7      4     2     0
## Zotu33:Sediminibacterium_spc               12      0     10      0     0     0
## Zotu42:Halomonas_desiderata                 6      0     12      0     0     0
## Zotu39:Acinetobacter_indicus                2      0     15      0     2     0
## Zotu41:Lactobacillus_spc                    0      0     16      0     1     0
## Zotu40:Undibacterium_oligocarboniphilum     3      0      7      0     0     0
##                                         B10-01 B10-06 B10-09a B10-09b B10-18
## Zotu19:Streptomyces_xanthophaeus            71      0       0       1      4
## Zotu20:Pseudomonas_brenneri                 68      0       0       4      2
## Zotu28:Halomonadaceae_spc                   25      0       0       0      1
## Zotu36:Pelomonas_aquatica                   12      2       0       0      2
## Zotu17:Staphylococcus_aureus                14      1       0       0      1
## Zotu33:Sediminibacterium_spc                16      0       0       0      0
## Zotu42:Halomonas_desiderata                  9      0       0       0      3
## Zotu39:Acinetobacter_indicus                 4      0       0       1      0
## Zotu41:Lactobacillus_spc                     8      0       0       0      0
## Zotu40:Undibacterium_oligocarboniphilum     14      1       0       0      0
##                                         B10-24 B10-25a B10-25b B10-26a B15-01a
## Zotu19:Streptomyces_xanthophaeus             0       3       1       1       1
## Zotu20:Pseudomonas_brenneri                  0       2       1       0       5
## Zotu28:Halomonadaceae_spc                    0       0       0       0       1
## Zotu36:Pelomonas_aquatica                    3       0       1       2      10
## Zotu17:Staphylococcus_aureus                 3      10       6       3      12
## Zotu33:Sediminibacterium_spc                10       0       0       1       0
## Zotu42:Halomonas_desiderata                  0       0       2       0       0
## Zotu39:Acinetobacter_indicus                 0       0       0       0       0
## Zotu41:Lactobacillus_spc                     0       1       0       0       1
## Zotu40:Undibacterium_oligocarboniphilum      5       0       0       0       0
##                                         B15-01b B15-06 B15-07a B15-07b B15-11
## Zotu19:Streptomyces_xanthophaeus              0      0       0       0      1
## Zotu20:Pseudomonas_brenneri                   9      0       0       0      1
## Zotu28:Halomonadaceae_spc                     0      0       0       2      0
## Zotu36:Pelomonas_aquatica                     4      2       3       0      1
## Zotu17:Staphylococcus_aureus                  0      0       2       1      4
## Zotu33:Sediminibacterium_spc                 13     13       2       0      3
## Zotu42:Halomonas_desiderata                   0      0       0       0      0
## Zotu39:Acinetobacter_indicus                  1      0       0       0      0
## Zotu41:Lactobacillus_spc                      0      0       0       0      0
## Zotu40:Undibacterium_oligocarboniphilum      13      8       5       3      6
##                                         B15-20 B15-24 B15-25a B15-25b B16-01a
## Zotu19:Streptomyces_xanthophaeus            33      2       7       0       3
## Zotu20:Pseudomonas_brenneri                 30      2       3       0       1
## Zotu28:Halomonadaceae_spc                   10      0       1       0       0
## Zotu36:Pelomonas_aquatica                    8      0       6       0       3
## Zotu17:Staphylococcus_aureus                 8      0       2       0       0
## Zotu33:Sediminibacterium_spc                17      0      16       1       6
## Zotu42:Halomonas_desiderata                  5      0       0       0       0
## Zotu39:Acinetobacter_indicus                 5      0       0       0       0
## Zotu41:Lactobacillus_spc                     0      1       2       1       0
## Zotu40:Undibacterium_oligocarboniphilum      7      0      19       0       3
##                                         B16-04 B16-31a B16-31b B20-04a B20-08a
## Zotu19:Streptomyces_xanthophaeus             0      13       9       2       0
```

```
## Zotu20:Pseudomonas_brenneri                  0       0       1       1       0
## Zotu28:Halomonadaceae_spc                    0       4       1       0       0
## Zotu36:Pelomonas_aquatica                    0       1       1       3       0
## Zotu17:Staphylococcus_aureus                 0       0       3       0       0
## Zotu33:Sediminibacterium_spc                 0       0       7       1       0
## Zotu42:Halomonas_desiderata                  0       0       0       0       0
## Zotu39:Acinetobacter_indicus                 0       0       2       0       0
## Zotu41:Lactobacillus_spc                     0       1       1       0       0
## Zotu40:Undibacterium_oligocarboniphilum      0       4       1       0       1
##                                         B20-08b B20-11 B23-21 B23-23a B24-02a
## Zotu19:Streptomyces_xanthophaeus              0      0      1       0       0
## Zotu20:Pseudomonas_brenneri                   0      0      3       5       0
## Zotu28:Halomonadaceae_spc                     0      0      0       0       0
## Zotu36:Pelomonas_aquatica                     0      0      3      20       0
## Zotu17:Staphylococcus_aureus                  0      0      1      11       0
## Zotu33:Sediminibacterium_spc                  0      0      0       0       0
## Zotu42:Halomonas_desiderata                   0      0      0       0       0
## Zotu39:Acinetobacter_indicus                  0      0      0       0       0
## Zotu41:Lactobacillus_spc                      0      0      0       0       0
## Zotu40:Undibacterium_oligocarboniphilum       0      0      0      26       0
##                                         B24-26a B24-26b B24-28a B24-29 B36-14a
## Zotu19:Streptomyces_xanthophaeus              0       1       0      0       0
## Zotu20:Pseudomonas_brenneri                   0       1       0      0       0
## Zotu28:Halomonadaceae_spc                     0       0       0      1       0
## Zotu36:Pelomonas_aquatica                     0       1       0      1       0
## Zotu17:Staphylococcus_aureus                  0       1       0      1       0
## Zotu33:Sediminibacterium_spc                  0       0       2      0       1
## Zotu42:Halomonas_desiderata                   0       0       0      0       0
## Zotu39:Acinetobacter_indicus                  0       0       0      0       0
## Zotu41:Lactobacillus_spc                      0       0       0      0       0
## Zotu40:Undibacterium_oligocarboniphilum       0       0       2      0       2
##                                         B36-14b B36-30 B36-31a B36-32 B39-15
## Zotu19:Streptomyces_xanthophaeus             15      0       1      0      0
## Zotu20:Pseudomonas_brenneri                   8      1       0      0      1
## Zotu28:Halomonadaceae_spc                     4      0       0      0      0
## Zotu36:Pelomonas_aquatica                     2      2       0      0      4
## Zotu17:Staphylococcus_aureus                  1      0       0      0      0
## Zotu33:Sediminibacterium_spc                  8      8       1      0      1
## Zotu42:Halomonas_desiderata                   1      0       1      0      0
## Zotu39:Acinetobacter_indicus                  2      0       0      0      0
## Zotu41:Lactobacillus_spc                      3      0       0      0      0
## Zotu40:Undibacterium_oligocarboniphilum       1      8       3      0      0
##                                         B39-16 B39-17a B46-03 B7-07 B7-08a
## Zotu19:Streptomyces_xanthophaeus             19       0      0     9      0
## Zotu20:Pseudomonas_brenneri                   6       2      0     3      0
## Zotu28:Halomonadaceae_spc                     5       0      0     5      0
## Zotu36:Pelomonas_aquatica                     9       0      0     4      0
## Zotu17:Staphylococcus_aureus                  3       0      8     4      0
## Zotu33:Sediminibacterium_spc                 19       0      0     5      0
## Zotu42:Halomonas_desiderata                   1       0      0     0      0
## Zotu39:Acinetobacter_indicus                  6       0      0     0      0
## Zotu41:Lactobacillus_spc                      6       1      2     0      0
## Zotu40:Undibacterium_oligocarboniphilum       3       0      0     1      1
##                                         medium1 medium2 negative1 negative2
```

```
## Zotu19:Streptomyces_xanthophaeus            444     859       352       532
## Zotu20:Pseudomonas_brenneri                 426     883       348       524
## Zotu28:Halomonadaceae_spc                   185     410       194       219
## Zotu36:Pelomonas_aquatica                   113     255       120       130
## Zotu17:Staphylococcus_aureus                129     193        81       120
## Zotu33:Sediminibacterium_spc                 73     234        67        83
## Zotu42:Halomonas_desiderata                  60     147        78       116
## Zotu39:Acinetobacter_indicus                 86     107        68       136
## Zotu41:Lactobacillus_spc                     50     146        60        94
## Zotu40:Undibacterium_oligocarboniphilum      57     143        35        76
##                                        standard1 standard2
## Zotu19:Streptomyces_xanthophaeus               0         0
## Zotu20:Pseudomonas_brenneri                    0         0
## Zotu28:Halomonadaceae_spc                      1         0
## Zotu36:Pelomonas_aquatica                      1         0
## Zotu17:Staphylococcus_aureus                1104       962
## Zotu33:Sediminibacterium_spc                   0         0
## Zotu42:Halomonas_desiderata                    0         0
## Zotu39:Acinetobacter_indicus                   0         0
## Zotu41:Lactobacillus_spc                       0         0
## Zotu40:Undibacterium_oligocarboniphilum        0         0
```

only very low read numbers of most abundant ZOTUS in our samples we let ZOTUS from neg. controls in the samples and choose they way of low abundance filtering/decontam!

check for ten most abundant ZOTUs in pos.controls

```
pos.controls<-subset_samples(dataset.16S.ordi, Treatment=="standard")
sample_names(pos.controls)
```

```
## [1] "standard1" "standard2"
```

```
filtaxa <- names (sort(rowSums(otu_table(pos.controls)) ,decreasing=T))[1:10]
tax_table(dataset.16S.ordi)[filtaxa]
```

```
## Taxonomy Table:     [10 taxa by 8 taxonomic ranks]:
##                               Domain     Phylum
## Zotu15:Salmonella_enterica    "Bacteria" "Proteobacteria"
## Zotu14:Escherichia_coli       "Bacteria" "Proteobacteria"
## Zotu18:Enterococcus_faecalis  "Bacteria" "Firmicutes"
## Zotu17:Staphylococcus_aureus  "Bacteria" "Firmicutes"
## Zotu25:Bacillus_subtilis      "Bacteria" "Firmicutes"
## Zotu27:Lactobacillus_fermentum "Bacteria" "Firmicutes"
## Zotu23:Pseudomonas_aeruginosa "Bacteria" "Proteobacteria"
## Zotu29:Listeria_monocytogenes "Bacteria" "Firmicutes"
## Zotu52:Enterobacteriaceae_spc "Bacteria" "Proteobacteria"
## Zotu147:Enterobacteriaceae_spc "Bacteria" "Proteobacteria"
##                               Class                Order
## Zotu15:Salmonella_enterica    "Gammaproteobacteria" "Enterobacteriales"
## Zotu14:Escherichia_coli       "Gammaproteobacteria" "Enterobacteriales"
## Zotu18:Enterococcus_faecalis  "Bacilli"             "Lactobacillales"
## Zotu17:Staphylococcus_aureus  "Bacilli"             "Bacillales"
## Zotu25:Bacillus_subtilis      "Bacilli"             "Bacillales"
```

```
## Zotu27:Lactobacillus_fermentum "Bacilli"             "Lactobacillales"
## Zotu23:Pseudomonas_aeruginosa  "Gammaproteobacteria" "Pseudomonadales"
## Zotu29:Listeria_monocytogenes  "Bacilli"             "Bacillales"
## Zotu52:Enterobacteriaceae_spc  "Gammaproteobacteria" "Enterobacteriales"
## Zotu147:Enterobacteriaceae_spc "Gammaproteobacteria" "Enterobacteriales"
##                                Family               Genus
## Zotu15:Salmonella_enterica     "Enterobacteriaceae" "Salmonella"
## Zotu14:Escherichia_coli        "Enterobacteriaceae" "Escherichia"
## Zotu18:Enterococcus_faecalis   "Enterococcaceae"    "Enterococcus"
## Zotu17:Staphylococcus_aureus   "Staphylococcaceae"  "Staphylococcus"
## Zotu25:Bacillus_subtilis       "Bacillaceae_1"      "Bacillus"
## Zotu27:Lactobacillus_fermentum "Lactobacillaceae"   "Lactobacillus"
## Zotu23:Pseudomonas_aeruginosa  "Pseudomonadaceae"   "Pseudomonas"
## Zotu29:Listeria_monocytogenes  "Listeriaceae"       "Listeria"
## Zotu52:Enterobacteriaceae_spc  "Enterobacteriaceae" "Enterobacteriaceae_spc"
## Zotu147:Enterobacteriaceae_spc "Enterobacteriaceae" "Enterobacteriaceae_spc"
##                                Species
## Zotu15:Salmonella_enterica     "Salmonella_enterica"
## Zotu14:Escherichia_coli        "Escherichia_coli"
## Zotu18:Enterococcus_faecalis   "Enterococcus_faecalis"
## Zotu17:Staphylococcus_aureus   "Staphylococcus_aureus"
## Zotu25:Bacillus_subtilis       "Bacillus_subtilis"
## Zotu27:Lactobacillus_fermentum "Lactobacillus_fermentum"
## Zotu23:Pseudomonas_aeruginosa  "Pseudomonas_aeruginosa"
## Zotu29:Listeria_monocytogenes  "Listeria_monocytogenes"
## Zotu52:Enterobacteriaceae_spc  "Enterobacteriaceae_spc"
## Zotu147:Enterobacteriaceae_spc "Enterobacteriaceae_spc"
##                                best_hit
## Zotu15:Salmonella_enterica     "Zotu15:Salmonella_enterica"
## Zotu14:Escherichia_coli        "Zotu14:Escherichia_coli"
## Zotu18:Enterococcus_faecalis   "Zotu18:Enterococcus_faecalis"
## Zotu17:Staphylococcus_aureus   "Zotu17:Staphylococcus_aureus"
## Zotu25:Bacillus_subtilis       "Zotu25:Bacillus_subtilis"
## Zotu27:Lactobacillus_fermentum "Zotu27:Lactobacillus_fermentum"
## Zotu23:Pseudomonas_aeruginosa  "Zotu23:Pseudomonas_aeruginosa"
## Zotu29:Listeria_monocytogenes  "Zotu29:Listeria_monocytogenes"
## Zotu52:Enterobacteriaceae_spc  "Zotu52:Enterobacteriaceae_spc"
## Zotu147:Enterobacteriaceae_spc "Zotu147:Enterobacteriaceae_spc"
```

```
round(otu_table(dataset.16S.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
## Zotu15:Salmonella_enterica          3      0      0      0    10      0      1
## Zotu14:Escherichia_coli             2      0      1      0     0      3      0
## Zotu18:Enterococcus_faecalis        0      0      0      0     0      0      0
## Zotu17:Staphylococcus_aureus        7      0      7      4     2      0     14
## Zotu25:Bacillus_subtilis            0      0      0      0     0      0      0
## Zotu27:Lactobacillus_fermentum      1      0      0      0     0      0      0
## Zotu23:Pseudomonas_aeruginosa       1      0      2      0     0      0      3
## Zotu29:Listeria_monocytogenes       0      0      0      0     0      0      0
## Zotu52:Enterobacteriaceae_spc       0      0      0      0     0      0      0
## Zotu147:Enterobacteriaceae_spc      0      0      0      0     0      0      0
```

```
##                                B10-06 B10-09a B10-09b B10-18 B10-24 B10-25a
## Zotu15:Salmonella_enterica          1       4       1      0      2       0
## Zotu14:Escherichia_coli             0       1       0      0      0       1
## Zotu18:Enterococcus_faecalis        0       0       0      0      0       0
## Zotu17:Staphylococcus_aureus        1       0       0      1      3      10
## Zotu25:Bacillus_subtilis            0       0       0      0      2       0
## Zotu27:Lactobacillus_fermentum      0       0       0      0      0       0
## Zotu23:Pseudomonas_aeruginosa       0       0       3      0      0       1
## Zotu29:Listeria_monocytogenes       0       0       1      0      0       0
## Zotu52:Enterobacteriaceae_spc       0       0       0      0      0       0
## Zotu147:Enterobacteriaceae_spc      0       0       0      0      0       0
##                                B10-25b B10-26a B15-01a B15-01b B15-06 B15-07a
## Zotu15:Salmonella_enterica           0       1       0       1      1       1
## Zotu14:Escherichia_coli              0       0       0       0      0       0
## Zotu18:Enterococcus_faecalis         0       0       0       0      0       0
## Zotu17:Staphylococcus_aureus         6       3      12       0      0       2
## Zotu25:Bacillus_subtilis             0       3       0       0      0       0
## Zotu27:Lactobacillus_fermentum       0       0       0       0      0       0
## Zotu23:Pseudomonas_aeruginosa        0       0       0       0      0       0
## Zotu29:Listeria_monocytogenes        0       0       0       0      0       0
## Zotu52:Enterobacteriaceae_spc        0       0       0       0      0       0
## Zotu147:Enterobacteriaceae_spc       0       0       0       0      0       0
##                                B15-07b B15-11 B15-20 B15-24 B15-25a B15-25b
## Zotu15:Salmonella_enterica           0      1      1      0       1       1
## Zotu14:Escherichia_coli              0      1      0      0       0       0
## Zotu18:Enterococcus_faecalis         0      0      0      0       0       0
## Zotu17:Staphylococcus_aureus         1      4      8      0       2       0
## Zotu25:Bacillus_subtilis             0      0      0      0       0       0
## Zotu27:Lactobacillus_fermentum       0      0      0      0       1       0
## Zotu23:Pseudomonas_aeruginosa        0      0      3      0       0       0
## Zotu29:Listeria_monocytogenes        0      0      0      0       0       0
## Zotu52:Enterobacteriaceae_spc        0      0      0      0       0       0
## Zotu147:Enterobacteriaceae_spc       0      0      0      0       0       0
##                                B16-01a B16-04 B16-31a B16-31b B20-04a B20-08a
## Zotu15:Salmonella_enterica           1      0       0       0       0       0
## Zotu14:Escherichia_coli              0      0       0       0       0       0
## Zotu18:Enterococcus_faecalis         0      0       0       0       0       0
## Zotu17:Staphylococcus_aureus         0      0       0       3       0       0
## Zotu25:Bacillus_subtilis             1      0       0       0       0       0
## Zotu27:Lactobacillus_fermentum       0      0       0       0       0       0
## Zotu23:Pseudomonas_aeruginosa        1      0       0       0       0       0
## Zotu29:Listeria_monocytogenes        0      0       0       0       0       0
## Zotu52:Enterobacteriaceae_spc        0      0       0       0       0       0
## Zotu147:Enterobacteriaceae_spc       0      0       0       0       0       0
##                                B20-08b B20-11 B23-21 B23-23a B24-02a B24-26a
## Zotu15:Salmonella_enterica           0      0      0       0       0       0
## Zotu14:Escherichia_coli              0      0      0       0       0       0
## Zotu18:Enterococcus_faecalis         0      0      0       0       0       0
## Zotu17:Staphylococcus_aureus         0      0      1      11       0       0
## Zotu25:Bacillus_subtilis             0      0      0       2       0       0
## Zotu27:Lactobacillus_fermentum       0      0      0       0       0       0
## Zotu23:Pseudomonas_aeruginosa        0      0      0       0       0       0
## Zotu29:Listeria_monocytogenes        0      0      0       0       0       0
## Zotu52:Enterobacteriaceae_spc        0      0      0       0       0       0
```

```
## Zotu147:Enterobacteriaceae_spc       0      0      0       0       0       0
##                                 B24-26b B24-28a B24-29 B36-14a B36-14b B36-30
## Zotu15:Salmonella_enterica            0       0      0       0       0      0
## Zotu14:Escherichia_coli               0       0      0       0       0      0
## Zotu18:Enterococcus_faecalis          0       0      0       0       0      0
## Zotu17:Staphylococcus_aureus          1       0      1       0       1      0
## Zotu25:Bacillus_subtilis              0       0      0       0       0      0
## Zotu27:Lactobacillus_fermentum        0       0      0       0       1      0
## Zotu23:Pseudomonas_aeruginosa         0       0      0       0       0      0
## Zotu29:Listeria_monocytogenes         0       0      0       0       0      0
## Zotu52:Enterobacteriaceae_spc         0       0      0       0       0      0
## Zotu147:Enterobacteriaceae_spc        0       0      0       0       0      0
##                                 B36-31a B36-32 B39-15 B39-16 B39-17a B46-03
## Zotu15:Salmonella_enterica            0      0      0      0       1      0
## Zotu14:Escherichia_coli               0      0      0      0       0      0
## Zotu18:Enterococcus_faecalis          0      0      0      0       0      0
## Zotu17:Staphylococcus_aureus          0      0      0      3       0      8
## Zotu25:Bacillus_subtilis              0      0      0      0       0      0
## Zotu27:Lactobacillus_fermentum        0      0      0      0       0      0
## Zotu23:Pseudomonas_aeruginosa         0      0      0      0       0      0
## Zotu29:Listeria_monocytogenes         0      0      0      0       0      0
## Zotu52:Enterobacteriaceae_spc         0      0      0      0       0      0
## Zotu147:Enterobacteriaceae_spc        0      0      0      0       0      0
##                                 B7-07 B7-08a medium1 medium2 negative1 negative2
## Zotu15:Salmonella_enterica          0      0       2       2         0         0
## Zotu14:Escherichia_coli             0      0       0       0         5         0
## Zotu18:Enterococcus_faecalis        0      0       0       0         0         1
## Zotu17:Staphylococcus_aureus        4      0     129     193        81       120
## Zotu25:Bacillus_subtilis            0      0       0       0         0         2
## Zotu27:Lactobacillus_fermentum      0      0       0       0         0         0
## Zotu23:Pseudomonas_aeruginosa       0      0      18      60        24        19
## Zotu29:Listeria_monocytogenes       0      0       0       0         2         0
## Zotu52:Enterobacteriaceae_spc       0      0       0       0         0         0
## Zotu147:Enterobacteriaceae_spc      0      0       0       0         0         0
##                                 standard1 standard2
## Zotu15:Salmonella_enterica           2527      2083
## Zotu14:Escherichia_coli              2414      2130
## Zotu18:Enterococcus_faecalis         1138      1035
## Zotu17:Staphylococcus_aureus         1104       962
## Zotu25:Bacillus_subtilis              664       598
## Zotu27:Lactobacillus_fermentum        615       550
## Zotu23:Pseudomonas_aeruginosa         599       507
## Zotu29:Listeria_monocytogenes         443       399
## Zotu52:Enterobacteriaceae_spc         183       165
## Zotu147:Enterobacteriaceae_spc        103       109
```

==> except Zotu 52 + 147 all Species were supposed to be present in Zymo Standard Community, but those two are not fully identified

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

define color bar for the next plots

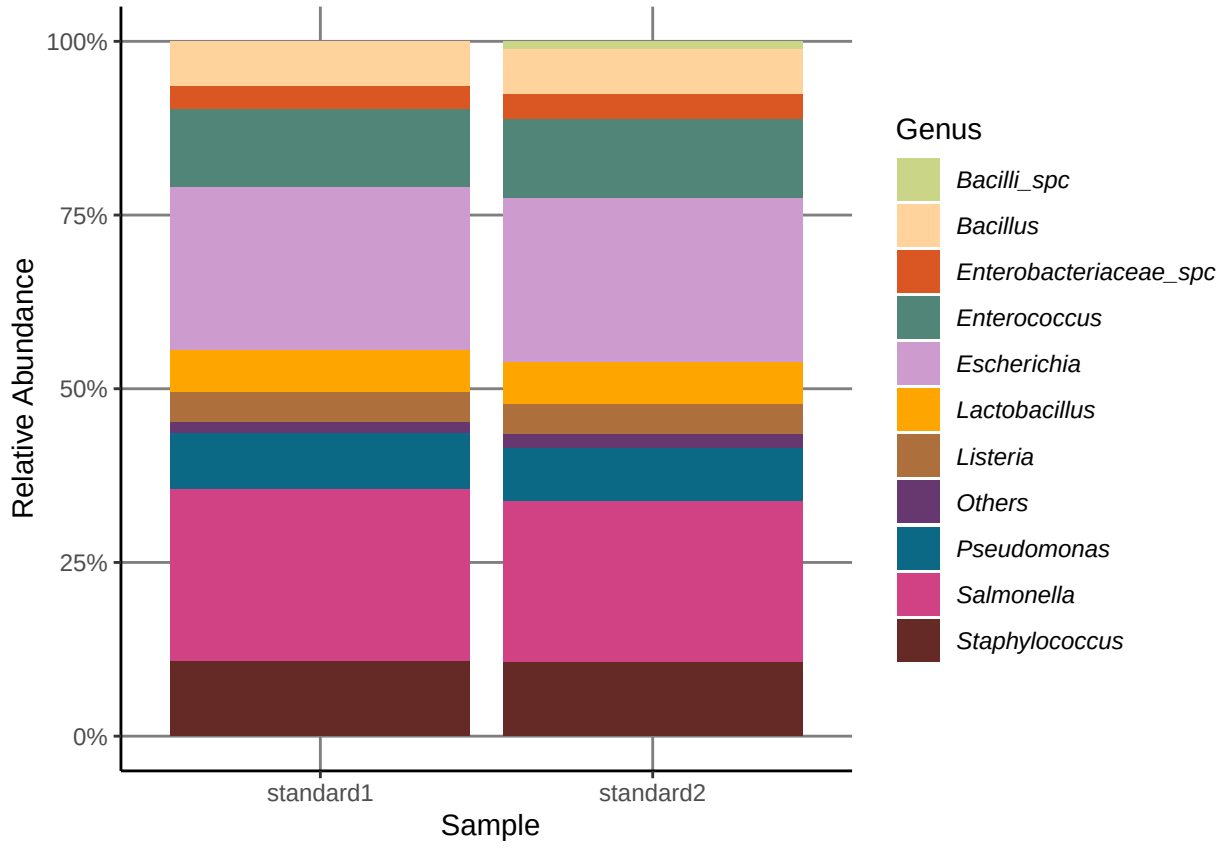
plot controls

prepare data for plotting

```
negs <- subset_samples(dataset.16S.ordi, Treatment == "negative" | Treatment == "medium")

pos <- subset_samples(dataset.16S.ordi, Treatment == "standard")

Bacteria_Genus.pos <- pos %>%
  tax_glom(taxrank = "Genus") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Genus)

Bacteria_Genus.pos$Genus<-as.character(Bacteria_Genus.pos$Genus)
Bacteria_Genus.pos$Genus[Bacteria_Genus.pos$Abundance<0.01]<-"Others"
Bacteria_Genus.pos$Class<-as.character(Bacteria_Genus.pos$Class)
Bacteria_Genus.pos$Class[Bacteria_Genus.pos$Abundance<0.01]<-"Others"

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

Bacteria_Genus.neg$Genus<-as.character(Bacteria_Genus.neg$Genus)
Bacteria_Genus.neg$Genus[Bacteria_Genus.neg$Abundance<0.02]<-"Others"
Bacteria_Genus.neg$Class<-as.character(Bacteria_Genus.neg$Class)
Bacteria_Genus.neg$Class[Bacteria_Genus.neg$Abundance<0.02]<-"Others"
```

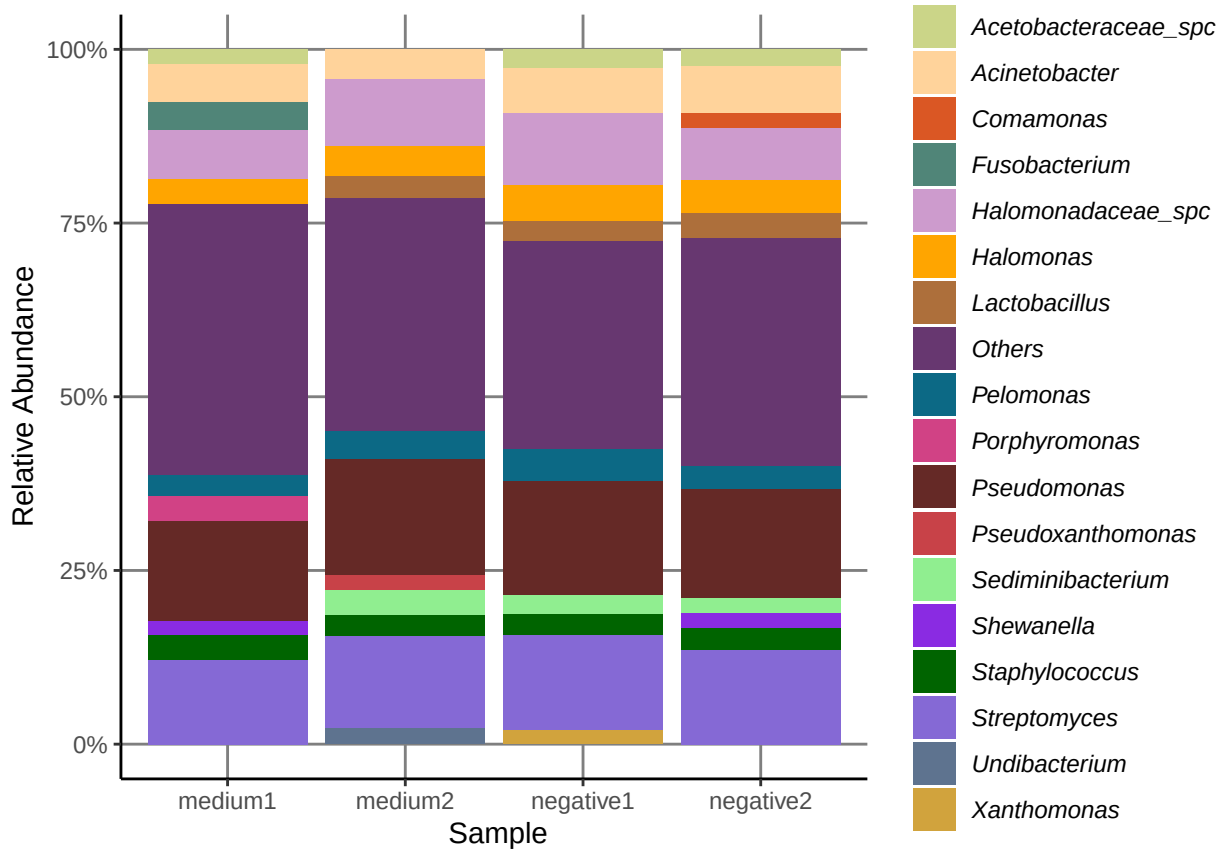
plot standard controls prae decontam

```
Bacteria_Genus.pos_plot <-ggplot(Bacteria_Genus.pos, aes(x = Sample, y = Abundance, fill = Genus)) +
  scale_fill_manual(values = Family_colors, name = "Genus")

g1<-Bacteria_Genus.pos_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
```



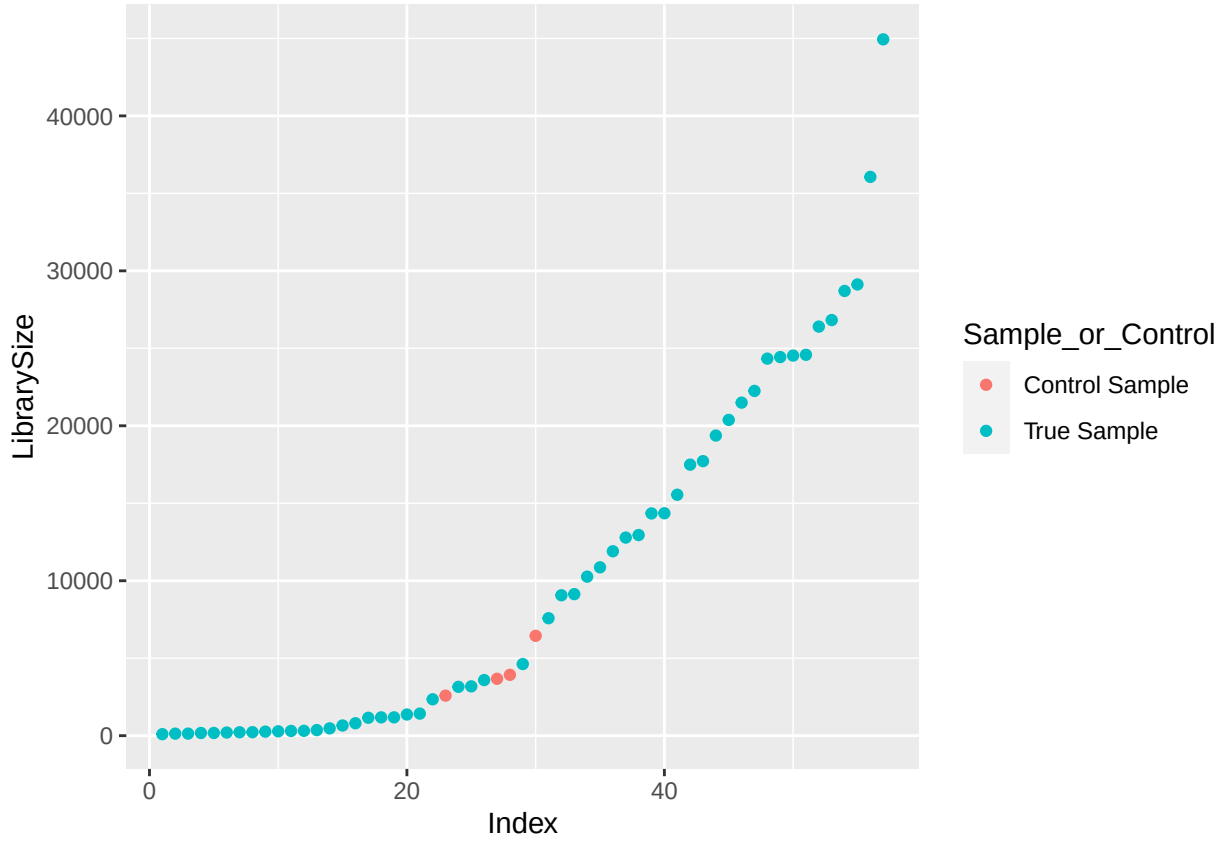
negative controls prae decontam

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

g2<-Bacteria_Genus.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"))

g2
```



run decontam to filter contaminating taxa

```
library(decontam)
df <- as.data.frame(sample_data(dataset.16S.ordi)) # Put sample_data into a ggplot-friendly data.frame
df$LibrarySize <- sample_sums(dataset.16S.ordi)
df <- df[order(df$LibrarySize),]
df$Index <- seq(nrow(df))
ggplot(data=df, aes(x=Index, y=LibrarySize, color=Sample_or_Control)) + geom_point()
```



