

Project_IRAS

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guides used: <https://www.nicholas-ollberding.com/post/introduction-to-the-statistical-analysis-of-microbiome-data-in-r/>, <https://rpubs.com/lconteville/713954>, <https://mibwurrepo.github.io/Microbial-bioinformatics-introductory-course-Material-2018/beta-diversity-metrics.html>, <https://rfunctions.blogspot.com/2019/03/betadisper-and-adonis-homogeneity-of.html>, <https://david-barnett.github.io/microViz/articles/web-only/compositions.html>, <https://microbiome.github.io/OMA/viz-chapter.html>, <https://microbiome.github.io/OMA/clustering.html>, https://microucph.github.io/amplicon_data_analysis/html/cluster.html, <https://www.datacamp.com/tutorial/hierarchical-clustering-R>, <https://rpubs.com/TBrach/68544>

```
library(phyloseq) # Data analysis and visualisation, also the basis of data object.
library(DT) # Interactive tables in html and markdown.
library(data.table) # Giving overview of data.
library(tidyverse) # Data handling and much more.
library(readxl) # Reading in excel files.
library(ape) # Phylogenetic package, used for creating random trees and as dependency for other package.
library(magrittr) # Data handling, specifically assignment pipes.
library(microViz) # Both analysis and visualisation.
library(plyr) # to apply functions, transform data.
library(microbiome) # For data analysis and visualisation, reading phyloseq object.
library(ggpubr) # Publication quality figures, based on ggplot2.
library(RColorBrewer) # Color options.
library(microbiomeutilities) # Some utility tools for microbiome package.
library(mia) # microbiome analysis package, making tse objects.
library(sechm) # Used for plotting heatmaps.
library(ggtree) # For creating trees, hierarchical clustering for heatmaps
library(pheatmap) # Creating heatmaps.
library(viridis) # Creating colour pallettes.
library(patchwork) # Used to add plots together into the same plot.
library(data.table) # Alternative to data.frame
library(picante) # Used for calculating Phylogenetic diversities
library(lme4) # Repeated measures, add to report if used
library(QsRutils) # For the goods() function, to estimate coverage
library(scater) # plotReducedDim
library(vegan) # used to run simper
library(nlme) # for usage of llply(), to apply functions over lists
library(mia) # Broad package, includes clustering functions.
library(bluster) # Used for clustering.
library(scater) # visualisation, reduced dimensions.
library(scran) # A wrapper for bluster and tse objects.
```

```
library(NbClust) # To find out the optimal number of clusters.
library(dendextend) # For creating dendrograms with additional options, labeling etc.
library(factoextra) # Visualize optimal number of clusters.
library(cluster) # For clustering algorithms, specifically used for PAM.
```

Load packages

R Markdown

Metataxonomics

Loading in metataxonomic data

```
pseq <- read_phyloseq(otu.file= "ASV.biom1",
                      taxonomy.file = NULL,
                      metadata.file = "MetaData.csv",
                      type="biom", sep =";" )

treefile <- read_tree("all_asvTREE.tree")
ps <-merge_phyloseq(pseq, treefile)
ps # 180 samples
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 6249 taxa and 180 samples ]
## sample_data() Sample Data: [ 180 samples by 26 sample variables ]
## tax_table() Taxonomy Table: [ 6249 taxa by 6 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 6249 tips and 6248 internal nodes ]
```

```
sort(sample_sums(ps))
```

```
##      14.F2S1.20.06      4.F2S1.11.07      13.F2S2.11.07
##      46850             51537             56421
##      5.F2S2.20.06      8.F5.S2.CA.21.9.17      24.F2S1.11.07
##      57164             58212             59365
##      7.F4.S2.CA.8.8.2017      24.F2S2.20.06      7.F2S2.11.07
##      60691             64111             65002
##      26.F2S2.11.07      17.F2S1.11.07      2.F5.S1.CA.21.9.17
##      72476             73085             76540
##      9.F4.S2.CA.29.8.2017      4.F6.S2.CA.21.9.17      3.F2S2.11.07
##      77277             81063             82316
##      3.F6.S1.CA.21.9.17      3.F6.S2.CA.21.9.17      27.F1S2.31.08
##      85263             85370             85864
##      4.F1S1.21.09      34.F1S1.21.09      9.F5.S1.CA.21.9.17
##      86303             90167             94098
##      20.F1S1.31.08      2.F6.S2.CA.21.9.17      4.F6.S1.CA.21.9.17
##      94757             94800             95509
##      6.F4.S2.CA.8.8.2017      19.F2S2.11.07      7.F5.S1.CA.31.8.17
##      97472             99631             100430
##      28.F1S1.21.09      5.F5.S1.CA.21.9.17      1.F6.S1.CA.21.9.17
##      101299            104417            107531
```

##	3.F5.S1.CA.21.9.17	33.F1S2.21.09	7.F4.S1.CA.29.8.2017
##	107684	110305	115371
##	31.F1S2.31.08	9.F4.S2.CA.8.8.2017	3.F1S2.31.08
##	117379	117431	118155
##	35.F1S2.31.08	4.F5.S2.CA.31.8.17	1.F1S2.21.09
##	119253	119720	121074
##	18.F2S1.20.06	4.F4.S1.CA.29.8.2017	10.F2S1.20.06
##	121143	122331	123221
##	11.F1S2.21.09	6.F5.S1.CA.21.9.17	14.F1S1.21.09
##	123322	123520	126120
##	8.F4.S2.CA.8.8.2017	12.F1S1.21.09	6.F5.S2.CA.21.9.17
##	126501	128514	128935
##	25.F2S2.20.06	6.F1S1.21.09	10.F2S1.11.07
##	129532	130408	132808
##	8.F4.S1.CA.29.8.2017	14.F1S1.31.08	9.F2S2.20.06
##	135194	135303	135373
##	21.F1S2.31.08	15.F1S2.21.09	6.F4.S1.CA.29.8.2017
##	135703	135968	136635
##	8.F6.S1.CA.21.9.17	9.F4.S1.CA.29.8.2017	6.F4.S1.CA.8.8.2017
##	138151	138463	138738
##	4.F5.S1.CA.21.9.17	7.F4.S1.CA.8.8.2017	1.F5.S1.CA.21.9.17
##	140635	140868	142741
##	19.F2S2.20.06	1.F4.S1.CA.8.8.2017	1.F5.S2.CA.21.9.17
##	144528	146133	146908
##	5.F4.S1.CA.8.8.2017	25.F2S1.11.07	7.F5.S1.CA.21.9.17
##	148633	149461	150709
##	2.F6.S1.CA.21.9.17	2.F5.S2.CA.21.9.17	8.F4.S1.CA.8.8.2017
##	150730	151425	153407
##	8.F4.S2.CA.29.8.2017	6.F4.S2.CA.29.8.2017	2.F2S2.20.06
##	153433	153692	153925
##	7.F1S2.21.09	2.F5.S2.CA.31.8.17	19.F1S2.21.09
##	155378	156224	156652
##	1.F4.S1.CA.29.8.2017	2.F1S1.31.08	18.F2S2.11.07
##	157292	158039	158392
##	2.F4.S2.CA.29.8.2017	30.F1S1.21.09	4.F2S2.11.07
##	158954	160633	160749
##	3.F5.S1.CA.31.8.17	8.F5.S1.CA.21.9.17	21.F1S2.21.09
##	161067	163383	163995
##	4.F5.S2.CA.21.9.17	5.F6.S1.CA.21.9.17	8.F6.S1.CA.31.8.17
##	166497	166953	167207
##	5.F4.S2.CA.8.8.2017	22.F1S1.21.09	9.F5.S2.CA.21.9.17
##	167899	169419	170464
##	13.F2S1.11.07	1.F6.S2.CA.21.9.17	5.F4.S1.CA.29.8.2017
##	171477	173496	173499
##	9.F1S2.31.08	3.F4.S2.CA.29.8.2017	20.F2S1.11.07
##	173638	175652	175734
##	6.F1S1.31.08	9.F2S1.11.07	10.F1S1.31.08
##	176305	178651	178746
##	10.F2S2.11.07	3.F4.S1.CA.29.8.2017	5.F5.S2.CA.21.9.17
##	179318	180443	180662
##	8.F2S1.20.06	2.F4.S1.CA.8.8.2017	25.F2S1.20.06
##	182277	188508	189251
##	17.F2S2.20.06	1.F5.S2.CA.31.8.17	9.F4.S1.CA.8.8.2017
##	189516	189756	192242

```

##          1.F2S1.11.07 1.F4.S2.CA.29.8.2017 4.F4.S2.CA.8.8.2017
##          192332          192909          194069
##    3.F5.S2.CA.21.9.17          2.F2S1.20.06 3.F4.S2.CA.8.8.2017
##          194734          195641          199046
##    7.F5.S2.CA.21.9.17    7.F5.S2.CA.31.8.17 2.F4.S2.CA.8.8.2017
##          199400          199807          201158
##          22.F1S1.31.08 5.F4.S2.CA.29.8.2017 2.F4.S1.CA.29.8.2017
##          201793          203638          204571
##          34.F1S1.31.08          5.F1S2.31.08 7.F4.S2.CA.29.8.2017
##          204694          207273          209008
##    1.F6.S2.CA.31.8.17    9.F6.S2.CA.31.8.17 3.F6.S2.CA.31.8.17
##          209323          221264          221983
##    1.F4.S2.CA.8.8.2017          22.F2S2.11.07 3.F5.S2.CA.31.8.17
##          221999          226798          228397
##    5.F5.S1.CA.31.8.17          20.F1S1.21.09 3.F6.S1.CA.31.8.17
##          228481          230243          231433
##    2.F6.S1.CA.31.8.17          13.F1S2.31.08          19.F1S2.31.08
##          232543          233573          235914
##    8.F5.S1.CA.31.8.17          27.F1S2.21.09 6.F6.S2.CA.31.8.17
##          239846          242410          244741
##    3.F4.S1.CA.8.8.2017    9.F6.S1.CA.21.9.17 5.F6.S1.CA.31.8.17
##          246719          247130          248915
##    6.F5.S1.CA.31.8.17          32.F1S1.31.08 2.F5.S1.CA.31.8.17
##          249576          249739          251208
##    4.F4.S1.CA.8.8.2017          22.F2S1.20.06 9.F6.S1.CA.31.8.17
##          251679          251715          257402
##    5.F5.S2.CA.31.8.17    6.F6.S1.CA.21.9.17 9.F5.S2.CA.31.8.17
##          257528          258237          259497
##    6.F5.S2.CA.31.8.17          31.F1S2.21.09 7.F6.S2.CA.31.8.17
##          260095          260175          261493
##    4.F6.S1.CA.31.8.17    4.F5.S1.CA.31.8.17 7.F6.S2.CA.21.9.17
##          263338          264570          269513
##    7.F6.S1.CA.21.9.17    9.F5.S1.CA.31.8.17 8.F5.S2.CA.31.8.17
##          269892          277381          278536
##    8.F6.S2.CA.21.9.17    6.F6.S1.CA.31.8.17          12.F2S2.20.06
##          279210          281640          282992
##    1.F6.S1.CA.31.8.17    8.F6.S2.CA.31.8.17          14.F2S2.20.06
##          283033          283726          289533
##    4.F6.S2.CA.31.8.17          4.F2S1.20.06 4.F4.S2.CA.29.8.2017
##          296588          297958          303543
##    1.F5.S1.CA.31.8.17          28.F1S1.31.08 7.F6.S1.CA.31.8.17
##          304766          305042          307360
##          19.F2S1.20.06    9.F6.S2.CA.21.9.17 5.F6.S2.CA.31.8.17
##          308959          320077          338720
##    2.F6.S2.CA.31.8.17    5.F6.S2.CA.21.9.17 6.F6.S2.CA.21.9.17
##          368702          393697          395258

```

```
### overview data
```

```
datatable(tax_table(ps))
```

```
### remove some contamination to filter out plant and eukaryote data that had chloroplast and mitochondria
```

```
subset <- subset_taxa(ps, Domain != "NA")
```

```
subset <- subset_taxa(subset, Family != "f__Mitochondria=*")
```

```
subset <- subset_taxa(subset, Family != "f__Mitochondria")
```

```
subset <- subset_taxa(subset, Order != "o__Chloroplast")
subset <- subset_taxa(subset, Domain != "k__Archaea")
```

```
### remove taxa with zeros
```

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

```
### subset phyloseq object n=120 metagenomics data
```

```
subset16S <- subset_samples(subset, Metagenomics == "yes" ) #n=120
```

```
subset16S <- prune_taxa(taxa_sums(subset16S) > 0, subset16S)
```

```
subset16S # 120 samples
```

```
## phyloseq-class experiment-level object
```

```
## otu_table() OTU Table: [ 1536 taxa and 120 samples ]
```

```
## sample_data() Sample Data: [ 120 samples by 26 sample variables ]
```

```
## tax_table() Taxonomy Table: [ 1536 taxa by 6 taxonomic ranks ]
```

```
## phy_tree() Phylogenetic Tree: [ 1536 tips and 1535 internal nodes ]
```

```
# cleaning out all kinds of overlapping names from taxonomy table, removing ~*, == and <empty>, this h
subset16S@tax_table = gsub("=\\*|~\\*|\\*|<empty>", "", subset16S@tax_table)
```

```
# overview data
```

```
datatable(tax_table(subset16S))
```

```
rank_names(subset16S) # Shows classes and ARGs
```

```
## [1] "Domain" "Phylum" "Class" "Order" "Family" "Genus"
```

```
sort(get_taxa_unique(subset16S, "Genus")) # Shows unique genera
```

```
## [1] "g__"
## [2] "g__[Eubacterium]_coprostanoligenes_group"
## [3] "g__[Eubacterium]_hallii_group"
## [4] "g__[Eubacterium]_nodatum_group"
## [5] "g__[Eubacterium]_ventriosum_group"
## [6] "g__[Ruminococcus]_gauvreauii_group"
## [7] "g__[Ruminococcus]_torques_group"
## [8] "g__Akkermansia"
## [9] "g__Alistipes"
## [10] "g__Anaerofilum"
## [11] "g__Anaerofustis"
## [12] "g__Anaeroplasma"
## [13] "g__Anaerostipes"
## [14] "g__Anaerotruncus"
## [15] "g__Angelakisella"
## [16] "g__ASF356"
## [17] "g__Bacillus"
## [18] "g__Bacteroides"
## [19] "g__Bifidobacterium"
## [20] "g__Bilophila"
## [21] "g__Blautia"
## [22] "g__Brachybacterium"
```

```

## [23] "g__Butyricicoccus"
## [24] "g__Caldalkalibacillus"
## [25] "g__Candidatus_Soleaferrea"
## [26] "g__CHKCI001"
## [27] "g__CHKCI002"
## [28] "g__Christensenellaceae_R-7_group"
## [29] "g__Coprobacter"
## [30] "g__Coproccoccus_3"
## [31] "g__Corynebacterium_1"
## [32] "g__Defluviitaleaceae_UCG-011"
## [33] "g__Dielma"
## [34] "g__DTU089"
## [35] "g__Eisenbergiella"
## [36] "g__Enterococcus"
## [37] "g__Erysipelatoclostridium"
## [38] "g__Escherichia-Shigella"
## [39] "g__Faecalibacterium"
## [40] "g__Family_XIII_AD3011_group"
## [41] "g__Flavonifractor"
## [42] "g__Fournierella"
## [43] "g__Fusicatenibacter"
## [44] "g__GCA-900066225"
## [45] "g__GCA-900066575"
## [46] "g__Gordonibacter"
## [47] "g__Hydrogenoanaerobacterium"
## [48] "g__Intestinimonas"
## [49] "g__Lachnoclostridium"
## [50] "g__Lachnospiraceae_FCS020_group"
## [51] "g__Lachnospiraceae_FE2018_group"
## [52] "g__Lachnospiraceae_UCG-004"
## [53] "g__Lachnospiraceae_UCG-006"
## [54] "g__Lachnospiraceae_UCG-008"
## [55] "g__Lactobacillus"
## [56] "g__Marvinbryantia"
## [57] "g__Merdibacter"
## [58] "g__Negativibacillus"
## [59] "g__Odoribacter"
## [60] "g__Oscillibacter"
## [61] "g__Oscillospira"
## [62] "g__Parabacteroides"
## [63] "g__Paraeggerthella"
## [64] "g__Parasutterella"
## [65] "g__Phascolarctobacterium"
## [66] "g__Phoceia"
## [67] "g__Ralstonia"
## [68] "g__Romboutsia"
## [69] "g__Roseburia"
## [70] "g__Ruminiclostridium"
## [71] "g__Ruminiclostridium_5"
## [72] "g__Ruminiclostridium_9"
## [73] "g__Ruminococcaceae_NK4A214_group"
## [74] "g__Ruminococcaceae_UCG-005"
## [75] "g__Ruminococcaceae_UCG-009"
## [76] "g__Ruminococcaceae_UCG-010"