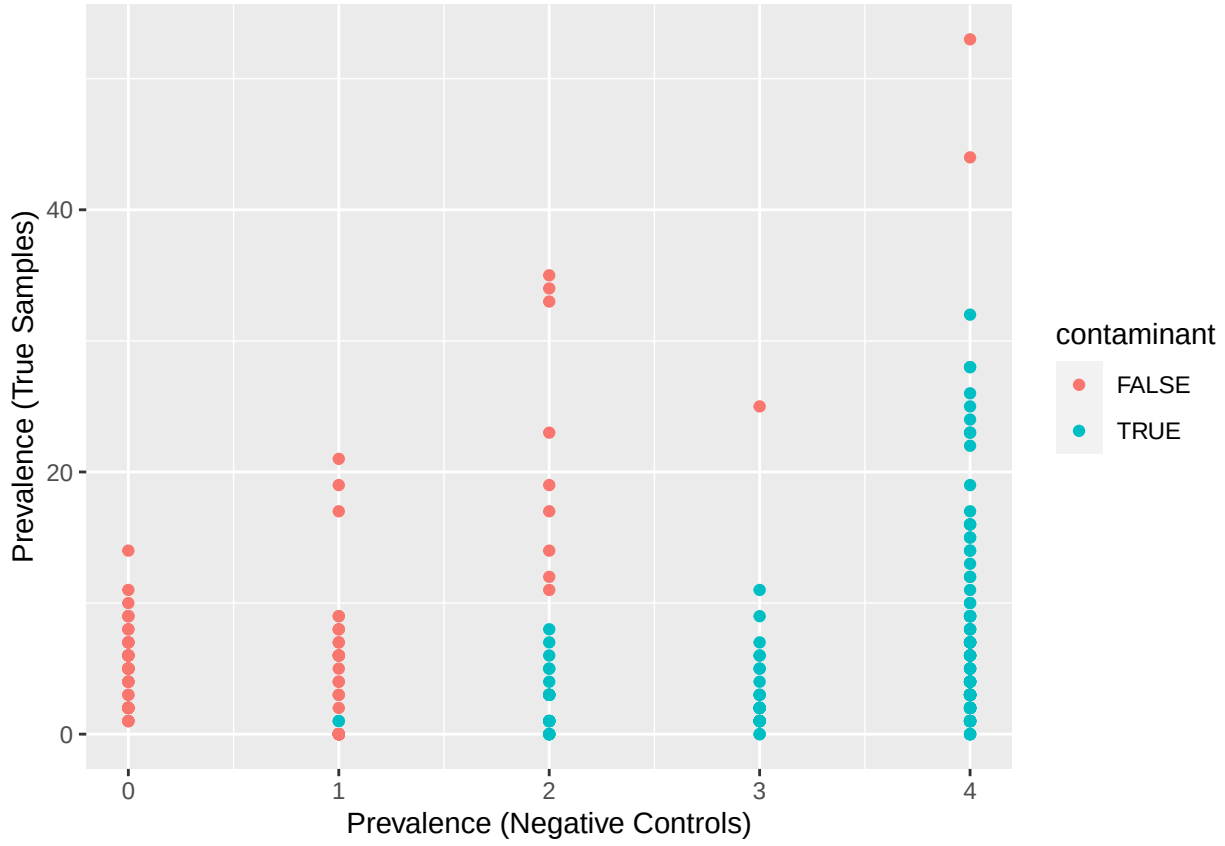

```
sample_data(dataset.16S.ordi)$is.neg <- sample_data(dataset.16S.ordi)$Sample_or_Control == "Control Samp
contamdf.prev <- isContaminant(dataset.16S.ordi, method="prevalence", neg="is.neg")
table(contamdf.prev$contaminant)
```

```
##
## FALSE  TRUE
##   114   178
```

```
ps.pa <- transform_sample_counts(dataset.16S.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$
ggplot(data=df.pa, aes(x=pa.neg, y=pa.pos, color=contaminant)) + geom_point() + xlab("Prevalence (Negati
```




```
ps.noncontam_dataset.16S.ordi <- prune_taxa(!contamdf.prev$contaminant, dataset.16S.ordi)
ps.noncontam_dataset.16S.ordi
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:         [ 114 taxa and 57 samples ]
## sample_data() Sample Data:       [ 57 samples by 17 sample variables ]
## tax_table()   Taxonomy Table:    [ 114 taxa by 8 taxonomic ranks ]
```

```
smin <- min(sample_sums(ps.noncontam_dataset.16S.ordi))
smean <- mean(sample_sums(ps.noncontam_dataset.16S.ordi))
smax <- max(sample_sums(ps.noncontam_dataset.16S.ordi))
cat("The minimum sample read count is:",smin)
```

```
## The minimum sample read count is: 46
```

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

```
## The average sample read count is: 9828.246
```

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

```
## The maximum sample read count is: 44924
```

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

create a list of all excluded contaminants

check and plot controls again

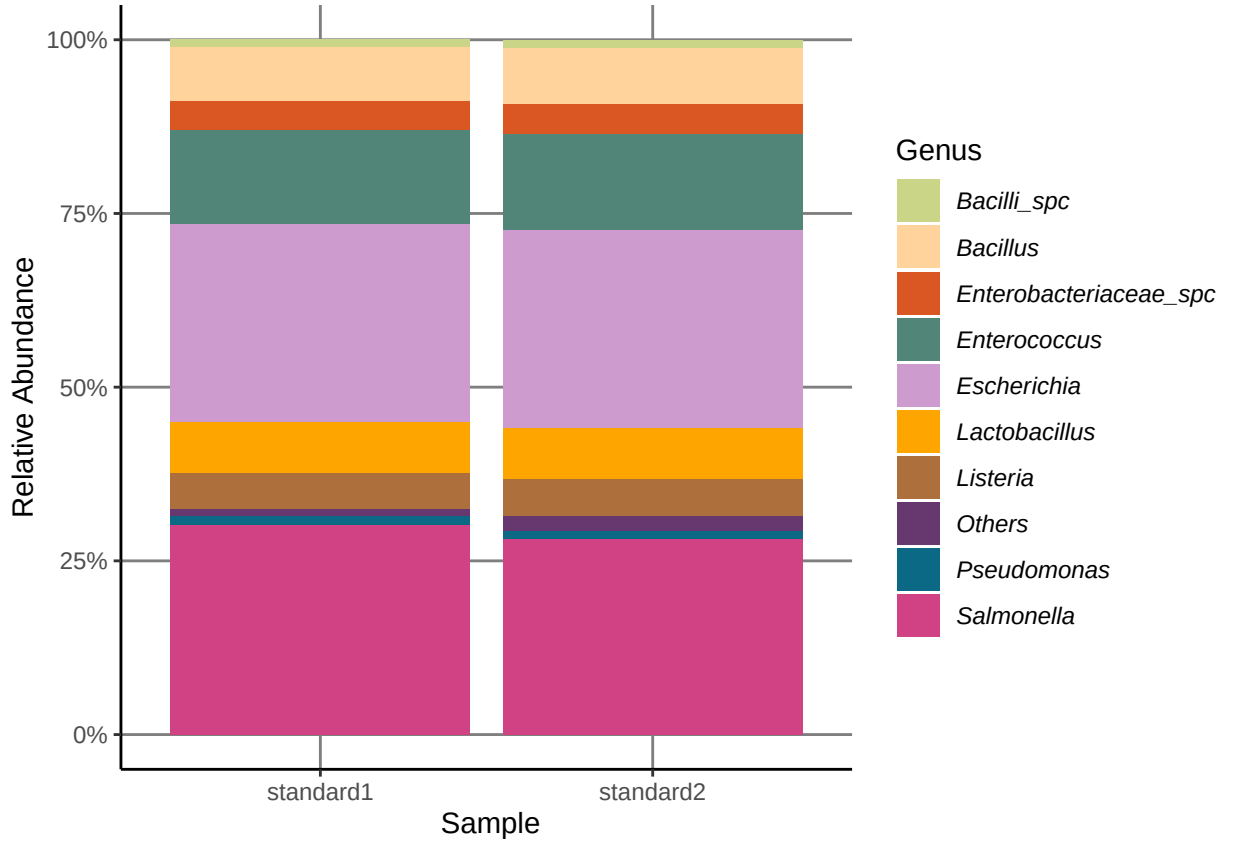
prepare data for plotting

```
negs2 <- subset_samples(ps.noncontam_dataset.16S.ordi, Treatment == "negative" | Treatment == "medium")
negs2 <- prune_taxa(taxa_sums(negs2) > 0, negs2)

pos2 <- subset_samples(ps.noncontam_dataset.16S.ordi, Treatment == "standard")
pos2 <- prune_taxa(taxa_sums(pos2) > 0, pos2)

Bacteria_Genus.pos2 <- pos2 %>%
  tax_glom(taxrank = "Genus") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Genus)

Bacteria_Genus.pos2$Genus<-as.character(Bacteria_Genus.pos2$Genus)
Bacteria_Genus.pos2$Genus[Bacteria_Genus.pos2$Abundance<0.01]<-"Others"
Bacteria_Genus.pos2$Class<-as.character(Bacteria_Genus.pos2$Class)
Bacteria_Genus.pos2$Class[Bacteria_Genus.pos2$Abundance<0.01]<-"Others"

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

Bacteria_Genus.neg2$Genus<-as.character(Bacteria_Genus.neg2$Genus)
Bacteria_Genus.neg2$Genus[Bacteria_Genus.neg2$Abundance<0.03]<-"Others"
Bacteria_Genus.neg2$Class<-as.character(Bacteria_Genus.neg2$Class)
Bacteria_Genus.neg2$Class[Bacteria_Genus.neg2$Abundance<0.03]<-"Others"
```

positive controls post decontam

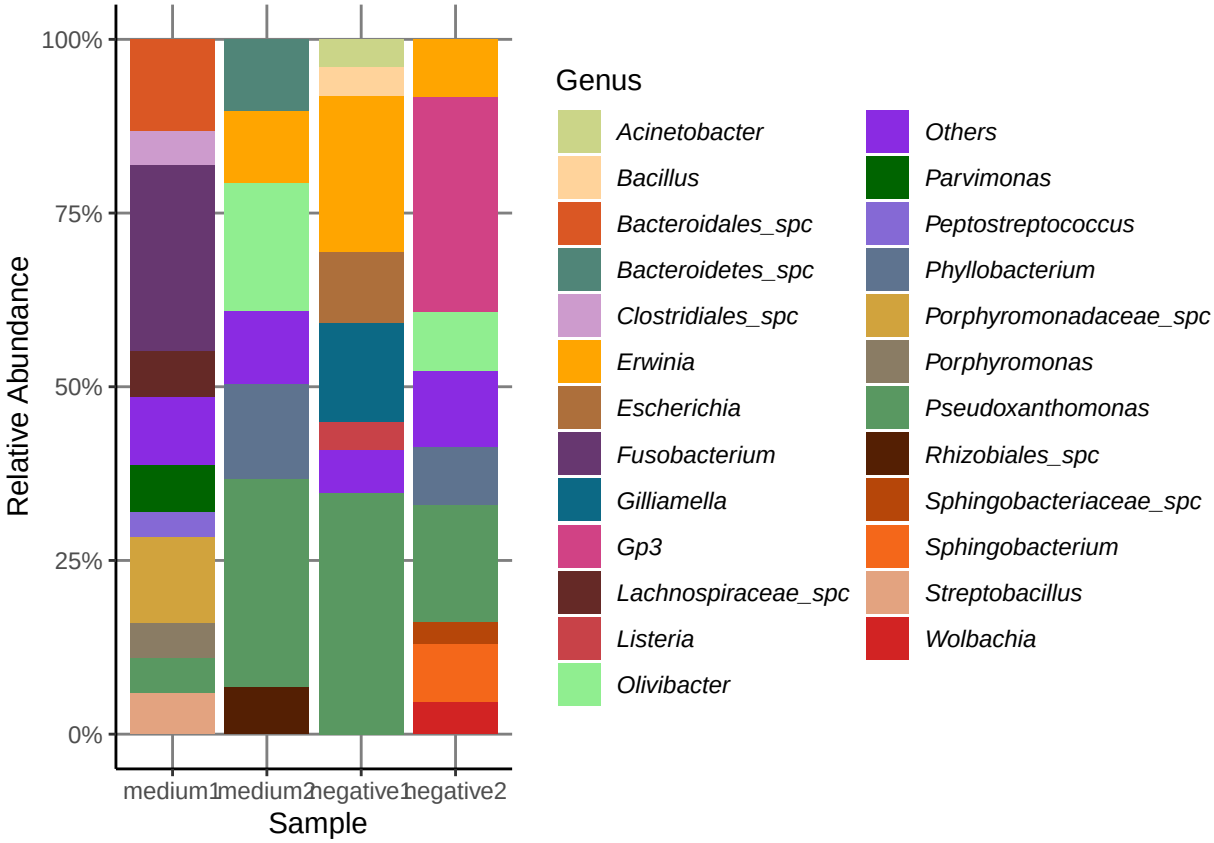
```
Bacteria_Genus.pos2_plot <-ggplot(Bacteria_Genus.pos2, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", position="fill") +
  scale_fill_manual(values = Family_colors, name = "Genus")

g3<-Bacteria_Genus.pos2_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
```



negative controls post decontam

```
Bacteria_Genus.neg2_plot <-ggplot(Bacteria_Genus.neg2, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", position="fill") +
  scale_fill_manual(values = Family_colors, name = "Genus")

g4<-Bacteria_Genus.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"))

g4
```



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

```
bac.without.controls <- subset_samples(ps.noncontam_dataset.16S.ordi, Treatment!="medium")
bac.without.controls2 <- subset_samples(bac.without.controls, Treatment!="negative")
bwc <- subset_samples(bac.without.controls2, Treatment!="standard")
```

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

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

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

```
## Taxonomy Table:     [15 taxa by 8 taxonomic ranks]:
##                                  Domain     Phylum
## Zotu2:Pseudoxanthomonas_spadix   "Bacteria" "Proteobacteria"
## Zotu5:Erwinia_spc                "Bacteria" "Proteobacteria"
## Zotu8:Ochrobactrum_spc           "Bacteria" "Proteobacteria"
## Zotu7:Wolbachia_spc              "Bacteria" "Proteobacteria"
## Zotu21:Microbacterium_pygmaeum   "Bacteria" "Actinobacteria"
```

```
## Zotu24:Wolbachia_spc              "Bacteria" "Proteobacteria"
## Zotu110:Enterobacteriaceae_spc    "Bacteria" "Proteobacteria"
## Zotu45:Pseudoxanthomonas_spc      "Bacteria" "Proteobacteria"
## Zotu49:Microbacterium_pygmaeum    "Bacteria" "Actinobacteria"
## Zotu9:Erwinia_spc                 "Bacteria" "Proteobacteria"
## Zotu48:Microbacterium_spc         "Bacteria" "Actinobacteria"
## Zotu93:Stenotrophomonas_spc       "Bacteria" "Proteobacteria"
## Zotu89:Pseudoxanthomonas_spc      "Bacteria" "Proteobacteria"
## Zotu51:Microbacterium_spc         "Bacteria" "Actinobacteria"
## Zotu43:Phyllobacterium_catacumbae "Bacteria" "Proteobacteria"
##                                   Class                 Order
## Zotu2:Pseudoxanthomonas_spadix    "Gammaproteobacteria" "Xanthomonadales"
## Zotu5:Erwinia_spc                 "Gammaproteobacteria" "Enterobacteriales"
## Zotu8:Ochrobactrum_spc            "Alphaproteobacteria" "Rhizobiales"
## Zotu7:Wolbachia_spc               "Alphaproteobacteria" "Rickettsiales"
## Zotu21:Microbacterium_pygmaeum    "Actinobacteria"      "Actinomycetales"
## Zotu24:Wolbachia_spc              "Alphaproteobacteria" "Rickettsiales"
## Zotu110:Enterobacteriaceae_spc    "Gammaproteobacteria" "Enterobacteriales"
## Zotu45:Pseudoxanthomonas_spc      "Gammaproteobacteria" "Xanthomonadales"
## Zotu49:Microbacterium_pygmaeum    "Actinobacteria"      "Actinomycetales"
## Zotu9:Erwinia_spc                 "Gammaproteobacteria" "Enterobacteriales"
## Zotu48:Microbacterium_spc         "Actinobacteria"      "Actinomycetales"
## Zotu93:Stenotrophomonas_spc       "Gammaproteobacteria" "Xanthomonadales"
## Zotu89:Pseudoxanthomonas_spc      "Gammaproteobacteria" "Xanthomonadales"
## Zotu51:Microbacterium_spc         "Actinobacteria"      "Actinomycetales"
## Zotu43:Phyllobacterium_catacumbae "Alphaproteobacteria" "Rhizobiales"
##                                   Family                Genus
## Zotu2:Pseudoxanthomonas_spadix    "Xanthomonadaceae"    "Pseudoxanthomonas"
## Zotu5:Erwinia_spc                 "Enterobacteriaceae"  "Erwinia"
## Zotu8:Ochrobactrum_spc            "Brucellaceae"        "Ochrobactrum"
## Zotu7:Wolbachia_spc               "Rickettsiaceae"      "Wolbachia"
## Zotu21:Microbacterium_pygmaeum    "Microbacteriaceae"   "Microbacterium"
## Zotu24:Wolbachia_spc              "Rickettsiaceae"      "Wolbachia"
## Zotu110:Enterobacteriaceae_spc    "Enterobacteriaceae"  "Enterobacteriaceae_spc"
## Zotu45:Pseudoxanthomonas_spc      "Xanthomonadaceae"    "Pseudoxanthomonas"
## Zotu49:Microbacterium_pygmaeum    "Microbacteriaceae"   "Microbacterium"
## Zotu9:Erwinia_spc                 "Enterobacteriaceae"  "Erwinia"
## Zotu48:Microbacterium_spc         "Microbacteriaceae"   "Microbacterium"
## Zotu93:Stenotrophomonas_spc       "Xanthomonadaceae"    "Stenotrophomonas"
## Zotu89:Pseudoxanthomonas_spc      "Xanthomonadaceae"    "Pseudoxanthomonas"
## Zotu51:Microbacterium_spc         "Microbacteriaceae"   "Microbacterium"
## Zotu43:Phyllobacterium_catacumbae "Phyllobacteriaceae" "Phyllobacterium"
##                                   Species
## Zotu2:Pseudoxanthomonas_spadix    "Pseudoxanthomonas_spadix"
## Zotu5:Erwinia_spc                 "Erwinia_spc"
## Zotu8:Ochrobactrum_spc            "Ochrobactrum_spc"
## Zotu7:Wolbachia_spc               "Wolbachia_spc"
## Zotu21:Microbacterium_pygmaeum    "Microbacterium_pygmaeum"
## Zotu24:Wolbachia_spc              "Wolbachia_spc"
## Zotu110:Enterobacteriaceae_spc    "Enterobacteriaceae_spc"
## Zotu45:Pseudoxanthomonas_spc      "Pseudoxanthomonas_spc"
## Zotu49:Microbacterium_pygmaeum    "Microbacterium_pygmaeum"
## Zotu9:Erwinia_spc                 "Erwinia_spc"
## Zotu48:Microbacterium_spc         "Microbacterium_spc"
```

```
## Zotu93:Stenotrophomonas_spc       "Stenotrophomonas_spc"
## Zotu89:Pseudoxanthomonas_spc      "Pseudoxanthomonas_spc"
## Zotu51:Microbacterium_spc         "Microbacterium_spc"
## Zotu43:Phyllobacterium_catacumbae "Phyllobacterium_catacumbae"
##                                   best_hit
## Zotu2:Pseudoxanthomonas_spadix    "Zotu2:Pseudoxanthomonas_spadix"
## Zotu5:Erwinia_spc                 "Zotu5:Erwinia_spc"
## Zotu8:Ochrobactrum_spc            "Zotu8:Ochrobactrum_spc"
## Zotu7:Wolbachia_spc               "Zotu7:Wolbachia_spc"
## Zotu21:Microbacterium_pygmaeum    "Zotu21:Microbacterium_pygmaeum"
## Zotu24:Wolbachia_spc              "Zotu24:Wolbachia_spc"
## Zotu110:Enterobacteriaceae_spc    "Zotu110:Enterobacteriaceae_spc"
## Zotu45:Pseudoxanthomonas_spc      "Zotu45:Pseudoxanthomonas_spc"
## Zotu49:Microbacterium_pygmaeum    "Zotu49:Microbacterium_pygmaeum"
## Zotu9:Erwinia_spc                 "Zotu9:Erwinia_spc"
## Zotu48:Microbacterium_spc         "Zotu48:Microbacterium_spc"
## Zotu93:Stenotrophomonas_spc       "Zotu93:Stenotrophomonas_spc"
## Zotu89:Pseudoxanthomonas_spc      "Zotu89:Pseudoxanthomonas_spc"
## Zotu51:Microbacterium_spc         "Zotu51:Microbacterium_spc"
## Zotu43:Phyllobacterium_catacumbae "Zotu43:Phyllobacterium_catacumbae"
```

```
round(otu_table(bwc)[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
## Zotu2:Pseudoxanthomonas_spadix     16671  17059     42     16   319    13
## Zotu5:Erwinia_spc                      0      0     33     10    44     3
## Zotu8:Ochrobactrum_spc                 2      1      1      0    23     0
## Zotu7:Wolbachia_spc                    1      0    895   2281   679   746
## Zotu21:Microbacterium_pygmaeum       323    293     25      9     3    15
## Zotu24:Wolbachia_spc                   0     22      0      0    32     0
## Zotu110:Enterobacteriaceae_spc         0      0      0      0     0     0
## Zotu45:Pseudoxanthomonas_spc          21    124      0      0     0     0
## Zotu49:Microbacterium_pygmaeum        76     46      0      0     1     0
## Zotu9:Erwinia_spc                      0      0      0      0     0     0
## Zotu48:Microbacterium_spc             58     40      0      0     0     0
## Zotu93:Stenotrophomonas_spc            0      0      0      0     0     0
## Zotu89:Pseudoxanthomonas_spc           0      0      0      0     0     0
## Zotu51:Microbacterium_spc             62     40      0      0     0     0
## Zotu43:Phyllobacterium_catacumbae      1      2      1      1    23     3
##                                   B10-01 B10-06 B10-09a B10-09b B10-18 B10-24
## Zotu2:Pseudoxanthomonas_spadix        16     35      76      29     24     35
## Zotu5:Erwinia_spc                      0      4       9       2     10      0
## Zotu8:Ochrobactrum_spc                 1      1       1       0      1      0
## Zotu7:Wolbachia_spc                    0      2       1       1      6      0
## Zotu21:Microbacterium_pygmaeum         0      0       0       0      0      0
## Zotu24:Wolbachia_spc                 222    121       2       0    100    135
## Zotu110:Enterobacteriaceae_spc         0      0       0       0      0      0
## Zotu45:Pseudoxanthomonas_spc           0      0       0       0      0      0
## Zotu49:Microbacterium_pygmaeum         0      0       0       0      0      0
## Zotu9:Erwinia_spc                      1      0       0       0      0      0
## Zotu48:Microbacterium_spc              0      0       0       0      0      0
## Zotu93:Stenotrophomonas_spc            0      0       0       0      0      0
```

```
## Zotu89:Pseudoxanthomonas_spc           0      0       0       0      0      0
## Zotu51:Microbacterium_spc              0      0       0       0      0      0
## Zotu43:Phyllobacterium_catacumbae      0      0       0       0      4      0
##                                  B10-25a B10-25b B10-26a B15-01a B15-01b
## Zotu2:Pseudoxanthomonas_spadix        29      28      60     181      11
## Zotu5:Erwinia_spc                      2       2      89      85       0
## Zotu8:Ochrobactrum_spc                 1       0       1       1       0
## Zotu7:Wolbachia_spc                    0       0       1       0     163
## Zotu21:Microbacterium_pygmaeum         0       1       0       0       0
## Zotu24:Wolbachia_spc                   1     147      58       0       0
## Zotu110:Enterobacteriaceae_spc         0       0       0       4       0
## Zotu45:Pseudoxanthomonas_spc           0       0       1       0       0
## Zotu49:Microbacterium_pygmaeum         0       0       0       0       0
## Zotu9:Erwinia_spc                      0       0       0       2       0
## Zotu48:Microbacterium_spc              0       0       0       0       0
## Zotu93:Stenotrophomonas_spc            0       0       0       0       0
## Zotu89:Pseudoxanthomonas_spc           0       0       0       2       0
## Zotu51:Microbacterium_spc              0       0       0       0       0
## Zotu43:Phyllobacterium_catacumbae      0       2       1       3       0
##                                  B15-06 B15-07a B15-07b B15-11 B15-20 B15-24
## Zotu2:Pseudoxanthomonas_spadix       55      86      22     54    282     39
## Zotu5:Erwinia_spc                    14       2       2      0      2      1
## Zotu8:Ochrobactrum_spc                0       0      23      0      0      2
## Zotu7:Wolbachia_spc                3467    3018    1124   3099   4079   1105
## Zotu21:Microbacterium_pygmaeum        0       1       0      0      0      0
## Zotu24:Wolbachia_spc                  0       0       0      0      0      0
## Zotu110:Enterobacteriaceae_spc        0       0       0      0      0      0
## Zotu45:Pseudoxanthomonas_spc          0       0       0      0      0      0
## Zotu49:Microbacterium_pygmaeum        0       0       0      0      0      0
## Zotu9:Erwinia_spc                     0       0       0      0      0      0
## Zotu48:Microbacterium_spc             0       0       0      0      0      0
## Zotu93:Stenotrophomonas_spc           0       0       0      0      1      0
## Zotu89:Pseudoxanthomonas_spc          0       0       0      0      0      0
## Zotu51:Microbacterium_spc             0       0       0      0      0      0
## Zotu43:Phyllobacterium_catacumbae     1       2       1      0      3      0
##                                  B15-25a B15-25b B16-01a B16-04 B16-31a
## Zotu2:Pseudoxanthomonas_spadix       120      43   24526  24421   35797
## Zotu5:Erwinia_spc                      0      11       3      4     195
## Zotu8:Ochrobactrum_spc                 0       0      18      2       0
## Zotu7:Wolbachia_spc                    3    1091       0      0       0
## Zotu21:Microbacterium_pygmaeum         0       2       0      0       3
## Zotu24:Wolbachia_spc                   0       0       0      2       1
## Zotu110:Enterobacteriaceae_spc         0       0       0      0       1
## Zotu45:Pseudoxanthomonas_spc           0       0       0      0       0
## Zotu49:Microbacterium_pygmaeum         0       0       0      0       0
## Zotu9:Erwinia_spc                      0       0       0      0       1
## Zotu48:Microbacterium_spc              0       0       2      0       0
## Zotu93:Stenotrophomonas_spc            0       0       0      0       1
## Zotu89:Pseudoxanthomonas_spc           0       0       0      0       2
## Zotu51:Microbacterium_spc              0       0       1      0       0
## Zotu43:Phyllobacterium_catacumbae      4       0       0      0       2
##                                  B16-31b B20-04a B20-08a B20-08b B20-11 B23-21
## Zotu2:Pseudoxanthomonas_spadix     20325   21259    7488   13954   7613     41
## Zotu5:Erwinia_spc                      2       0      27       1      0     13
```

```
## Zotu8:Ochrobactrum_spc                3    7424       7       6   14591      10
## Zotu7:Wolbachia_spc                   6       0      15     370      20       0
## Zotu21:Microbacterium_pygmaeum        0       0       0       0       0       0
## Zotu24:Wolbachia_spc                  0       2       2       0       0     162
## Zotu110:Enterobacteriaceae_spc        0       0       0       0       0       0
## Zotu45:Pseudoxanthomonas_spc          0       0       0       0       0       0
## Zotu49:Microbacterium_pygmaeum        0       0       0       0       0       0
## Zotu9:Erwinia_spc                     0       0       0       0       0       0
## Zotu48:Microbacterium_spc             0       0       0       0       0       0
## Zotu93:Stenotrophomonas_spc           0       0       0       0       0       0
## Zotu89:Pseudoxanthomonas_spc          0       0       0       0       0       0
## Zotu51:Microbacterium_spc             0       0       0       0       0       0
## Zotu43:Phyllobacterium_catacumbae     0       1       2       0       2       2
##                                  B23-23a B24-02a B24-26a B24-26b B24-28a
## Zotu2:Pseudoxanthomonas_spadix        73    1292   28365    3492   13767
## Zotu5:Erwinia_spc                     46   19969   15147    5361      27
## Zotu8:Ochrobactrum_spc                 9      11      18       2       0
## Zotu7:Wolbachia_spc                    0       2       4       0       0
## Zotu21:Microbacterium_pygmaeum         0       2       2      19     278
## Zotu24:Wolbachia_spc                   0       0       1       0       0
## Zotu110:Enterobacteriaceae_spc         0      25     316      50       0
## Zotu45:Pseudoxanthomonas_spc           0       0       0       1      20
## Zotu49:Microbacterium_pygmaeum         0       1       0       2      64
## Zotu9:Erwinia_spc                      0      49     247      21       1
## Zotu48:Microbacterium_spc              0       3       0       1      42
## Zotu93:Stenotrophomonas_spc            1      34     110      43       0
## Zotu89:Pseudoxanthomonas_spc           0      31     124      32       0
## Zotu51:Microbacterium_spc              2       0       0       2      42
## Zotu43:Phyllobacterium_catacumbae     34      10      26       0      31
##                                  B24-29 B36-14a B36-14b B36-30 B36-31a B36-32
## Zotu2:Pseudoxanthomonas_spadix        80   12863   10757  11835   23964  23573
## Zotu5:Erwinia_spc                      3      11       5      5      39      3
## Zotu8:Ochrobactrum_spc                40       0       0      0       0      2
## Zotu7:Wolbachia_spc                    2       0       1      1       1      0
## Zotu21:Microbacterium_pygmaeum        25       0       0      0     194    386
## Zotu24:Wolbachia_spc                   2       0       0      0       0      0
## Zotu110:Enterobacteriaceae_spc         1       0       0      0       0      0
## Zotu45:Pseudoxanthomonas_spc           0       0       0      0       2     50
## Zotu49:Microbacterium_pygmaeum         0       0       0      0      64     93
## Zotu9:Erwinia_spc                      0       0       0      0       0      0
## Zotu48:Microbacterium_spc              0       0       0      0      49     67
## Zotu93:Stenotrophomonas_spc            0       0       0      0       0      0
## Zotu89:Pseudoxanthomonas_spc           0       0       0      0       0      0
## Zotu51:Microbacterium_spc              0       0       0      0      30     40
## Zotu43:Phyllobacterium_catacumbae      0      22       0      3      19      1
##                                  B39-15 B39-16 B39-17a B46-03 B7-07 B7-08a
## Zotu2:Pseudoxanthomonas_spadix    26359  26612    4077  14748 19215  12684
## Zotu5:Erwinia_spc                     3      6   24556     18     4      7
## Zotu8:Ochrobactrum_spc                5      1       4      3     4     18
## Zotu7:Wolbachia_spc                   5     28       2      1     9      1
## Zotu21:Microbacterium_pygmaeum        0      0       1    391     0      0
## Zotu24:Wolbachia_spc                  1     19       0     26    77      0
## Zotu110:Enterobacteriaceae_spc        0      0      42      0     0      0
## Zotu45:Pseudoxanthomonas_spc          0      0       0    161     2      0
```

```
## Zotu49:Microbacterium_pygmaeum         0      0       0     34      0      0
## Zotu9:Erwinia_spc                      0      0      56      0      0      0
## Zotu48:Microbacterium_spc              0      0       0     29      0      0
## Zotu93:Stenotrophomonas_spc            0      0      88      0      0      0
## Zotu89:Pseudoxanthomonas_spc           0      0      83      0      0      0
## Zotu51:Microbacterium_spc              0      0       0     40      0      0
## Zotu43:Phyllobacterium_catacumbae      0      1      27      2      1      2
```

create subset of dataset

subset dataset bwc and exclude samples with low read counts (<500 reads)

```
sample_data(bwc)$ColSums <- colSums(otu_table(bwc))
high.reads <- subset_samples(bwc, ColSums>500)
```

reduce metadata

```
sample_data(high.reads) <- sample_data(high.reads)[,c("Sample","Nest","Linage","Treatment", "age_samplin
```

get rid of no read taxa in dataset

```
high.reads <- prune_taxa(taxa_sums(high.reads) > 0, high.reads)
```

extract general information on data

```
microbiome::summarize_phyloseq(high.reads)
```

```
## Compositional = NO2

## 1] Min. number of reads = 7932] Max. number of reads = 449243] Total number of reads = 5404224] Avera
##         (i.e. exactly one read detected across all samples)7.246376811594210] Number of sample varial

## [[1]]
## [1] "1] Min. number of reads = 793"
##
## [[2]]
## [1] "2] Max. number of reads = 44924"
##
## [[3]]
## [1] "3] Total number of reads = 540422"
##
## [[4]]
## [1] "4] Average number of reads = 15011.7222222222"
##
## [[5]]
## [1] "5] Median number of reads = 14342"
##
## [[6]]
## [1] "7] Sparsity = 0.799919484702093"
##
## [[7]]
```

```
## [1] "6] Any OTU sum to 1 or less? YES"
##
## [[8]]
## [1] "8] Number of singletons = 5"
##
## [[9]]
## [1] "9] Percent of OTUs that are singletons \n        (i.e. exactly one read detected across all samp
##
## [[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(high.reads))
table(replicates$Treatment)
```

```
##
## 2nd-foundation        control        removal
##              8             14             14
```

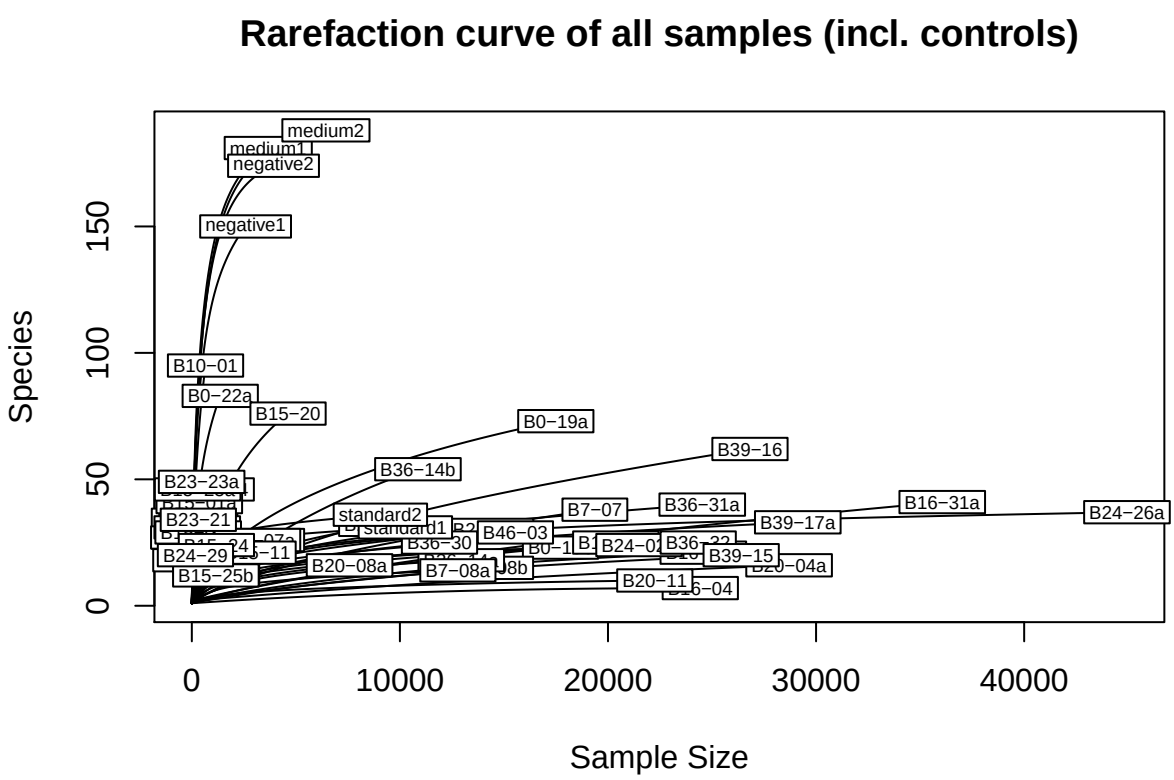
Analysis

Rarefaction curves

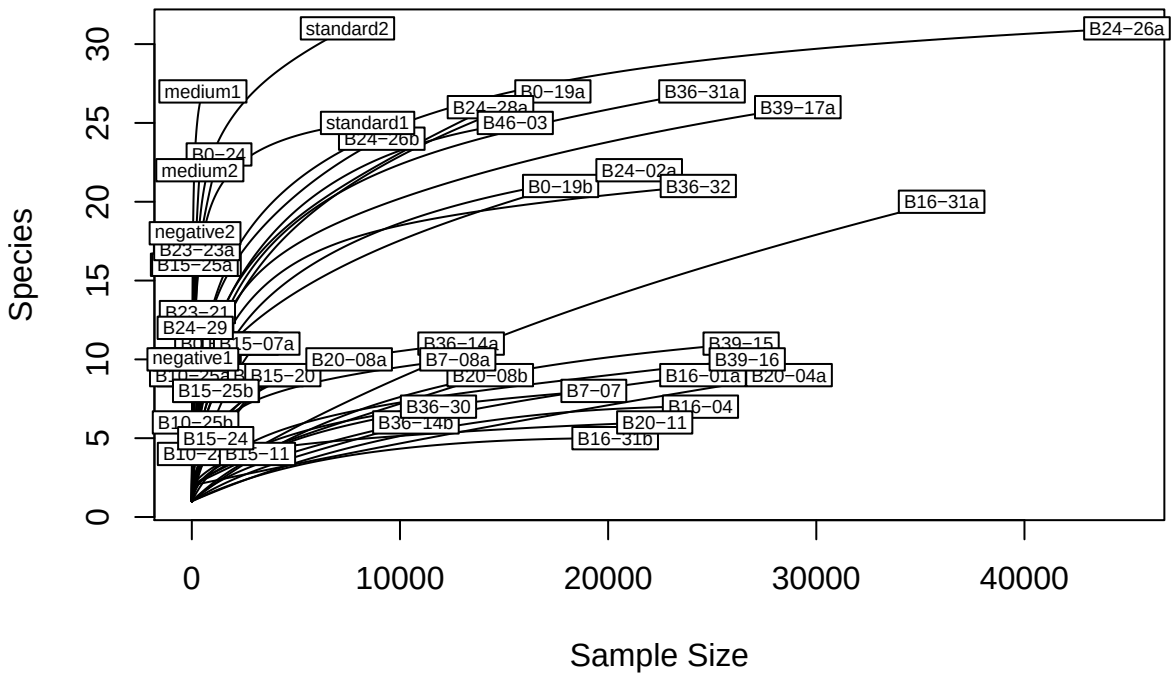
1. all samples with controls
2. all samples with controls decontaminated
3. all samples without controls
4. all samples >500 reads

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

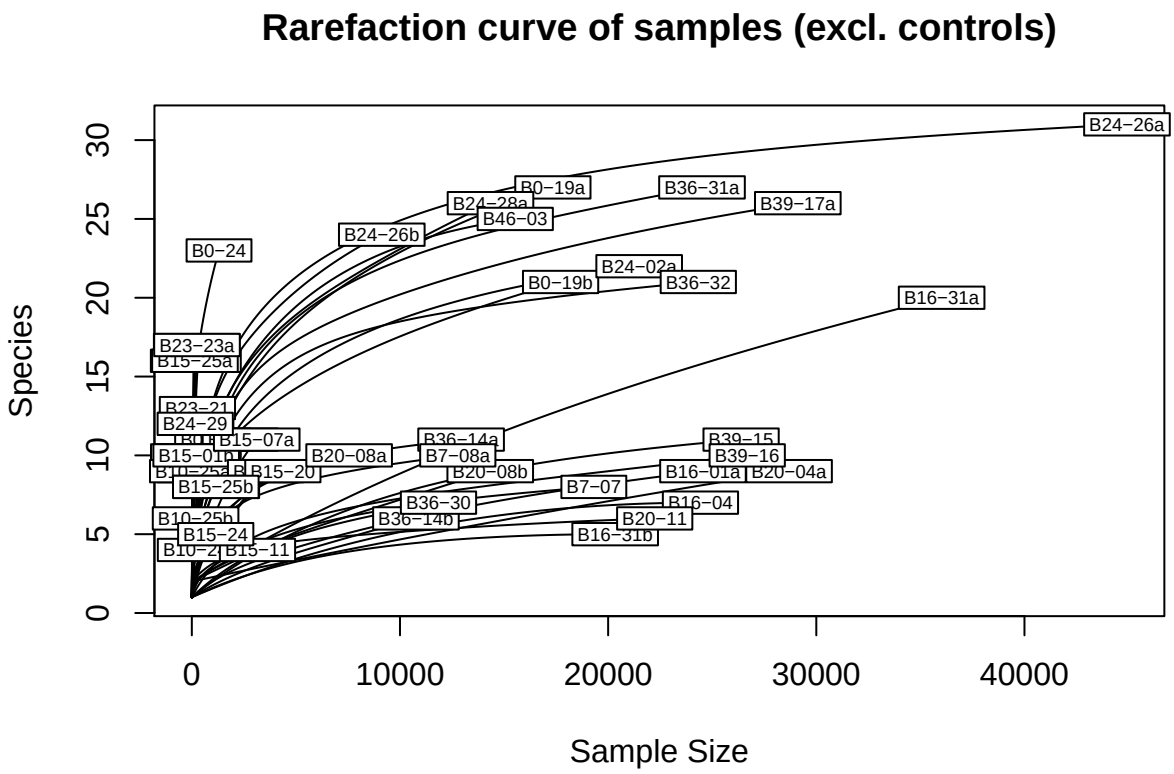
```
rarecurve(t(otu_table(dataset.16S.ordi)), cex=0.6, step = 20, main = "Rarefaction curve of all samples
```



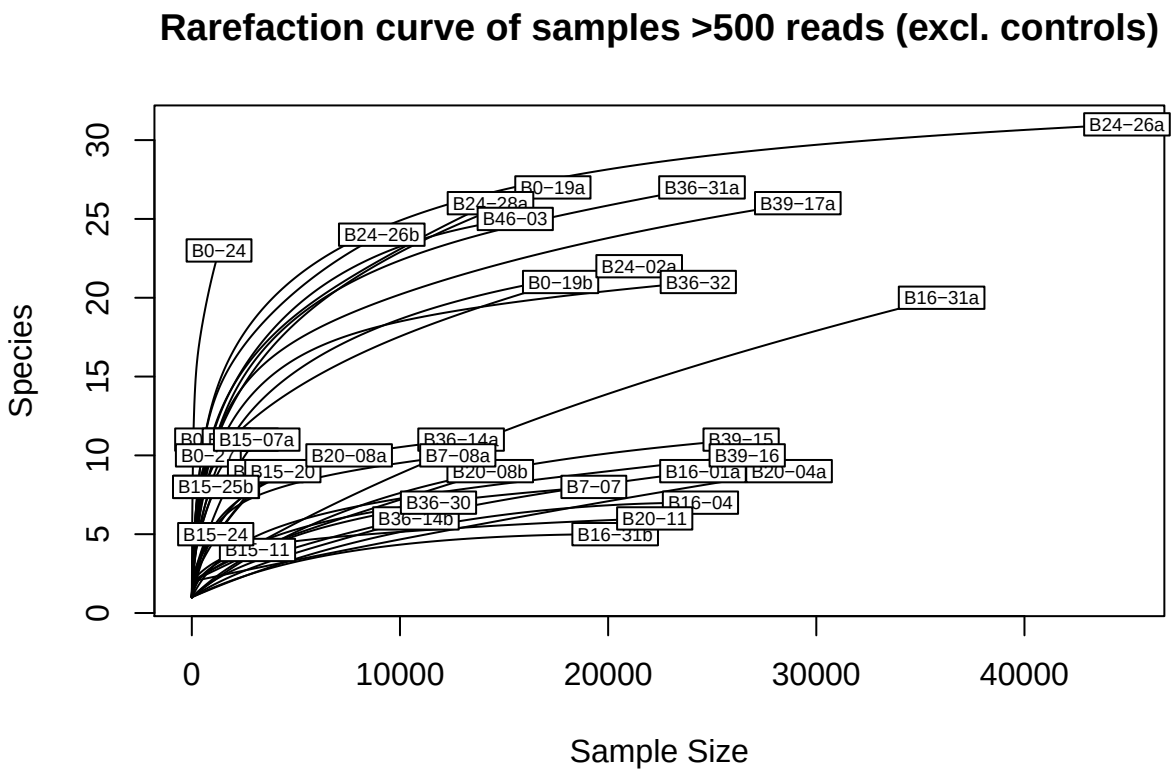

```
rarecurve(t(otu_table(ps.noncontam_dataset.16S.ordi)), cex=0.6, step = 20, main = "Rarefaction curve of
```




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

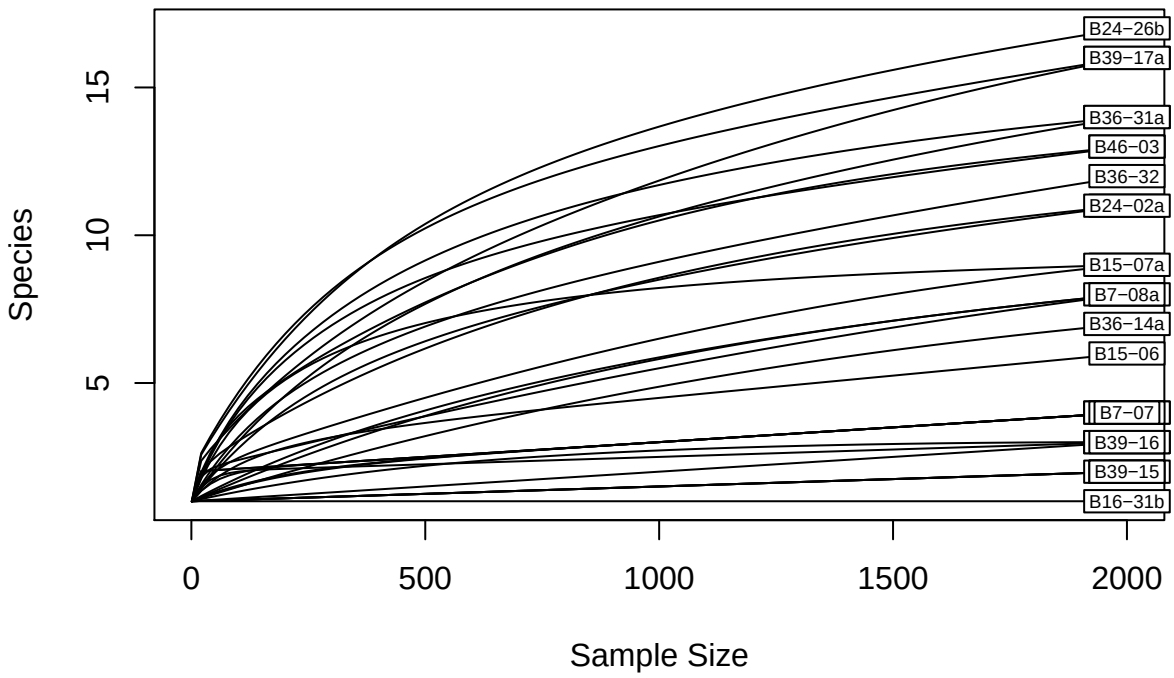



```
rarecurve(t(otu_table(high.reads)), cex=0.6, step = 20, main = "Rarefaction curve of samples >500 reads
```



rarefy dataset to 2000 reads

```
ps2 <-high.reads
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)
```



–> 6 samples removed because they contained fewer reads than `sample.size`. Up to first five removed samples are: B0-22a B0-24 B0-27 B15-07b B15-24 ...

15OTUs were removed because they are no longer present in any sample after random subsampling

subset data to two comparison groups

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

#removal vs. 2^nd^ attempt
R2nd <- subset_samples(high.reads, 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    0.1539935  0.1977253
## 2   removal    0.2550152  0.2528587
```

```
p1 <- ggplot(ps1.meta.CR, aes(x = Treatment, y = Shannon))
shan_bac <- 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
    theme_bw()+
    ylim(NA, 0.95)+
    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      5.454545    3.933539
## 2   removal      9.769231    4.585373
```

```
p2 <- ggplot(ps1.meta.CR, aes(x = Treatment, y = Observed))
obs_bac <- 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-Obse
    theme_bw()+
    ylim(NA, 17.5)+
    labs(x = "Treatment group", y = "Observed richness")+
  theme(axis.title = element_text(size = 18, face = "bold"), axis.text = element_text(size = 16))
```

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"), labe

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

```
##     Treatment Shannon.mean Shannon.sd
## 1     removal    0.2550152  0.2528587
## 2 2nd-attempt    0.2270820  0.3171305
```

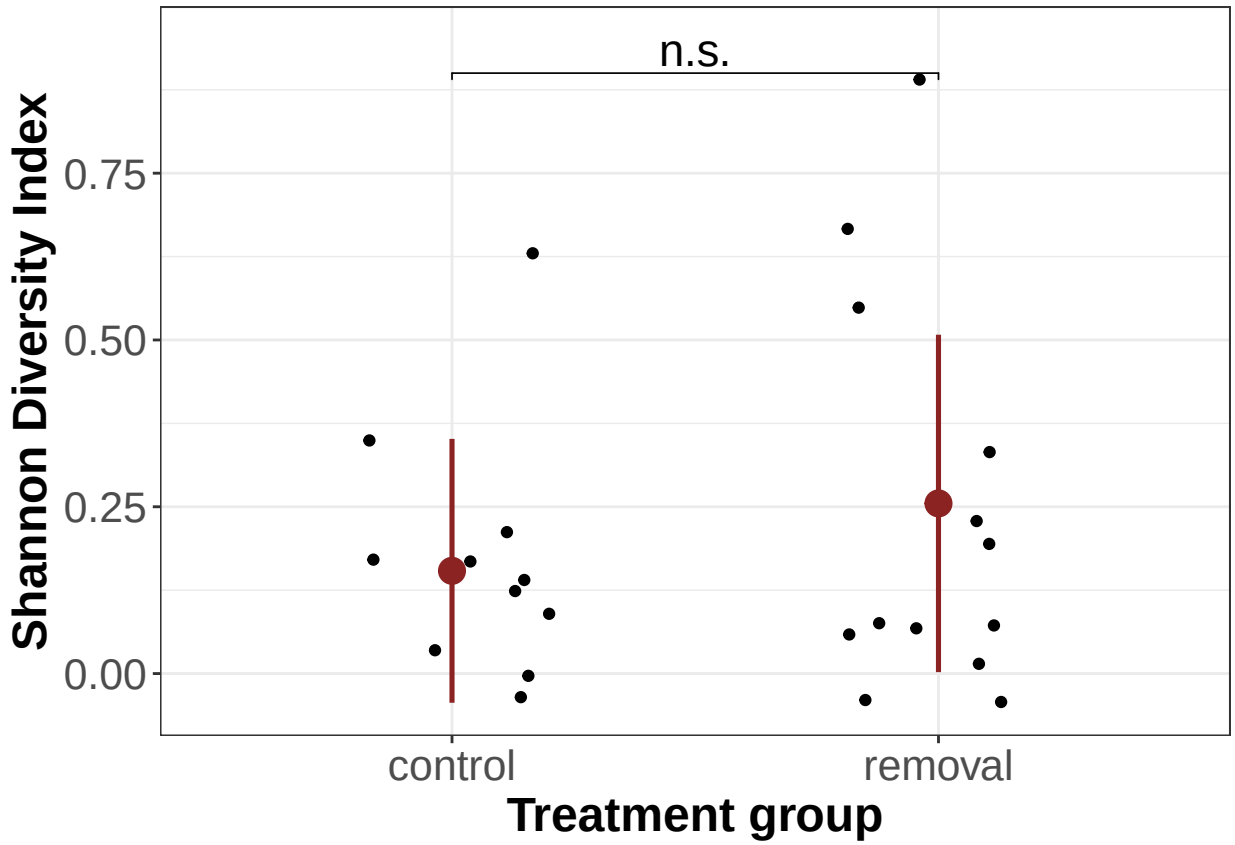
```
p3 <- ggplot(ps1.meta.R2nd, aes(x = Treatment, y = Shannon))
shan_bac2<- p3 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
    geom_pointrange(data = c, mapping = aes(x = Treatment, y = Shannon.mean, ymin = Shannon.mean-Shannon
    theme_bw()+
    ylim(NA, 1)+
    labs(x = "Treatment group", 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      9.769231    4.585373
## 2 2nd-attempt      7.166667    6.274286
```

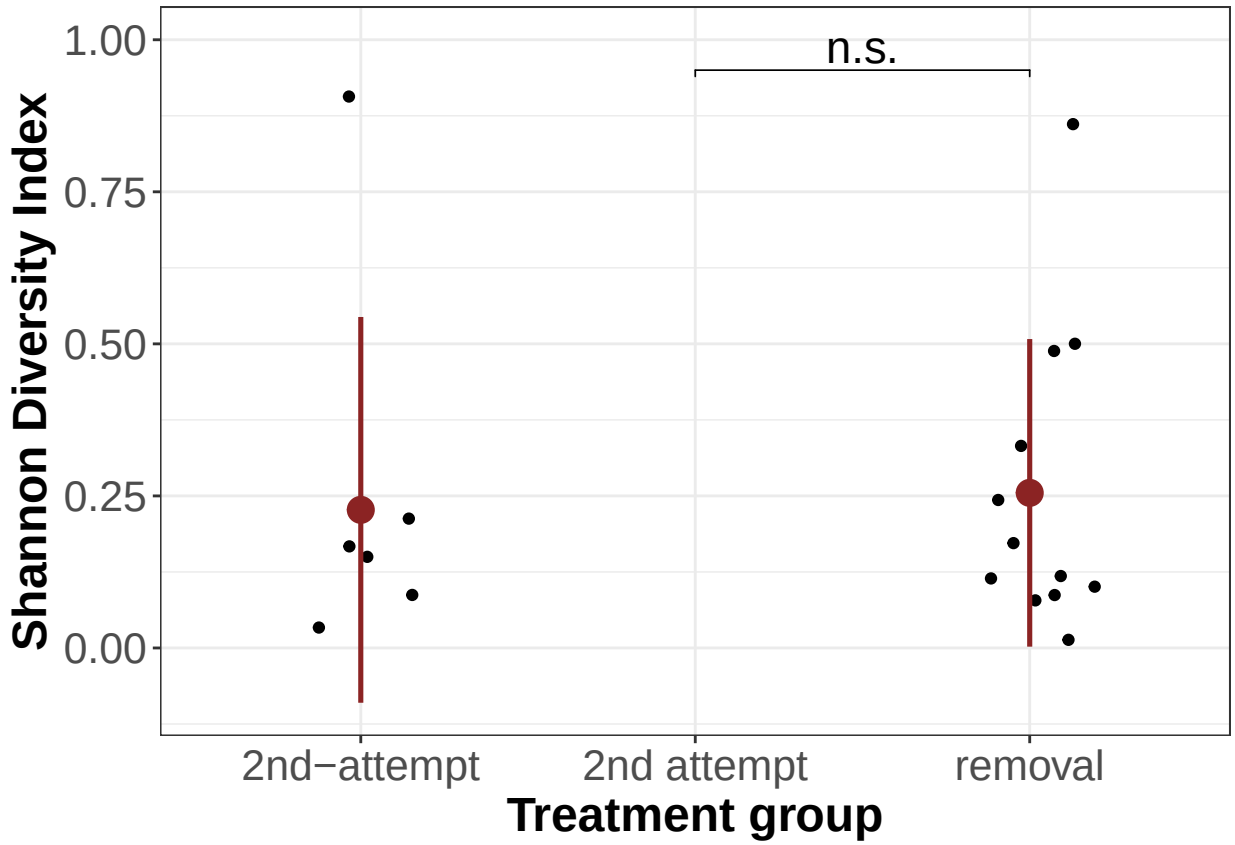
```
p4 <- ggplot(ps1.meta.R2nd, aes(x = Treatment, y = Observed))
obs_bac2 <- p4 + geom_jitter(position = position_jitter(w=0.2, h=0.1))+
    geom_pointrange(data = d, mapping = aes(x = Treatment, y = Observed.mean, ymin = Observed.mean-Obse
    theme_bw()+
    ylim(NA, 18.5)+
    labs(x = "Treatment 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

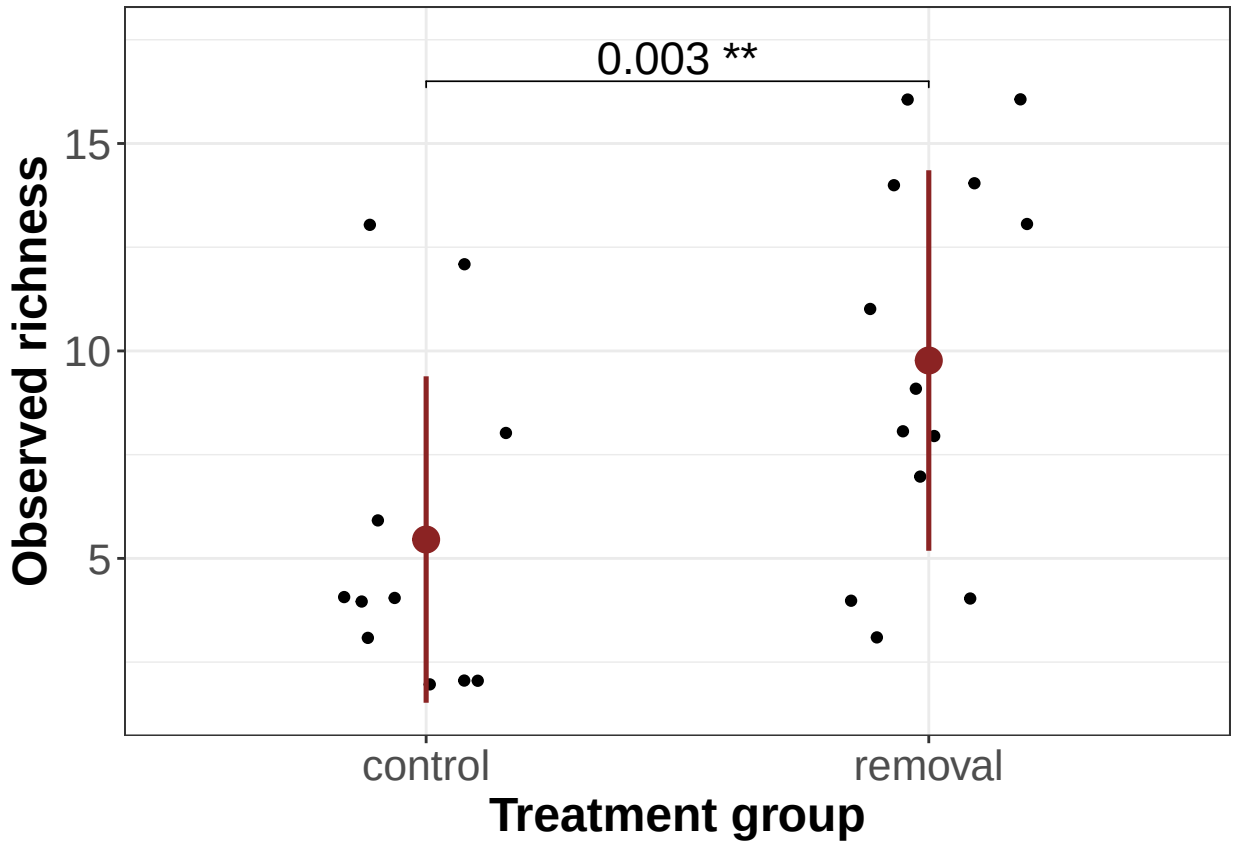
```
shan_bac <- shan_bac + font("axis.title", size = 18)+
        font("xylab", size = 18)+
        geom_bracket(
            xmin = "control", xmax = "removal", y.position = 0.9, label = "n.s.", label.size = 6,tip.le

shan_bac
```



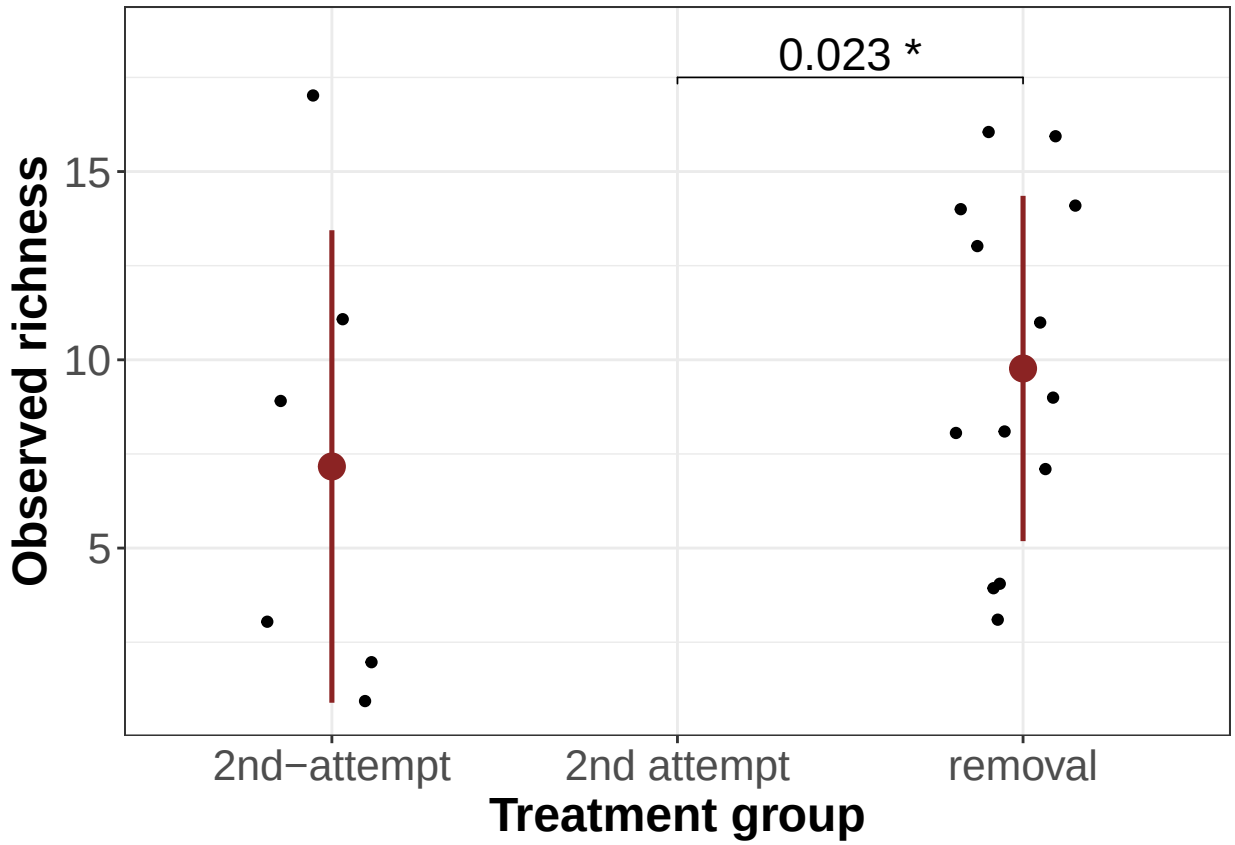

```
shan_bac2 <- shan_bac2 + font("axis.title", size = 18)+
        font("xylab", size = 18)+
        geom_bracket(
            xmin = "removal", xmax = "2nd attempt", y.position = 0.95, label = "n.s.", label.size = 6,t

shan_bac2
```




```
obs_bac <- obs_bac + font("axis.title", size = 18)+
        font("xylab", size = 18)+
        geom_bracket(
            xmin = "control", xmax = "removal", y.position = 16.5, label = "0.003 **", label.size = 6,t

obs_bac
```




```
obs_bac2 <- obs_bac2 + font("axis.title", size = 18)+
        font("xylab", size = 18)+
        geom_bracket(
            xmin = "removal", xmax = "2nd attempt", y.position = 17.5, label = "0.023 *", label.size = 

obs_bac2
```



Statistics

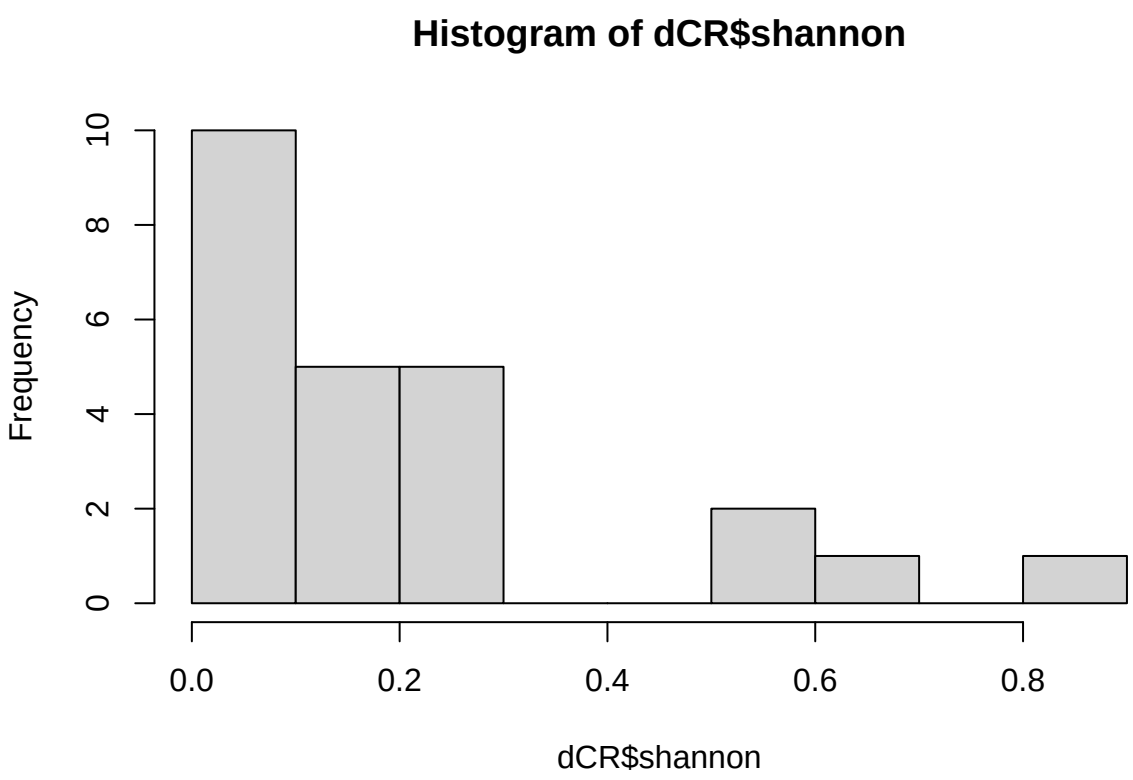
Testing differences in alpha diversity

create dataframe for analysis

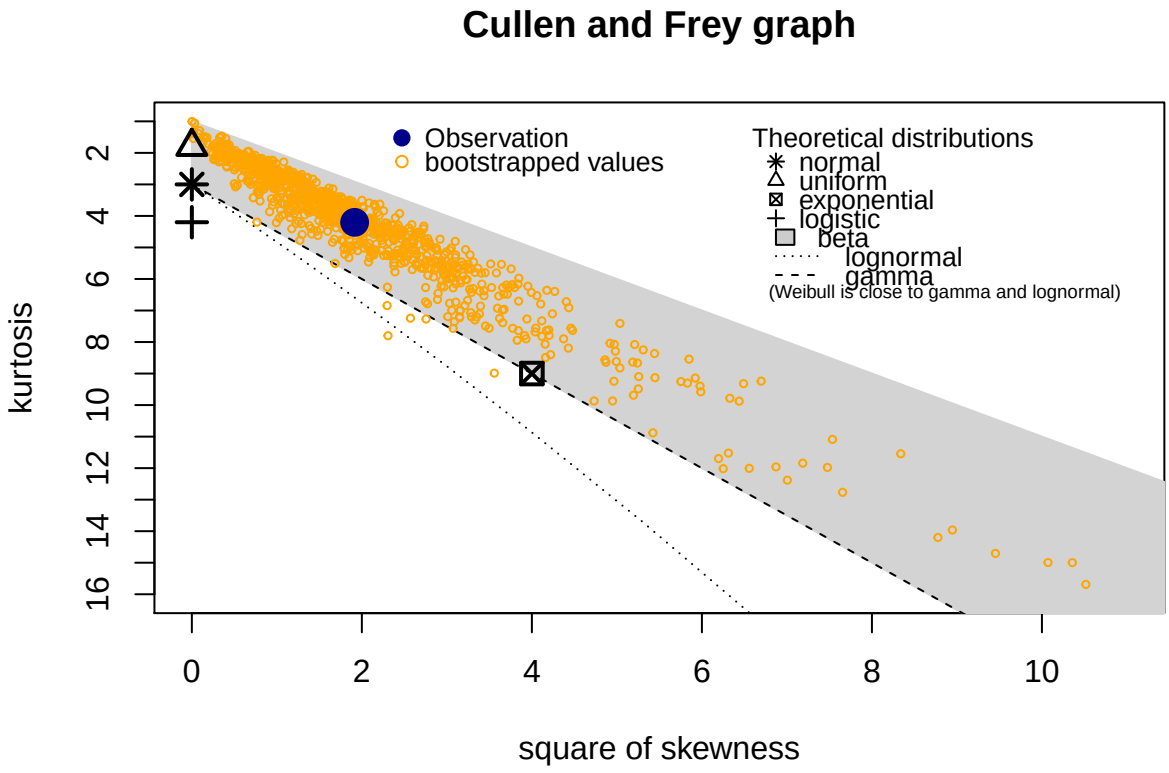
```
#control vs. removal
dCR <- meta(CRrf)
shannon <- diversity(CRrf, "shannon")
dCR$shannon <- shannon$shannon
observed <- alpha(CRrf, index = "observed", zeroes = TRUE)
dCR$observed <- observed$observed

#removal vs. 2^nd^ 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)
```

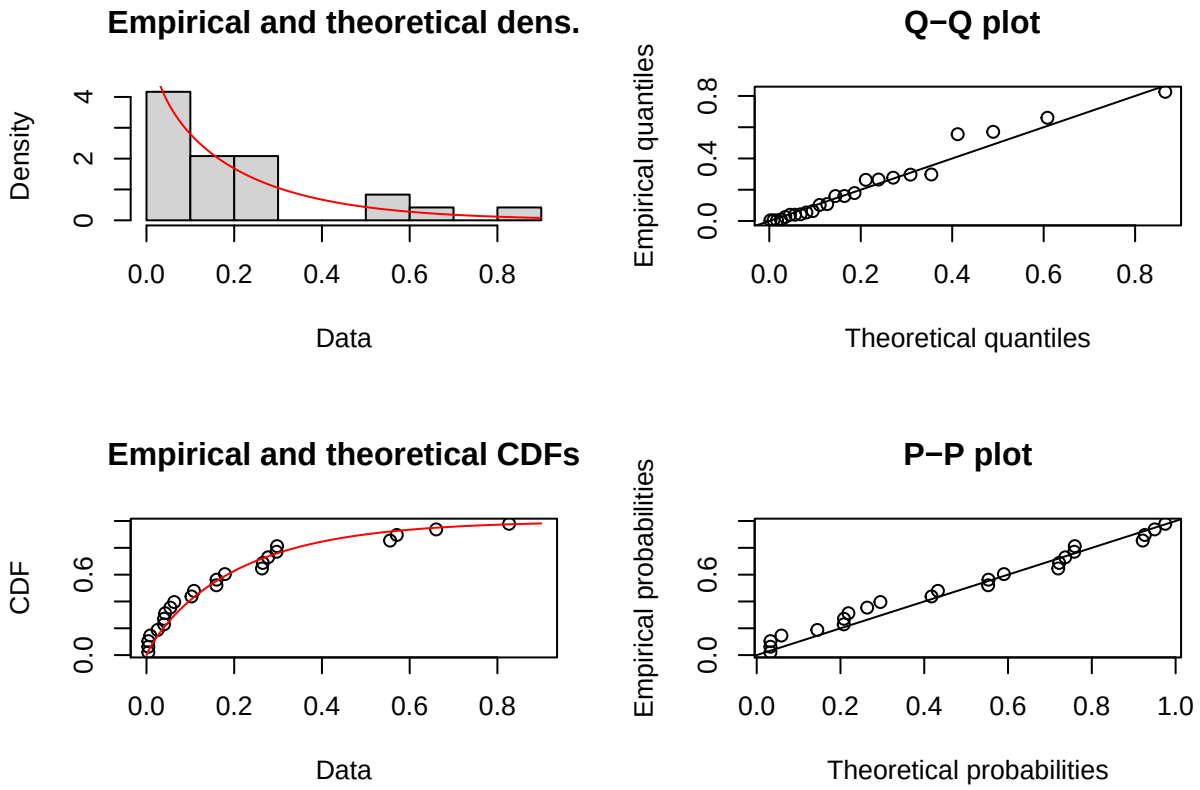



```
descdist(dCR$shannon, boot = 1000)
```




```
## summary statistics
## ------
## min:  0.004300326   max:  0.8266148
## median:  0.1338609
## mean:  0.2087136
## estimated sd:  0.2302183
## estimated skewness:  1.383827
## estimated kurtosis:  4.203764
```

```
fit.gamma <- fitdist(dCR$shannon, distr = "gamma", method = "mme")
plot(fit.gamma)
```