```

```
## [77] "g__Ruminococcaceae_UCG-013"
## [78] "g__Ruminococcaceae_UCG-014"
## [79] "g__Ruminococcus_1"
## [80] "g__Ruminococcus_2"
## [81] "g__Sellimonas"
## [82] "g__Shuttleworthia"
## [83] "g__Staphylococcus"
## [84] "g__Streptococcus"
## [85] "g__Subdoligranulum"
## [86] "g__Tyzzerella"
## [87] "g__Tyzzerella_3"
## [88] "g__UBA1819"
## [89] "g__UC5-1-2E3"
## [90] "g__uncultured"
## [91] "g__uncultured_bacterium"
## [92] "g__uncultured_organism"
## [93] "g__Weissella"
```

```
sort(sample_sums(subset16S)) # Amount of unique taxa"per sample, the min is 46731 and max 393697, which
```

```
##      14.F2S1.20.06      4.F2S1.11.07      13.F2S2.11.07
##      46731      51537      55002
##      5.F2S2.20.06      24.F2S1.11.07      7.F2S2.11.07
##      57164      59138      65002
##      2.F5.S1.CA.21.9.17      4.F6.S2.CA.21.9.17      3.F6.S1.CA.21.9.17
##      76540      80838      85041
##      3.F6.S2.CA.21.9.17      27.F1S2.31.08      4.F1S1.21.09
##      85370      85751      86303
##      20.F1S1.31.08      2.F6.S2.CA.21.9.17      4.F6.S1.CA.21.9.17
##      94222      94800      95509
##      6.F4.S2.CA.8.8.2017      19.F2S2.11.07      7.F5.S1.CA.31.8.17
##      96497      99631      100277
##      5.F5.S1.CA.21.9.17      1.F6.S1.CA.21.9.17      3.F5.S1.CA.21.9.17
##      104268      107531      107684
##      10.F2S1.20.06      31.F1S2.31.08      35.F1S2.31.08
##      112768      117379      119253
##      4.F5.S2.CA.31.8.17      18.F2S1.20.06      1.F1S2.21.09
##      119720      120688      121074
##      4.F4.S1.CA.29.8.2017      6.F5.S1.CA.21.9.17      11.F1S2.21.09
##      121879      123314      123322
##      14.F1S1.21.09      12.F1S1.21.09      6.F5.S2.CA.21.9.17
##      126120      128514      128935
##      6.F1S1.21.09      10.F2S1.11.07      8.F4.S1.CA.29.8.2017
##      130408      132808      134681
##      9.F2S2.20.06      14.F1S1.31.08      21.F1S2.31.08
##      134990      135080      135703
##      15.F1S2.21.09      6.F4.S1.CA.29.8.2017      8.F6.S1.CA.21.9.17
##      135968      136635      137890
##      6.F4.S1.CA.8.8.2017      4.F5.S1.CA.21.9.17      7.F4.S1.CA.8.8.2017
##      138088      140635      140868
##      1.F5.S1.CA.21.9.17      1.F4.S1.CA.8.8.2017      1.F5.S2.CA.21.9.17
##      141444      146133      146908
##      2.F6.S1.CA.21.9.17      2.F5.S2.CA.21.9.17      7.F1S2.21.09
##      150121      151425      153367
```

```
## 6.F4.S2.CA.29.8.2017      2.F2S2.20.06      2.F5.S2.CA.31.8.17
##          153497          153925          155053
##          19.F1S2.21.09 1.F4.S1.CA.29.8.2017      2.F1S1.31.08
##          156652          157292          158039
##          18.F2S2.11.07 2.F4.S2.CA.29.8.2017      3.F5.S1.CA.31.8.17
##          158392          158632          159579
##          21.F1S2.21.09      4.F5.S2.CA.21.9.17      8.F6.S1.CA.31.8.17
##          163719          165742          166565
##          5.F4.S2.CA.8.8.2017      22.F1S1.21.09      20.F2S1.11.07
##          167240          169419          171242
##          1.F6.S2.CA.21.9.17      9.F1S2.31.08 3.F4.S2.CA.29.8.2017
##          173063          173638          175120
##          6.F1S1.31.08      9.F2S1.11.07      10.F1S1.31.08
##          175950          177762          178746
##          3.F4.S1.CA.29.8.2017      10.F2S2.11.07      5.F5.S2.CA.21.9.17
##          179206          179318          180662
##          8.F2S1.20.06      2.F4.S1.CA.8.8.2017      1.F5.S2.CA.31.8.17
##          182277          188508          189411
##          17.F2S2.20.06      1.F2S1.11.07 1.F4.S2.CA.29.8.2017
##          189516          191885          192096
##          4.F4.S2.CA.8.8.2017      3.F5.S2.CA.21.9.17      2.F2S1.20.06
##          194069          194327          194902
##          3.F4.S2.CA.8.8.2017      2.F4.S2.CA.8.8.2017      5.F4.S2.CA.29.8.2017
##          199046          201158          203364
##          2.F4.S1.CA.29.8.2017      5.F1S2.31.08      1.F6.S2.CA.31.8.17
##          203912          207273          209323
##          3.F6.S2.CA.31.8.17      1.F4.S2.CA.8.8.2017      22.F2S2.11.07
##          221983          221999          225672
##          3.F5.S2.CA.31.8.17      20.F1S1.21.09      3.F6.S1.CA.31.8.17
##          227443          230243          231433
##          2.F6.S1.CA.31.8.17      8.F5.S1.CA.31.8.17      6.F6.S2.CA.31.8.17
##          232543          238256          244741
##          3.F4.S1.CA.8.8.2017      2.F5.S1.CA.31.8.17      4.F4.S1.CA.8.8.2017
##          246719          250484          251679
##          5.F5.S2.CA.31.8.17      6.F5.S2.CA.31.8.17      4.F6.S1.CA.31.8.17
##          257528          260095          263338
##          4.F5.S1.CA.31.8.17      7.F6.S1.CA.21.9.17      7.F6.S2.CA.21.9.17
##          264570          269468          269513
##          12.F2S2.20.06      1.F6.S1.CA.31.8.17      14.F2S2.20.06
##          282992          283033          289533
##          4.F6.S2.CA.31.8.17      4.F2S1.20.06      4.F4.S2.CA.29.8.2017
##          296588          297958          303543
##          28.F1S1.31.08      1.F5.S1.CA.31.8.17      7.F6.S1.CA.31.8.17
##          304246          304446          307360
##          5.F6.S2.CA.31.8.17      2.F6.S2.CA.31.8.17      5.F6.S2.CA.21.9.17
##          338720          368702          393697
```

```
summary(sample_sums(subset16S)) # summary of the sampling depths
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 46731 125421 161649 173742 212488 393697
```



```
sample_variables(subset16S) # metadata variables
```

```
## [1] "LibraryNumber" "Sample_Unique" "LibraryName" "Farm"
## [5] "Farm2" "Stable" "FarmRoundStable" "Days"
## [9] "Age" "Sname" "WeightAnimal" "Gender"
## [13] "AgeParentStock" "Hatchery" "Researcher" "AB"
## [17] "Abday" "FlockSize" "FeedF" "FeedType"
## [21] "FeedProducent" "Cox" "OPG" "Cluster"
## [25] "LitterType" "Metagenomics"
```

```
# Rewriting sampleIDs as sample_unique rownames to align with the other datasets
```

```
sample_names(subset16S) = sample_data(subset16S)$Sample_Unique
sample_names(subset16S)
```

```
## [1] "2_23" "2_24" "2_25" "2_26" "2_27" "2_29" "2_36" "2_39" "2_40"
## [10] "2_41" "2_42" "2_47" "2_48" "2_49" "2_50" "2_51" "2_52" "2_56"
## [19] "2_57" "2_58" "2_59" "2_60" "2_61" "4_36" "4_37" "4_38" "4_39"
## [28] "4_40" "4_41" "4_54" "4_55" "4_56" "4_57" "4_65" "5_39" "5_40"
## [37] "5_41" "5_54" "5_55" "5_59" "6_36" "6_37" "6_38" "6_54" "6_55"
## [46] "6_56" "6_57" "6_58" "9_16" "9_17" "9_18" "9_19" "9_21" "9_22"
## [55] "9_34" "9_35" "9_36" "9_37" "9_38" "9_39" "10_1" "10_2" "10_3"
## [64] "10_4" "10_7" "10_8" "10_10" "10_11" "10_12" "10_13" "10_14" "10_15"
## [73] "10_19" "10_20" "10_21" "10_22" "10_25" "10_26" "10_28" "10_29" "10_30"
## [82] "10_33" "10_34" "10_35" "10_39" "10_40" "10_41" "10_42" "10_43" "10_44"
## [91] "10_48" "10_49" "10_50" "10_51" "10_52" "10_53" "10_57" "10_58" "10_59"
## [100] "10_60" "10_63" "10_64" "10_66" "10_67" "10_68" "10_69" "11_1" "11_3"
## [109] "14_20" "14_21" "14_22" "14_23" "14_25" "14_27" "14_29" "14_30" "14_33"
## [118] "14_34" "14_35" "14_36"
```

```
# Stable "Farm2R1S1" has the three lowest sampling depths of the dataset, the other nine samples are f
subset16S %>% ps_filter(FarmRoundStable == c("Farm2R1S1")) %>% sample_sums() %>% sort()
```

```
## 4_40 4_55 5_55 4_39 4_41 4_57 5_54 4_56 4_38 4_54 4_36
## 46731 51537 59138 112768 120688 132808 171242 177762 182277 191885 194902
## 4_37
## 297958
```

```
# Amount of different taxa present.
```

```
sort(table(tax_table(subset16S)[, "Phylum"])))
```

```
##
## p__Verrucomicrobia p__Cyanobacteria p__Proteobacteria p__Bacteroidetes
## 2 13 14 22
## p__Actinobacteria p__Tenericutes p__Firmicutes
## 48 81 1356
```

```
sort(table(tax_table(subset16S)[, "Order"])))
```

```
##
```

##	o__Selenomonadales	o__Corynebacteriales	o__Desulfovibrionales
##	1	2	2
##	o__Izimaplasmatales	o__Micrococcales	o__Verrucomicrobiales
##	2	2	2
##	o__	o__Anaeroplasmatales	o__Enterobacteriales
##	4	4	5
##	o__Bacillales	o__Betaproteobacteriales	o__Coriobacteriales
##	6	7	12
##	o__Gastranaerophilales	o__Bacteroidales	o__Bifidobacteriales
##	13	22	32
##	o__Erysipelotrichales	o__Lactobacillales	o__Mollicutes_RF39
##	39	45	71
##	o__Clostridiales		
##	1265		

```
sort(table(tax_table(subset16S)[, "Family"])))
```