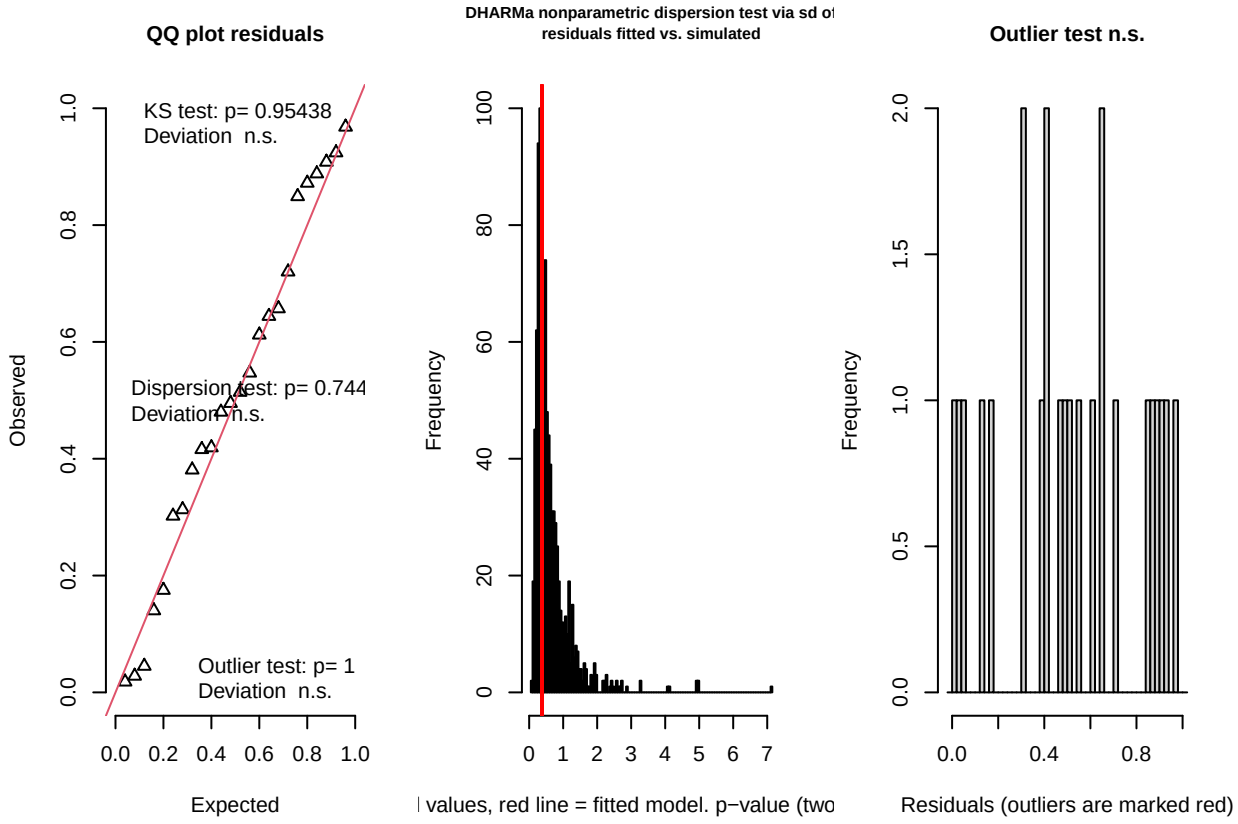
–> gamma distribution fits well

test with subset 1

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

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

```
## Analysis of Deviance Table (Type II tests)
##
## Response: shannon
##           LR Chisq Df Pr(>Chisq)
## Treatment   1.5711  1    0.21005
## Linage     19.2578  8    0.01354 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

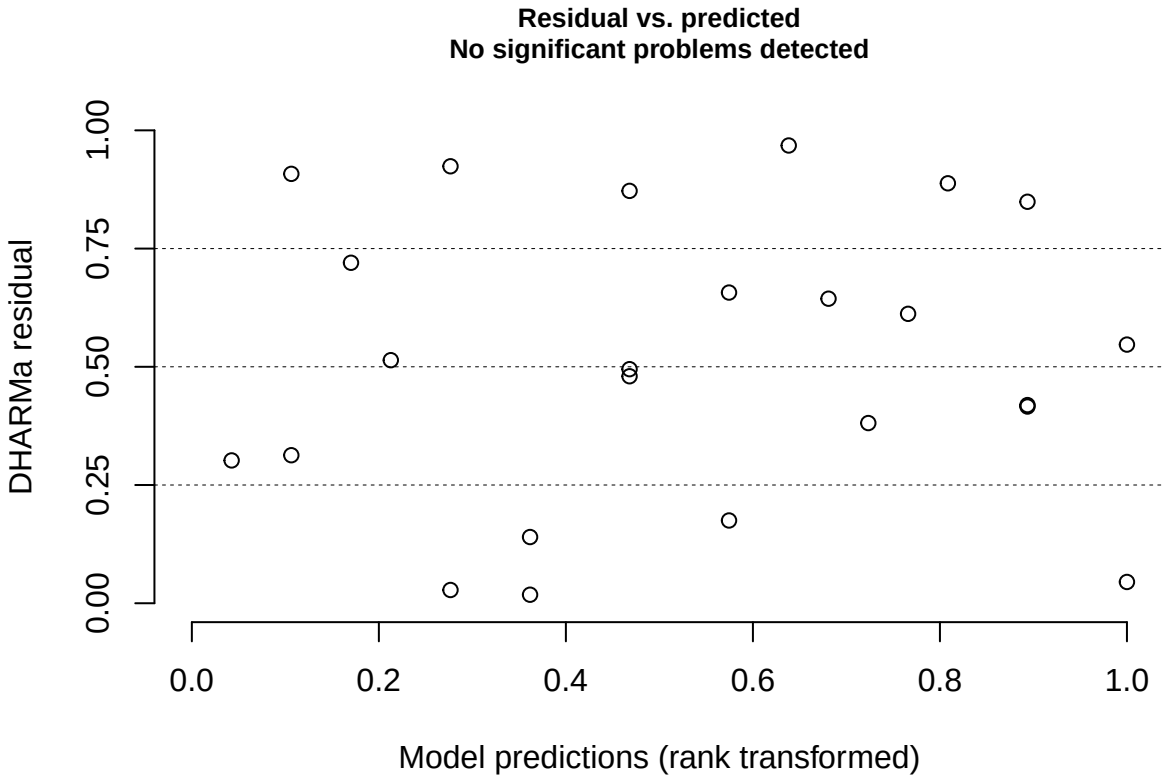
```
res_shan1 <- simulateResiduals(shan1, n = 1000)
testResiduals(res_shan1)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.099, p-value = 0.9544
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.59005, p-value = 0.744
## 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 = 24, p-value = 1
```

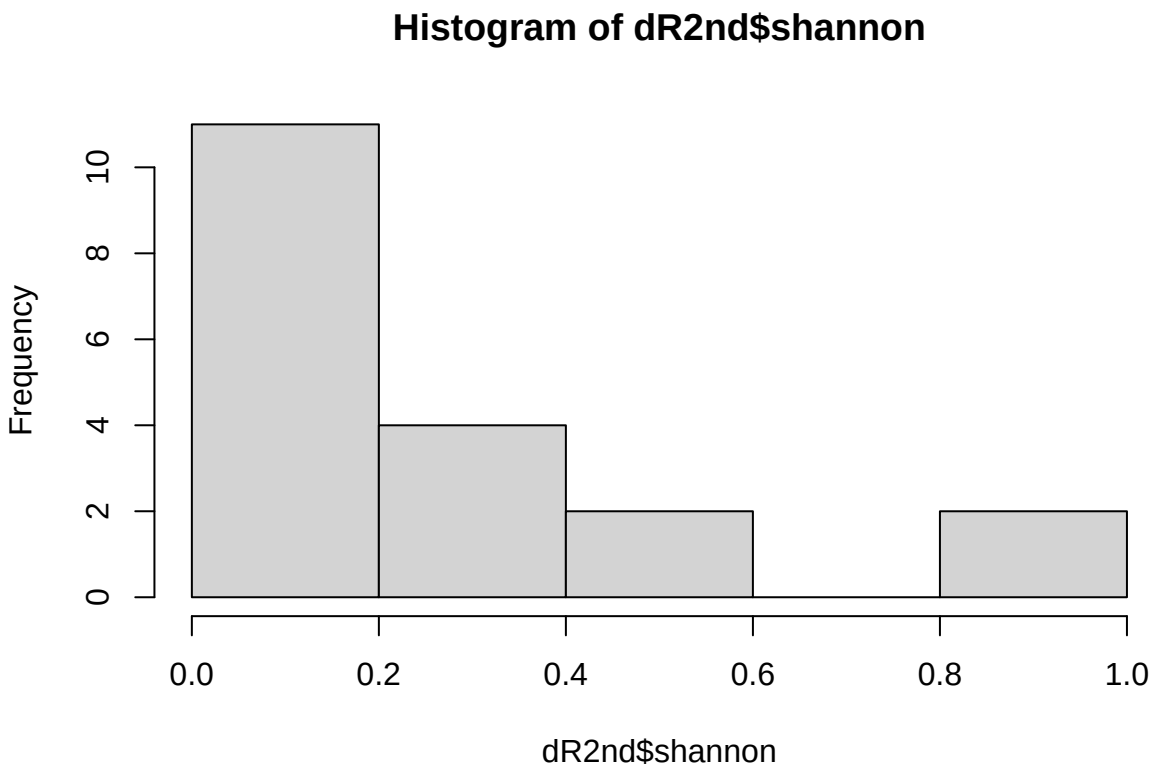
```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1424736
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.099, p-value = 0.9544
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.59005, p-value = 0.744
## 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 = 24, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1424736
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

```
plotResiduals(res_shan1)
```

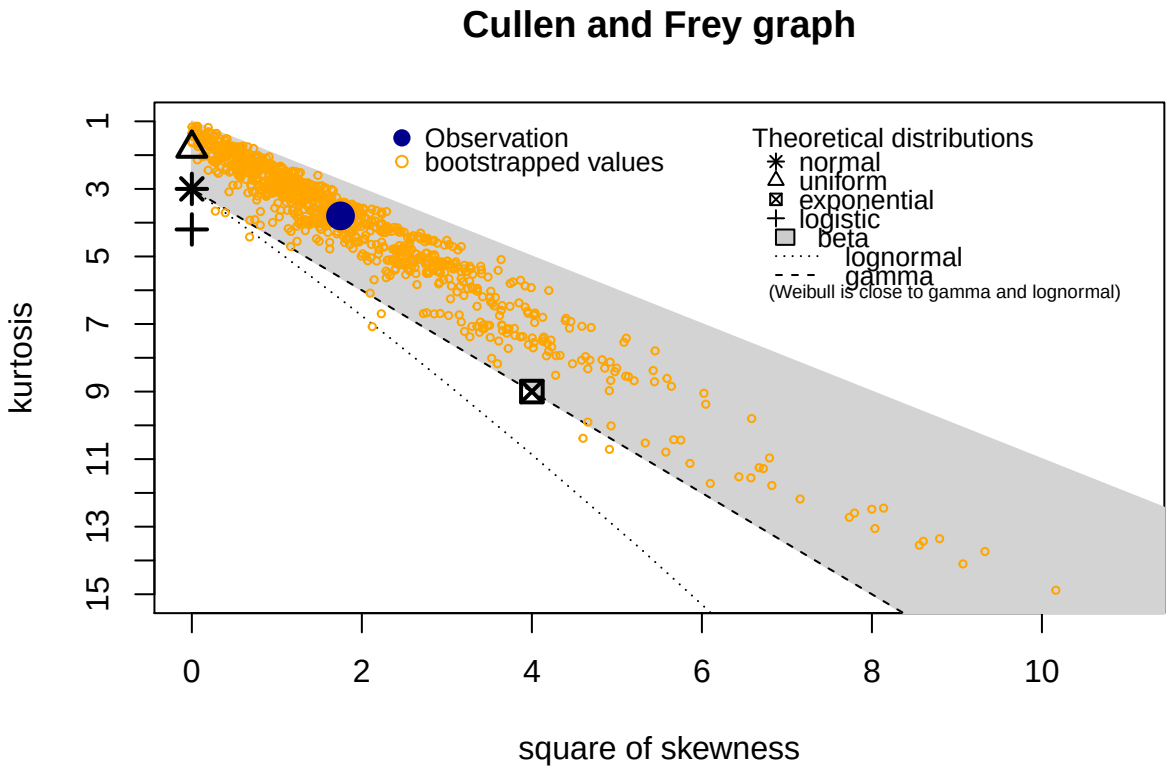


test with subset 2

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




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

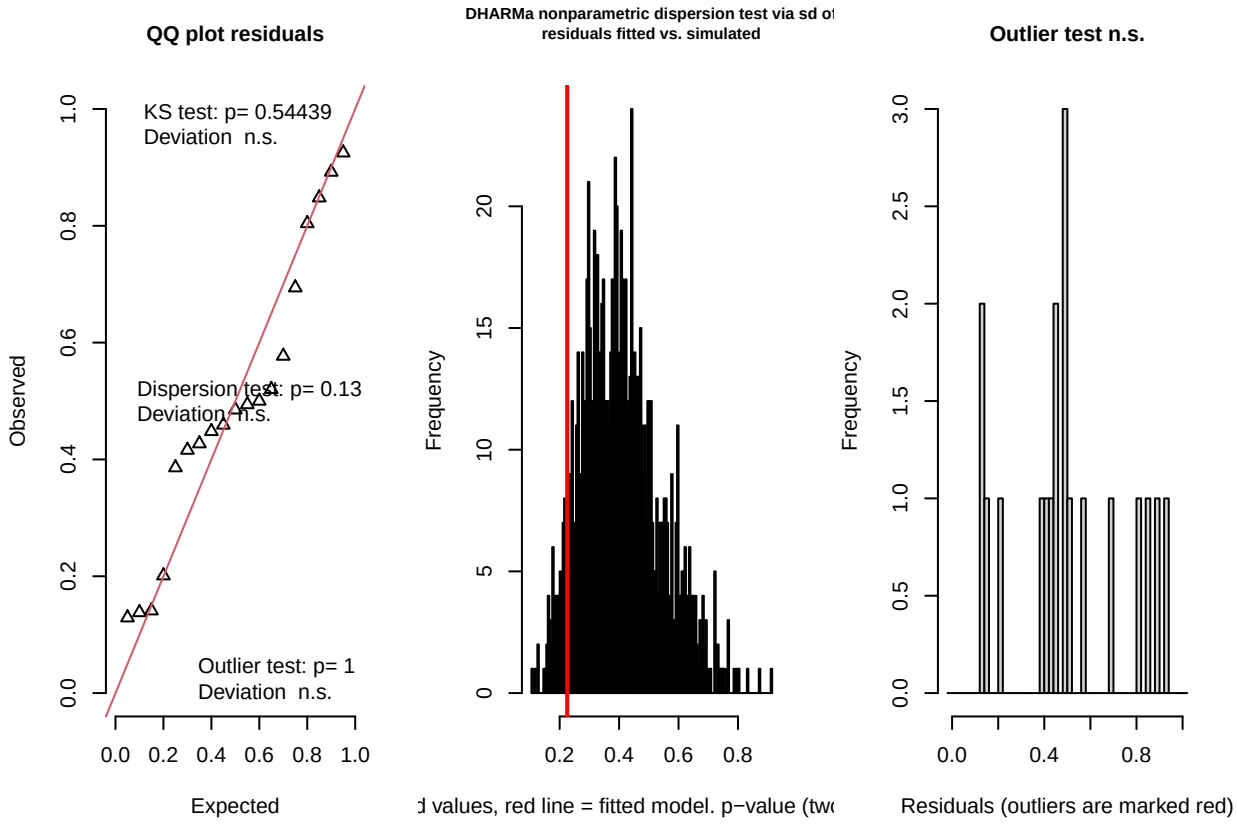



```
## summary statistics
## ------
## min:  0   max:  0.8483415
## median:  0.1594503
## mean:  0.2461942
## estimated sd:  0.2659691
## estimated skewness:  1.322517
## estimated kurtosis:  3.799409
```

```
logistic <- function(p) log(p / (1-p) +0.01)
shan1 <- lm(logistic(shannon) ~ Treatment + Linage, data = dR2nd)
Anova(shan1, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(shannon)
##           Sum Sq Df F value  Pr(>F)
## Treatment  0.622  1  0.4061 0.53829
## Linage    40.785  7  3.8062 0.02808 *
## Residuals 15.308 10
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

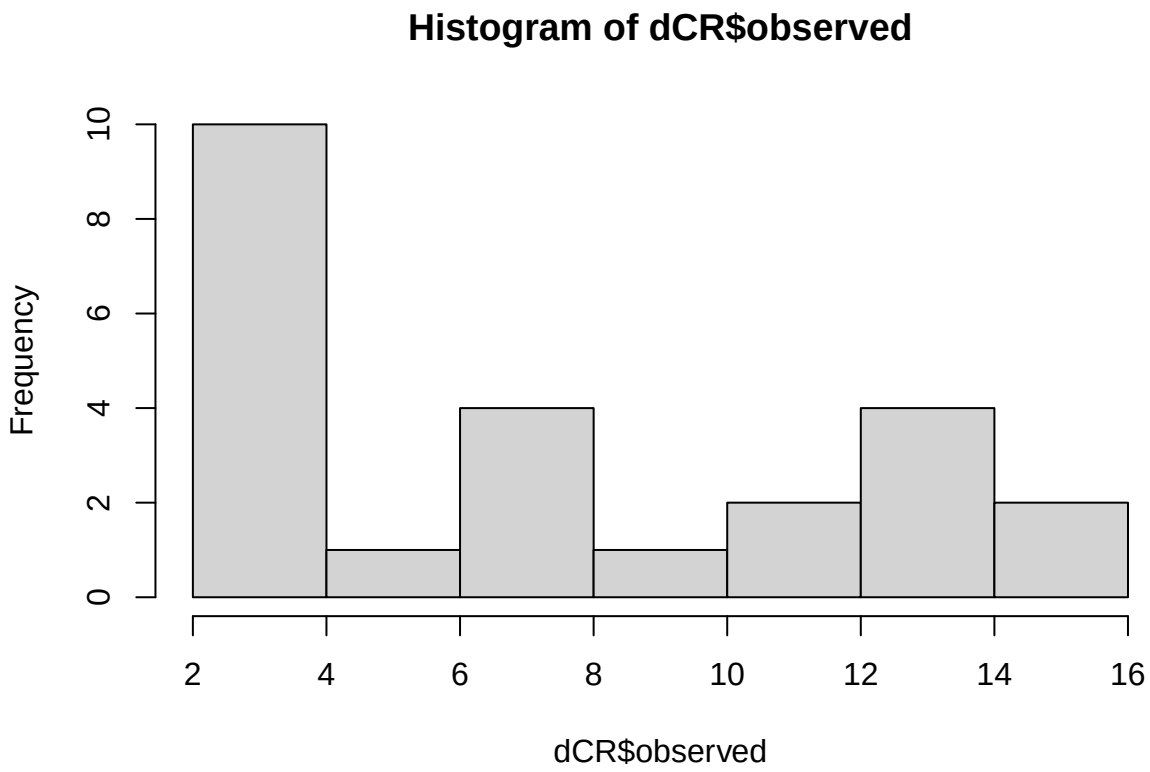
```
res_shan1 <- simulateResiduals(shan1, n = 1000)
testResiduals(res_shan1)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.17547, p-value = 0.5444
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.55459, p-value = 0.13
## alternative hypothesis: two.sided
##
##
## $outliers
##
##  DHARMa outlier test based on exact binomial test with approximate
```

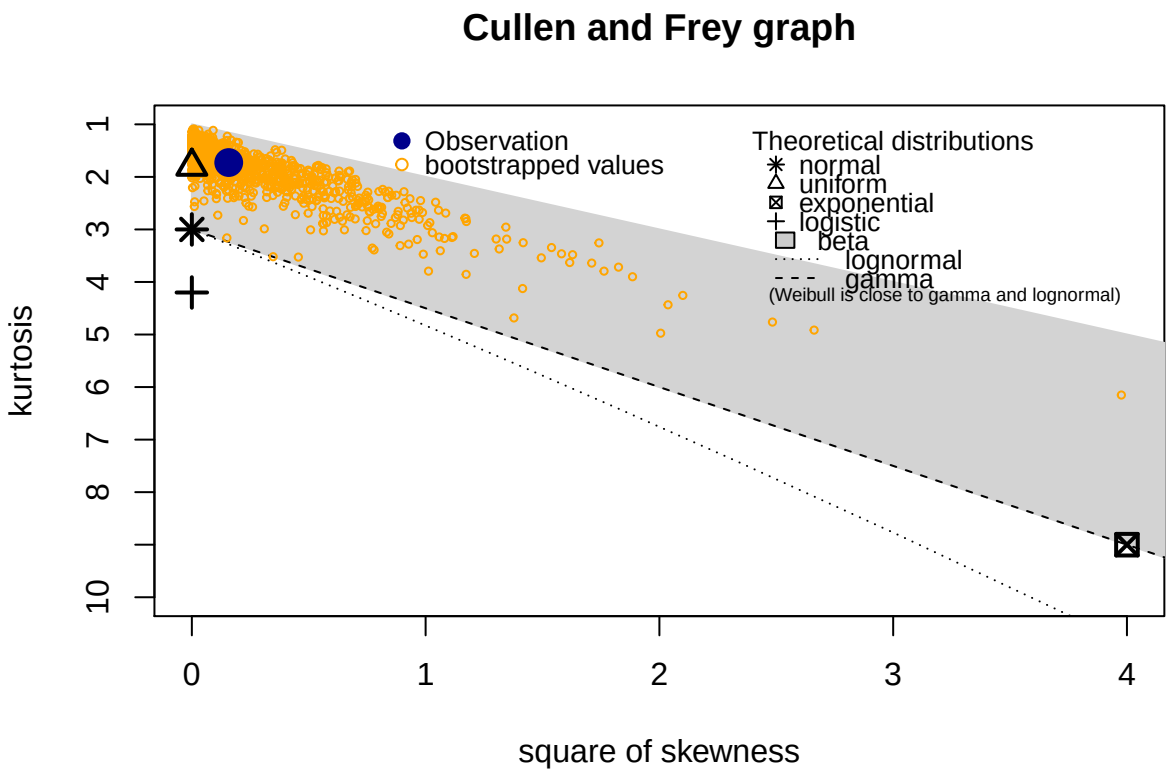
```
##  expectations
##
## data:  simulationOutput
## outliers at both margin(s) = 0, observations = 19, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1764669
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                    0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.17547, p-value = 0.5444
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.55459, p-value = 0.13
## 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 = 19, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1764669
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                    0
```

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



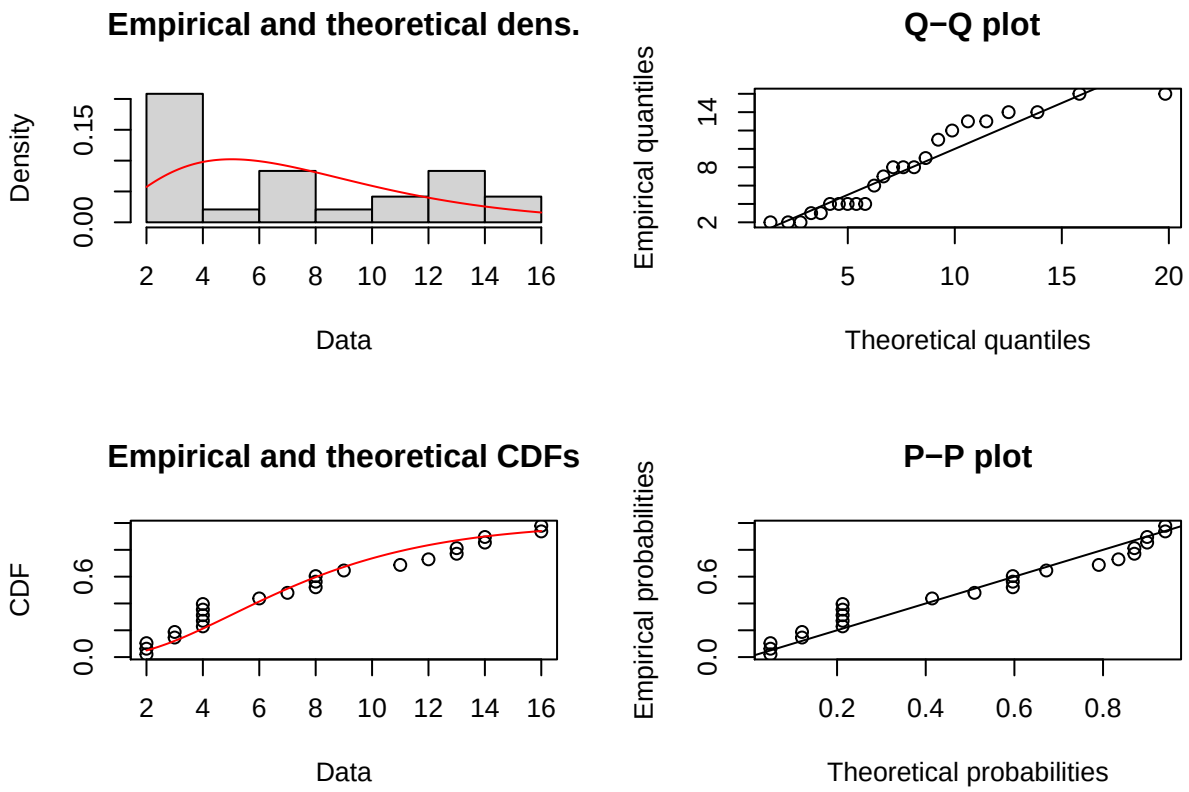
Observed richness

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




```
## summary statistics
## ------
## min:  2   max:  16
## median:  7.5
## mean:  7.791667
## estimated sd:  4.745517
## estimated skewness:  0.3971958
## estimated kurtosis:  1.7269
```

```
fit.gamma <- fitdist(dCR$observed, distr = "gamma", method = "mme")
plot(fit.gamma)
```



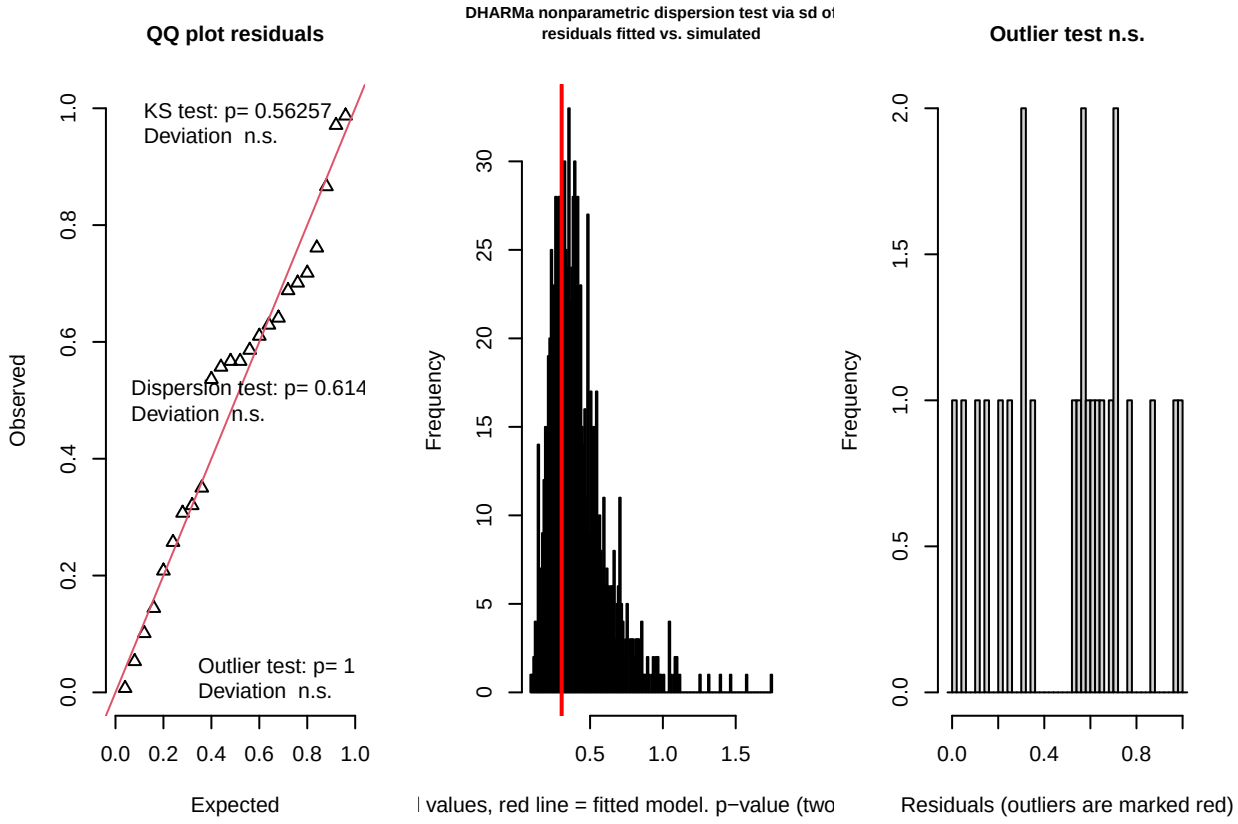
–> gamma distribution fits well

test with subset 1

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

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

```
## Analysis of Deviance Table (Type II tests)
##
## Response: observed
##           LR Chisq Df Pr(>Chisq)
## Treatment   8.8115  1   0.002993 **
## Linage     19.3559  8   0.013068 *
## ---
## 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 = 0.161, p-value = 0.5626
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.72767, p-value = 0.614
## 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 = 24, p-value = 1
```

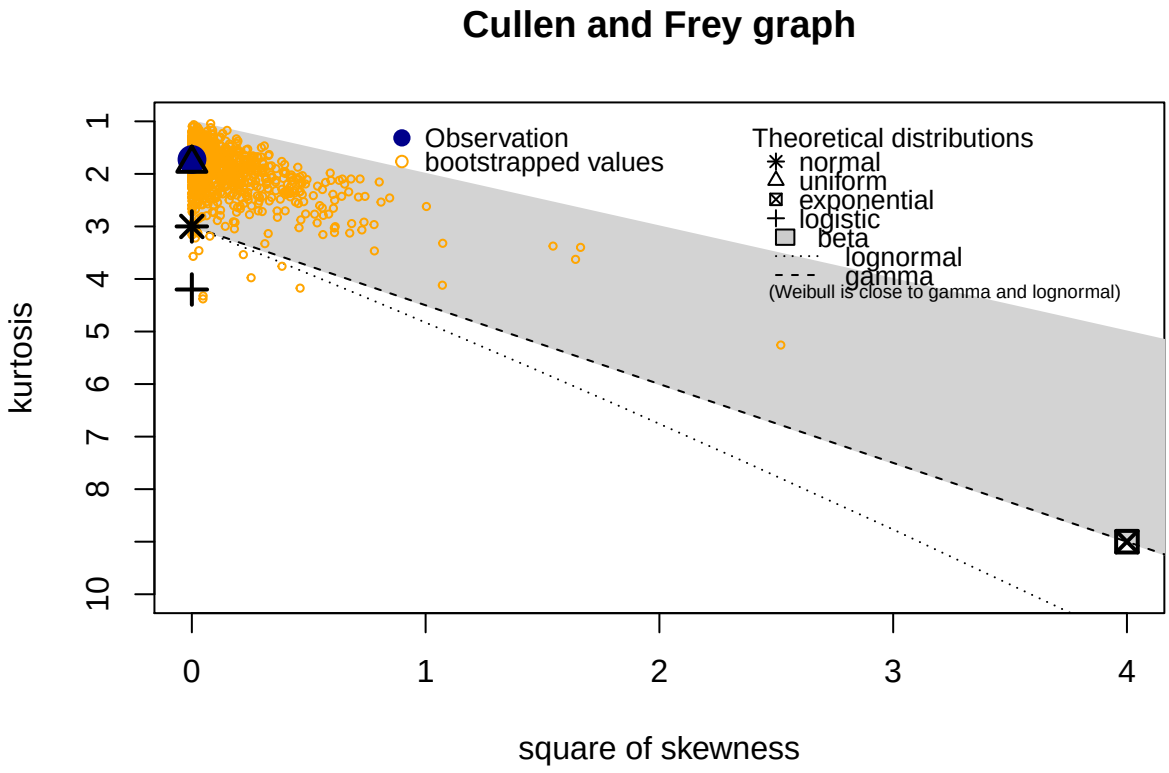
```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1424736
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.161, p-value = 0.5626
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.72767, p-value = 0.614
## 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 = 24, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1424736
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

test with subset 2

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


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

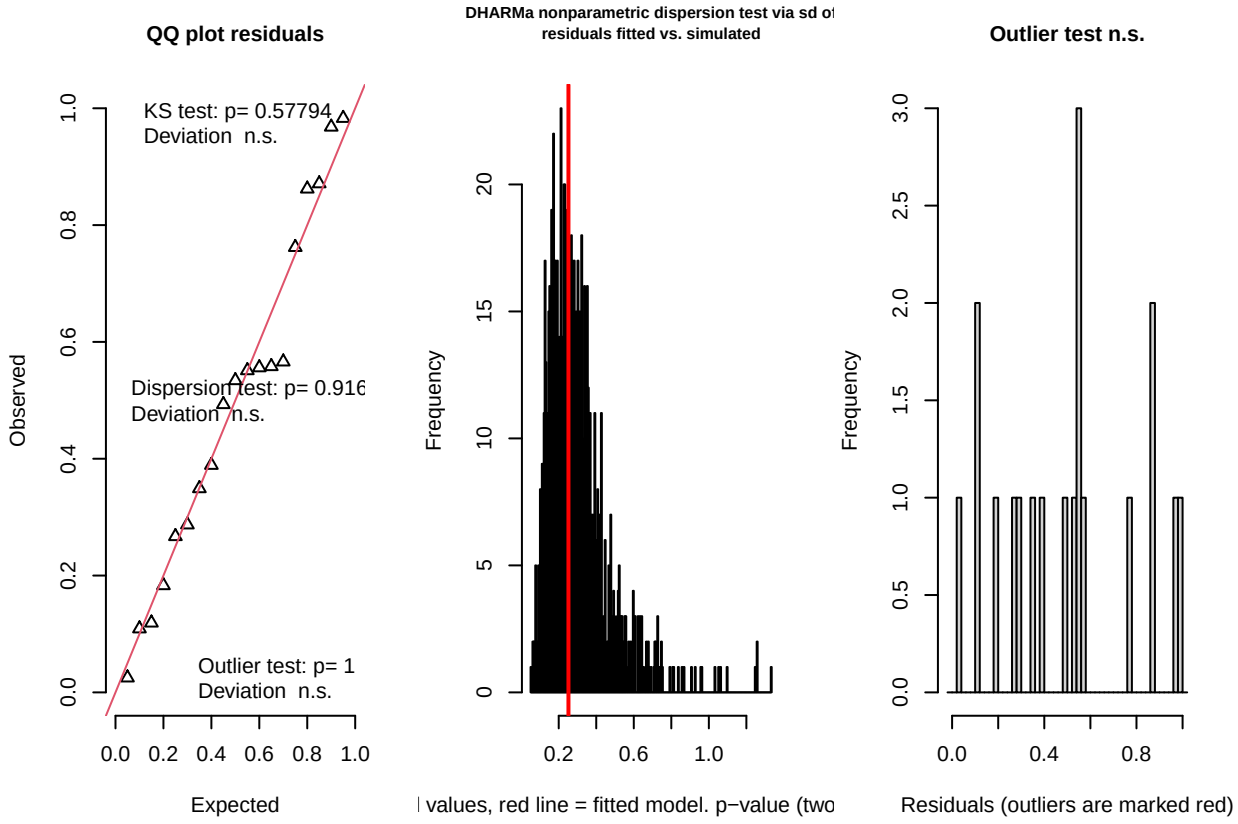



```
## summary statistics
## ------
## min:  1   max:  17
## median:  9
## mean:  8.947368
## estimated sd:  5.147531
## estimated skewness:  0.02563437
## estimated kurtosis:  1.725505
```

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

```
## Analysis of Deviance Table (Type II tests)
##
## Response: observed
##           LR Chisq Df Pr(>Chisq)
## Treatment   5.1938  1  0.0226677 *
## Linage     29.2485  7  0.0001303 ***
## ---
## 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 = 0.17084, p-value = 0.5779
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.84443, p-value = 0.916
## 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 = 19, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1764669
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                    0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.17084, p-value = 0.5779
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.84443, p-value = 0.916
## 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 = 19, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1764669
## 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.

transform total abundance data into compositional data

```
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 Genus level, transform to rel. abundance, melt long format and sort data frame alph. by Genus

```
Bacteria_Genus1 <- CR %>%
  tax_glom(taxrank = "Genus") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Genus)

Bacteria_Genus2 <- R2nd %>%
  tax_glom(taxrank = "Genus") %>%
  transform_sample_counts(function(x) {x/sum(x)} ) %>%
  psmelt() %>%
  arrange(Genus)
```

find the mean and standard deviation by Genus

```
mean.high.reads <- tapply(Bacteria_Genus2$Abundance, Bacteria_Genus2$Genus, mean)
mean.high.reads
```

```
##           Acinetobacter           Aeromicrobium            Azospirillum
##            1.457811e-05            1.265484e-05            9.036689e-05
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            2.620614e-06            5.171980e-05            1.810834e-04
##        Chryseobacterium                Demetria  Enterobacteriaceae_spc
##            7.768506e-05            6.872956e-05            1.142967e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            1.542575e-04            1.260451e-01            5.042467e-05
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            2.573011e-05            1.561368e-06            7.392703e-06
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            8.414631e-05            1.774612e-05            3.289585e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            6.574305e-03            4.222046e-06            1.292654e-02
##             Olivibacter              Pedobacter         Phyllobacterium
##            3.579871e-04            1.766378e-04            4.638771e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            2.414314e-04            6.327369e-01            2.091667e-05
##              Roseomonas             Rubrobacter              Salmonella
##            2.171624e-05            4.342752e-05            6.530945e-05
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            2.931454e-05            3.415974e-06            3.252822e-04
##          Staphylococcus        Stenotrophomonas             Veillonella
##            0.000000e+00            5.360464e-04            5.855029e-05
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            2.168947e-01            1.523172e-04            4.980774e-06
##             Xanthomonas
##            4.420340e-06
```

```
rem <- subset(Bacteria_Genus2, Treatment == "removal")
mean.rem <- tapply(rem$Abundance, rem$Genus, mean)
mean.rem
```

```
##           Acinetobacter           Aeromicrobium           Azospirillum
##            2.290846e-05            1.988618e-05            1.420051e-04
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            4.118107e-06            8.127397e-05            2.225556e-04
##        Chryseobacterium                Demetria  Enterobacteriaceae_spc
##            9.140731e-05            1.039717e-04            1.177764e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            1.563085e-04            1.547910e-01            7.923877e-05
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            3.340888e-05            2.453578e-06            0.000000e+00
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            4.118107e-06            2.788675e-05            4.327392e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            7.720834e-03            0.000000e+00            1.885603e-02
##             Olivibacter              Pedobacter         Phyllobacterium
##            5.318819e-04            1.765518e-04            6.294782e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            0.000000e+00            6.803950e-01            3.286906e-05
##              Roseomonas             Rubrobacter              Salmonella
##            3.412552e-05            7.504659e-06            4.062516e-05
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            4.606571e-05            5.367960e-06            5.061753e-04
##          Staphylococcus        Stenotrophomonas             Veillonella
##            0.000000e+00            5.058005e-04            0.000000e+00
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            1.330016e-01            1.141247e-04            0.000000e+00
##             Xanthomonas
##            2.914381e-06
```

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

```
##           Acinetobacter           Aeromicrobium           Azospirillum
##            8.571560e-05            3.922692e-05            5.313345e-04
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            1.540855e-05            2.548339e-04            4.411361e-04
##        Chryseobacterium                Demetria  Enterobacteriaceae_spc
##            2.627674e-04            3.890266e-04            3.213563e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            4.637591e-04            3.240921e-01            2.650834e-04
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            1.100232e-04            9.180449e-06            0.000000e+00
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            1.540855e-05            8.630467e-05            8.646594e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            1.312521e-02            0.000000e+00            6.904979e-02
##             Olivibacter              Pedobacter         Phyllobacterium
##            1.043470e-03            3.478402e-04            6.520588e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            0.000000e+00            4.157842e-01            5.922467e-05
##              Roseomonas             Rubrobacter              Salmonella
##            8.435820e-05            2.140827e-05            9.302948e-05
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            8.497767e-05            1.370364e-05            9.229474e-04
```

```
##          Staphylococcus        Stenotrophomonas             Veillonella
##            0.000000e+00            1.039782e-03            0.000000e+00
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            3.378380e-01            3.257274e-04            0.000000e+00
##             Xanthomonas
##            1.090462e-05
```

```
sec <- subset(Bacteria_Genus2, Treatment == "2nd-foundation")
mean.sec <- tapply(sec$Abundance, sec$Genus, mean)
mean.sec
```

```
##           Acinetobacter           Aeromicrobium            Azospirillum
##            0.000000e+00            0.000000e+00            0.000000e+00
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            0.000000e+00            0.000000e+00            1.085069e-04
##        Chryseobacterium                Demetria  Enterobacteriaceae_spc
##            5.367110e-05            7.055769e-06            1.082073e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            1.506684e-04            7.573989e-02            0.000000e+00
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            1.229226e-05            0.000000e+00            2.032993e-05
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            2.241957e-04            0.000000e+00            1.473423e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            4.567880e-03            1.161063e-05            2.549932e-03
##             Olivibacter              Pedobacter         Phyllobacterium
##            5.367110e-05            1.767884e-04            1.740752e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            6.639364e-04            5.493352e-01            0.000000e+00
##              Roseomonas             Rubrobacter              Salmonella
##            0.000000e+00            1.062925e-04            1.085069e-04
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            0.000000e+00            0.000000e+00            8.719308e-06
##          Staphylococcus        Stenotrophomonas             Veillonella
##            0.000000e+00            5.889766e-04            1.610133e-04
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            3.637075e-01            2.191541e-04            1.369713e-05
##             Xanthomonas
##            7.055769e-06
```

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

```
##           Acinetobacter           Aeromicrobium            Azospirillum
##            0.000000e+00            0.000000e+00            0.000000e+00
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            0.000000e+00            0.000000e+00            3.069040e-04
##        Chryseobacterium                Demetria  Enterobacteriaceae_spc
##            1.518048e-04            1.995673e-05            3.060565e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            4.261546e-04            2.077148e-01            0.000000e+00
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            3.476776e-05            0.000000e+00            3.814795e-05
```

```
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            5.974746e-04            0.000000e+00            3.370360e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            9.421317e-03            3.283981e-05            6.873753e-03
##             Olivibacter              Pedobacter         Phyllobacterium
##            1.518048e-04            4.499583e-04            3.111645e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            9.962397e-04            4.818013e-01            0.000000e+00
##              Roseomonas             Rubrobacter              Salmonella
##            0.000000e+00            3.006406e-04            3.069040e-04
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            0.000000e+00            0.000000e+00            2.466193e-05
##          Staphylococcus       Stenotrophomonas             Veillonella
##            0.000000e+00            1.665877e-03            4.554144e-04
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            4.945458e-01            6.198613e-04            3.874133e-05
##             Xanthomonas
##            1.995673e-05
```

```
control <- subset(Bacteria_Genus2, Treatment == "control")
mean.control <- tapply(control$Abundance, control$Genus, mean)
mean.control
```

```
## logical(0)
```

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

```
## logical(0)
```

```
SD.high.reads <- tapply(Bacteria_Genus2$Abundance, Bacteria_Genus2$Genus, sd)
SD.high.reads
```

```
##           Acinetobacter           Aeromicrobium            Azospirillum
##            6.837739e-05            3.237946e-05            4.238583e-04
##             Bacilli_spc                Bacillus       Bacteroidetes_spc
##            1.229177e-05            2.044566e-04            3.937223e-04
##        Chryseobacterium               Demetria  Enterobacteriaceae_spc
##            2.253220e-04            3.099959e-04            3.085039e-03
##   Enterobacteriales_spc                 Erwinia             Escherichia
##            4.400951e-04            2.844623e-01            2.121842e-04
##          Flavobacterium Gammaproteobacteria_spc             Gilliamella
##            8.946884e-05            7.323465e-06            2.419264e-05
##           Lactobacillus        Methylobacterium   Microbacteriaceae_spc
##            3.617742e-04            6.927843e-05            7.214100e-04
##          Microbacterium         Novosphingobium            Ochrobactrum
##            1.177459e-02            1.980315e-05            5.506131e-02
##             Olivibacter              Pedobacter         Phyllobacterium
##            8.585786e-04            3.773431e-04            5.880122e-04
##             Pseudomonas       Pseudoxanthomonas         Rhizobiales_spc
##            6.615849e-04            4.342354e-01            4.932805e-05
##              Roseomonas             Rubrobacter              Salmonella
```

```
##            6.846640e-05           1.810464e-04           1.946055e-04
## Sphingobacteriaceae_spc    Sphingobacteriia_spc        Sphingobacterium
##            7.060245e-05           1.110119e-05           7.664973e-04
##          Staphylococcus        Stenotrophomonas             Veillonella
##            0.000000e+00           1.263332e-03           2.746252e-04
##               Wolbachia    Xanthomonadaceae_spc     Xanthomonadales_spc
##            4.063040e-01           4.432044e-04           2.336190e-05
##             Xanthomonas
##            1.450952e-05
```

edit object for plotting

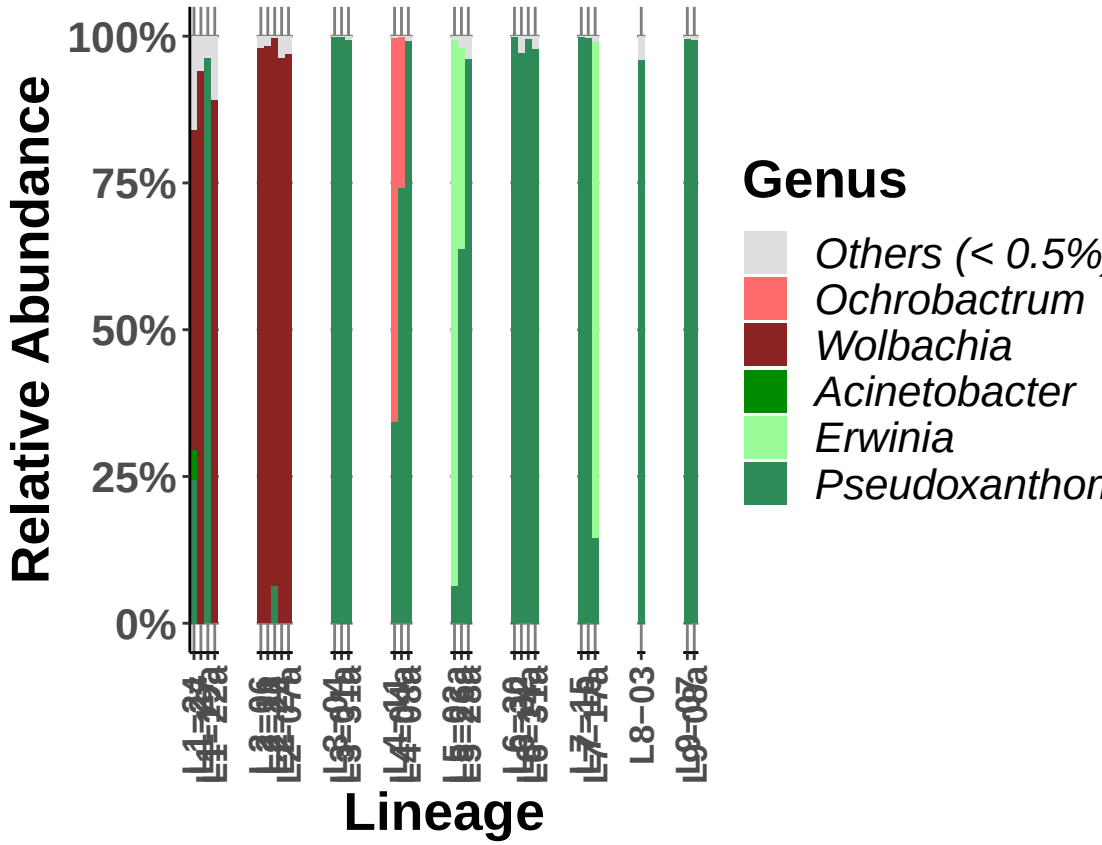
```
#control vs. removal
Bacteria_Genus1$Genus<-as.character(Bacteria_Genus1$Genus)
Bacteria_Genus1$Genus[Bacteria_Genus1$Abundance<0.05]<-"Others"
Bacteria_Genus1$Class<-as.character(Bacteria_Genus1$Class)
Bacteria_Genus1$Class[Bacteria_Genus1$Abundance<0.05]<-"Others"
Bacteria_Genus1$Genus<-factor(Bacteria_Genus1$Genus)
Bacteria_Genus1<-droplevels(Bacteria_Genus1)
Bacteria_Genus1$Treatment<-factor(Bacteria_Genus1$Treatment,levels=c("control", "removal"))
Bacteria_Genus1$Genus <- factor(Bacteria_Genus1$Genus, levels = c("Others",  "Ochrobactrum", "Wolbachia

#removal vs. 2^nd^ attempt
Bacteria_Genus2$Genus<-as.character(Bacteria_Genus2$Genus)
Bacteria_Genus2$Genus[Bacteria_Genus2$Abundance<0.05]<-"Others"
Bacteria_Genus2$Class<-as.character(Bacteria_Genus2$Class)
Bacteria_Genus2$Class[Bacteria_Genus2$Abundance<0.05]<-"Others"
Bacteria_Genus2$Genus<-factor(Bacteria_Genus2$Genus)
Bacteria_Genus2<-droplevels(Bacteria_Genus2)
Bacteria_Genus2$Treatment<-factor(Bacteria_Genus2$Treatment,levels=c("removal", "2nd-foundation"))
Bacteria_Genus2$Genus <- factor(Bacteria_Genus2$Genus, levels = c("Others",  "Ochrobactrum", "Wolbachia
```

define color bar

```
Plot_colors_g <- c("grey87", "indianred1", "brown4", "green4", "palegreen", "seagreen")
Plot_colors_g2 <- c("grey87", "indianred1","brown4", "palegreen", "seagreen")
```

```
Bac <- Bacteria_Genus1
Bac$Sample <- factor(Bac$Sample, levels = c("B0-24", "B0-27", "B0-19a", "B0-22a", "B15-06", "B15-11", "

Bacteria_Genus_plot <- ggplot(Bac, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", color = NA,  position="fill") +
  scale_fill_manual(values = Plot_colors_g, name = "Genus")

relAB_bac <-Bacteria_Genus_plot +
  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="Lineage", y="Relative Abundance")+
```

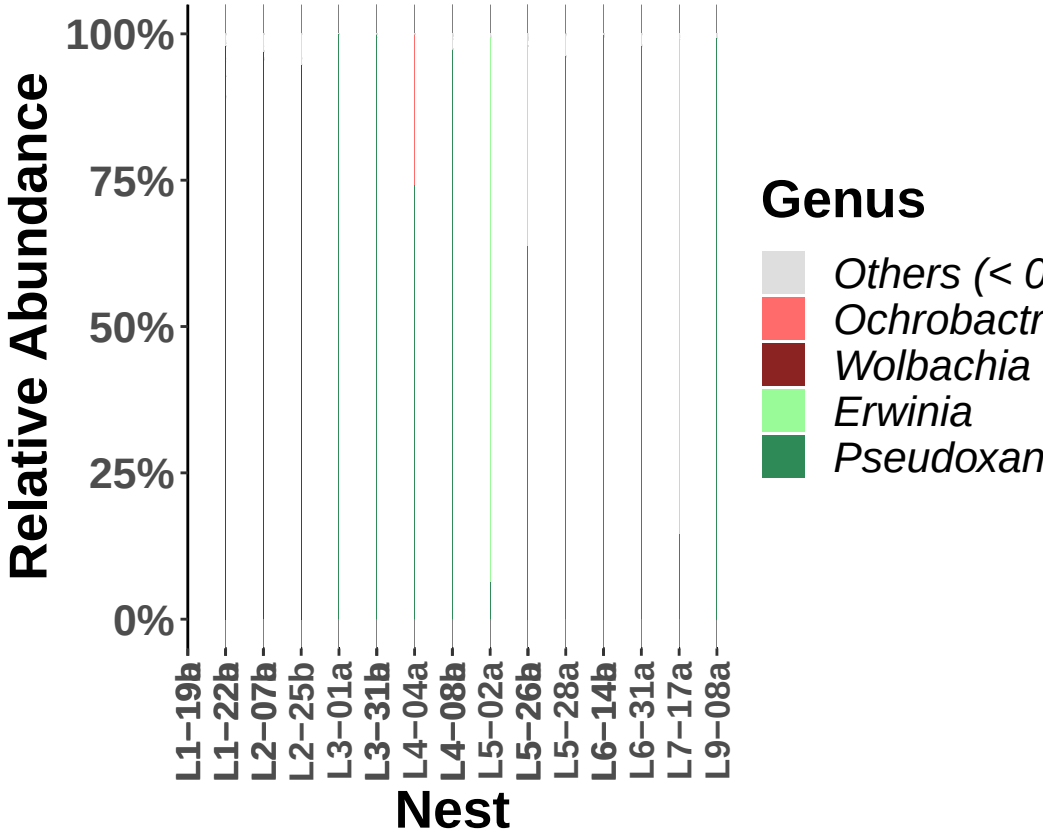
```
  scale_y_continuous(labels=percent_format())+
  theme(axis.text.x=element_text(size = 13, angle = 90, vjust = 0.5))+
  theme(text = element_text(size=20, face = "bold"))+
  theme(legend.title = element_text(size = 20), legend.text = element_text(size = 16))+
  theme(legend.text = element_text(face = "italic"))+
  theme(strip.text.x = element_blank())

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

relAB_bac
```



plot control vs. removal

```
Bac2 <- Bacteria_Genus2
Bac2$Sample <- factor(Bac2$Sample, levels = c("B0-19a", "B0-19b", "B0-22a", "B0-22b", "B15-07a", "B15-07

Bacteria_Genus2_plot2 <- ggplot(Bac2, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", color = NA,  position="fill") +
  scale_fill_manual(values = Plot_colors_g2, name = "Genus")

g4<-Bacteria_Genus2_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_bac2 <- g4 + theme(panel.spacing.x = unit(0.5, "cm"))

relAb_bac2
```



plot removal vs. 2nd-attempt

Microbiome composition

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

carefull, plot was created with fungal data additionally

load fungal data and merge with bacteria

```
CR_fun <- readRDS("//NAS/home/Analysis Removal Experiment/LSU_stand_13.04.22/CR_fun.rds")
rel.CR_fun <- CR_fun %>%
  transform_sample_counts(function(x) {x/sum(x)} )
```

```
R2nd_fun <- readRDS("//NAS/home/Analysis Removal Experiment/LSU_stand_13.04.22/R2nd_fun.rds")
rel.R2nd_fun <- R2nd_fun %>%
  transform_sample_counts(function(x) {x/sum(x)} )
```

```
#Bacteria
pseqB <- aggregate_rare(rel.CR, level = "Species", detection = 5/100, prevalence = 10/100)
pseqBh <- pseqB %>%
  psmelt()

#Fungi
pseqF <- aggregate_rare(rel.CR_fun, level = "Species", detection = 5/100, prevalence = 10/100)
pseqFh <- pseqF %>%
  psmelt()
```

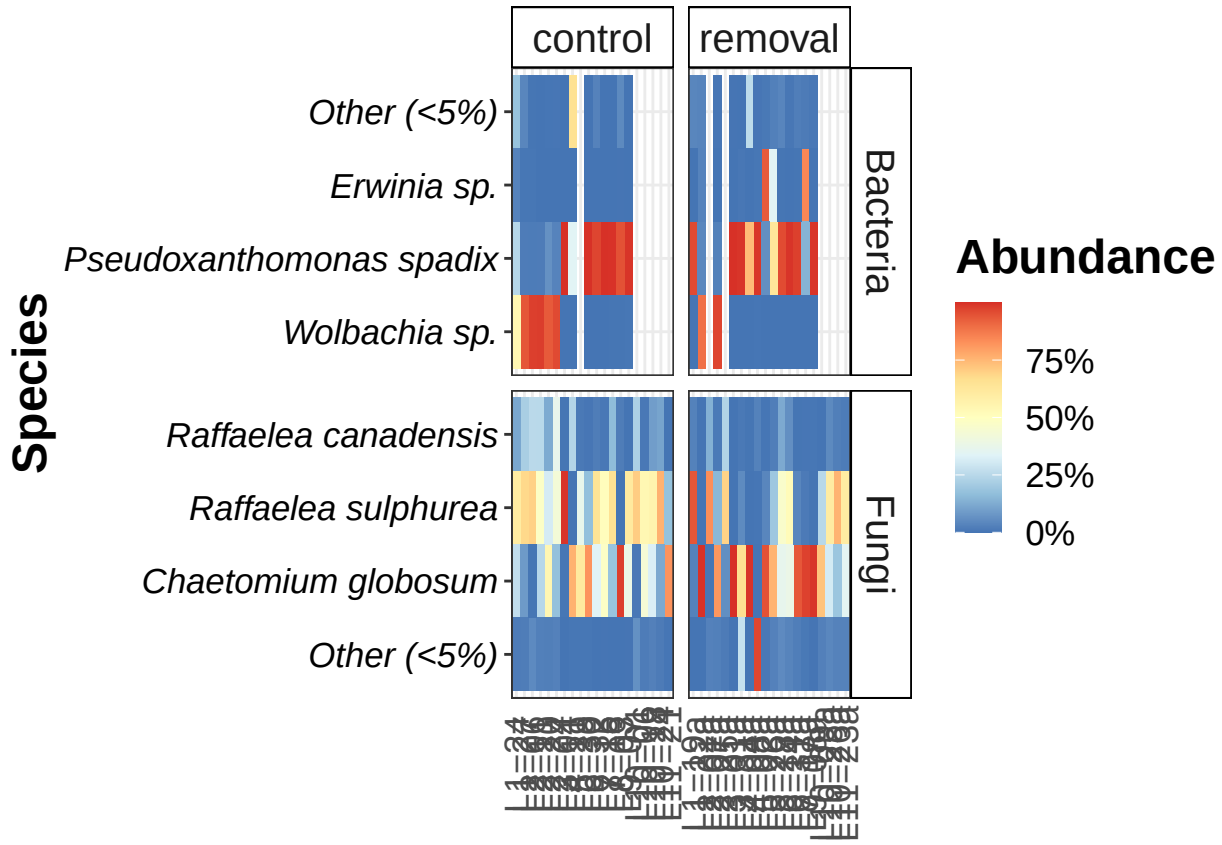
combine both dataframes and prepare for plotting

```
pseqh <- bind_rows(pseqBh, pseqFh)

pseqh$Treatment<-factor(pseqh$Treatment,levels=c("control", "removal"))
pseqh$Group<-factor(pseqh$Group,levels=c("Bacteria", "Fungi"))
pseqh$Species<-factor(pseqh$Species,levels=c("Wolbachia_spc", "Pseudoxanthomonas_spadix", "Erwinia_spc"
pseqh$Sample <- factor(pseqh$Sample, levels = c("B0-24", "B0-27", "B0-19a", "B0-22a", "B15-06", "B15-11
```

plot heatmap

```
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) + the

# 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(Group ~ Treatment, scales = "free", space = "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 <- 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"))
```

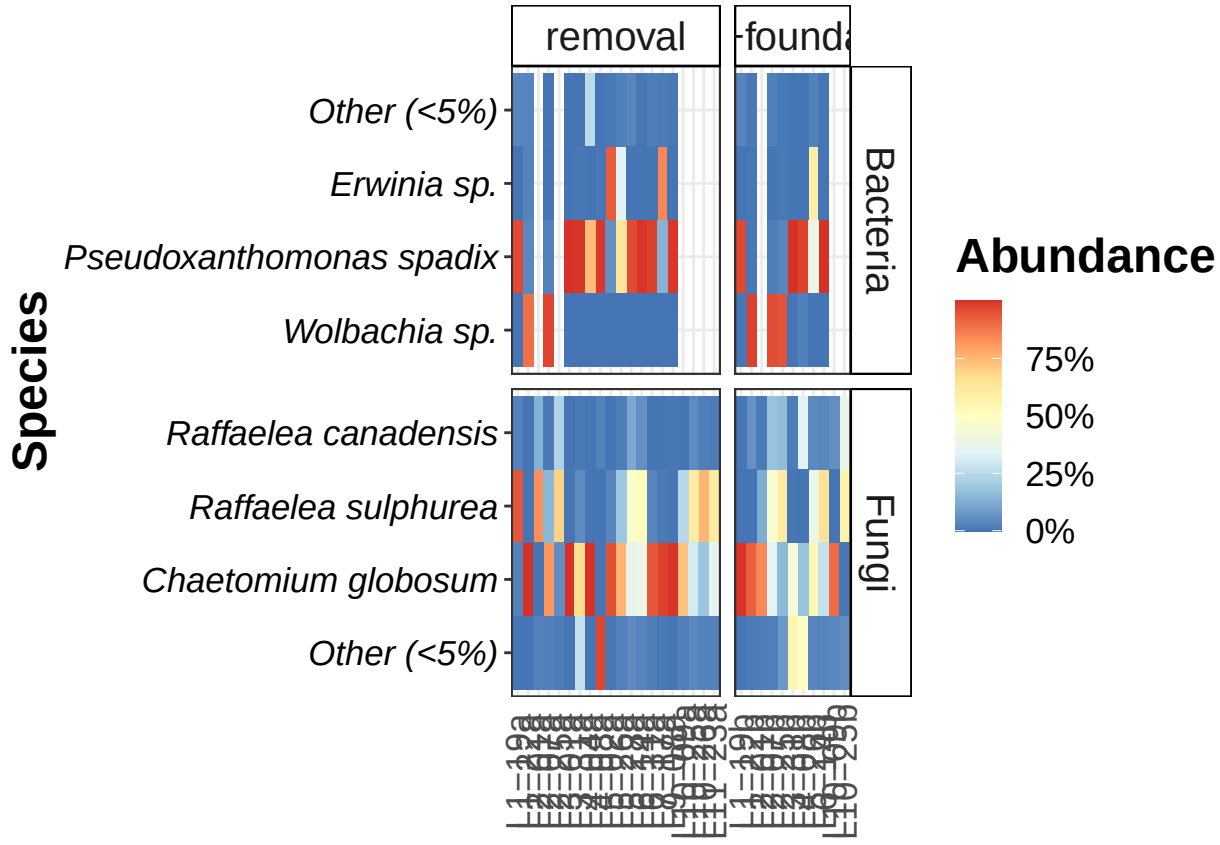
```
p.heat
```



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

```
pseqB2 <- aggregate_rare(rel.R2nd, level = "Species", detection = 5/100, prevalence = 10/100)
pseqBh2 <- pseqB2 %>%
  psmelt()

pseqF2 <- aggregate_rare(rel.R2nd_fun, level = "Species", detection = 5/100, prevalence = 10/100)
pseqFh2 <- pseqF2 %>%
  psmelt()

pseqh2 <- bind_rows(pseqBh2, pseqFh2)

pseqh2$Treatment<-factor(pseqh2$Treatment,levels=c("removal", "2nd-foundation"))
pseqh2$Group<-factor(pseqh2$Group,levels=c("Bacteria", "Fungi"))
pseqh2$Species<-factor(pseqh2$Species,levels=c("Wolbachia_spc",  "Pseudoxanthomonas_spadix",  "Erwinia_s
pseqh2$Sample <- factor(pseqh2$Sample, levels = c("B0-19a", "B0-19b", "B0-22a", "B0-22b", "B15-01a", "B

p.heat <- ggplot(pseqh2, 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) + the

# 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(Group ~ Treatment, scales = "free", space = "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 <- 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

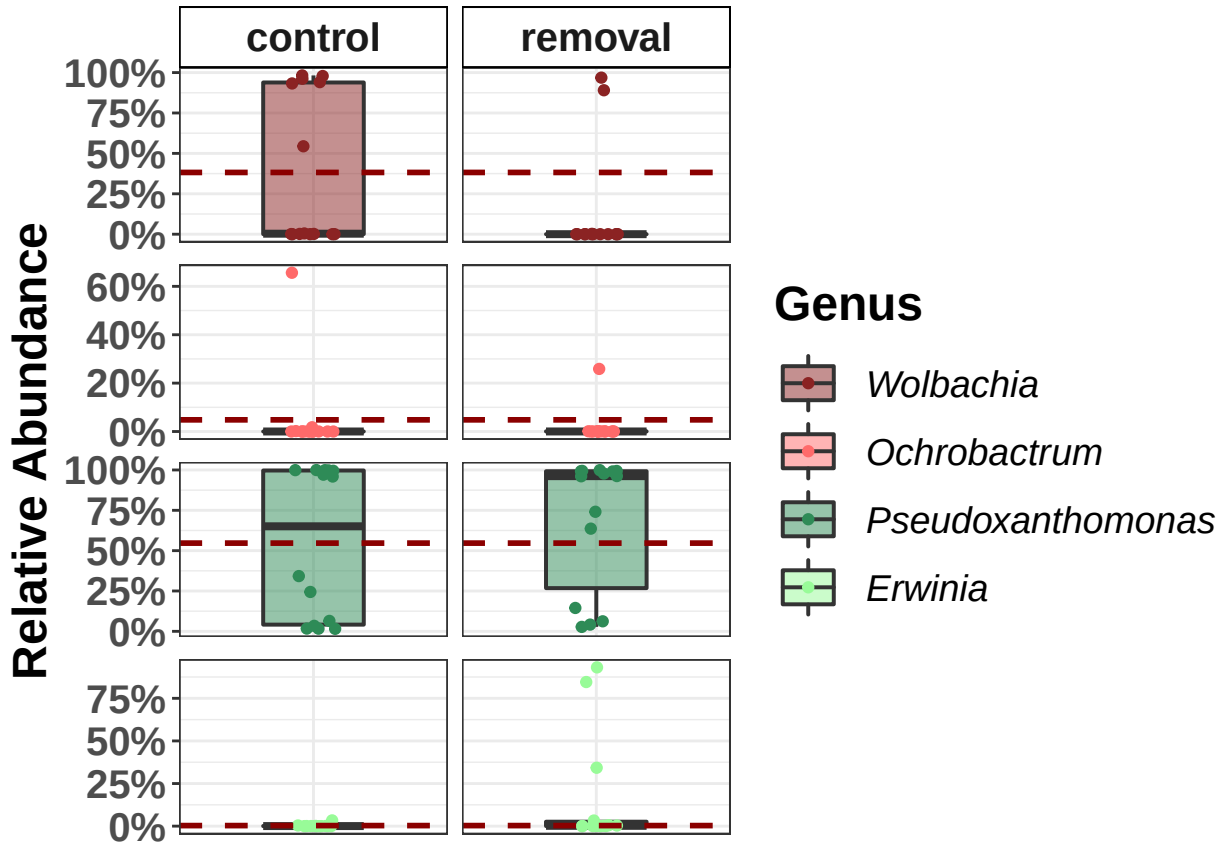
```
colpal_bac <- c("brown4", "indianred1", "seagreen", "palegreen")
colpal_bac2 <- c("indianred1", "seagreen", "palegreen")
```

plot core taxa of control vs. removal with relative abundance

```
ps4 <- prune_taxa(taxa_sums(rel.CR) > 0, rel.CR)
ps4 <- tax_glom(ps4, taxrank = 'Genus')
psOrd4 = subset_taxa(ps4, Genus=="Wolbachia" | Genus=="Pseudoxanthomonas" | Genus=="Ochrobactrum" | Genu
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$Genus <- factor(melt3$Genus, levels = c("Wolbachia", "Ochrobactrum", "Pseudoxanthomonas", "Erwinia
melt4$Treament <- factor(melt4$Treatment, levels = c("control","removal"))
melt4$Genus <- factor(melt4$Genus, levels = c("Wolbachia", "Ochrobactrum", "Pseudoxanthomonas", "Erwinia
a_mean <- melt4 %>%
  group_by(Genus) %>%
  summarize(mean_val = mean(Abundance))
print(a_mean)
```

```
## # A tibble: 4 x 2
##   Genus             mean_val
##   <fct>                <dbl>
## 1 Wolbachia          0.382
## 2 Ochrobactrum       0.0483
## 3 Pseudoxanthomonas  0.547
## 4 Erwinia            0.00322
```

```
p2<-ggplot(data = melt3, aes(x = Treatment, y = Abundance)) +
  geom_boxplot(aes(fill=Genus),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,ou
  scale_fill_manual(values = colpal_bac, name = "Genus")+
  scale_color_manual(values = colpal_bac, name = "Genus")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Genus~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
```

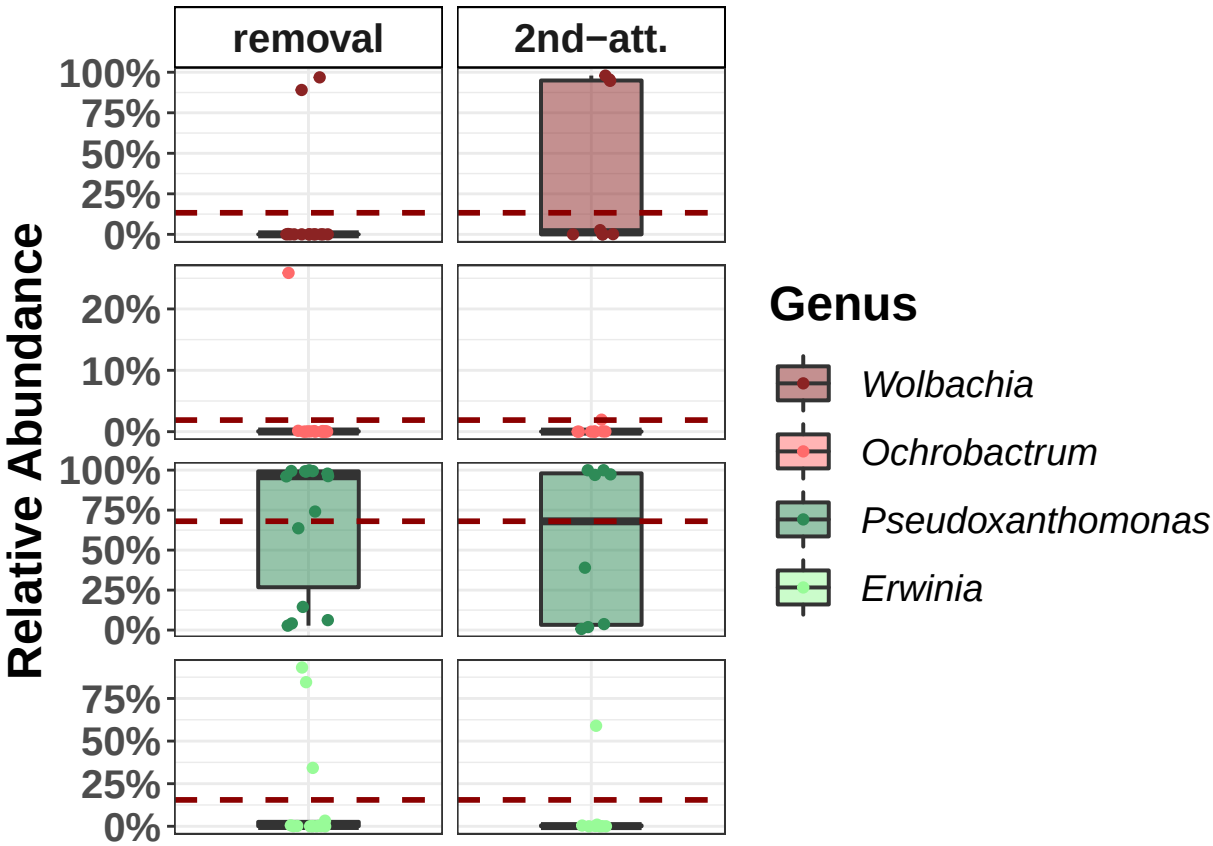


plot core taxa of removal vs. 2nd attempt with relative abundance