##		
##	f__Acidaminococcaceae	f__Barnesiellaceae
##	1	1
##	f__Dermabacteraceae	f__Eubacteriaceae
##	1	1
##	f__F082	f__Gracilibacteraceae
##	1	1
##	f__Leuconostocaceae	f__Marinifilaceae
##	1	1
##	f__Micrococcaceae	f__Akkermansiaceae
##	1	2
##	f__Corynebacteriaceae	f__Desulfovibrionaceae
##	2	2
##	f__Peptococcaceae	f__Streptococcaceae
##	2	2
##	f__uncultured_Firmicutes_bacterium	f__Bacillaceae
##	2	3
##	f__Rikenellaceae	f__Staphylococcaceae
##	3	3
##	f__Tannerellaceae	f__uncultured_rumen_bacterium
##	3	3
##	f__Anaeroplasmataceae	f__Defluviitaleaceae
##	4	4
##	f__Family_XIII	f__Enterobacteriaceae
##	4	5
##	f__Enterococcaceae	f__Burkholderiaceae
##	5	7
##	f__Peptostreptococcaceae	f__Eggerthellaceae
##	11	12
##	f__Bacteroidaceae	f__Christensenellaceae
##	13	24
##	f__Clostridiales_vadinBB60_group	f__Bifidobacteriaceae
##	28	32
##	f__	f__Lactobacillaceae
##	33	37
##	f__Erysipelotrichaceae	f__uncultured_bacterium
##	39	62

```
##                                f__Ruminococcaceae                                f__Lachnospiraceae
##                                549                                631

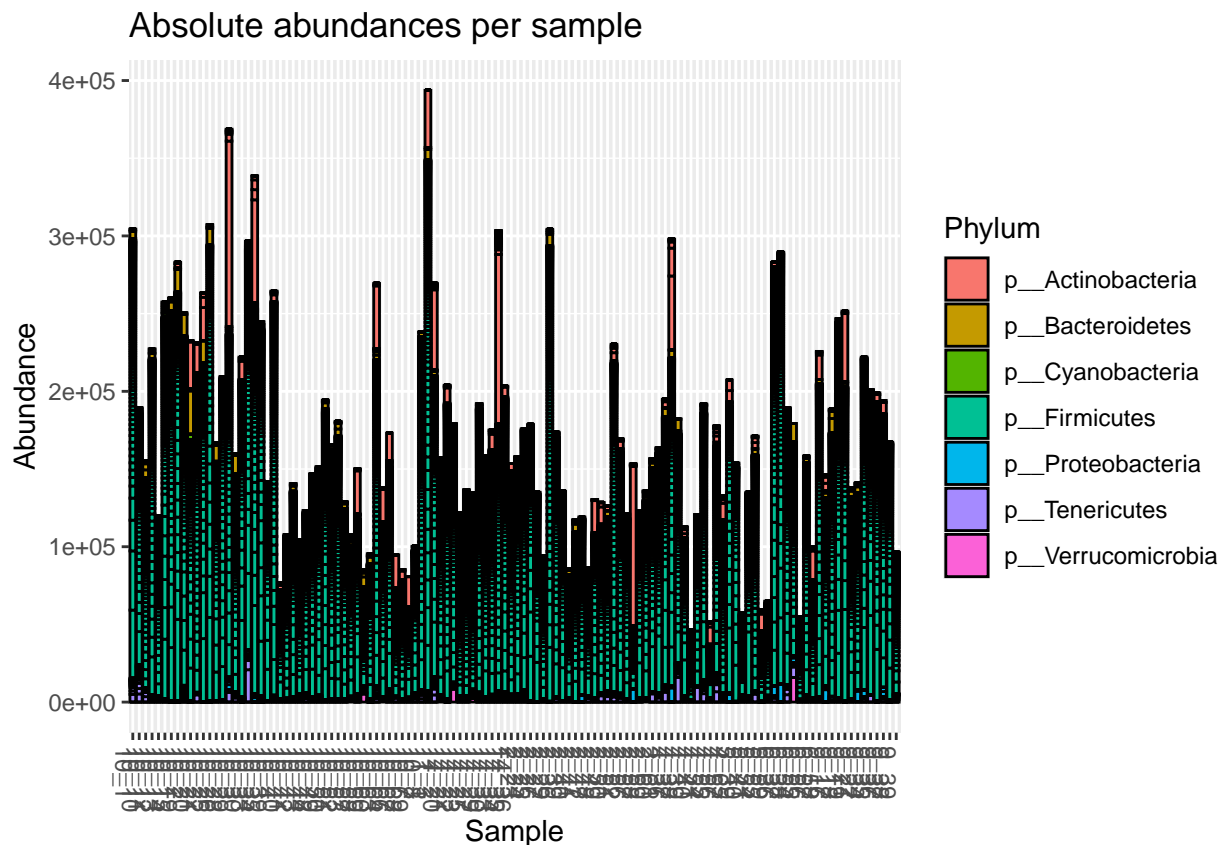
# factorizing variables as not to create problems with visualization later down the line
sample_data(subset16S)$Cluster = as.factor(sample_data(subset16S)$Cluster)
sample_data(subset16S)$FlockSize = as.factor(sample_data(subset16S)$FlockSize)
sample_data(subset16S)$AgeParentStock = as.factor(sample_data(subset16S)$AgeParentStock)
sample_data(subset16S)$Age = as.factor(sample_data(subset16S)$Age)
sample_data(subset16S)$LibraryNumber = as.factor(sample_data(subset16S)$LibraryNumber)

# add stable column with shorter names
sample_data(subset16S)$FarmRoundStable = as.factor(sample_data(subset16S)$FarmRoundStable)
subset16S@sam_data$Stables = revalue(sample_data(subset16S)$FarmRoundStable, c("Farm1R1S1"="Stable1", "Farm1R2S1"="Stable2", "Farm1R3S1"="Stable3", "Farm1R4S1"="Stable4", "Farm2R1S1"="Stable5", "Farm2R2S1"="Stable6", "Farm2R3S1"="Stable7", "Farm2R4S1"="Stable8", "Farm3R1S1"="Stable9", "Farm3R2S1"="Stable10", "Farm3R3S1"="Stable11", "Farm3R4S1"="Stable12", "Farm4R1S1"="Stable13", "Farm4R2S1"="Stable14", "Farm4R3S1"="Stable15", "Farm4R4S1"="Stable16"))

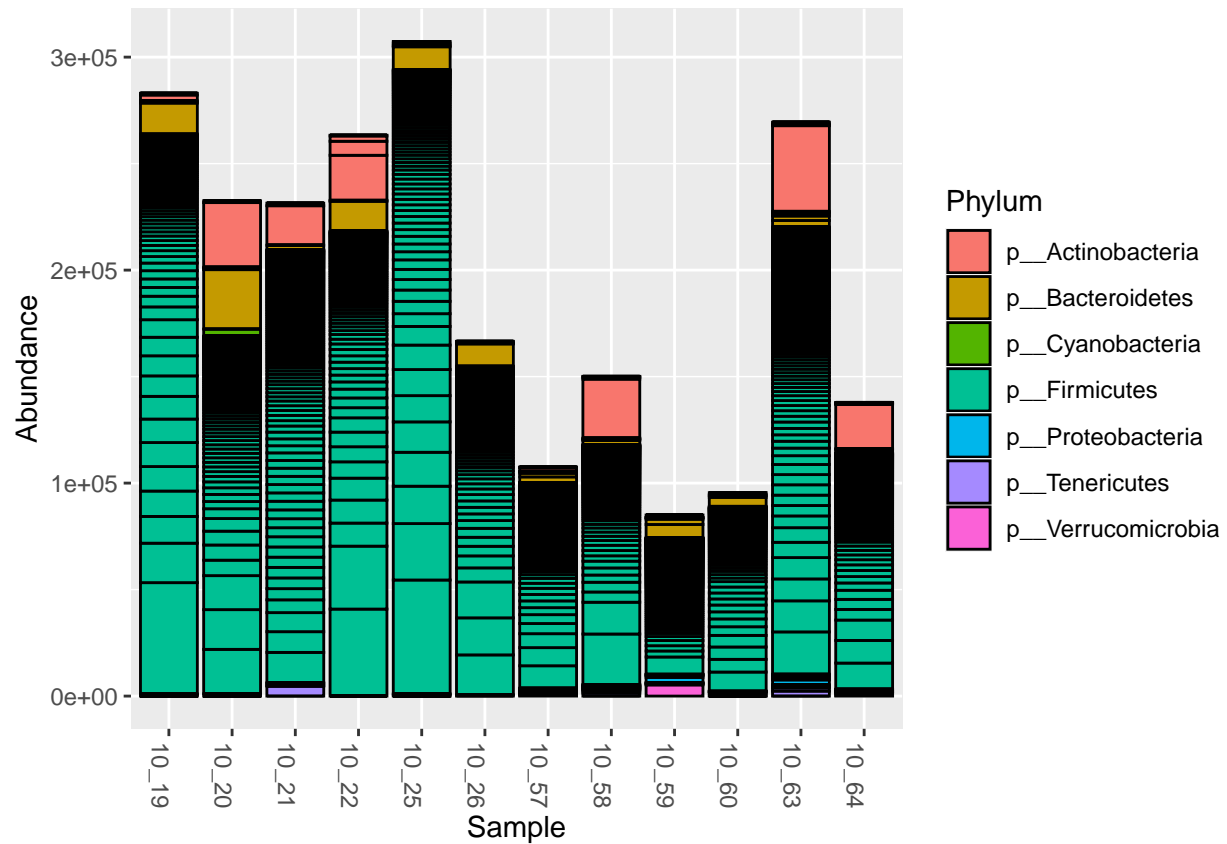
# Shortening agent names
subset16S@sam_data$Cox[subset16S@sam_data$Cox == "narasinandnicarbazin(maxiban)"] = "Maxiban"
subset16S@sam_data$Cox[subset16S@sam_data$Cox == "narasin(monteban)"] = "Monteban"
subset16S@sam_data$Cox[subset16S@sam_data$Cox == "salinomycin(Sacox120microGranulate)"] = "Sacox"
```

Abundances and heatmaps

```
# absolute abundances - phylum
plot_bar(subset16S, fill="Phylum", title = "Absolute abundances per sample")
```



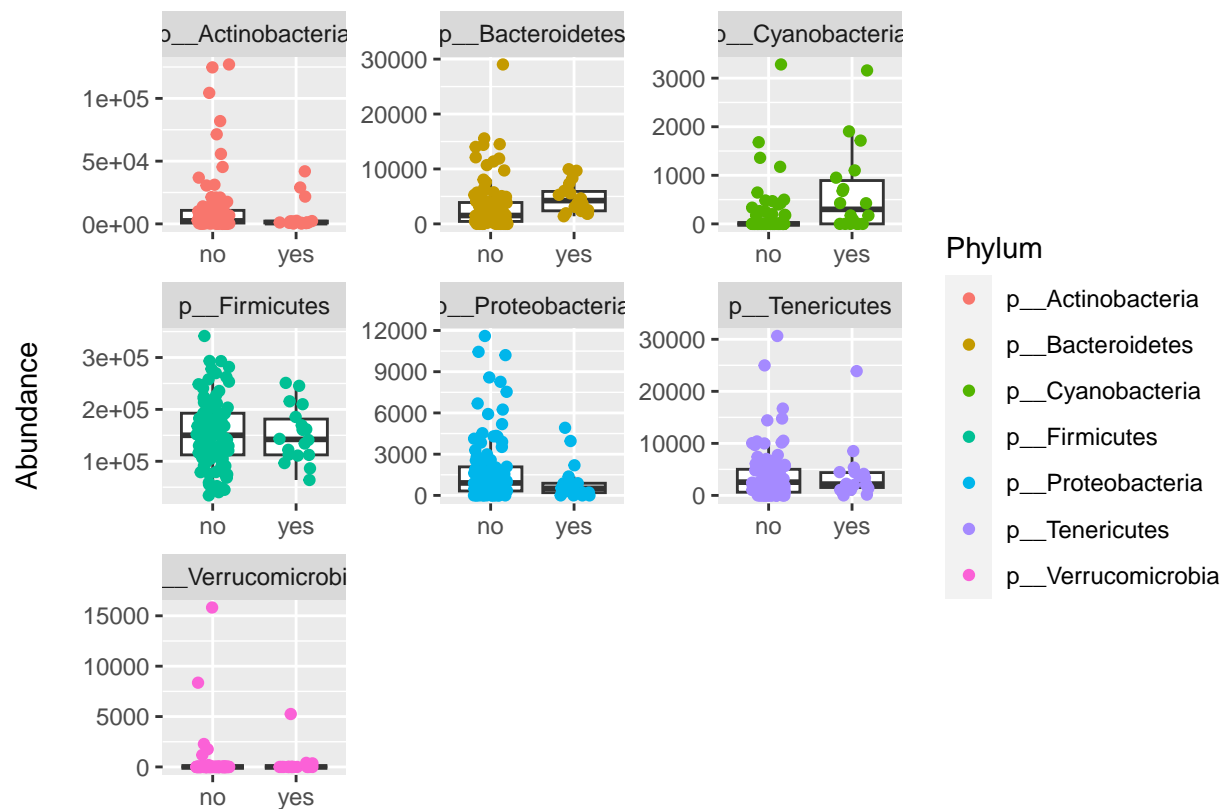
```
# for plotting abundances of specific stables
subset16S %>% ps_filter(Stables == c("Stable9")) %>% plot_bar(fill="Phylum")
```



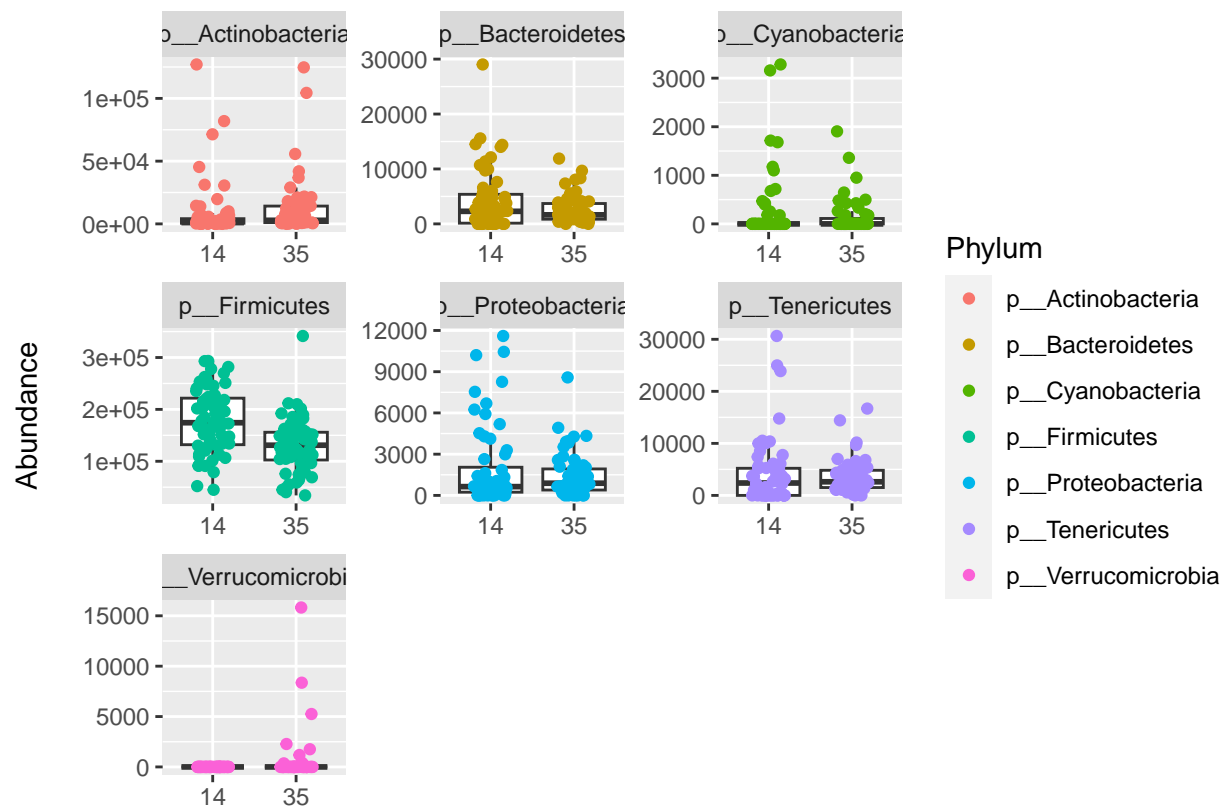
visualisation on AB at Phylum level, more data for samples which have not been treated with AB, but a

```
ps_prim <- phyloseq::tax_glom(subset16S, "Phylum")
taxa_names(ps_prim) <- phyloseq::tax_table(ps_prim)[, "Phylum"]

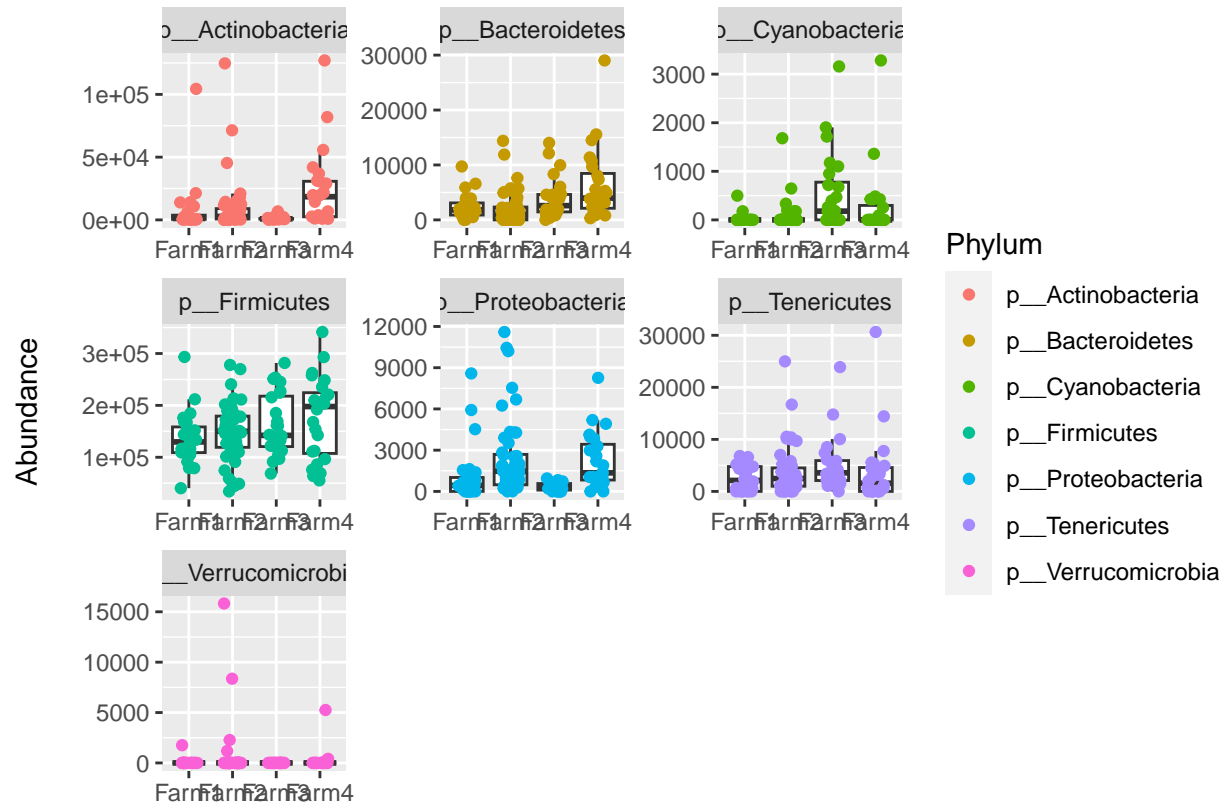
psmelt(ps_prim) %>% # AB
  ggplot(data = ., aes(x = AB, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Phylum), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Phylum, scales = "free")
```