```
Treats <- c('removal' = "removal", '2nd-foundation' = "2nd-att.")
ps4 <- prune_taxa(taxa_sums(rel.R2nd) > 0, rel.R2nd)
ps4 <- tax_glom(ps4, taxrank = 'Genus')
psOrd4 = subset_taxa(ps4, Genus=="Wolbachia" | Genus=="Pseudoxanthomonas" | Genus=="Ochrobactrum" | Genu
psctr = subset_samples(psOrd4, Treatment=="removal")
#Melt and plot
melt<-psmelt(psOrd4)
melt2<-psmelt(psctr)
melt$Treament <- factor(melt$Treatment, levels = c("removal", "2nd-foundation")) #, labels = c("removal
melt$Genus <- factor(melt$Genus, levels = c("Wolbachia", "Ochrobactrum", "Pseudoxanthomonas", "Erwinia"
melt2$Treament <- factor(melt2$Treatment, levels = c("removal", "2nd-foundation")) #, labels = c("remov
melt2$Genus <- factor(melt2$Genus, levels = c("Wolbachia", "Ochrobactrum", "Pseudoxanthomonas", "Erwini
a_mean <- melt2 %>%
  group_by(Genus) %>%
  summarize(mean_val = mean(Abundance))
print(a_mean)
```

```
## # A tibble: 4 x 2
##   Genus              mean_val
##   <fct>                 <dbl>
## 1 Wolbachia           0.133
## 2 Ochrobactrum        0.0189
## 3 Pseudoxanthomonas   0.680
## 4 Erwinia             0.155
```

```
p<-ggplot(data = melt, aes(x = Treatment, y = Abundance)) +
  geom_boxplot(aes(fill=Genus),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,ou
  scale_fill_manual(values = colpal_bac, name = "Genus")+
  scale_color_manual(values = colpal_bac, name = "Genus")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Genus~fct_relevel(Treatment, "removal", "2nd-foundation"), labeller = as_labeller(Treats),
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"))
TreatmentR2nd<-abu+theme(axis.title.y = element_text(size=18, face="bold"))+theme(axis.text.y = element_
  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_bac <- TreatmentR2nd + theme(panel.spacing.y = unit(0.3, "cm"))

relAbcoreR2nd_bac
```



Ordination analysis

Sample ordination

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

NMDS

```
## Run 0 stress 0.004101445
## Run 1 stress 0.004098865
## ... New best solution
## ... Procrustes: rmse 0.002824601  max resid 0.008262479
## ... Similar to previous best
## Run 2 stress 0.004117503
## ... Procrustes: rmse 0.001517589  max resid 0.003808871
## ... Similar to previous best
## Run 3 stress 0.004076859
## ... New best solution
## ... Procrustes: rmse 0.002901198  max resid 0.01008774
## Run 4 stress 0.004059221
## ... New best solution
## ... Procrustes: rmse 0.002873452  max resid 0.007736466
## ... Similar to previous best
## Run 5 stress 0.004082078
## ... Procrustes: rmse 0.001587272  max resid 0.004786337
## ... Similar to previous best
## Run 6 stress 0.004063495
## ... Procrustes: rmse 0.0007269976  max resid 0.002142316
## ... Similar to previous best
## Run 7 stress 0.006355146
## Run 8 stress 0.004066319
## ... Procrustes: rmse 0.001770016  max resid 0.004658518
## ... Similar to previous best
## Run 9 stress 0.004082557
## ... Procrustes: rmse 0.001916299  max resid 0.005164159
## ... Similar to previous best
## Run 10 stress 0.004104849
## ... Procrustes: rmse 0.001891373  max resid 0.004529891
## ... Similar to previous best
## Run 11 stress 0.00551348
## Run 12 stress 0.004050816
## ... New best solution
## ... Procrustes: rmse 0.002384241  max resid 0.007869152
## ... Similar to previous best
## Run 13 stress 0.004105757
## ... Procrustes: rmse 0.003220953  max resid 0.01171721
## Run 14 stress 0.00411733
## ... Procrustes: rmse 0.004184579  max resid 0.01101352
## Run 15 stress 0.004205084
## ... Procrustes: rmse 0.005207008  max resid 0.01382579
## Run 16 stress 0.004059522
## ... Procrustes: rmse 0.002206135  max resid 0.007141013
## ... Similar to previous best
## Run 17 stress 0.004066419
## ... Procrustes: rmse 0.002952615  max resid 0.009668699
```

```
## ... Similar to previous best
## Run 18 stress 0.004237588
## ... Procrustes: rmse 0.004326122  max resid 0.01153981
## Run 19 stress 0.007574037
## Run 20 stress 0.004062869
## ... Procrustes: rmse 0.002709043  max resid 0.00885975
## ... Similar to previous best
## *** Solution reached
```

```
ordi.CR$stress
```

```
## [1] 0.004050816
```

```
set.seed(1)
ordi.R2nd = ordinate(rel.R2nd, "NMDS", "bray", k=3, trymax=100)
```

```
## Run 0 stress 0.000497131
## Run 1 stress 0.00197463
## Run 2 stress 0.001703256
## Run 3 stress 0.001308364
## Run 4 stress 0.001248164
## Run 5 stress 0.001519522
## Run 6 stress 0.001343547
## Run 7 stress 0.001598612
## Run 8 stress 0.001721315
## Run 9 stress 0.001897331
## Run 10 stress 0.001720918
## Run 11 stress 0.001865885
## Run 12 stress 0.00113327
## Run 13 stress 0.002059709
## Run 14 stress 0.001943087
## Run 15 stress 0.001545742
## Run 16 stress 0.001559676
## Run 17 stress 0.001704741
## Run 18 stress 0.002029385
## Run 19 stress 0.001869968
## Run 20 stress 0.001005818
## Run 21 stress 0.002108947
## Run 22 stress 0.001967605
## Run 23 stress 0.001303404
## Run 24 stress 0.001647096
## Run 25 stress 0.001852263
## Run 26 stress 0.002365609
## Run 27 stress 0.001375455
## Run 28 stress 0.001762234
## Run 29 stress 0.001435289
## Run 30 stress 0.002406851
## Run 31 stress 0.001297131
## Run 32 stress 0.002199374
## Run 33 stress 0.001483006
## Run 34 stress 0.001361984
## Run 35 stress 0.00181785
## Run 36 stress 0.001490938
```

```
## Run 37 stress 0.002538429
## Run 38 stress 0.002129452
## Run 39 stress 0.002098761
## Run 40 stress 0.001472975
## Run 41 stress 0.001515251
## Run 42 stress 0.001708189
## Run 43 stress 0.002163114
## Run 44 stress 0.001851951
## Run 45 stress 0.0008179611
## ... Procrustes: rmse 0.006359988  max resid 0.01984187
## Run 46 stress 0.001648781
## Run 47 stress 0.001419709
## Run 48 stress 0.002341615
## Run 49 stress 0.002229777
## Run 50 stress 0.001347667
## Run 51 stress 0.002041472
## Run 52 stress 0.001705717
## Run 53 stress 0.001793895
## Run 54 stress 0.001430107
## Run 55 stress 0.001735565
## Run 56 stress 0.001664429
## Run 57 stress 0.002675406
## Run 58 stress 0.001438074
## Run 59 stress 0.002363232
## Run 60 stress 0.001713227
## Run 61 stress 0.001733833
## Run 62 stress 0.001718886
## Run 63 stress 0.001257307
## Run 64 stress 0.001601473
## Run 65 stress 0.001702062
## Run 66 stress 0.001763214
## Run 67 stress 0.001740427
## Run 68 stress 0.00202921
## Run 69 stress 0.001591445
## Run 70 stress 0.001584794
## Run 71 stress 0.00144915
## Run 72 stress 0.002198814
## Run 73 stress 0.001753384
## Run 74 stress 0.001338002
## Run 75 stress 0.002008779
## Run 76 stress 0.001476132
## Run 77 stress 0.002114631
## Run 78 stress 0.001935933
## Run 79 stress 0.001514252
## Run 80 stress 0.001411258
## Run 81 stress 0.001563949
## Run 82 stress 0.001646717
## Run 83 stress 0.001543691
## Run 84 stress 0.001656827
## Run 85 stress 0.00196587
## Run 86 stress 0.00180755
## Run 87 stress 0.001456862
## Run 88 stress 0.001799494
## Run 89 stress 0.001490406
```

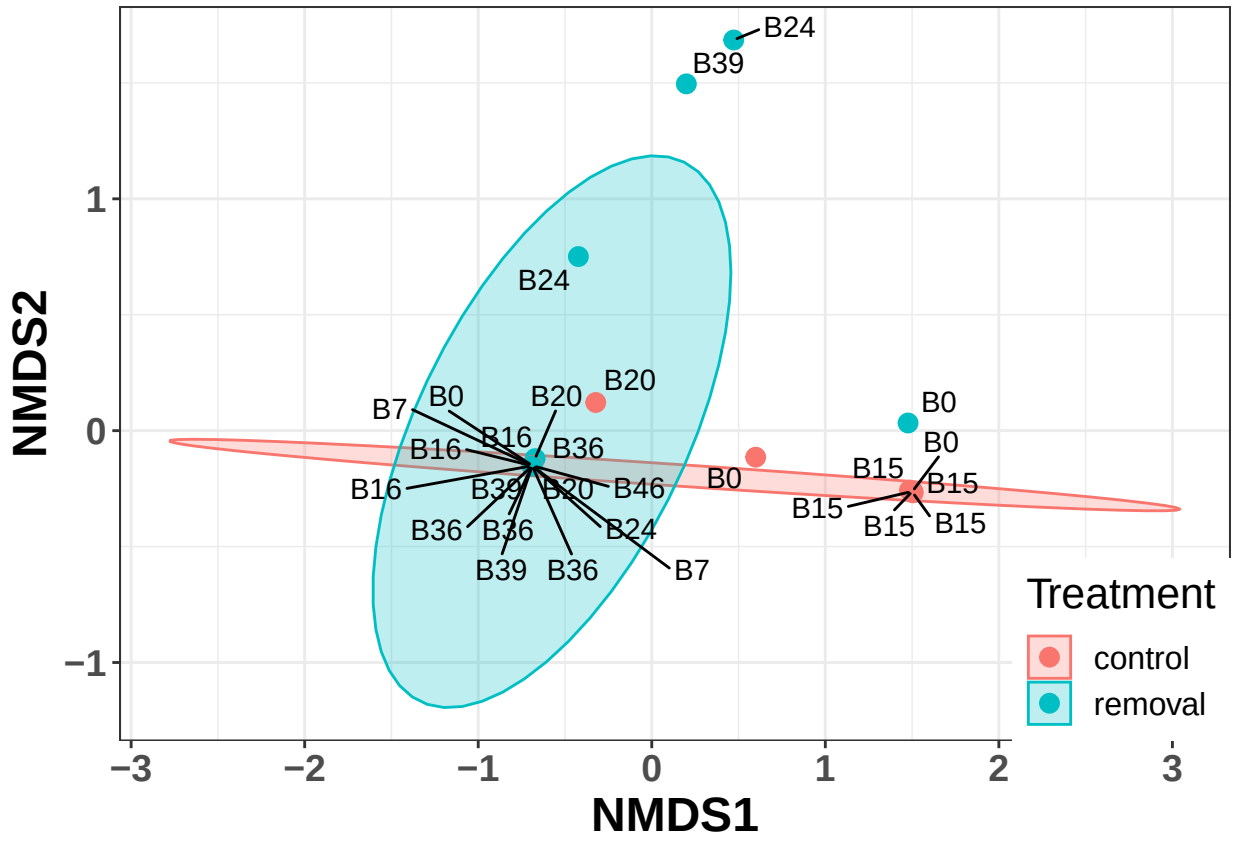
```
## Run 90 stress 0.001900829
## Run 91 stress 0.001626272
## Run 92 stress 0.00215964
## Run 93 stress 0.001492013
## Run 94 stress 0.00141128
## Run 95 stress 0.002279657
## Run 96 stress 0.002300291
## Run 97 stress 0.00135705
## Run 98 stress 0.002098906
## Run 99 stress 0.001471998
## Run 100 stress 0.0009480887
## ... Procrustes: rmse 0.008738421  max resid 0.02757519
## *** No convergence -- monoMDS stopping criteria:
##     100: no. of iterations >= maxit
```

```
ordi.R2nd$stress
```

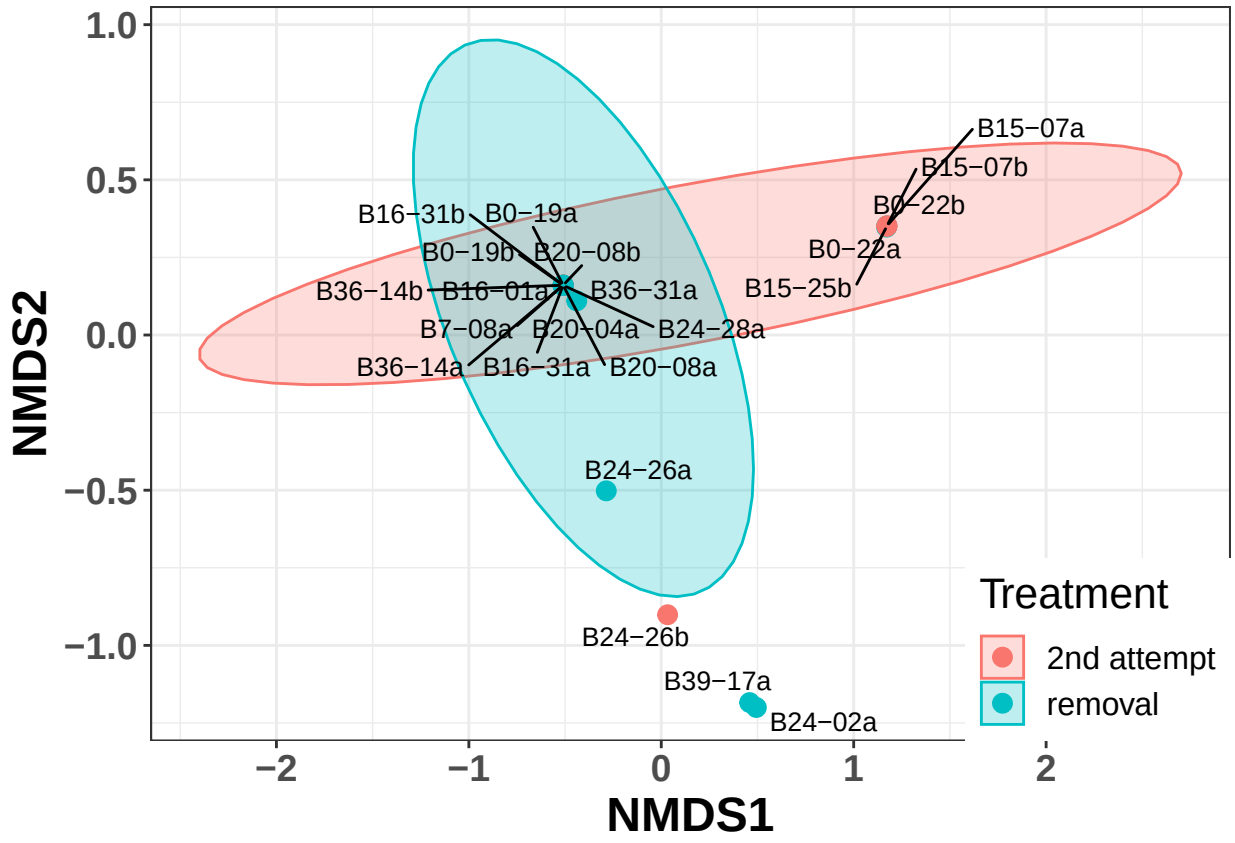
```
## [1] 0.000497131
```

plot NMDS

```
NMDS_CR_bac <- 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_bac
```




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

NMDS_R2nd_bac <- 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_bac
```



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   0.4260 0.06554 4.2998  0.038 *
## Linage     8   4.2905 0.66007 5.4126  0.001 ***
## Residual  18   1.7835 0.27439
## Total     27   6.5001 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

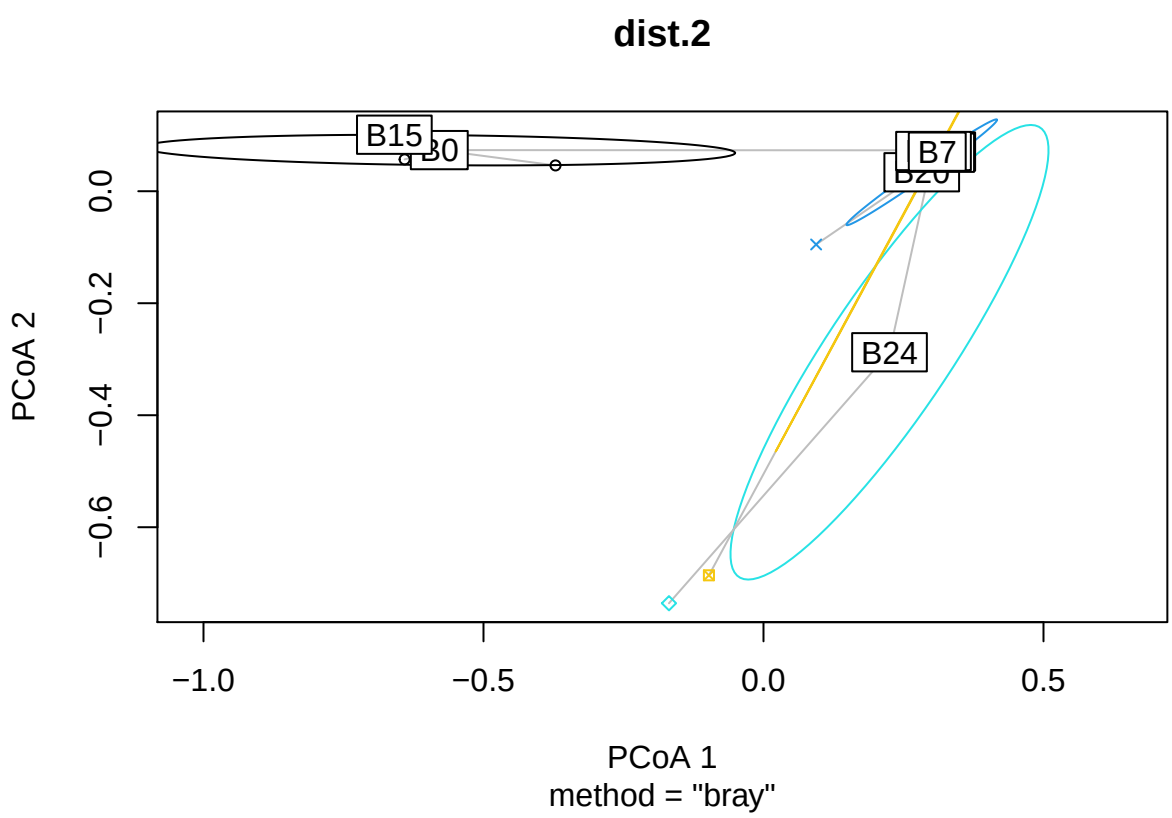
```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = distance(rel.R2nd, method = "bray") ~ Treatment + Linage, data = meta.R2nd)
##           Df SumOfSqs      R2      F Pr(>F)
## Treatment  1   0.2100 0.04241 1.8759  0.169
## Linage     7   3.2869 0.66369 4.1939  0.003 **
## Residual  13   1.4555 0.29390
## Total     21   4.9525 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Checking the homogeneity condition Check that variance homogeneity assumptions hold (to ensure the reliability of the results): Note the assumption of similar multivariate spread among the groups ie. analogous to variance homogeneity. Here the groups have signif. different spreads and permanova result may be potentially explained by that.

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

```
## Analysis of Variance Table
##
## Response: Distances
##           Df  Sum Sq  Mean Sq F value Pr(>F)
## Groups     8 0.60721 0.075901  1.1552  0.374
## Residuals 19 1.24843 0.065707
```

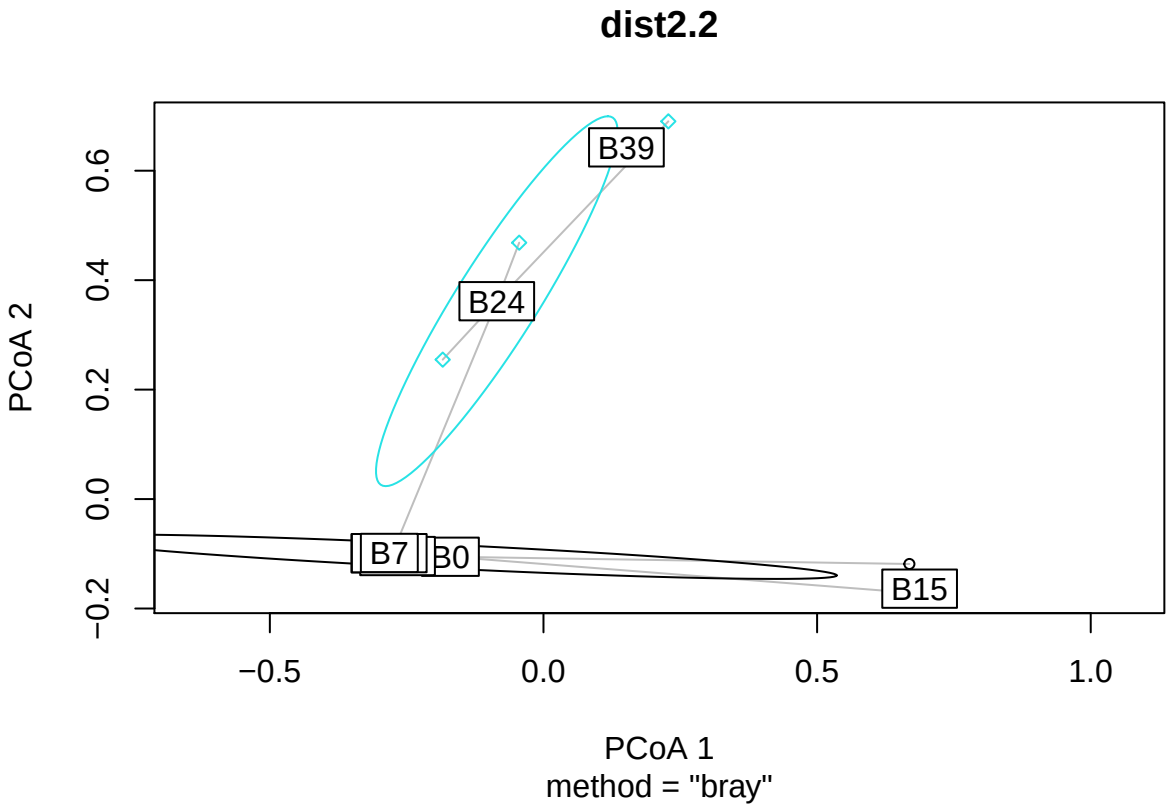
```
plot(dist.2, hull = FALSE, ellipse = TRUE)
```




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

```
## Analysis of Variance Table
##
## Response: Distances
##           Df  Sum Sq  Mean Sq F value Pr(>F)
## Groups     7 0.76889 0.109841  2.1272 0.1087
## Residuals 14 0.72292 0.051637
```

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



closer look at most abundant taxa

subset dataset Bacteria_Genus of both combinations to most abundant Geni (each *Wolbachia*, *Pseudoxanthomonas*) (high.reads with rel. abundance of total dataset)

```
WolbachiaCR <- subset_taxa(rel.CR, Genus == "Wolbachia")
dfWCR <- WolbachiaCR %>%
  tax_glom(taxrank = "Genus") %>%
  psmelt()


PseudoCR <- subset_taxa(rel.CR, Genus == "Pseudoxanthomonas")
dfPCR <- PseudoCR %>%
  tax_glom(taxrank = "Genus") %>%
  psmelt()


WolbachiaR2nd <- subset_taxa(rel.R2nd, Genus == "Wolbachia")
dfWR2nd <- WolbachiaR2nd %>%
  tax_glom(taxrank = "Genus") %>%
  psmelt()


PseudoR2nd <- subset_taxa(rel.R2nd, Genus == "Pseudoxanthomonas")
dfPR2nd <- PseudoR2nd %>%
```

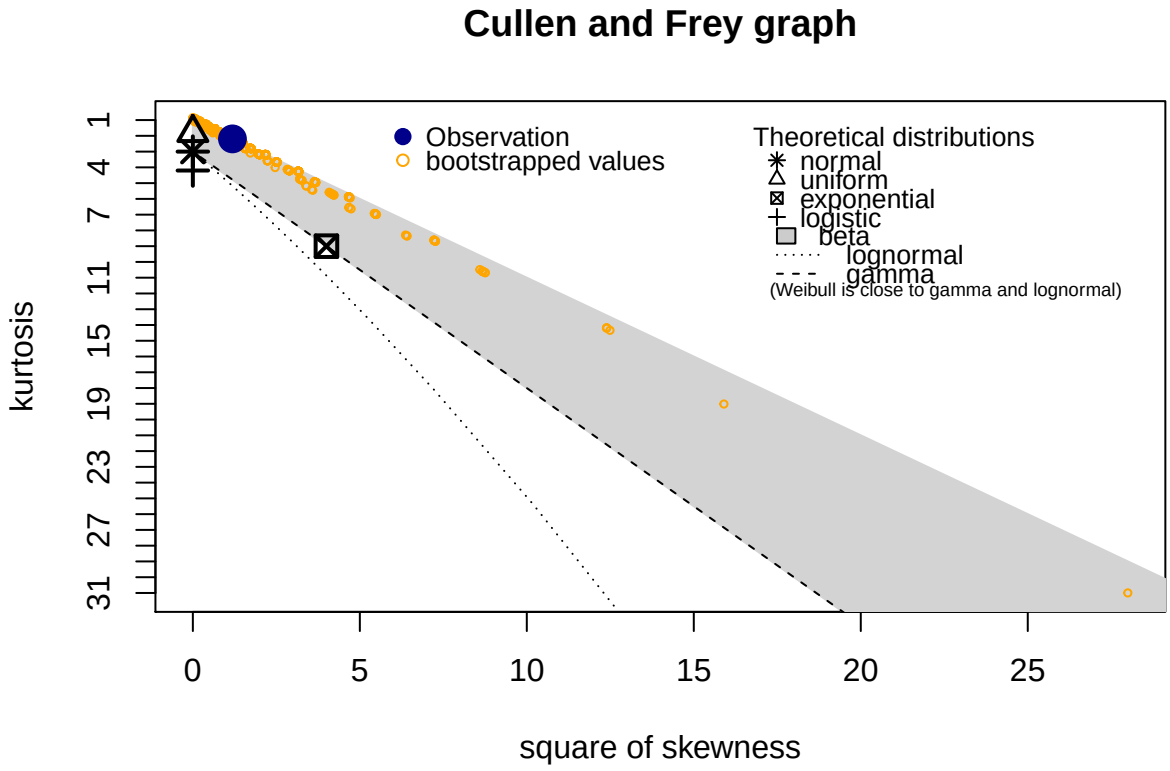
```
  tax_glom(taxrank = "Genus") %>%
  psmelt()
```

dataset *Wolbachia*

abundance between treatments

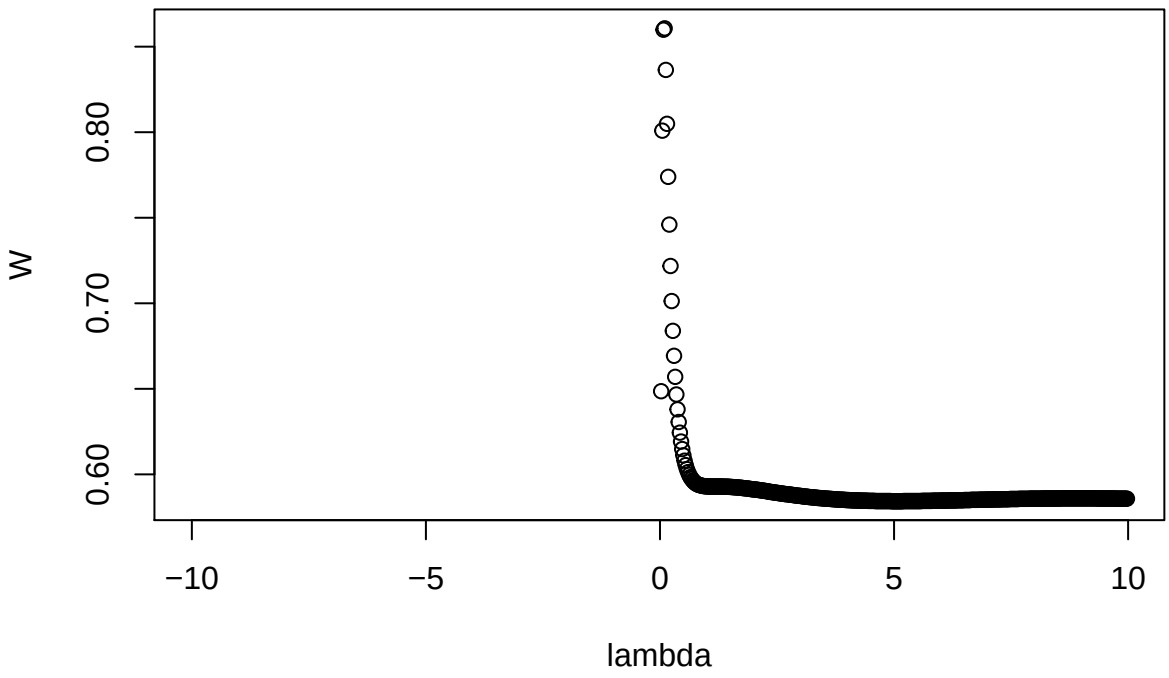
Tukey transformation of data

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

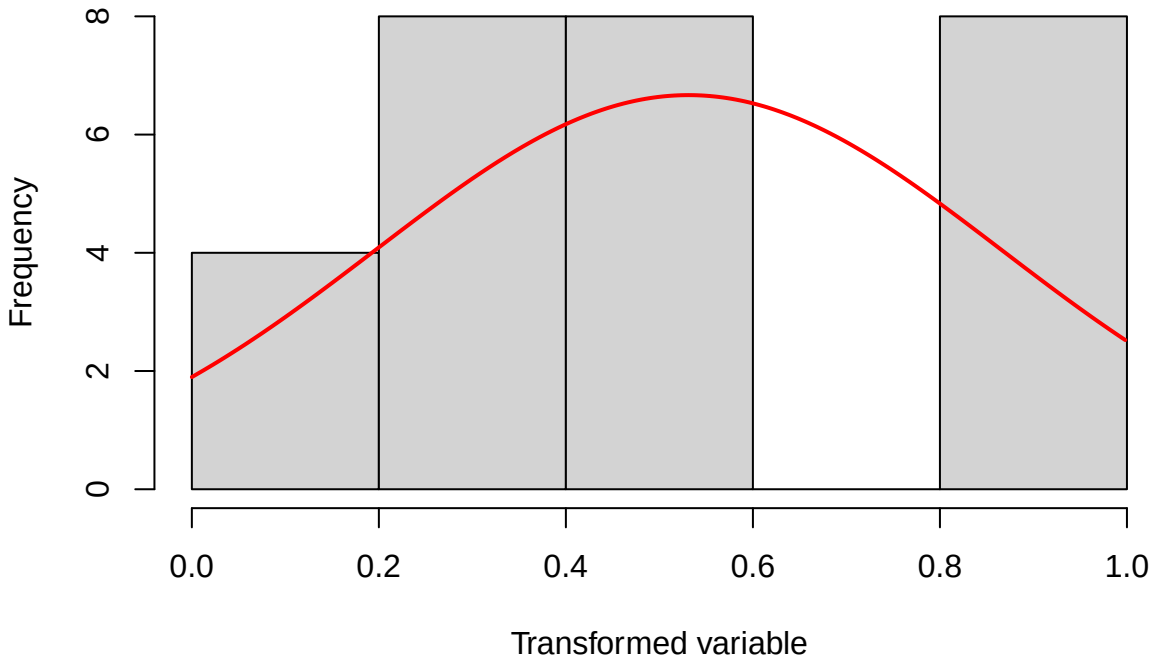


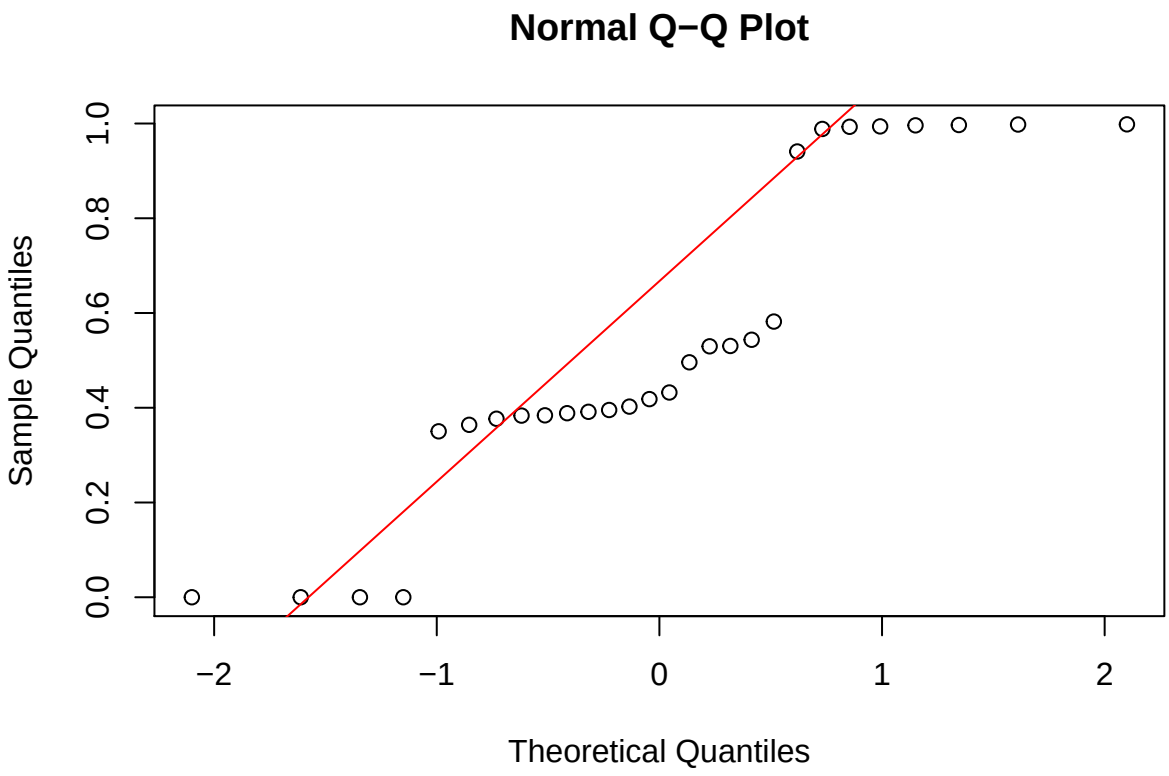

```
## summary statistics
## ------
## min:  0   max:  0.9822504
## median:  0.0001955422
## mean:  0.2575581
## estimated sd:  0.4204394
## estimated skewness:  1.088921
## estimated kurtosis:  2.194203
```

```
library(rcompanion)

dfWCR$Ab.tuk <- rcompanion::transformTukey(dfWCR$Abundance)
```

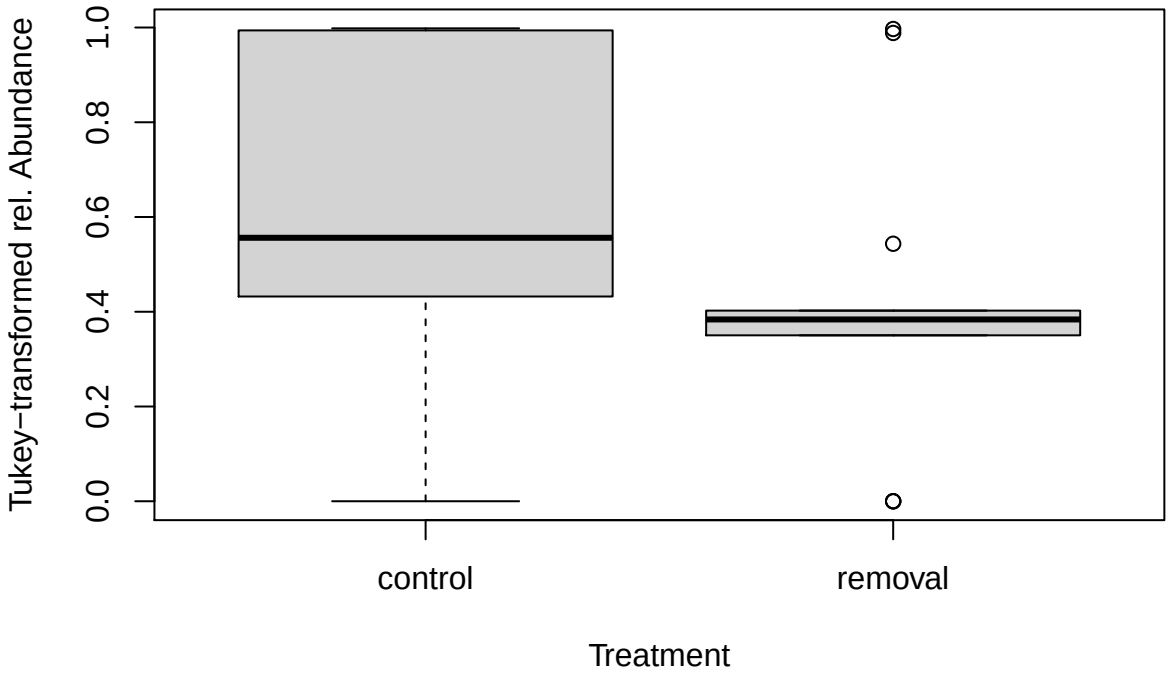



```
##
##     lambda      W Shapiro.p.value
## 405    0.1 0.8607        0.001548
##
## if (lambda >  0){TRANS = x ^ lambda}
## if (lambda == 0){TRANS = log(x)}
## if (lambda <  0){TRANS = -1 * x ^ lambda}
```


Frequency
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0
Transformed variable



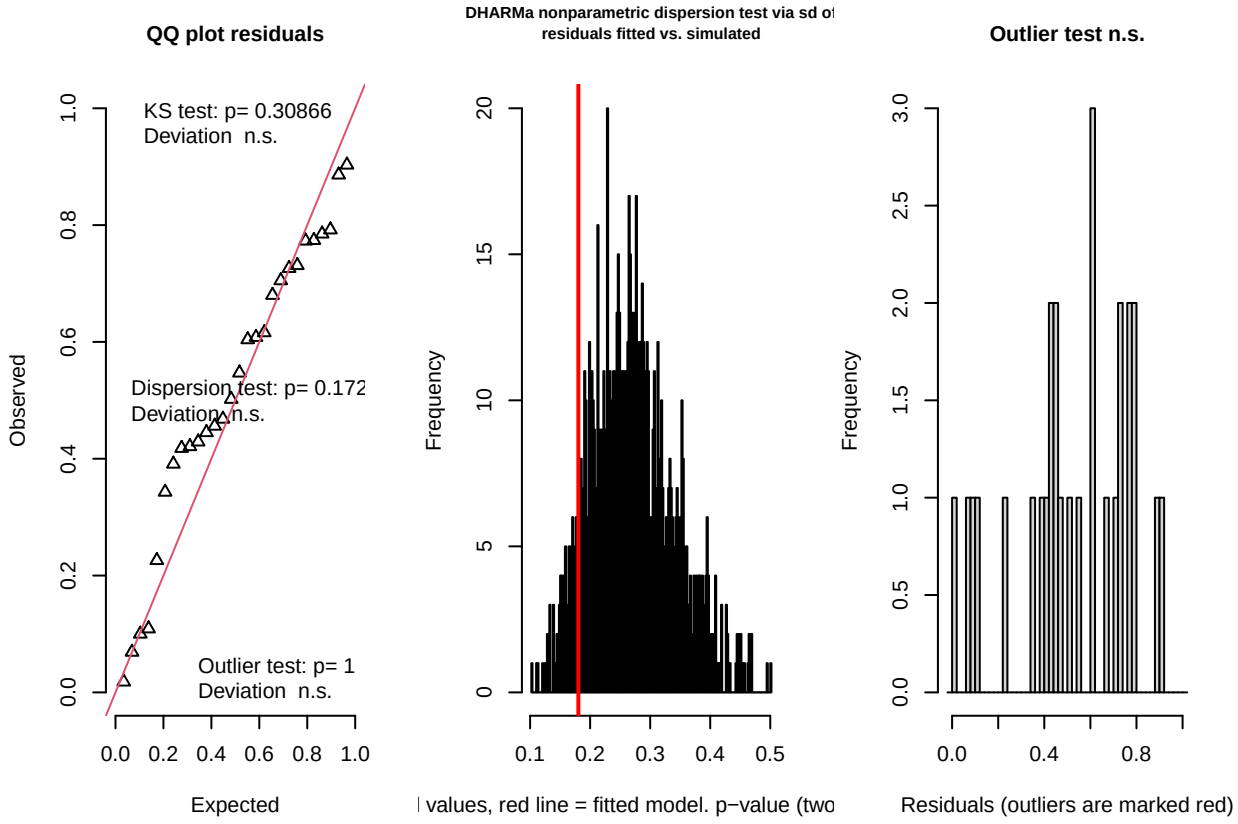

```
boxplot(Ab.tuk ~ Treatment, data = dfWCR, ylab = "Tukey-transformed rel. Abundance", xlab = "Treatment"
```




```
trans <- lm(Ab.tuk ~ Treatment + Linage, data = dfWCR)
Anova(trans, type="II")
```

```
## Anova Table (Type II tests)
##
## Response: Ab.tuk
##            Sum Sq Df F value    Pr(>F)
## Treatment 0.07815  1  2.4061 0.1382692
## Linage    1.95094  8  7.5085 0.0002021 ***
## Residuals 0.58462 18
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

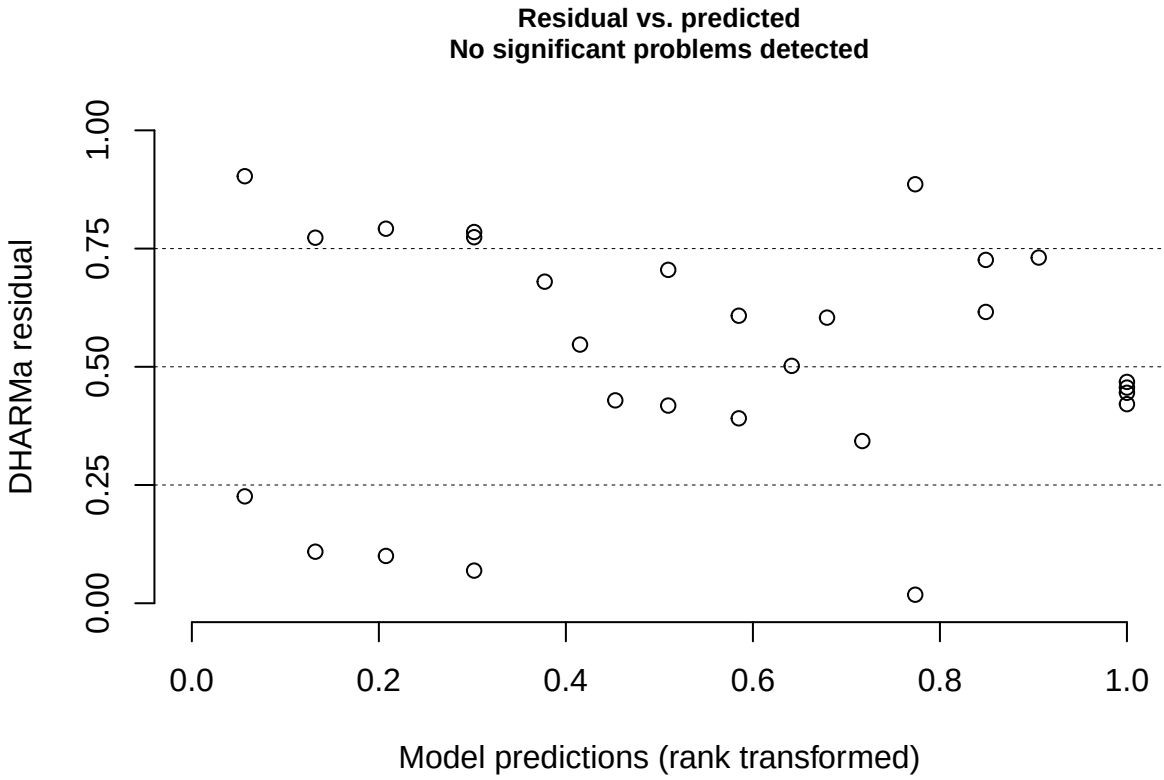
```
res_trans <- simulateResiduals(trans, n = 1000)
testResiduals(res_trans)
```



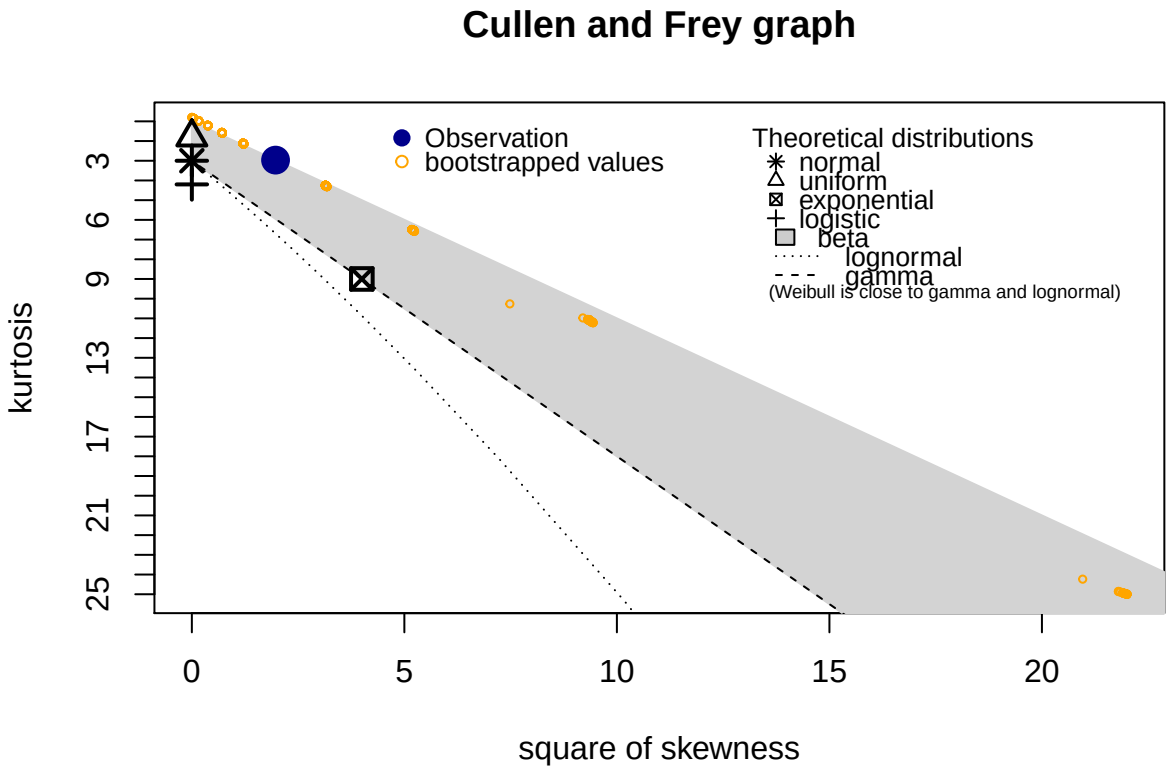

```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.17671, p-value = 0.3087
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.66538, 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 = 28, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1234361
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.17671, p-value = 0.3087
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.66538, 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 = 28, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1234361
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

```
plotResiduals(res_trans)
```

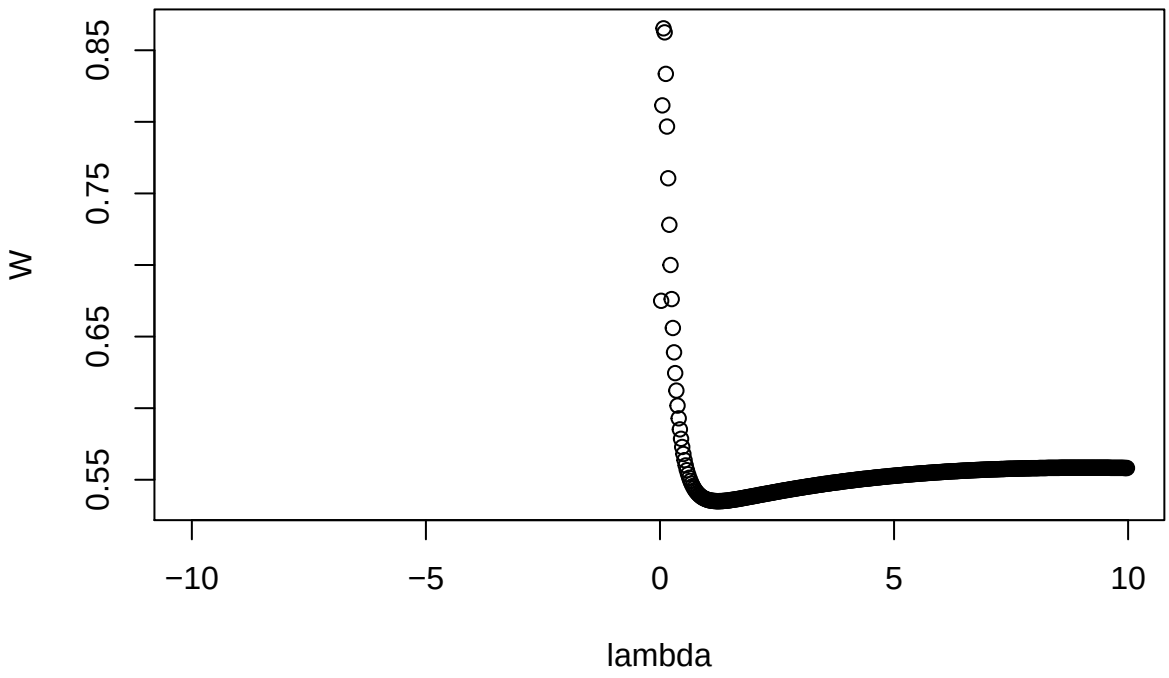



```
descdist(dfWR2nd$Abundance, boot = 1000)
```

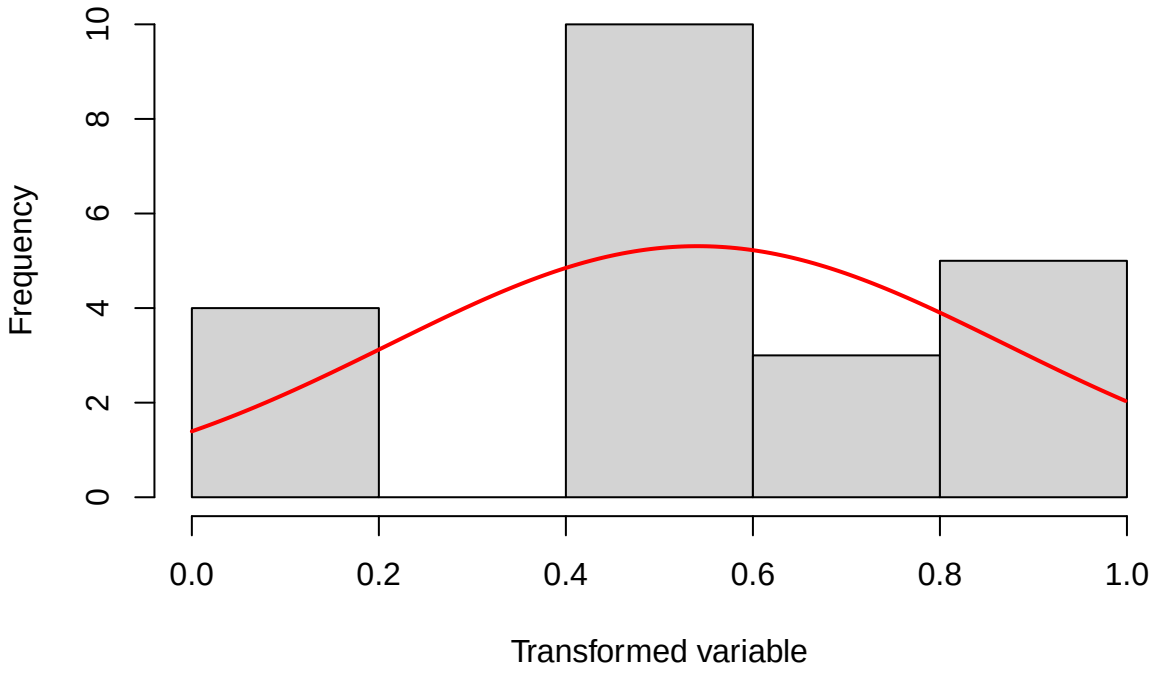


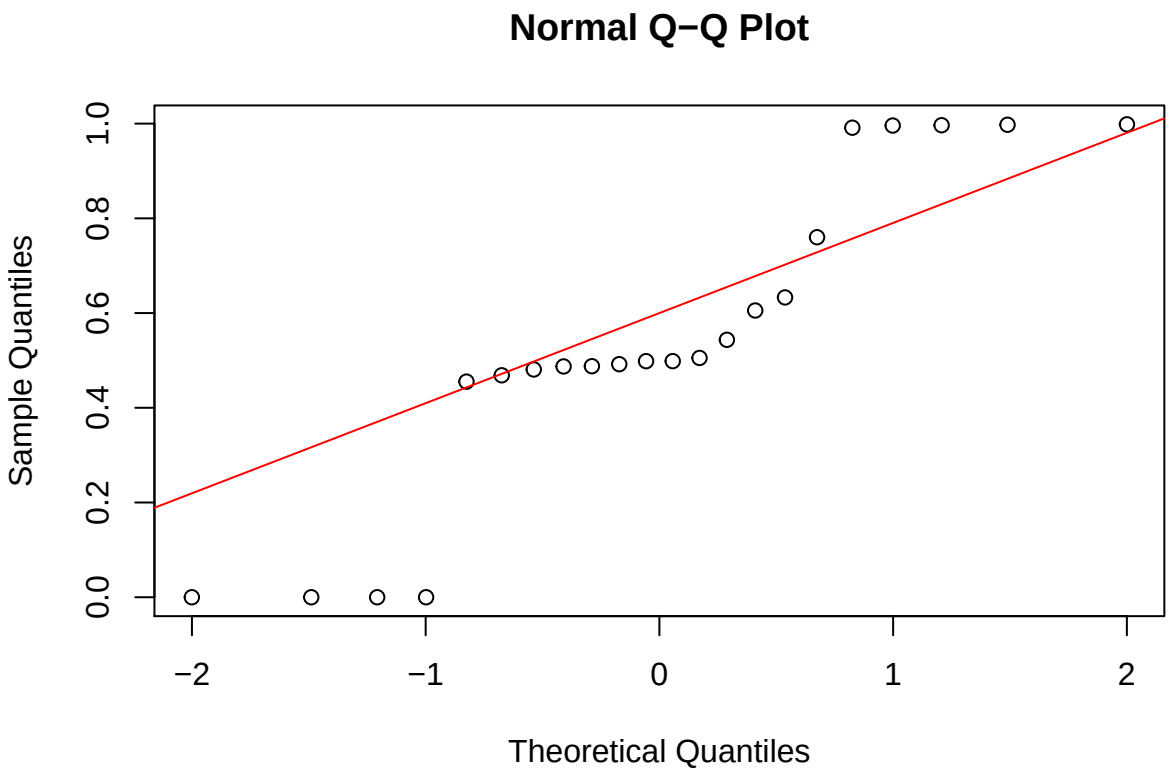

```
## summary statistics
## ------
## min:  0   max:  0.9793903
## median:  9.297361e-05
## mean:  0.2168947
## estimated sd:  0.406304
## estimated skewness:  1.403302
## estimated kurtosis:  2.967066
```

```
dfWR2nd$Ab.tuk <- rcompanion::transformTukey(dfWR2nd$Abundance)
```




```
##
##     lambda      W Shapiro.p.value
## 404  0.075 0.8653        0.006398
##
## if (lambda >  0){TRANS = x ^ lambda}
## if (lambda == 0){TRANS = log(x)}
## if (lambda <  0){TRANS = -1 * x ^ lambda}
```

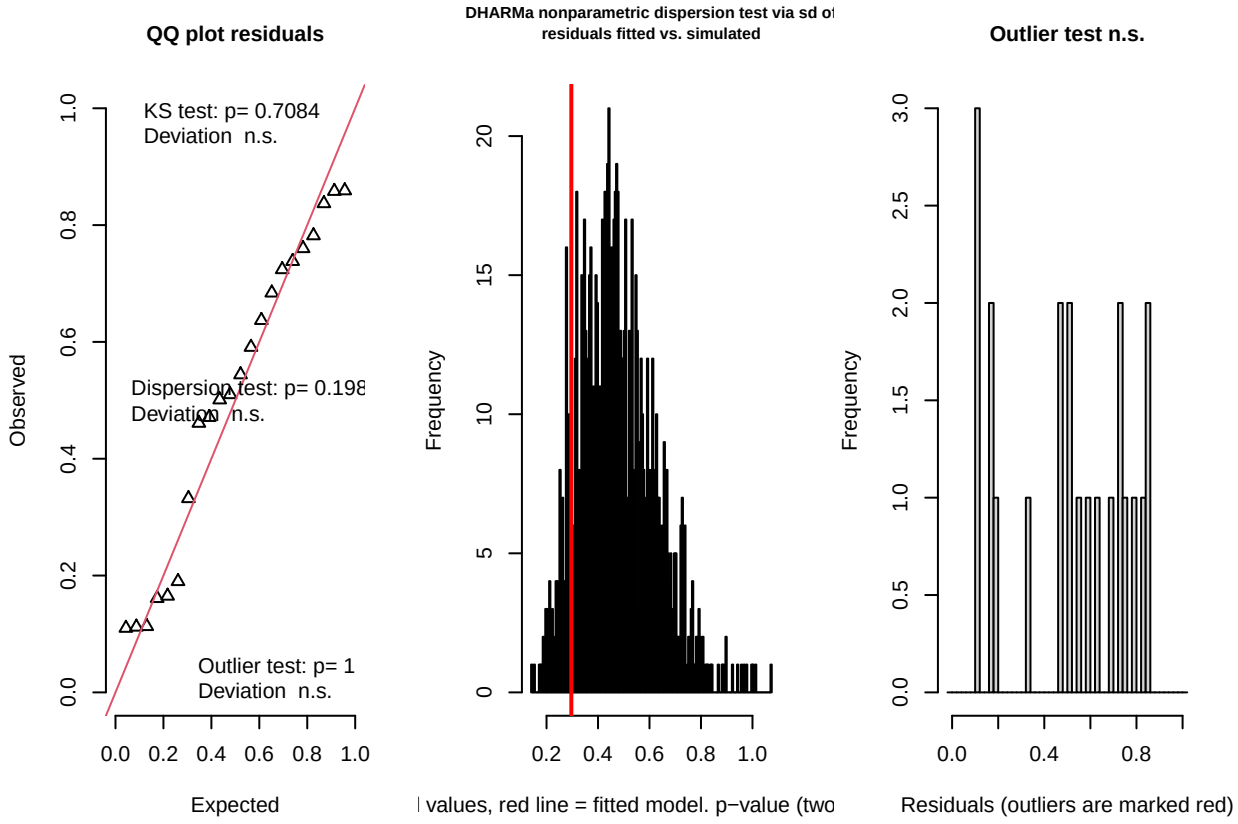

Frequency
0
2
4
6
8
10
0.0
0.2
0.4
0.6
0.8
1.0
Transformed variable




```
trans <- lm(Ab.tuk ~ Treatment + Linage, data = dfWR2nd)
Anova(trans, type="II")
```

```
## Anova Table (Type II tests)
##
## Response: Ab.tuk
##            Sum Sq Df F value  Pr(>F)
## Treatment 0.02523  1  0.4089 0.53363
## Linage    1.26758  7  2.9349 0.04463 *
## Residuals 0.80210 13
## ---
## Signif. codes:  0 ’***’ 0.001 ’**’ 0.01 ’*’ 0.05 ’.’ 0.1 ’ ’ 1
```

```
res_trans <- simulateResiduals(trans, n = 1000)
testResiduals(res_trans)
```

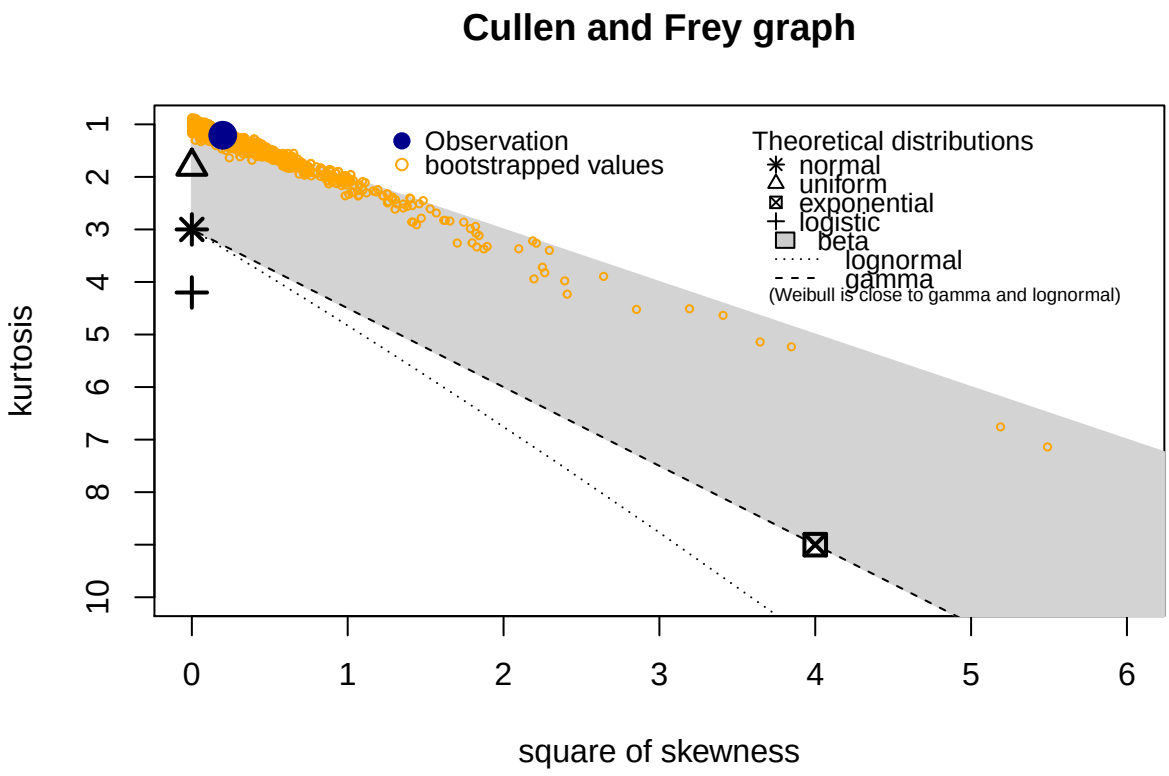



```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.14282, p-value = 0.7084
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.61762, p-value = 0.198
## 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 = 22, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1543725
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.14282, p-value = 0.7084
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.61762, p-value = 0.198
## 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 = 22, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1543725
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

dataset *Pseudoxanthomonas*

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

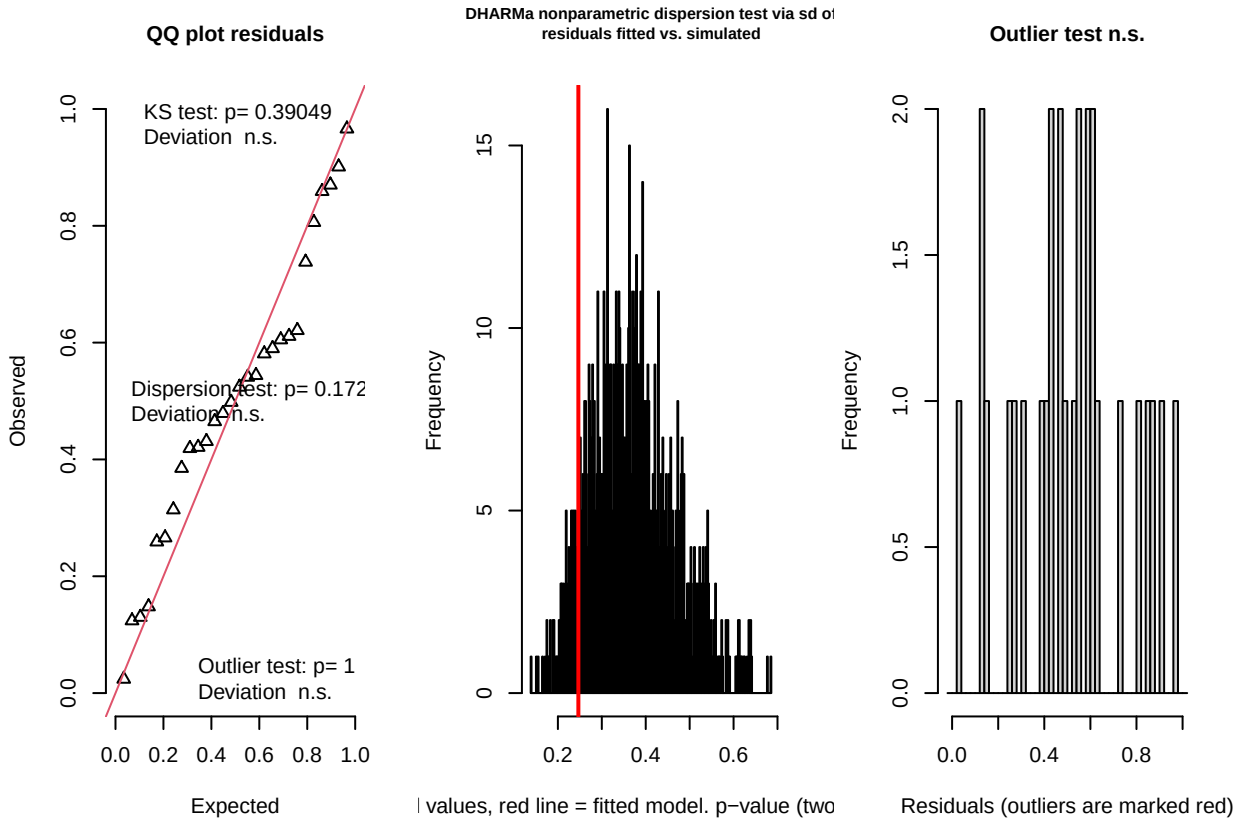



```
## summary statistics
## ------
## min:  0.01552795   max:  0.9994271
## median:  0.9602095
## mean:  0.613549
## estimated sd:  0.4394213
## estimated skewness:  -0.4451336
## estimated kurtosis:  1.207382
```

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

dfPCR.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = dfPCR)
Anova(dfPCR.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##            Sum Sq Df F value    Pr(>F)
## Treatment   1.116  1  0.1770 0.6789498
## Linage    299.461  8  5.9393 0.0008398 ***
## Residuals 113.445 18
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

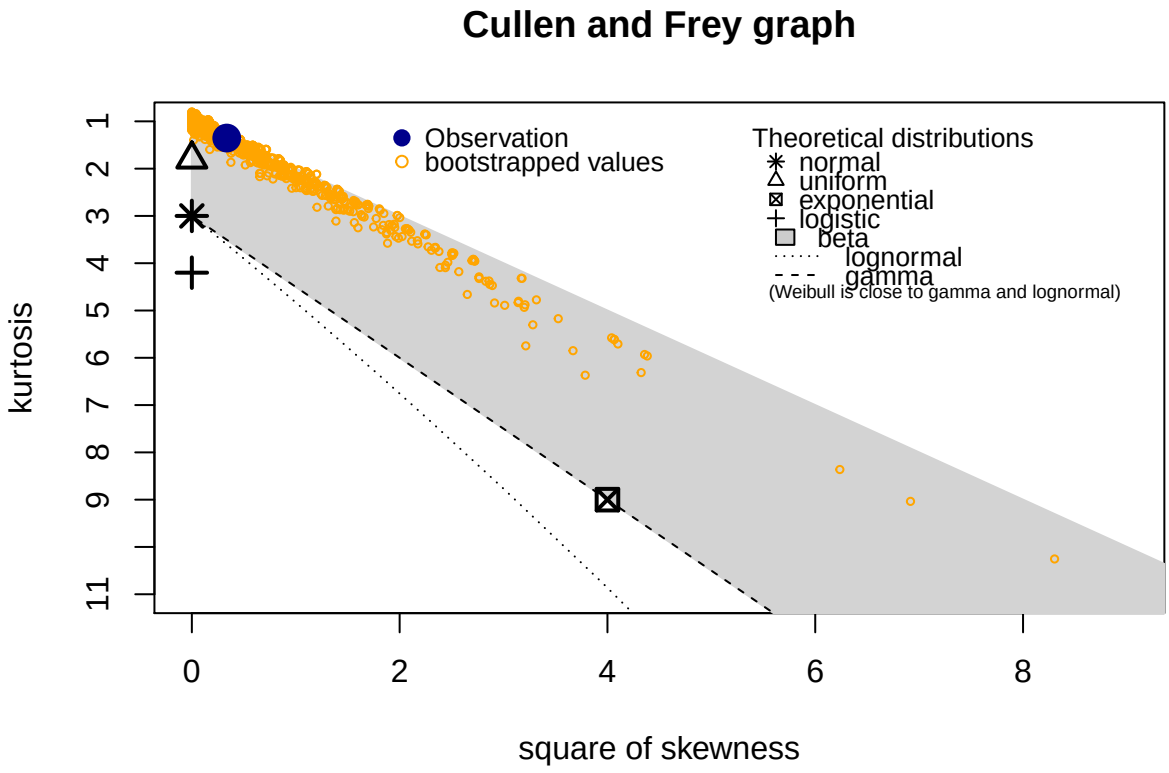
```
res_dfPCR.mod <- simulateResiduals(dfPCR.mod, n = 1000)
testResiduals(res_dfPCR.mod)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.16471, p-value = 0.3905
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.66538, 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 = 28, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1234361
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                    0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.16471, p-value = 0.3905
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.66538, 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 = 28, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1234361
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                    0
```

```
descdist(dfPR2nd$Abundance, boot = 1000)
```