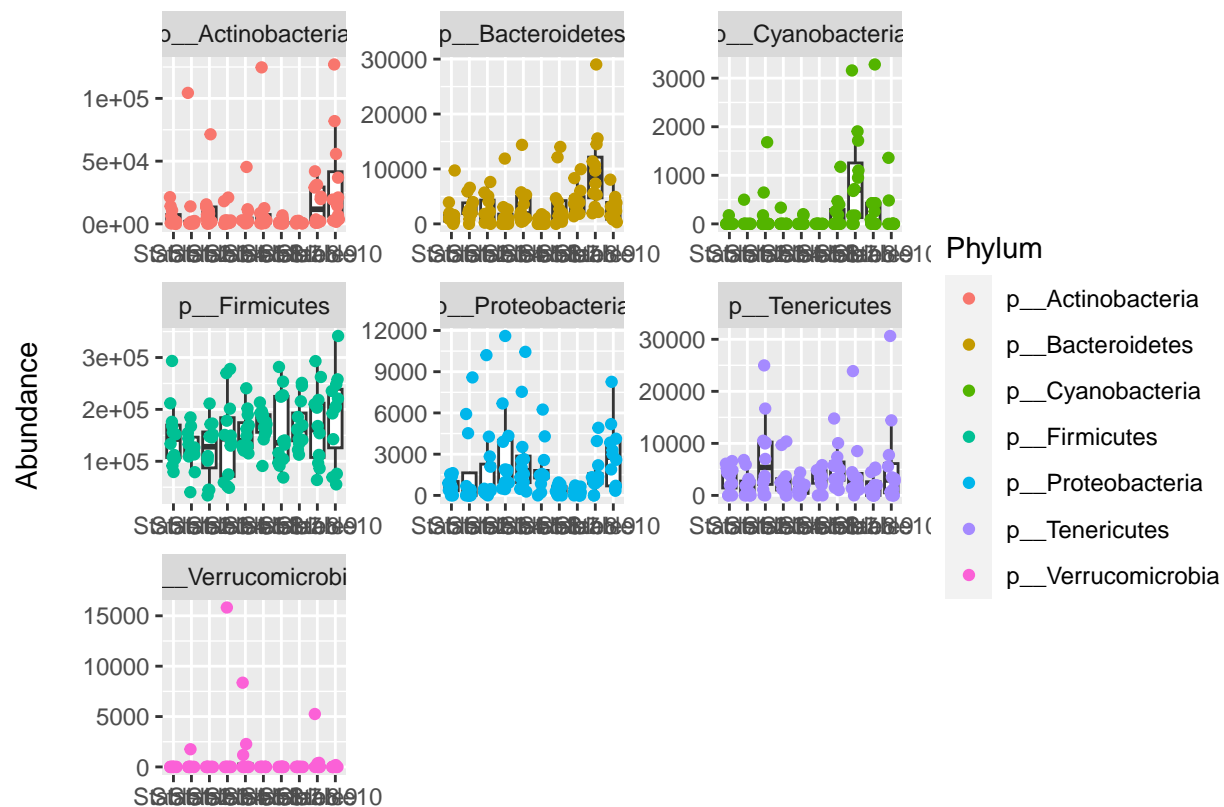
```
psmelt(ps_prim) %>% # Age
  ggplot(data = ., aes(x = Age, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Phylum), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Phylum, scales = "free")
```



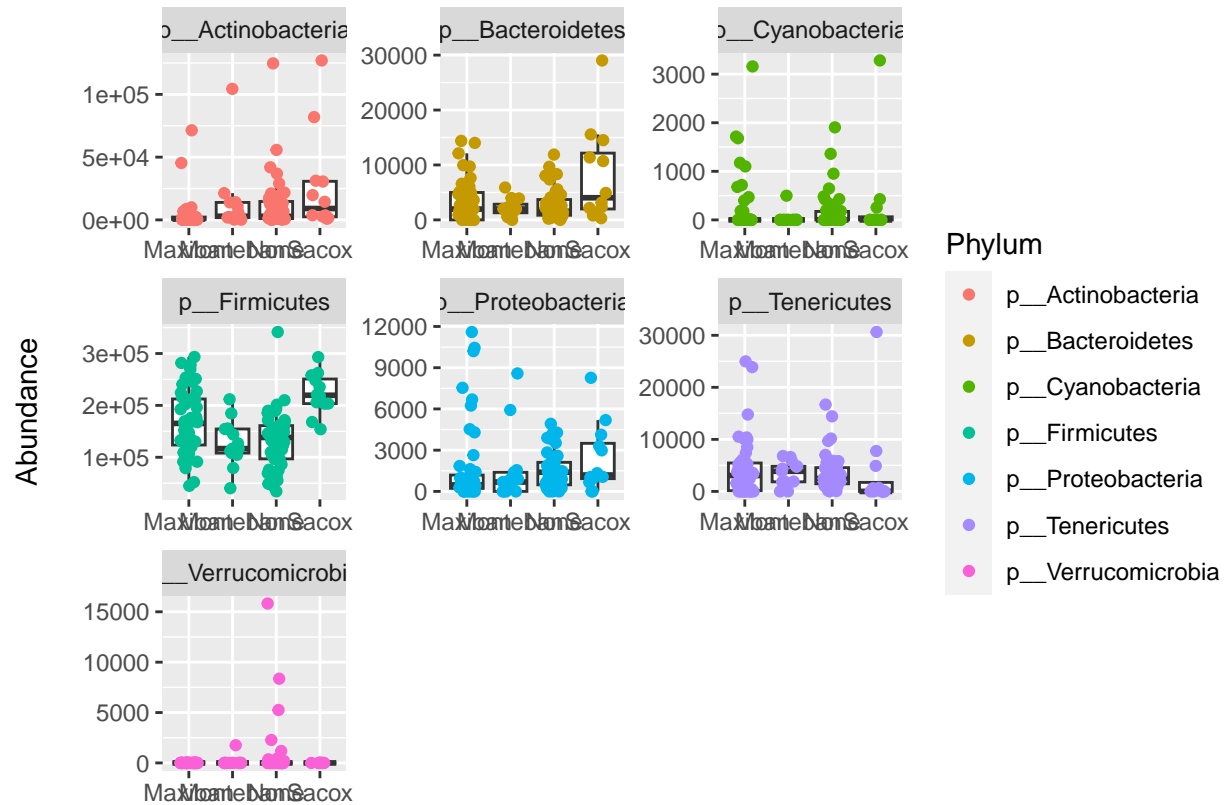
```
psmelt(ps_prim) %>% # Farm
  ggplot(data = ., aes(x = Farm2, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Phylum), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Phylum, scales = "free")
```



```
psmelt(ps_prim) %>% # Stable
  ggplot(data = ., aes(x = Stables, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Phylum), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Phylum, scales = "free")
```



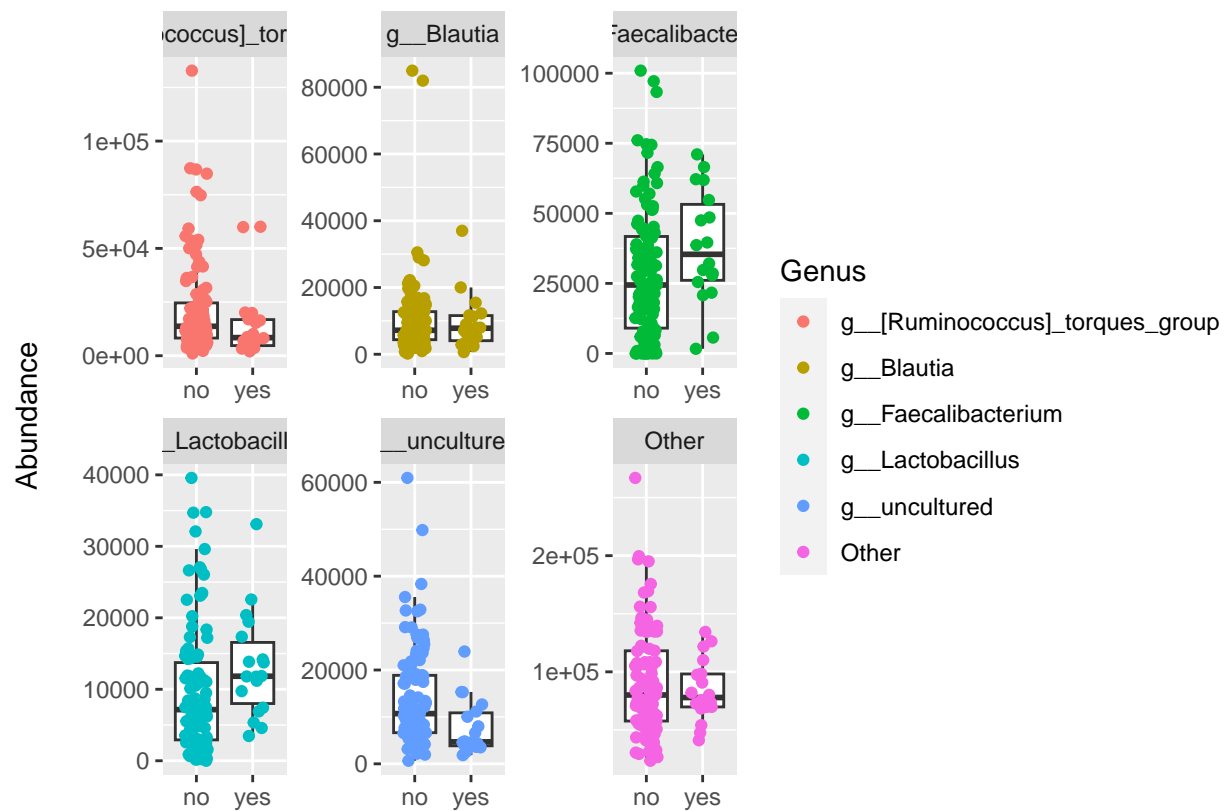
```
psmelt(ps_prim) %>% # Agent
  ggplot(data = ., aes(x = Cox, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Phylum), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Phylum, scales = "free")
```

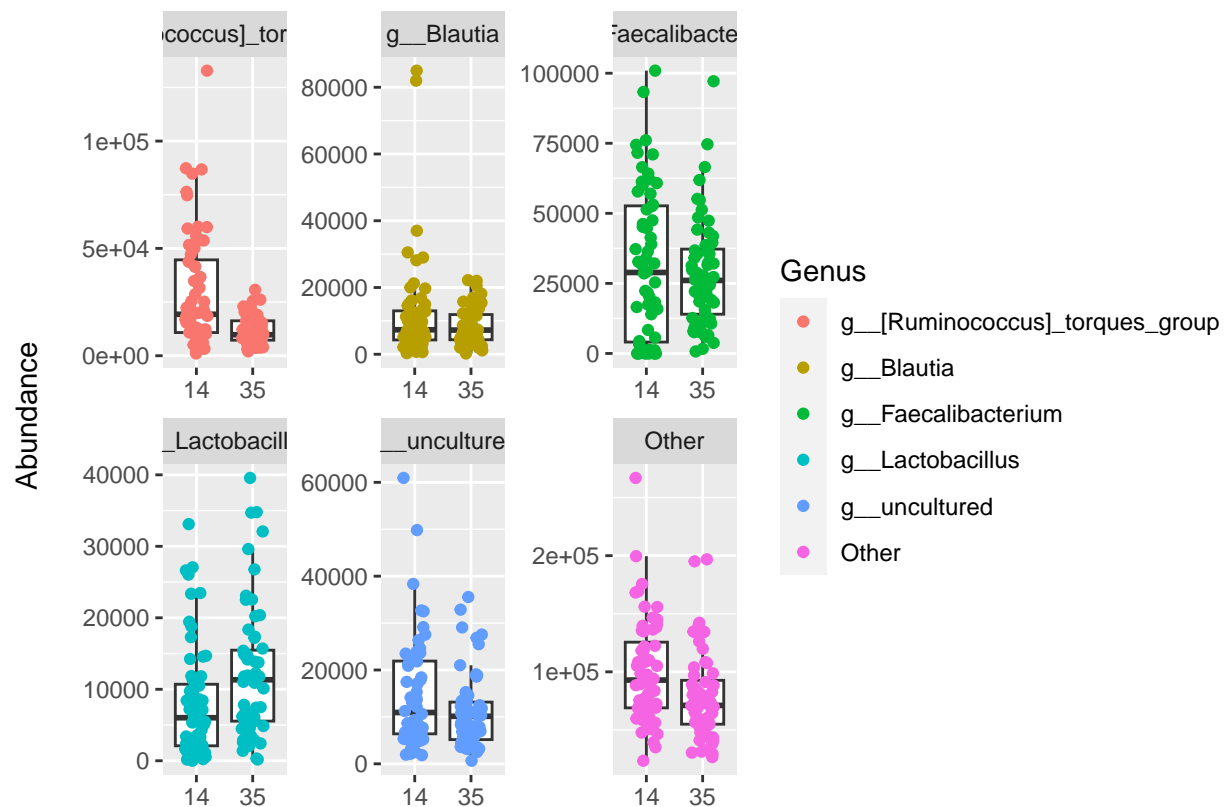
visualisation on AB at Genus level, more data for samples which have not been treated with AB, but al

```
ps_prim <- subset16S %>% aggregate_top_taxa2("Genus", top = 5) %>% phyloseq::tax_glom("Genus")
taxa_names(ps_prim) <- phyloseq::tax_table(ps_prim)[, "Genus"]

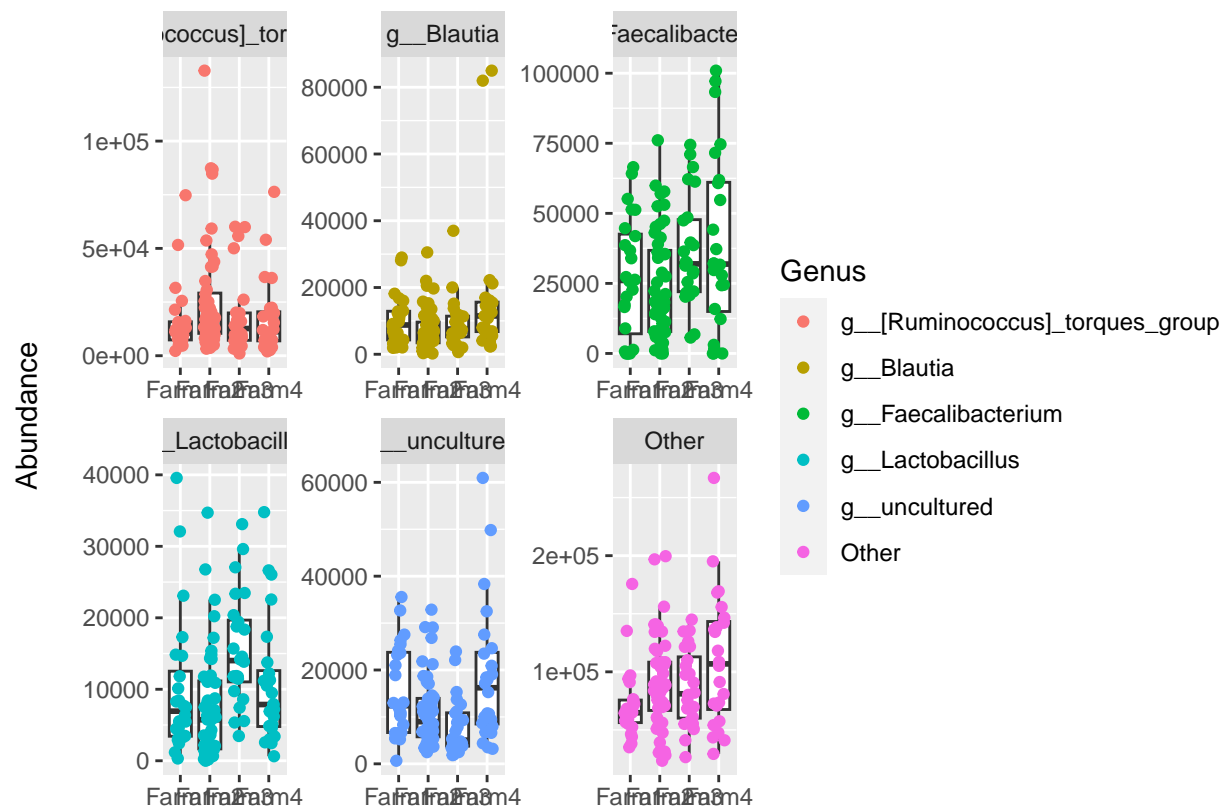
psmelt(ps_prim) %>% # AB
  ggplot(data = ., aes(x = AB, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Genus), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Genus, scales = "free")
```