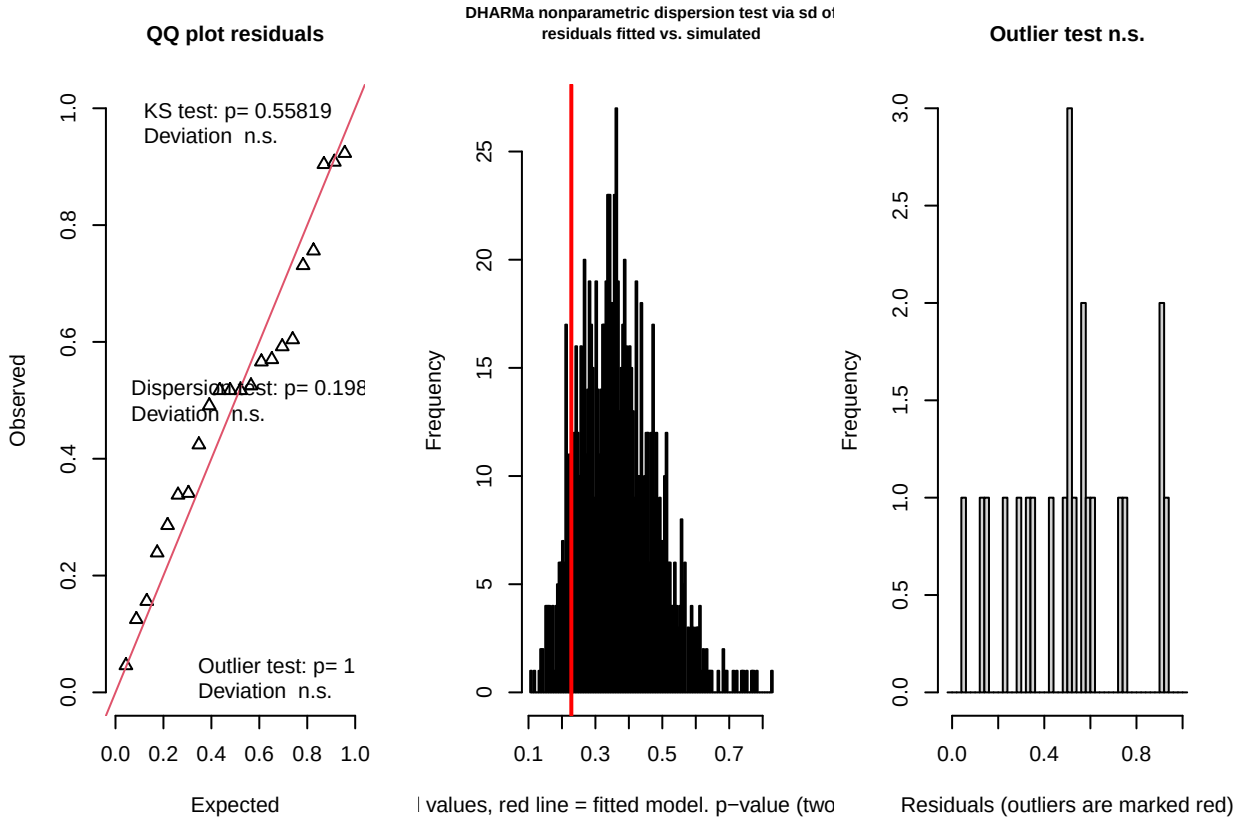



```
## summary statistics
## ------
## min:  0.006869901   max:  0.9993608
## median:  0.9616264
## mean:  0.6327369
## estimated sd:  0.4342354
## estimated skewness:  -0.5796202
## estimated kurtosis:  1.353467
```

```
dfPR2nd.mod <- lm(logistic(Abundance) ~ Treatment + Linage, data = dfPR2nd)
Anova(dfPR2nd.mod, type = "II")
```

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##             Sum Sq Df F value   Pr(>F)
## Treatment    0.996  1  0.1634 0.692660
## Linage     228.179  7  5.3447 0.004643 **
## Residuals   79.286 13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
res_dfPR2nd.mod <- simulateResiduals(dfPR2nd.mod, n = 1000)
testResiduals(res_dfPR2nd.mod)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.16873, p-value = 0.5582
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.61762, p-value = 0.198
## 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 = 22, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1543725
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.16873, p-value = 0.5582
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.61762, p-value = 0.198
## 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 = 22, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.1543725
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

testing difference of 'successful' 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
## B0-19a   B0-19a  B0-19     B0   removal             31 Bacteria
## B0-22a   B0-22a  B0-22     B0   removal             31 Bacteria
```

```
## B15-07a B15-07a B15-07    B15   removal             29 Bacteria
## B16-01a B16-01a B16-01    B16   removal             32 Bacteria
## B16-31a B16-31a B16-31    B16   removal             30 Bacteria
## B20-04a B20-04a B20-04    B20   removal             35 Bacteria
## B20-08a B20-08a B20-08    B20   removal             31 Bacteria
## B24-02a B24-02a B24-02    B24   removal             32 Bacteria
## B24-26a B24-26a B24-26    B24   removal             32 Bacteria
## B24-28a B24-28a B24-28    B24   removal             32 Bacteria
## B36-14a B36-14a B36-14    B36   removal             19 Bacteria
## B36-31a B36-31a B36-31    B36   removal             32 Bacteria
## B39-17a B39-17a B39-17    B39   removal             31 Bacteria
## B7-08a   B7-08a  B7-08     B7   removal             33 Bacteria
```

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

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

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

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

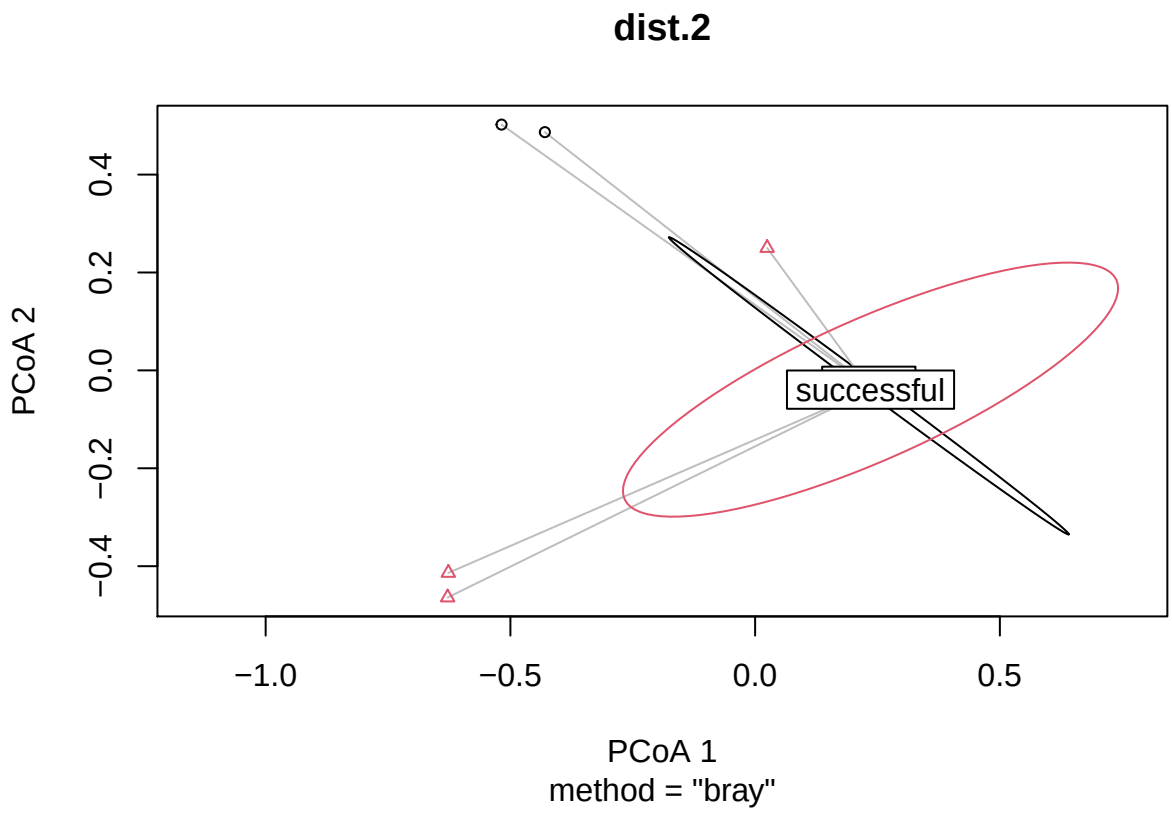
Permanova on data REM

```
## 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  0.19689 0.07083 0.9147  0.573
## Residual 12  2.58296 0.92917
## Total    13  2.77985 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 0.00368 0.003684  0.0205 0.8885
## Residuals 12 2.15427 0.179522
```

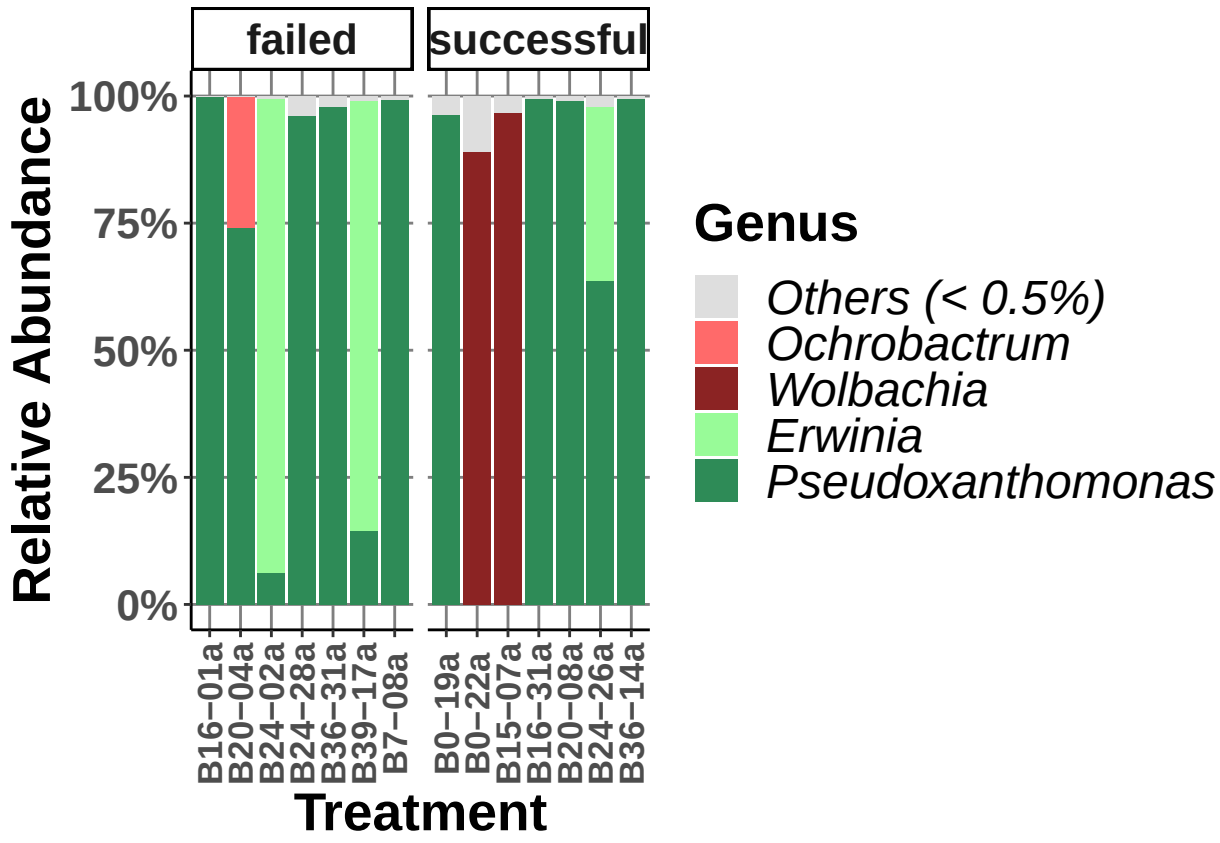
```
plot(dist.2, hull = FALSE, ellipse = TRUE)
```



prepare data and plot

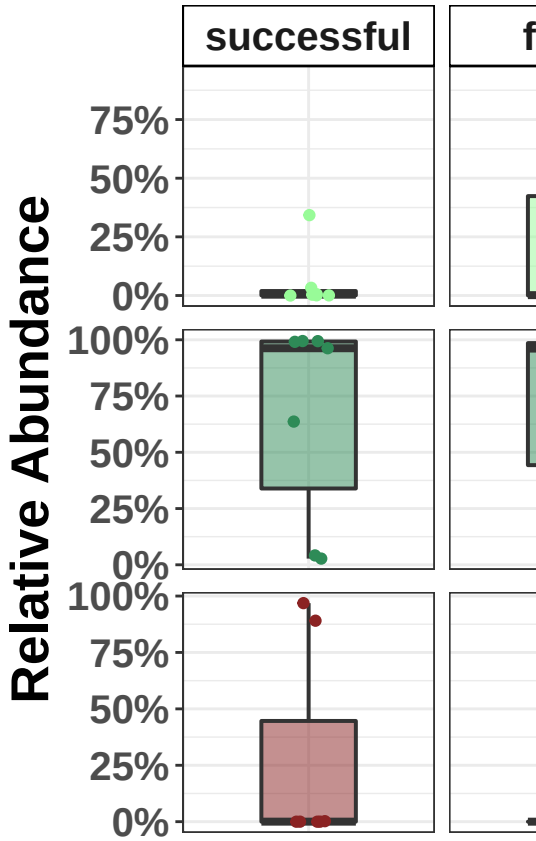
```
REM.plot <- dfREM
REM.plot$Success <- factor(REM.plot$success, levels = c("successful", "failed"))
REM.plot$Genus<-as.character(REM.plot$Genus)
REM.plot$Genus[REM.plot$Abundance<0.05]<-"Others"
REM.plot$Genus<-factor(REM.plot$Genus, levels = c("Others", "Ochrobactrum", "Wolbachia", "Erwinia","Pseu


Fungi_Success_plot <-ggplot(REM.plot, aes(x = Sample, y = Abundance, fill = Genus)) +
  geom_bar(stat = "identity", color = NA,  position="fill") +
  scale_fill_manual(values = Plot_colors_g2, name = "Genus") +
  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



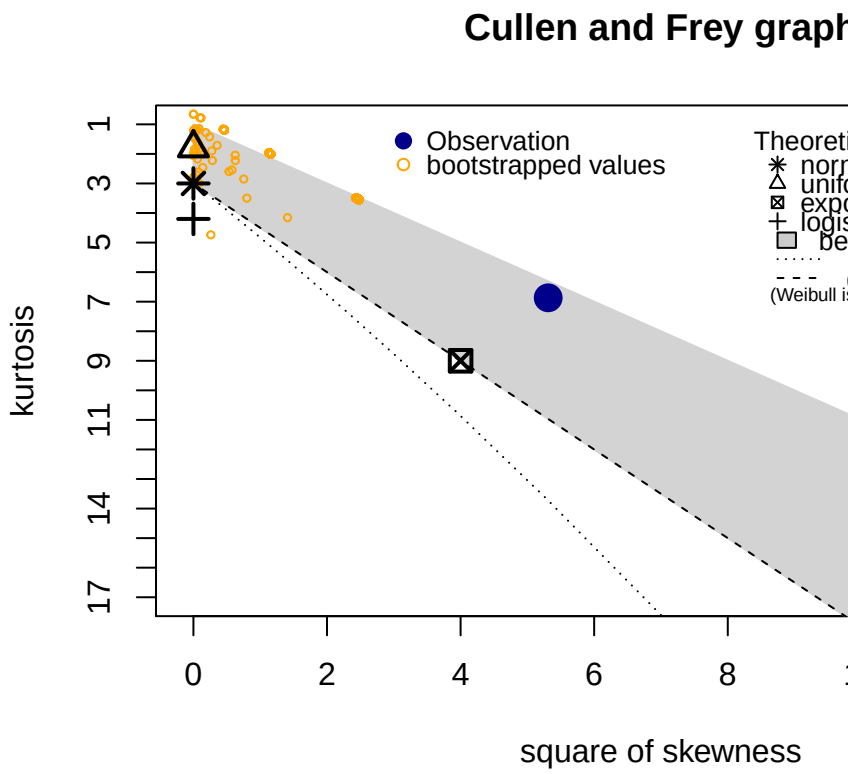

```
dfREM.sub <- subset(dfREM, Genus == "Wolbachia" | Genus == "Pseudoxanthomonas" | Genus == "Erwinia")
ptax_col <- c("palegreen", "seagreen", "brown4")
ptax<-ggplot(data = dfREM.sub, aes(x = success, y = Abundance)) +
  geom_boxplot(aes(fill=Genus),alpha=0.5,lwd=0.7, position = position_dodge(width = 0.3), width=0.45,ou
  scale_fill_manual(values = ptax_col, name = "Genus")+
  scale_color_manual(values = ptax_col, name = "Genus")+
  labs(x = "", y = "Abundance\n")+
  facet_grid(Genus~fct_relevel(success, "successful", "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_bac <- Success.plot + theme(panel.spacing.y = unit(0.3, "cm"))

Success.plot_bac
```



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

```
Wrem <- subset(dfREM, Genus == "Wolbachia")
descdist(Wrem$Abundance, boot = 1000)
```



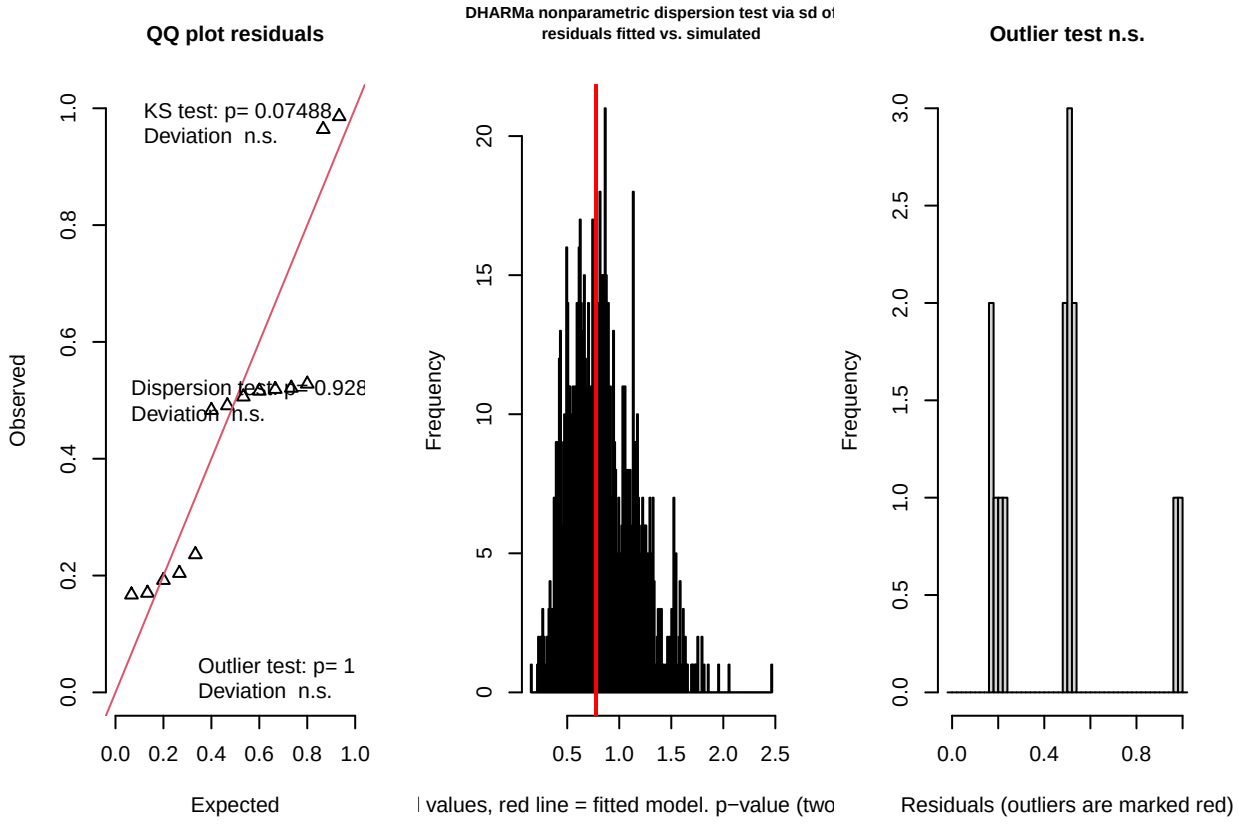
test if taxa are different in these two groups

```
## summary statistics
## ------
## min:  0   max:  0.9685696
## median:  6.920423e-05
## mean:  0.1330016
## estimated sd:  0.337838
## estimated skewness:  2.304651
## estimated kurtosis:  6.869043
```

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

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##           Sum Sq Df F value Pr(>F)
## success   15.912  1  2.4623 0.1426
## Residuals 77.548 12
```

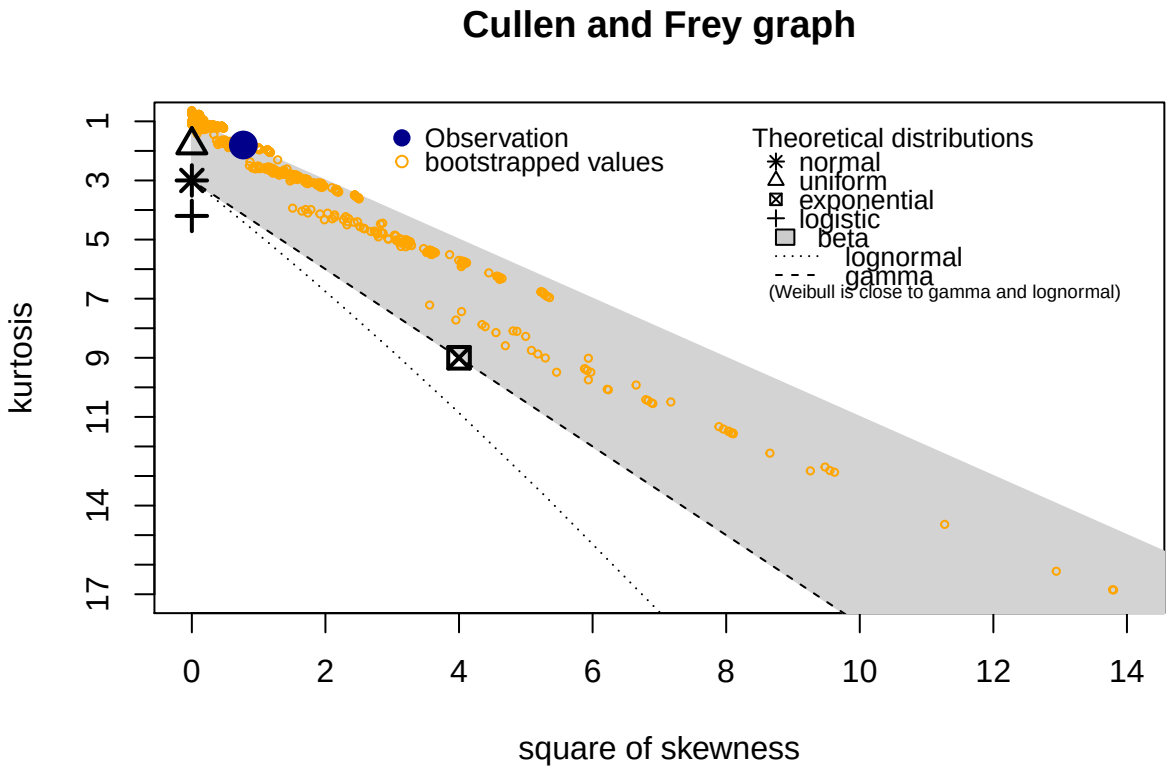
```
res_Wrem.mod <- simulateResiduals(Wrem.mod, n = 1000)
testResiduals(res_Wrem.mod)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.32914, p-value = 0.07488
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.32914, p-value = 0.07488
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

```
Prem <- subset(dfREM, Genus == "Pseudoxanthomonas")
descdist(Prem$Abundance, boot = 1000)
```

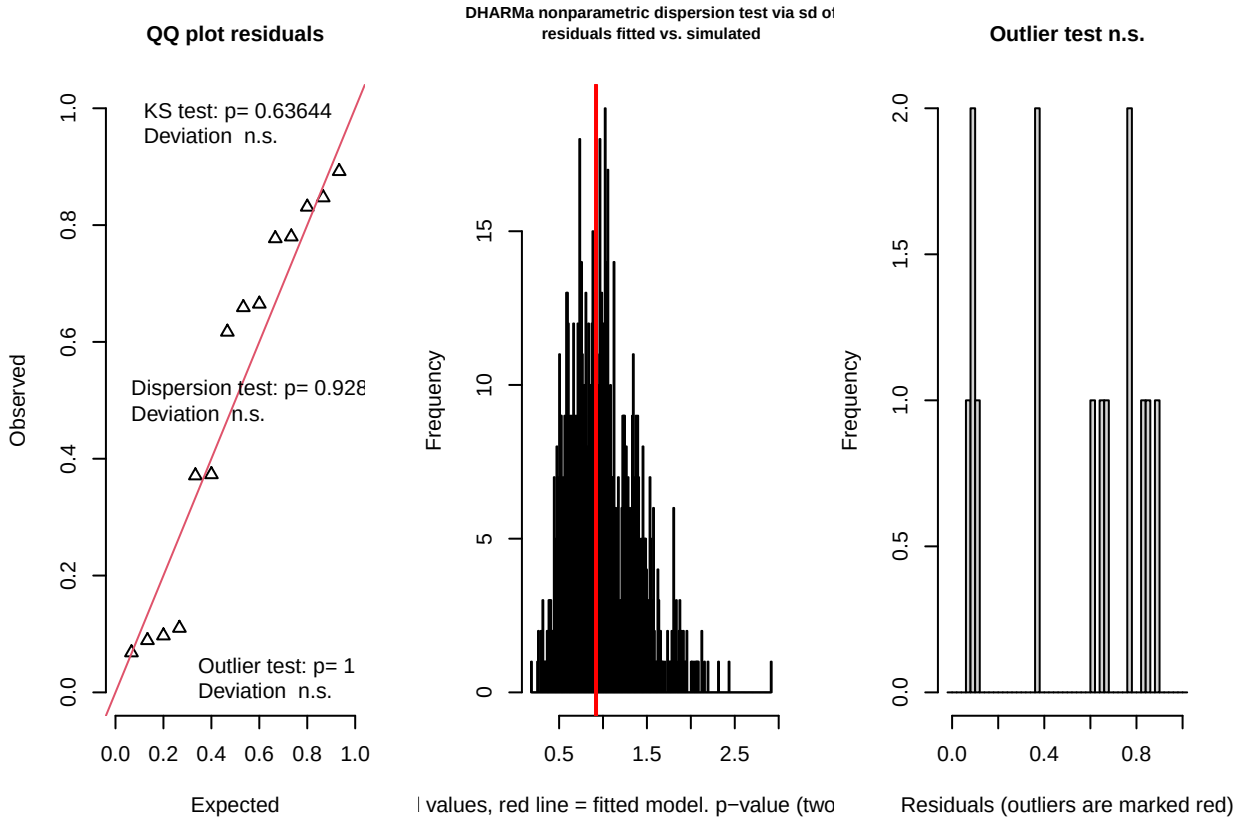



```
## summary statistics
## ------
## min:  0.02758178   max:  0.9987783
## median:  0.9616264
## mean:  0.680395
## estimated sd:  0.4157842
## estimated skewness:  -0.8779036
## estimated kurtosis:  1.800568
```

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

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##             Sum Sq Df F value Pr(>F)
## success      0.562  1  0.0441 0.8371
## Residuals 152.746 12
```

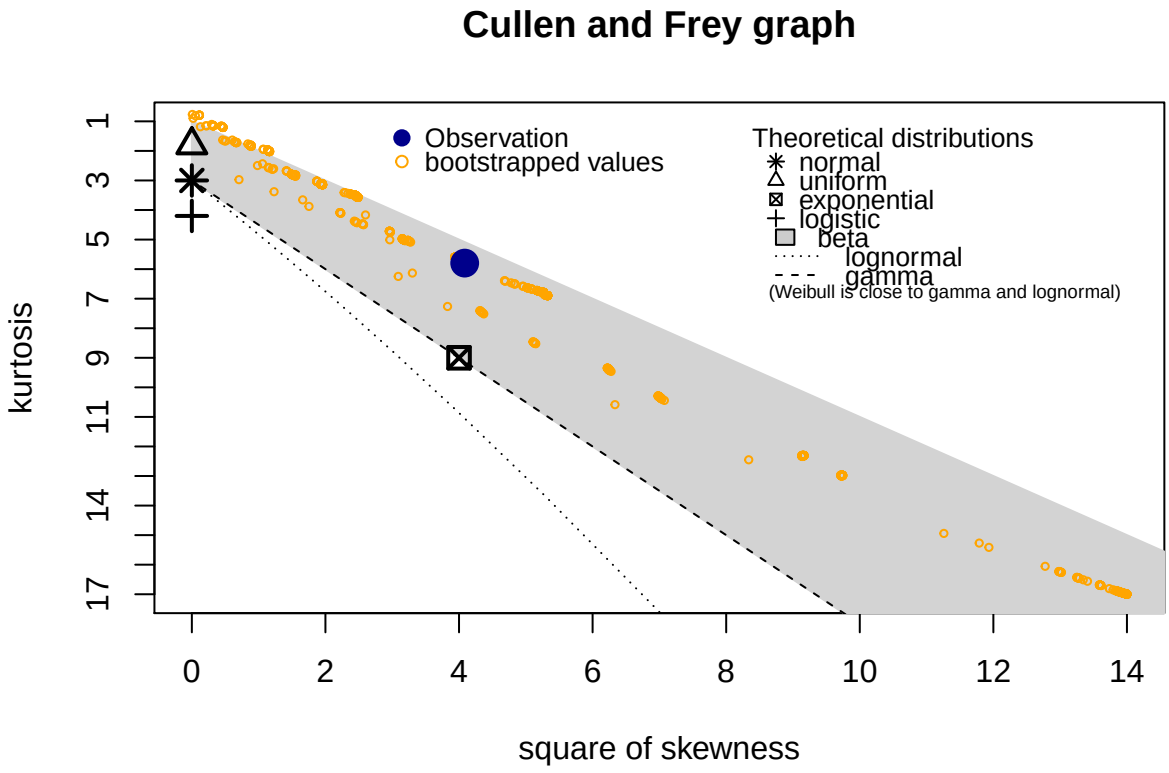
```
res_Prem.mod <- simulateResiduals(Prem.mod, n = 1000)
testResiduals(res_Prem.mod)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.18843, p-value = 0.6364
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.18843, p-value = 0.6364
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

```
Erem <- subset(dfREM, Genus == "Erwinia")
descdist(Erem$Abundance, boot = 1000)
```

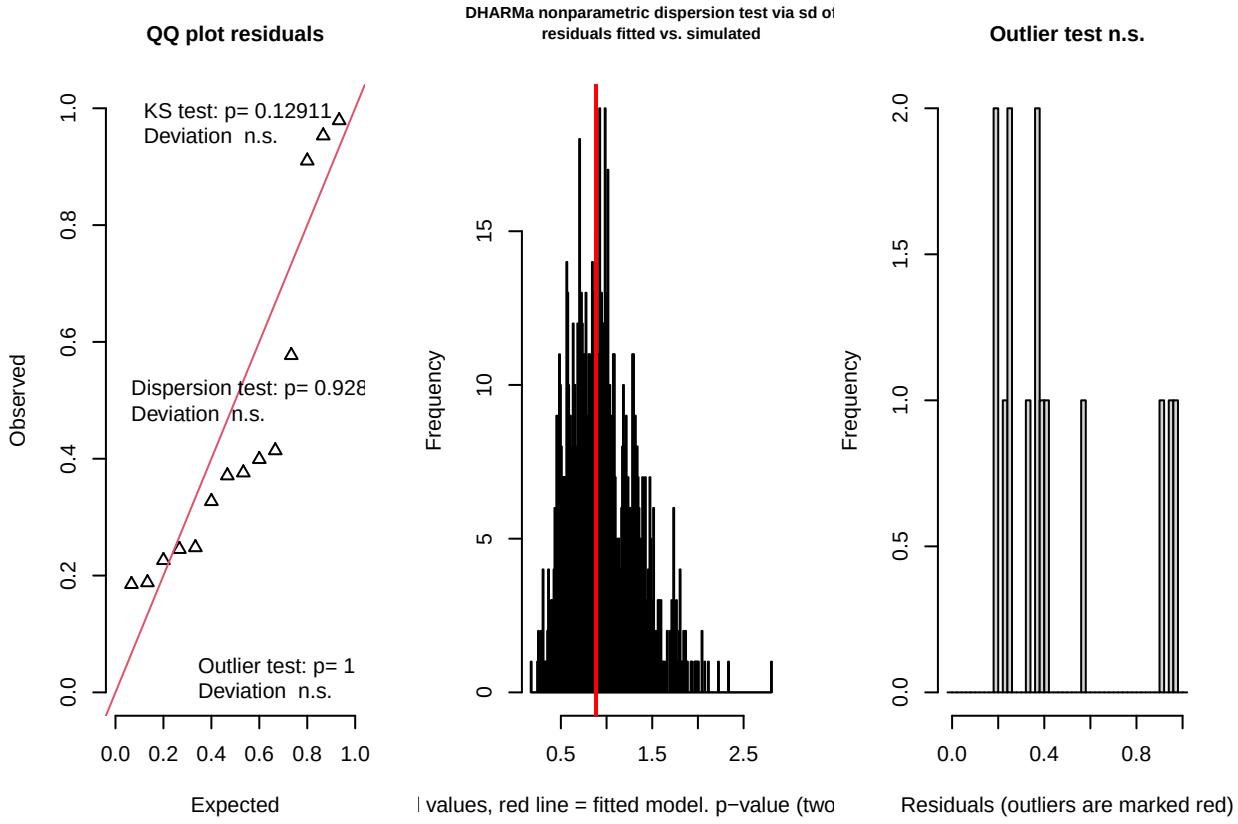



```
## summary statistics
## ------
## min:  0   max:  0.9314597
## median:  0.001771372
## mean:  0.154791
## estimated sd:  0.3240921
## estimated skewness:  2.021202
## estimated kurtosis:  5.796488
```

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

```
## Anova Table (Type II tests)
##
## Response: logistic(Abundance)
##           Sum Sq Df F value Pr(>F)
## success    4.098  1  0.6406  0.439
## Residuals 76.756 12
```

```
res_Erem.mod <- simulateResiduals(Erem.mod, n = 1000)
testResiduals(res_Erem.mod)
```




```
## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.30029, p-value = 0.1291
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
```

```
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0

## $uniformity
##
##  One-sample Kolmogorov-Smirnov test
##
## data:  simulationOutput$scaledResiduals
## D = 0.30029, p-value = 0.1291
## alternative hypothesis: two-sided
##
##
## $dispersion
##
##  DHARMa nonparametric dispersion test via sd of residuals fitted vs.
##  simulated
##
## data:  simulationOutput
## dispersion = 0.92226, p-value = 0.928
## 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 = 14, p-value = 1
## alternative hypothesis: true probability of success is not equal to 0.001998002
## 95 percent confidence interval:
##  0.0000000 0.2316358
## sample estimates:
## frequency of outliers (expected: 0.001998001998002 )
##                                                     0
```

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