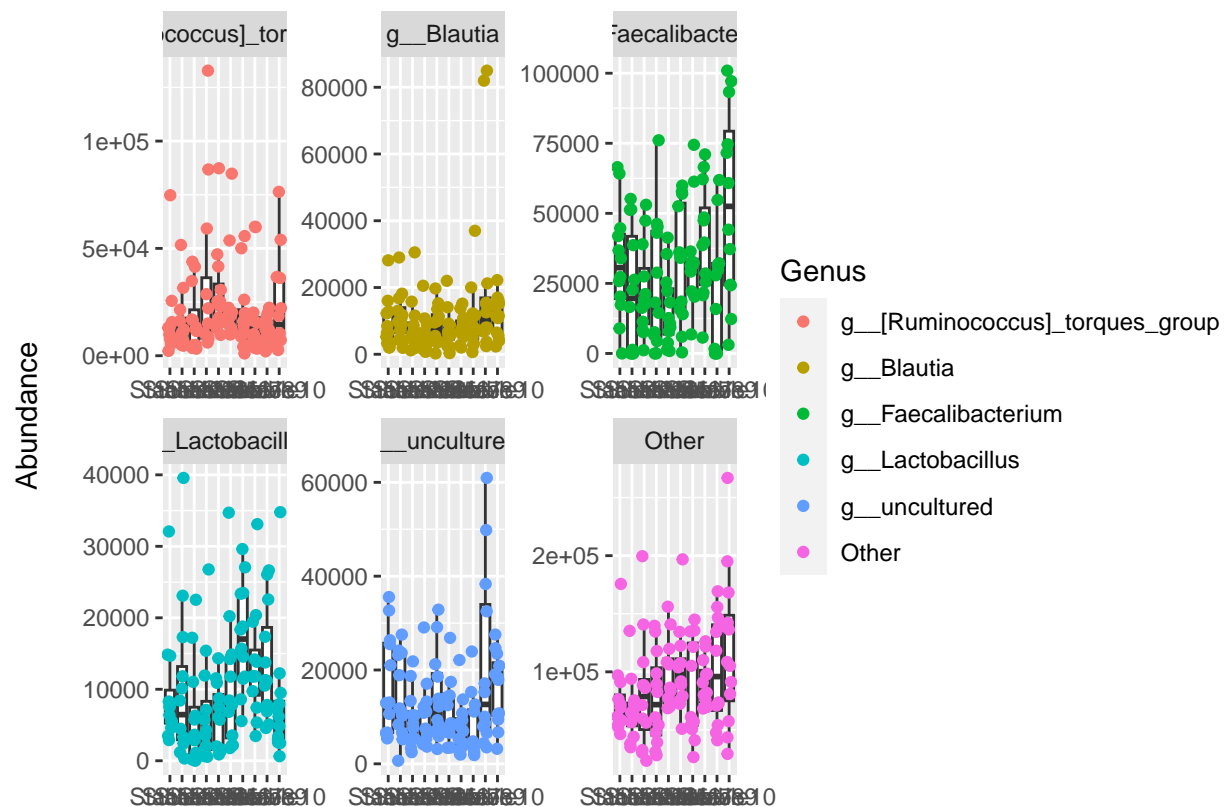
```
psmelt(ps_prim) %>% # Age
  ggplot(data = ., aes(x = Age, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Genus), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Genus, scales = "free")
```



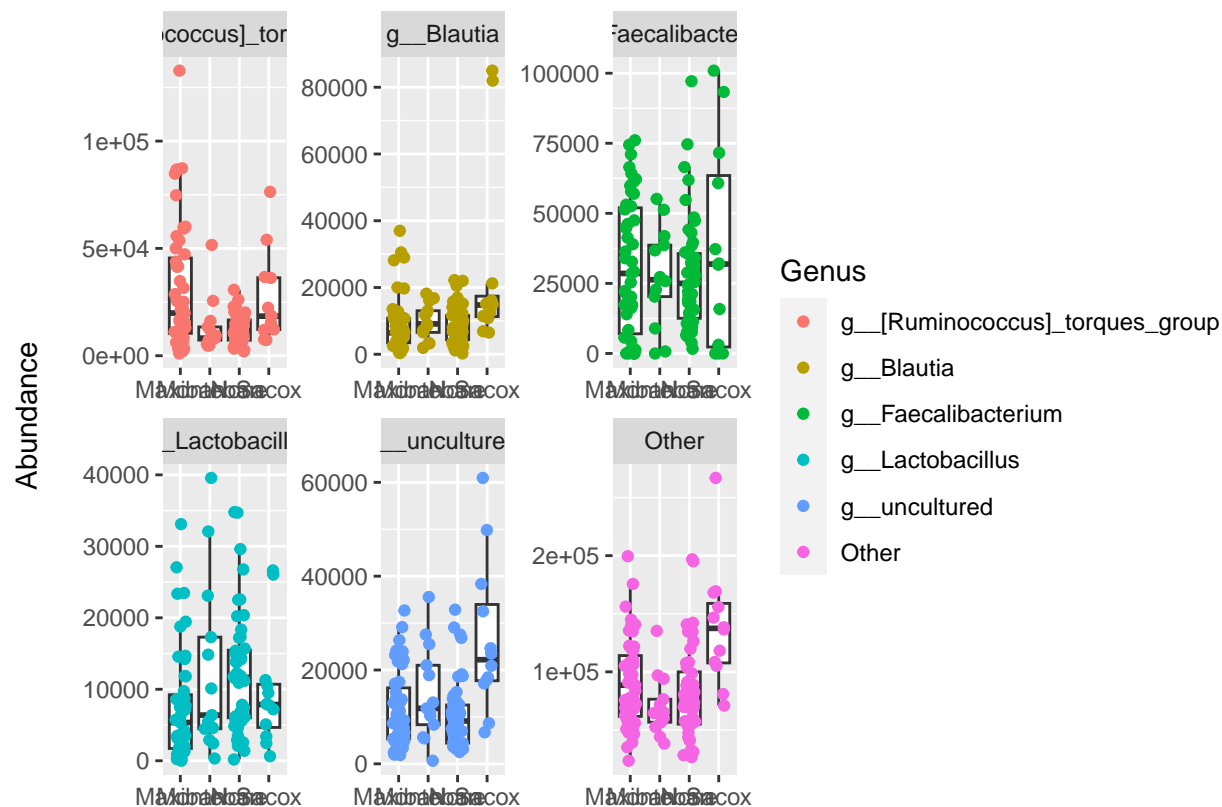
```
psmelt(ps_prim) %>% # Farm
  ggplot(data = ., aes(x = Farm2, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Genus), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Genus, scales = "free")
```



```
psmelt(ps_prim) %>% # Stable
  ggplot(data = ., aes(x = Stables, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Genus), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Genus, scales = "free")
```



```
psmelt(ps_prim) %>% # Agent
  ggplot(data = ., aes(x = Cox, y = Abundance)) +
  geom_boxplot(outlier.shape = NA) +
  geom_jitter(aes(color = Genus), height = 0, width = .2) +
  labs(x = "", y = "Abundance\n") +
  facet_wrap(~ Genus, scales = "free")
```



```
# Check the amount of unique genera in samples which have and have not been treated with antibiotics
subset16S %>% ps_filter(AB == "no") %>% get_taxa_unique("Genus") # 93 different genera for non AB treat
```

```
## [1] "g__"
## [2] "g__Dielma"
## [3] "g__uncultured"
## [4] "g__Anaeroplasma"
## [5] "g__Merdibacter"
## [6] "g__Erysipelatoclostridium"
## [7] "g__Blautia"
## [8] "g__Odoribacter"
## [9] "g__Parabacteroides"
## [10] "g__Copro bacter"
## [11] "g__Bacteroides"
## [12] "g__Alistipes"
## [13] "g__Bilophila"
## [14] "g__Parasutterella"
## [15] "g__Ralstonia"
## [16] "g__Escherichia-Shigella"
## [17] "g__Corynebacterium_1"
## [18] "g__Brachy bacterium"
## [19] "g__Bifidobacterium"
## [20] "g__Ruminococcaceae_UCG-014"
## [21] "g__Lachnospiraceae_UCG-008"
## [22] "g__CHKCI002"
## [23] "g__Gordonibacter"
```

```

## [24] "g__Akkermansia"
## [25] "g__Paraeggerthella"
## [26] "g__Christensenellaceae_R-7_group"
## [27] "g__uncultured_bacterium"
## [28] "g__Ruminococcaceae_UCG-010"
## [29] "g__Tyzzerella_3"
## [30] "g__Family_XIII_AD3011_group"
## [31] "g__Romboutsia"
## [32] "g__Staphylococcus"
## [33] "g__Caldalkalibacillus"
## [34] "g__Lactobacillus"
## [35] "g__Weissella"
## [36] "g__Bacillus"
## [37] "g__Enterococcus"
## [38] "g__Eisenbergiella"
## [39] "g__Streptococcus"
## [40] "g__Phascolarctobacterium"
## [41] "g__uncultured_organism"
## [42] "g__Anaerofustis"
## [43] "g__Ruminococcaceae_UCG-013"
## [44] "g__Butyricicoccus"
## [45] "g__Faecalibacterium"
## [46] "g__Phoceia"
## [47] "g__Subdoligranulum"
## [48] "g__GCA-900066225"
## [49] "g__[Eubacterium]_coprostanoligenes_group"
## [50] "g__Negativibacillus"
## [51] "g__Hydrogenoanaerobacterium"
## [52] "g__Angelakisella"
## [53] "g__Fournierella"
## [54] "g__Anaerofilum"
## [55] "g__Anaerotruncus"
## [56] "g__[Ruminococcus]_torques_group"
## [57] "g__Sellimonas"
## [58] "g__Lachnoclostridium"
## [59] "g__GCA-900066575"
## [60] "g__CHKCI001"
## [61] "g__UC5-1-2E3"
## [62] "g__Fusicatenibacter"
## [63] "g__Roseburia"
## [64] "g__Marvinbryantia"
## [65] "g__Coproccoccus_3"
## [66] "g__Lachnospiraceae_UCG-006"
## [67] "g__[Ruminococcus]_gauvreauui_group"
## [68] "g__Lachnospiraceae_UCG-004"
## [69] "g__Lachnospiraceae_FCS020_group"
## [70] "g__Lachnospiraceae_FE2018_group"
## [71] "g__[Eubacterium]_ventriosum_group"
## [72] "g__Shuttleworthia"
## [73] "g__[Eubacterium]_hallii_group"
## [74] "g__Anaerostipes"
## [75] "g__UBA1819"
## [76] "g__Ruminococcus_1"
## [77] "g__Defluviitaleaceae_UCG-011"

```

```
## [78] "g__ASF356"
## [79] "g__Tyzzerella"
## [80] "g__[Eubacterium]_nodatum_group"
## [81] "g__Ruminiclostridium_9"
## [82] "g__Ruminiclostridium_5"
## [83] "g__Ruminiclostridium"
## [84] "g__Oscillospira"
## [85] "g__Oscillibacter"
## [86] "g__Ruminococcus_2"
## [87] "g__Ruminococcaceae_UCG-009"
## [88] "g__Ruminococcaceae_UCG-005"
## [89] "g__DTU089"
## [90] "g__Candidatus_Soleaferrea"
## [91] "g__Ruminococcaceae_NK4A214_group"
## [92] "g__Flavonifractor"
## [93] "g__Intestinimonas"
```

```
subset16S %>% ps_filter(AB == "yes") %>% get_taxa_unique("Genus") # 74 different genera for AB treated
```

```
## [1] "g__"
## [2] "g__uncultured"
## [3] "g__Anaeroplasma"
## [4] "g__Merdibacter"
## [5] "g__Erysipelatoclostridium"
## [6] "g__Odoribacter"
## [7] "g__Bacteroides"
## [8] "g__Alistipes"
## [9] "g__uncultured_bacterium"
## [10] "g__Bilophila"
## [11] "g__Parasutterella"
## [12] "g__Escherichia-Shigella"
## [13] "g__Bifidobacterium"
## [14] "g__Ruminococcaceae_UCG-014"
## [15] "g__CHKC1002"
## [16] "g__Gordonibacter"
## [17] "g__Akkermansia"
## [18] "g__Christensenellaceae_R-7_group"
## [19] "g__Ruminococcaceae_UCG-010"
## [20] "g__Tyzzerella_3"
## [21] "g__Romboutsia"
## [22] "g__Lactobacillus"
## [23] "g__Bacillus"
## [24] "g__Enterococcus"
## [25] "g__Eisenbergiella"
## [26] "g__Streptococcus"
## [27] "g__Phascolarctobacterium"
## [28] "g__Anaerofustis"
## [29] "g__Ruminococcaceae_UCG-013"
## [30] "g__Butyricicoccus"
## [31] "g__Faecalibacterium"
## [32] "g__Phoceia"
## [33] "g__Subdoligranulum"
## [34] "g__GCA-900066225"
## [35] "g__Negativibacillus"
```



```

## [36] "g__Hydrogenoanaerobacterium"
## [37] "g__Anaerofilum"
## [38] "g__Anaerotruncus"
## [39] "g__Fournierella"
## [40] "g__[Ruminococcus]_torques_group"
## [41] "g__Sellimonas"
## [42] "g__GCA-900066575"
## [43] "g__Blautia"
## [44] "g__CHKCI001"
## [45] "g__UC5-1-2E3"
## [46] "g__Fusicatenibacter"
## [47] "g__Marvinbryantia"
## [48] "g__Coprococcus_3"
## [49] "g__Lachnospiraceae_UCG-006"
## [50] "g__Lachnospiraceae_UCG-008"
## [51] "g__Lachnoclostridium"
## [52] "g__Lachnospiraceae_UCG-004"
## [53] "g__Lachnospiraceae_FCS020_group"
## [54] "g__Shuttleworthia"
## [55] "g__Ruminiclostridium_9"
## [56] "g__[Eubacterium]_hallii_group"
## [57] "g__Anaerostipes"
## [58] "g__Roseburia"
## [59] "g__Defluviitaleaceae_UCG-011"
## [60] "g__ASF356"
## [61] "g__Tyzzerella"
## [62] "g__[Eubacterium]_nodatum_group"
## [63] "g__Ruminococcus_1"
## [64] "g__Oscillospira"
## [65] "g__Oscillibacter"
## [66] "g__Ruminiclostridium"
## [67] "g__[Eubacterium]_coprostanoligenes_group"
## [68] "g__Ruminococcaceae_UCG-005"
## [69] "g__DTU089"
## [70] "g__Ruminiclostridium_5"
## [71] "g__Ruminococcus_2"
## [72] "g__Candidatus_Soleaferrea"
## [73] "g__Ruminococcaceae_NK4A214_group"
## [74] "g__Flavonifractor"

```

```
subset16S %>% get_taxa_unique("Genus") # 93 different genes in total, which are all present in non-trea
```

```

## [1] "g__"
## [2] "g__Dielma"
## [3] "g__uncultured"
## [4] "g__Anaeroplasma"
## [5] "g__Merdibacter"
## [6] "g__Erysipelatoclostridium"
## [7] "g__Blautia"
## [8] "g__Odoribacter"
## [9] "g__Parabacteroides"
## [10] "g__Copro bacter"
## [11] "g__Bacteroides"
## [12] "g__Alistipes"

```

```

## [13] "g__uncultured_bacterium"
## [14] "g__Bilophila"
## [15] "g__Parasutterella"
## [16] "g__Ralstonia"
## [17] "g__Escherichia-Shigella"
## [18] "g__Corynebacterium_1"
## [19] "g__Brachybacterium"
## [20] "g__Bifidobacterium"
## [21] "g__Ruminococcaceae_UCG-014"
## [22] "g__Lachnospiraceae_UCG-008"
## [23] "g__CHKCI002"
## [24] "g__Gordonibacter"
## [25] "g__Akkermansia"
## [26] "g__Paraeggerthella"
## [27] "g__Christensenellaceae_R-7_group"
## [28] "g__Ruminococcaceae_UCG-010"
## [29] "g__Tyzzerella_3"
## [30] "g__Romboutsia"
## [31] "g__Family_XIII_AD3011_group"
## [32] "g__Staphylococcus"
## [33] "g__Caldalkalibacillus"
## [34] "g__Lactobacillus"
## [35] "g__Weissella"
## [36] "g__Bacillus"
## [37] "g__Enterococcus"
## [38] "g__Eisenbergiella"
## [39] "g__Streptococcus"
## [40] "g__Phascolarctobacterium"
## [41] "g__uncultured_organism"
## [42] "g__Anaerofustis"
## [43] "g__Ruminococcaceae_UCG-013"
## [44] "g__Butyricicoccus"
## [45] "g__Faecalibacterium"
## [46] "g__Phoceia"
## [47] "g__Subdoligranulum"
## [48] "g__GCA-900066225"
## [49] "g__[Eubacterium]_coprostanoligenes_group"
## [50] "g__Negativibacillus"
## [51] "g__Hydrogenoanaerobacterium"
## [52] "g__Angelakisella"
## [53] "g__Fournierella"
## [54] "g__Anaerofilum"
## [55] "g__Anaerotruncus"
## [56] "g__[Ruminococcus]_torques_group"
## [57] "g__Sellimonas"
## [58] "g__Lachnoclostridium"
## [59] "g__GCA-900066575"
## [60] "g__CHKCI001"
## [61] "g__UC5-1-2E3"
## [62] "g__Fusicatenibacter"
## [63] "g__Roseburia"
## [64] "g__Marvinbryantia"
## [65] "g__Coproccoccus_3"
## [66] "g__Lachnospiraceae_UCG-006"

```

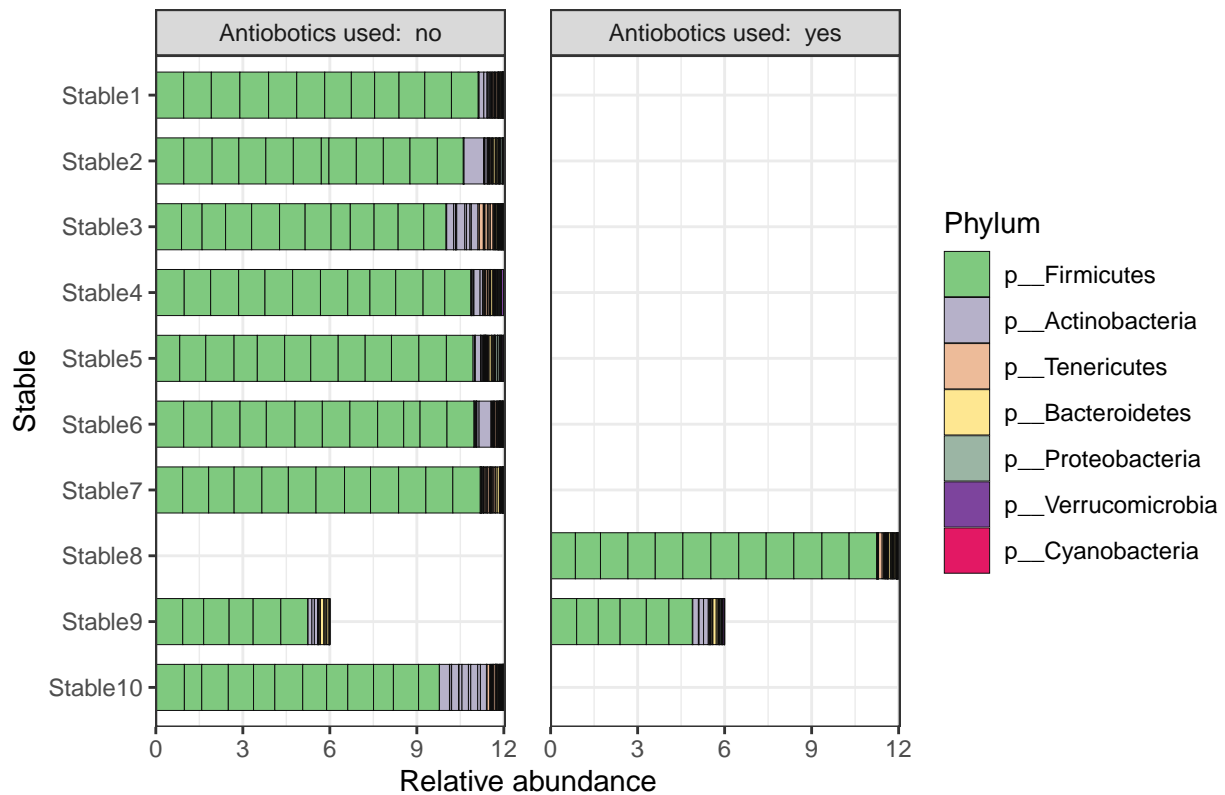
```
## [67] "g__[Ruminococcus]_gauvreauui_group"
## [68] "g__Lachnospiraceae_UCG-004"
## [69] "g__Lachnospiraceae_FCS020_group"
## [70] "g__Lachnospiraceae_FE2018_group"
## [71] "g__[Eubacterium]_ventriosum_group"
## [72] "g__Shuttleworthia"
## [73] "g__Ruminiclostridium_9"
## [74] "g__[Eubacterium]_hallii_group"
## [75] "g__Anaerostipes"
## [76] "g__UBA1819"
## [77] "g__Ruminococcus_1"
## [78] "g__Defluviitaleaceae_UCG-011"
## [79] "g__ASF356"
## [80] "g__Tyzzerella"
## [81] "g__[Eubacterium]_nodatum_group"
## [82] "g__Ruminiclostridium_5"
## [83] "g__Ruminiclostridium"
## [84] "g__Oscillospira"
## [85] "g__Oscillibacter"
## [86] "g__Ruminococcus_2"
## [87] "g__Ruminococcaceae_UCG-009"
## [88] "g__Ruminococcaceae_UCG-005"
## [89] "g__DTU089"
## [90] "g__Candidatus_Soleaferrea"
## [91] "g__Ruminococcaceae_NK4A214_group"
## [92] "g__Flavonifractor"
## [93] "g__Intestinimonas"
```

Plots of relative abundances, fixing some genes that are clustered in the data twice, showing top 12

Relative abundance for both stable and antibiotics used

```
subset16S %>%
  ps_arrange(Stables) %>%
  ps_mutate(
    Stables = factor(Stables, rev(unique(Stables)))
  ) %>%
  comp_barplot(
    tax_level = "Phylum", bar_width = 0.7, sample_order = "asis",
    palette = colorRampPalette(brewer.pal(8,"Accent"))(9),
    x = "Stables") +
  facet_wrap(
    facets = vars(AB), labeller = as_labeller(~ paste("Antibiotics used: ", .)),
    scales = "fixed"
  ) +
  coord_flip() +
  labs(x = "Stable", y = "Relative abundance") +
  scale_y_continuous(expand = expansion(add = c(0, 0.05))) +
  theme_bw() +
  theme(panel.spacing.x = unit(6, "mm")) +
  ggtitle("Relative abundance of Phyla by stable and antibiotics used")
```

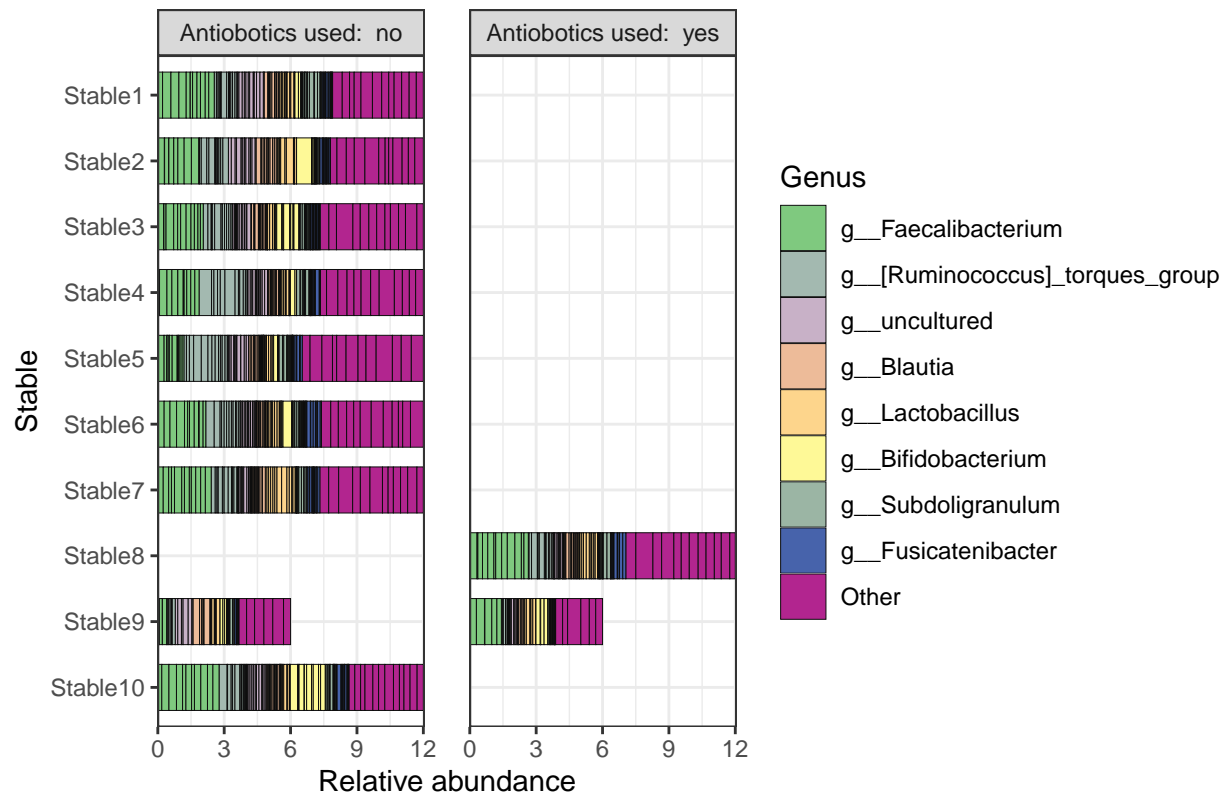
Relative abundance of Phyla by stable and antibiotics used



Same plot but with Genus

```
subset16S %>% aggregate_top_taxa2("Genus", top = 8) %>% phyloseq::tax_glom("Genus") %>%
  ps_arrange(Stables) %>%
  ps_mutate(
    Stables = factor(Stables, rev(unique(Stables)))
  ) %>%
  comp_barplot(
    tax_level = "Genus", bar_width = 0.7, sample_order = "asis",
    palette = colorRampPalette(brewer.pal(8,"Accent"))(13),
    x = "Stables",
    n_taxa = 12, other_name = "Other ARG", merge_other = F) +
  facet_wrap(
    facets = vars(AB), labeller = as_labeller(~ paste("Antibiotics used: ", .)),
    scales = "fixed"
  ) +
  coord_flip() +
  labs(x = "Stable", y = "Relative abundance") +
  scale_y_continuous(expand = expansion(add = c(0, 0.05))) +
  theme_bw() +
  theme(panel.spacing.x = unit(6, "mm")) +
  ggtitle("Relative abundance of Genera by stable and antibiotics used")
```

Relative abundance of Genera by stable and antibiotics used



Rel abundance Phyla

```
ps1.com <- subset16S

taxa_names(ps1.com) <- paste0("ASV_", rownames(tax_table(ps1.com)))

# set Palette
taxic <- as.data.frame(ps1.com@tax_table) # this will help in setting large color options
taxic$OTU <- rownames(taxic) # Add the OTU ids from OTU table into the taxa table at the end.
colnames(taxic) # You can see that we now have extra taxonomy levels.

## [1] "Domain" "Phylum" "Class" "Order" "Family" "Genus" "OTU"

taxmat <- as.matrix(taxic) # convert it into a matrix.
new.tax <- tax_table(taxmat) # convert into phyloseq compatible file.
tax_table(ps1.com) <- new.tax # incorporate into phyloseq Object

# now edit the unclassified taxa
tax_table(ps1.com)[tax_table(ps1.com)[, "Phylum"] == "", "Phylum"] <- "Unclassified phylum"

guide_italics <- guides(fill = guide_legend(label.theme = element_text(
  size = 15,
```

```

    face = "italic", colour = "Black", angle = 0
  )))

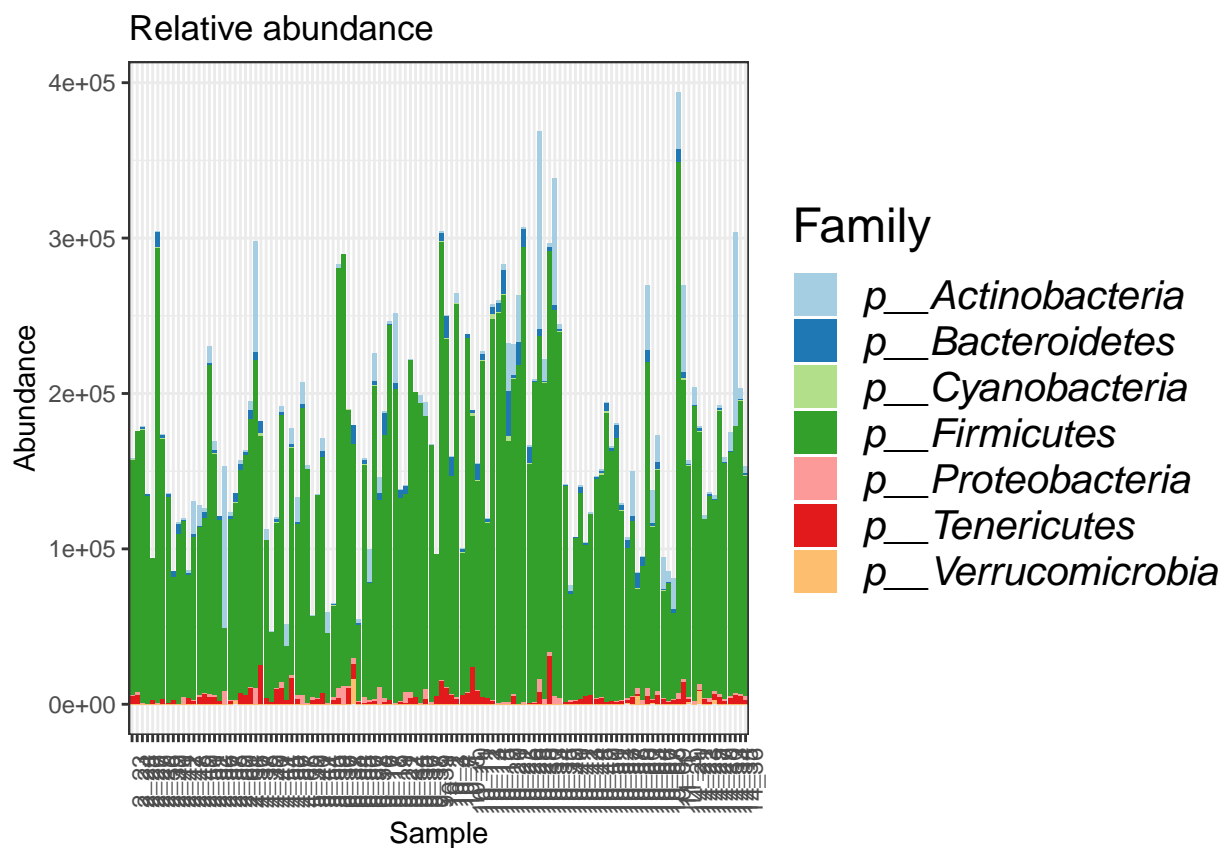
ps1.com@phy_tree <- NULL

# merge at phylum level

ps1.com.fam <- microbiomeutilities::aggregate_top_taxa2(ps1.com, "Phylum", top = 10)

plot_composition(ps1.com.fam) + theme(legend.position = "bottom") +
  scale_fill_brewer("Family", palette = "Paired") + theme_bw() +
  theme(axis.text.x = element_text(angle = 90)) +
  ggtitle("Relative abundance") + guide_italics + theme(legend.title = element_text(size = 18))

```



Other Phyla plots

```

tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)

tse <- transformCounts(tse, method = "relabundance")

tse_phylum <- agglomerateByRank(tse,
                                rank = "Phylum",
                                onRankOnly = TRUE)

tse_phylum <- transformCounts(tse_phylum,
                                assay.type = "counts",

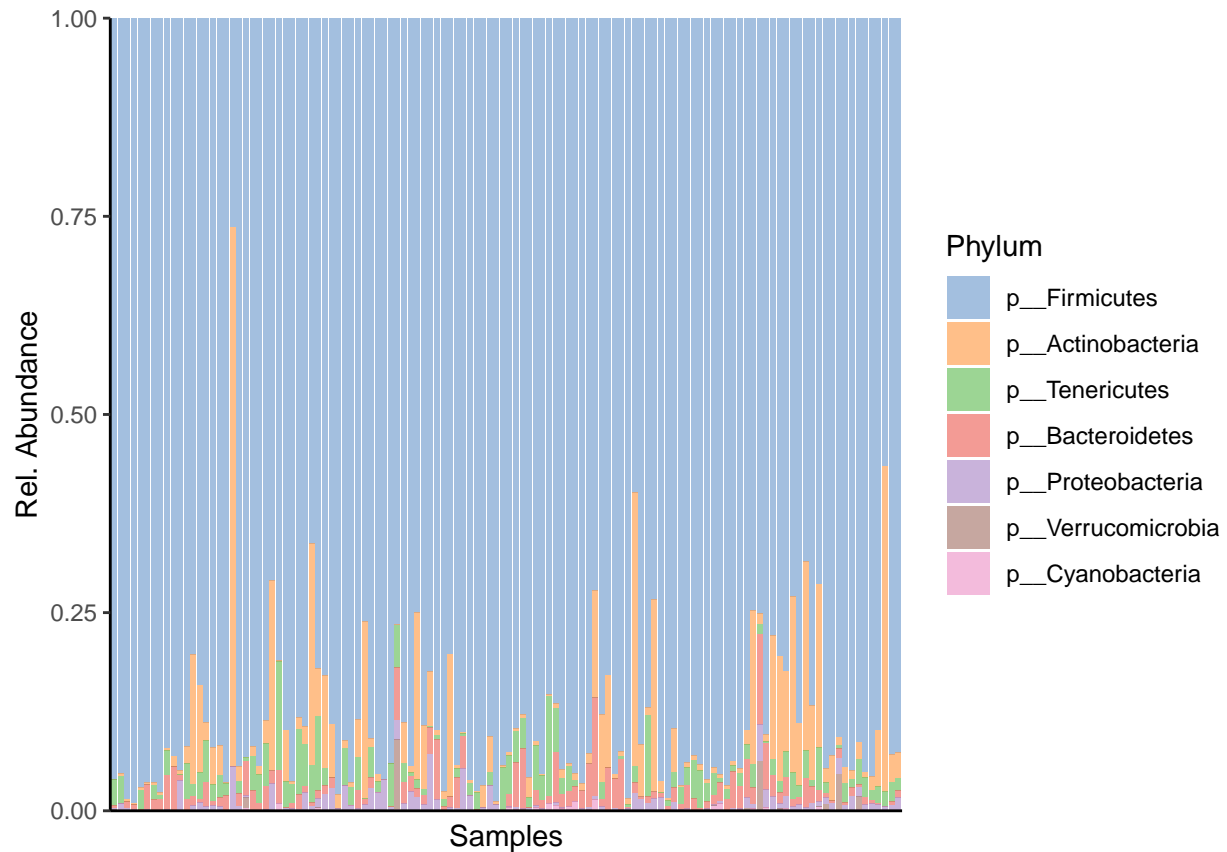
```

```

method = "relabundance")

miaViz::plotAbundance(tse_phylum,
                      assay.type = "relabundance",
                      rank = "Phylum",
                      order_rank_by = "abund")

```



Same plot but with antibiotic treatment added in

```

tse_phylum$Farm2 = as.factor(tse_phylum$Farm2)
tse_phylum$AB = as.factor(tse_phylum$AB)

plots <- miaViz::plotAbundance(tse_phylum,
                              assay.type = "relabundance",
                              rank = "Phylum",
                              order_sample_by = "AB",
                              features = "AB")

plots[[1]] <- plots[[1]] +
  theme(legend.key.size = unit(0.3, 'cm'),
        legend.text = element_text(size = 6),
        legend.title = element_text(size = 8))
plots[[2]] <- plots[[2]] +
  theme(legend.key.height = unit(0.3, 'cm'),
        legend.key.width = unit(0.3, 'cm'),
        legend.text = element_text(size = 6),

```

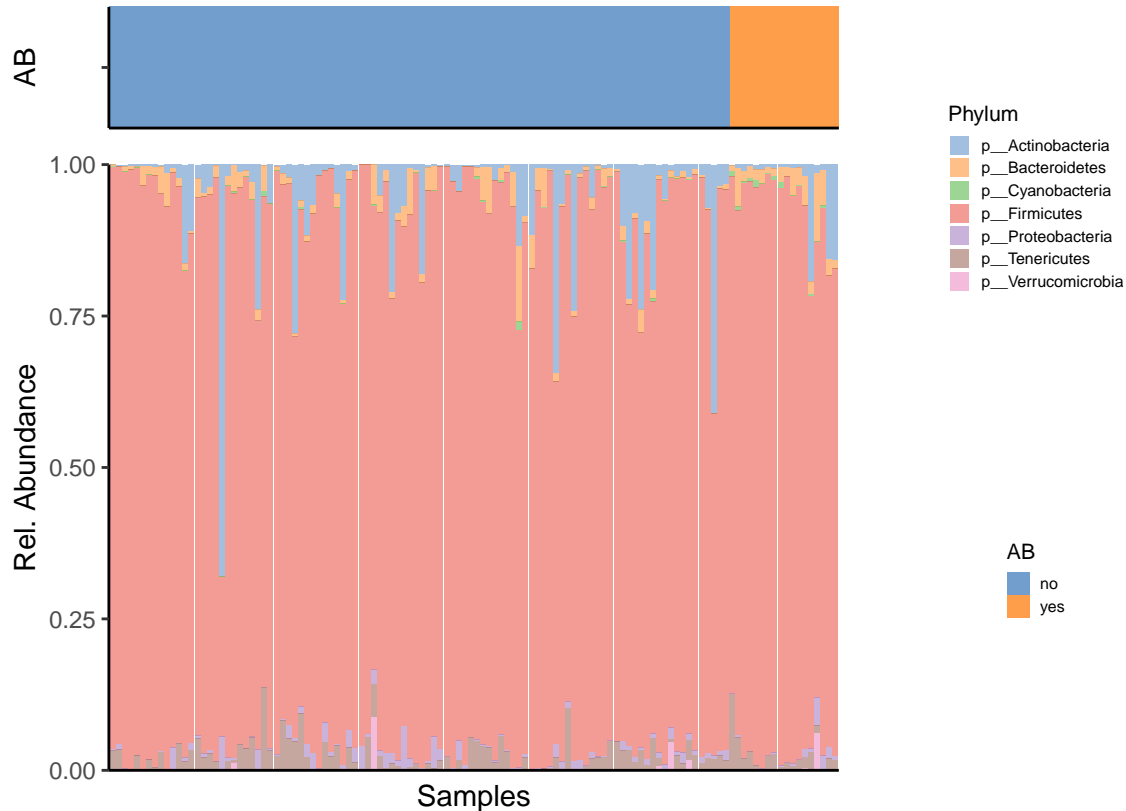
```

legend.title = element_text(size = 8),
legend.direction = "vertical")

legend <- wrap_plots(as_ggplot(get_legend(plots[[1]])), as_ggplot(get_legend(plots[[2]])), ncol = 1)
plots[[1]] <- plots[[1]] + theme(legend.position = "none")
plots[[2]] <- plots[[2]] + theme(legend.position = "none", axis.title.x=element_blank())

plot <- wrap_plots(plots[[2]], plots[[1]], ncol = 1, heights = c(2, 10))
wrap_plots(plot, legend, nrow = 1, widths = c(2, 1))

```



heatmaps on phylum level

```

tse_phylum <- agglomerateByRank(tse,
                                rank = "Phylum",
                                onRankOnly = TRUE)

tse_phylum <- transformCounts(tse_phylum, MARGIN = "samples", method = "clr", assay.type = "counts", ps
tse_phylum <- transformCounts(tse_phylum, assay.type = "clr",
                                MARGIN = "features",
                                method = "z", name = "clr_z")

#top_taxa <- getTopTaxa(tse_phylum, top = 20) there are few phyla in this data so no need to exclude some
#tse_phylum <- tse_phylum[top_taxa, ]

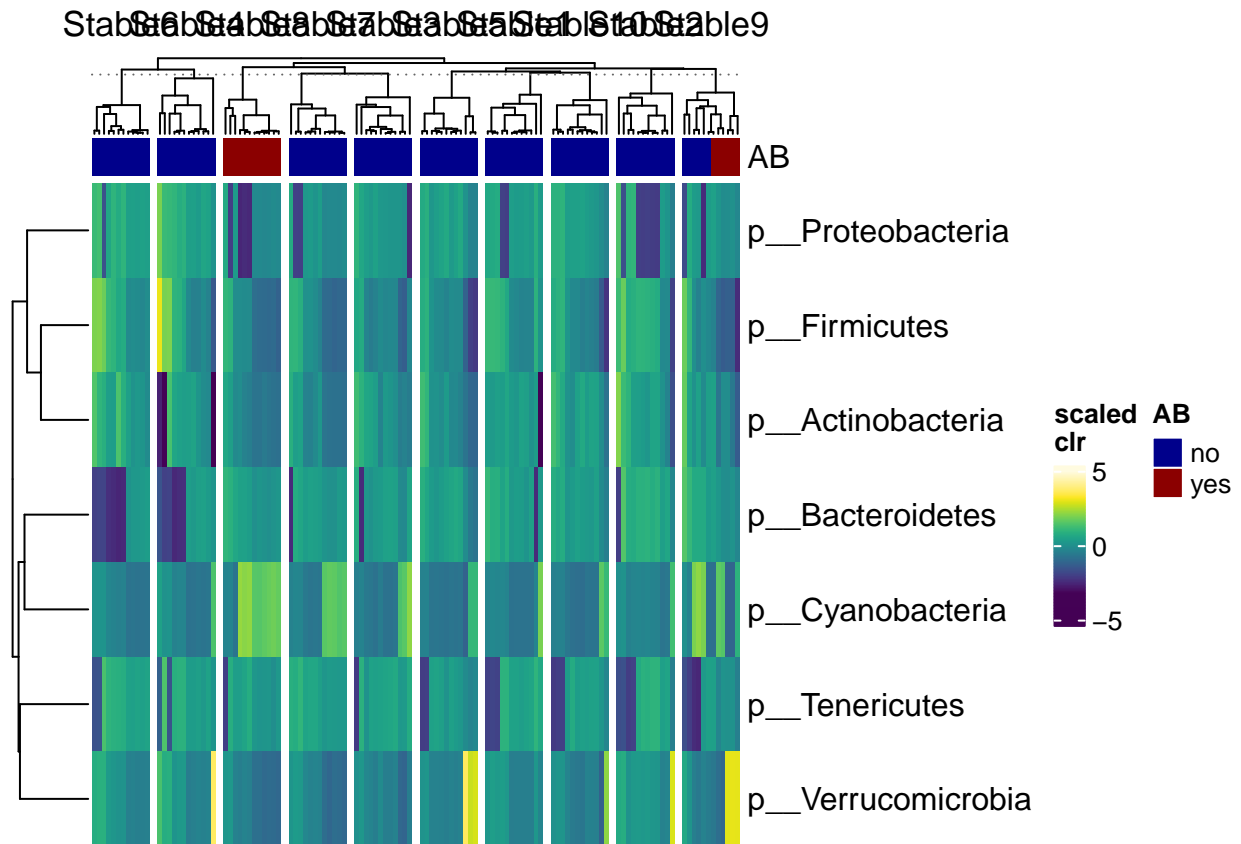
# Phylum AB heatmap

```



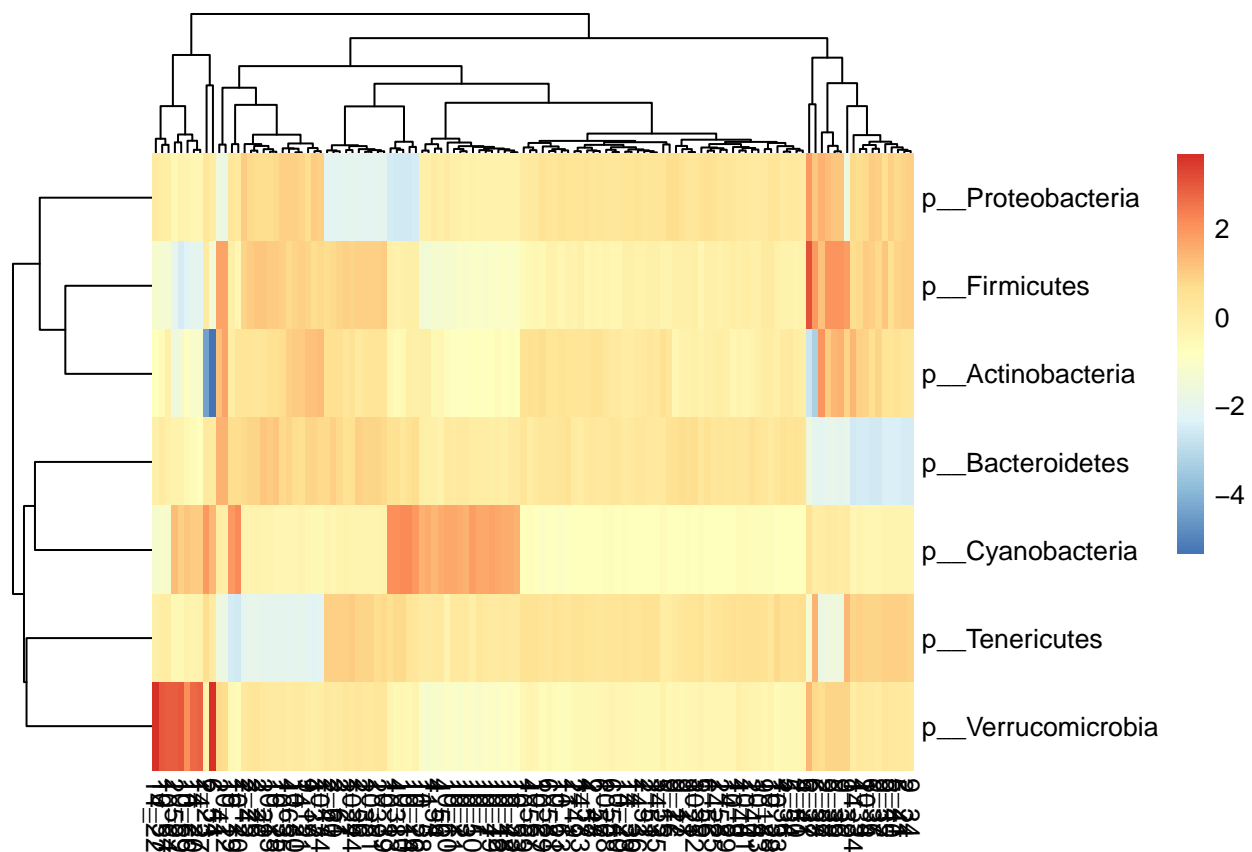
```
tse_phylum@metadata$anno_colors$AB = c(yes = "darkred",no = "darkblue")
```

```
sechm(tse_phylum,
  features = rownames(tse_phylum),
  assayName = "clr",
  do.scale = TRUE,
  top_annotation = "AB",
  gaps_at = "Stables",
  hmcols = viridis(256),
  cluster_cols = TRUE, cluster_rows = TRUE,
  sortRowsOn=NULL)
```



```
# Phylum heatmap
mat <- assay(tse_phylum, "clr_z")

pheatmap(mat)
```



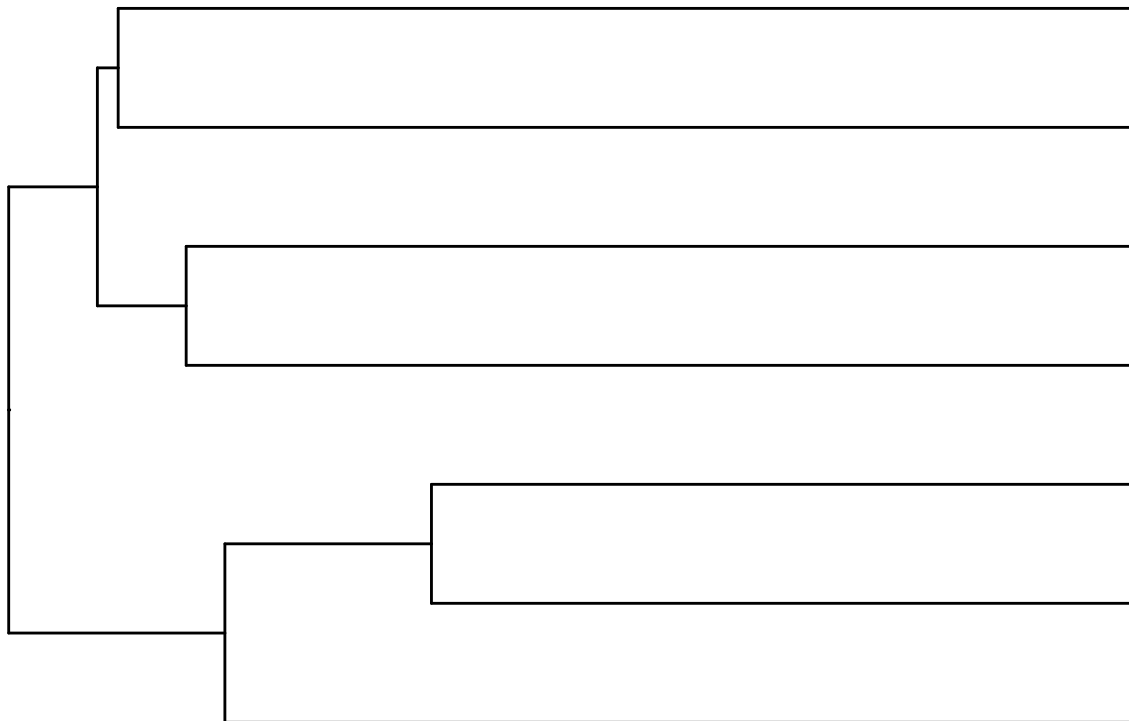
Phylum heatmap hierarchal clustering with AB Clustering both samples and features hierarchically

```
taxa_hclust <- hclust(dist(mat), method = "complete")

# Creates a phylogenetic tree
taxa_tree <- as.phylo(taxa_hclust)

# Plot taxa tree
taxa_tree <- ggtree(taxa_tree) +
  theme(plot.margin=margin(0,0,0,0)) # removes margins

taxa_tree # based on this three, we'll create two clusters
```



```

# Get order of taxa in plot
taxa_ordered <- get_taxa_name(taxa_tree)

taxa_clusters <- cutree(tree = taxa_hclust, k = 2) # 2 clusters based on tree figure

# Converts into data frame
taxa_clusters <- data.frame(clusters = taxa_clusters)
taxa_clusters$clusters <- factor(taxa_clusters$clusters)

# Order data so that it's same as in phylo tree
taxa_clusters <- taxa_clusters[taxa_ordered, , drop = FALSE]

# Prints taxa and their clusters
taxa_clusters

```

```

##                clusters
## p__Verrucomicrobia      1
## p__Tenericutes         1
## p__Cyanobacteria       1
## p__Bacteroidetes       1
## p__Actinobacteria       2
## p__Firmicutes          2
## p__Proteobacteria      2

```

```
rowData(tse_phylum)$clusters <- taxa_clusters[order(match(rownames(taxa_clusters), rownames(tse_phylum))
```

```
# Prints taxa and their clusters
```

```
rowData(tse_phylum)$clusters
```

```
## [1] 1 2 1 2 2 1 1
```

```
## Levels: 1 2
```

```
sample_hclust <- hclust(dist(t(mat)), method = "complete")
```

```
# Creates a phylogenetic tree
```

```
sample_tree <- as.phylo(sample_hclust)
```

```
# Plot sample tree
```

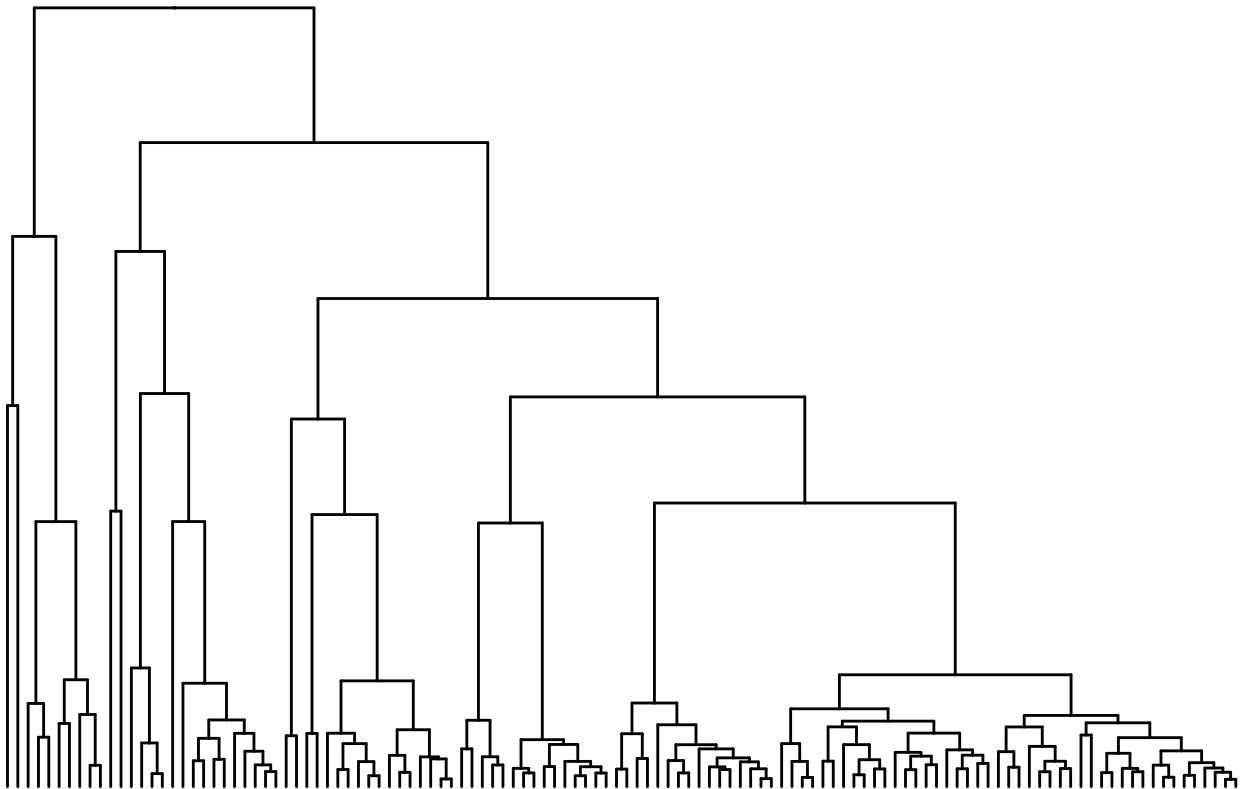
```
sample_tree <- ggtree(sample_tree) + layout_dendrogram() +  
  theme(plot.margin=margin(0,0,0,0)) # removes margins
```

```
# Get order of samples in plot
```

```
samples_ordered <- rev(get_taxa_name(sample_tree))
```

```
# to view the tree, run
```

```
sample_tree
```



```

# Creates clusters
sample_clusters <- factor(cutree(tree = sample_hclust, k = 2)) # 2 clusters based on methods in Cluster

# Converts into data frame
sample_data <- data.frame(clusters = sample_clusters)

# Order data so that it's same as in phylo tree
sample_data <- sample_data[samples_ordered, , drop = FALSE]

# Order data based on
tse_phylum <- tse_phylum[ , rownames(sample_data)]

# Add sample type data
sample_data$sample_types <- colData(tse_phylum)$AB

sample_data

```

```

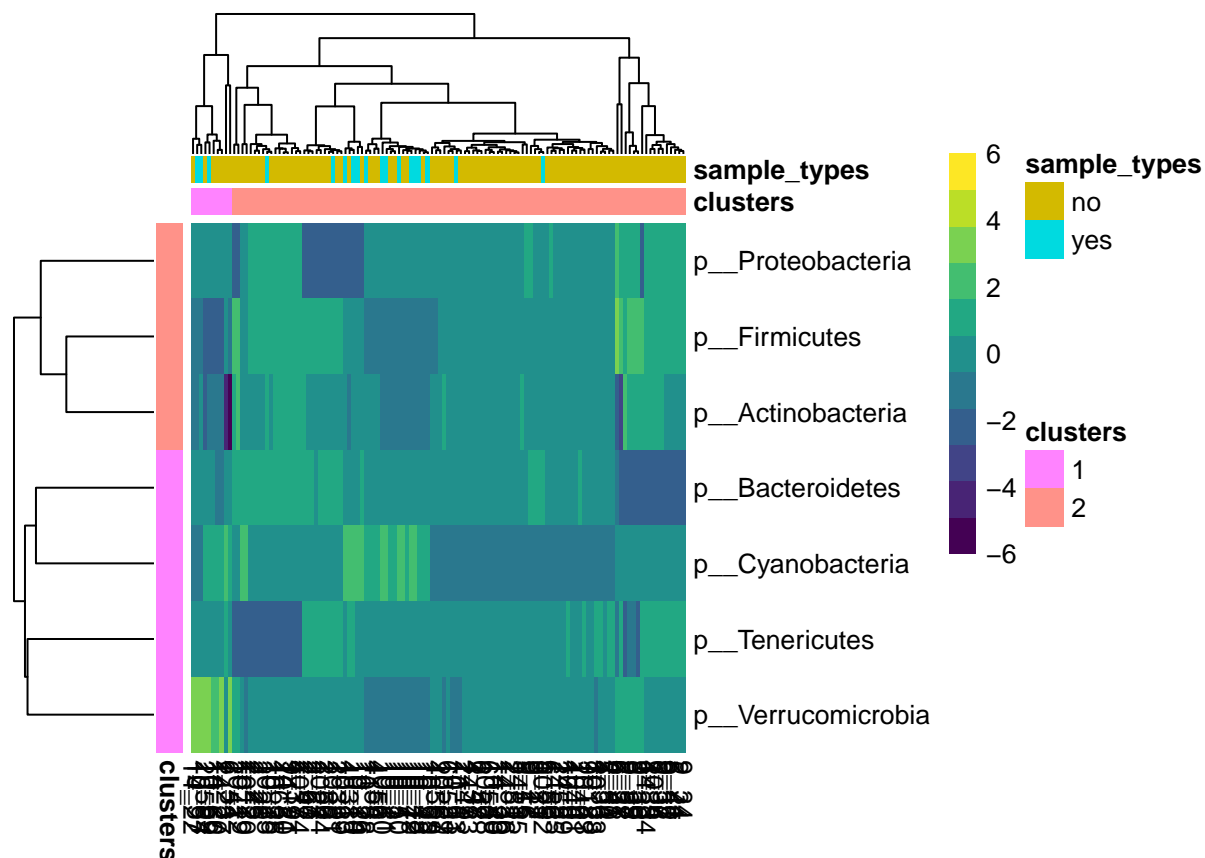
##      clusters sample_types
## 2_23         1         no
## 6_54         1         no
## 14_22        1         no
## 10_57        1         yes
## 10_64        1         yes
## 2_59         1         no
## 10_59        1         yes
## 10_66        1         no
## 14_20        1         no
## 14_27        1         no
## 6_37         2         no
## 6_38         2         no
## 2_57         2         no
## 9_38         2         no
## 5_39         2         no
## 9_36         2         no
## 9_39         2         no
## 14_34        2         no
## 4_39         2         no
## 10_4         2         no
## 6_36         2         no
## 9_37         2         no
## 9_35         2         no
## 5_40         2         no
## 5_41         2         no
## 2_24         2         no
## 9_34         2         no
## 2_41         2         no
## 10_22        2         no
## 10_19        2         no
## 10_20        2         no
## 2_42         2         no
## 2_25         2         no
## 2_26         2         no
## 2_29         2         no

```

## 10_15	2	yes
## 10_25	2	no
## 9_19	2	no
## 4_37	2	no
## 10_34	2	no
## 10_35	2	no
## 4_65	2	no
## 10_30	2	no
## 14_21	2	no
## 10_14	2	yes
## 10_26	2	no
## 10_13	2	yes
## 4_38	2	no
## 10_10	2	yes
## 2_47	2	no
## 2_60	2	no
## 2_61	2	no
## 2_27	2	no
## 10_44	2	no
## 2_56	2	no
## 2_36	2	no
## 10_51	2	yes
## 2_39	2	no
## 10_39	2	no
## 10_58	2	yes
## 11_3	2	no
## 4_54	2	no
## 4_56	2	no
## 10_60	2	yes
## 10_11	2	yes
## 10_2	2	no
## 10_3	2	no
## 10_50	2	yes
## 10_1	2	no
## 10_7	2	no
## 10_49	2	yes
## 10_12	2	yes
## 10_48	2	yes
## 10_42	2	no
## 10_53	2	yes
## 9_22	2	no
## 9_17	2	no
## 9_21	2	no
## 10_52	2	yes
## 9_18	2	no
## 10_28	2	no
## 10_33	2	no
## 4_36	2	no
## 5_54	2	no
## 2_50	2	no
## 4_41	2	no
## 5_59	2	no
## 6_56	2	no
## 14_25	2	no

```
## 2_58      2      no
## 14_29     2      no
## 10_8      2      no
## 4_40      2      no
## 10_41     2      no
## 2_40      2      no
## 10_43     2      no
## 10_69     2      no
## 4_55      2      no
## 10_67     2      no
## 10_29     2      no
## 6_58      2      no
## 10_21     2      no
## 10_63     2      yes
## 11_1      2      no
## 5_55      2      no
## 9_16      2      no
## 2_49      2      no
## 14_33     2      no
## 4_57      2      no
## 2_48      2      no
## 6_57      2      no
## 10_68     2      no
## 6_55      2      no
## 10_40     2      no
## 14_23     2      no
## 14_30     2      no
## 14_36     2      no
## 2_52      2      no
## 2_51      2      no
## 14_35     2      no
```

```
breaks <- seq(-ceiling(max(abs(mat))), ceiling(max(abs(mat))),
              length.out = ifelse( max(abs(mat))>5, 2*ceiling(max(abs(mat))), 10 ) )
#colors <- colorRampPalette(c("darkblue", "blue", "white", "red", "darkred"))(length(breaks)-1) replace
pheatmap(mat, annotation_row = taxa_clusters,
          annotation_col = sample_data,
          breaks = breaks,
          color = colorRampPalette(viridis(256))(length(breaks)-1))
```



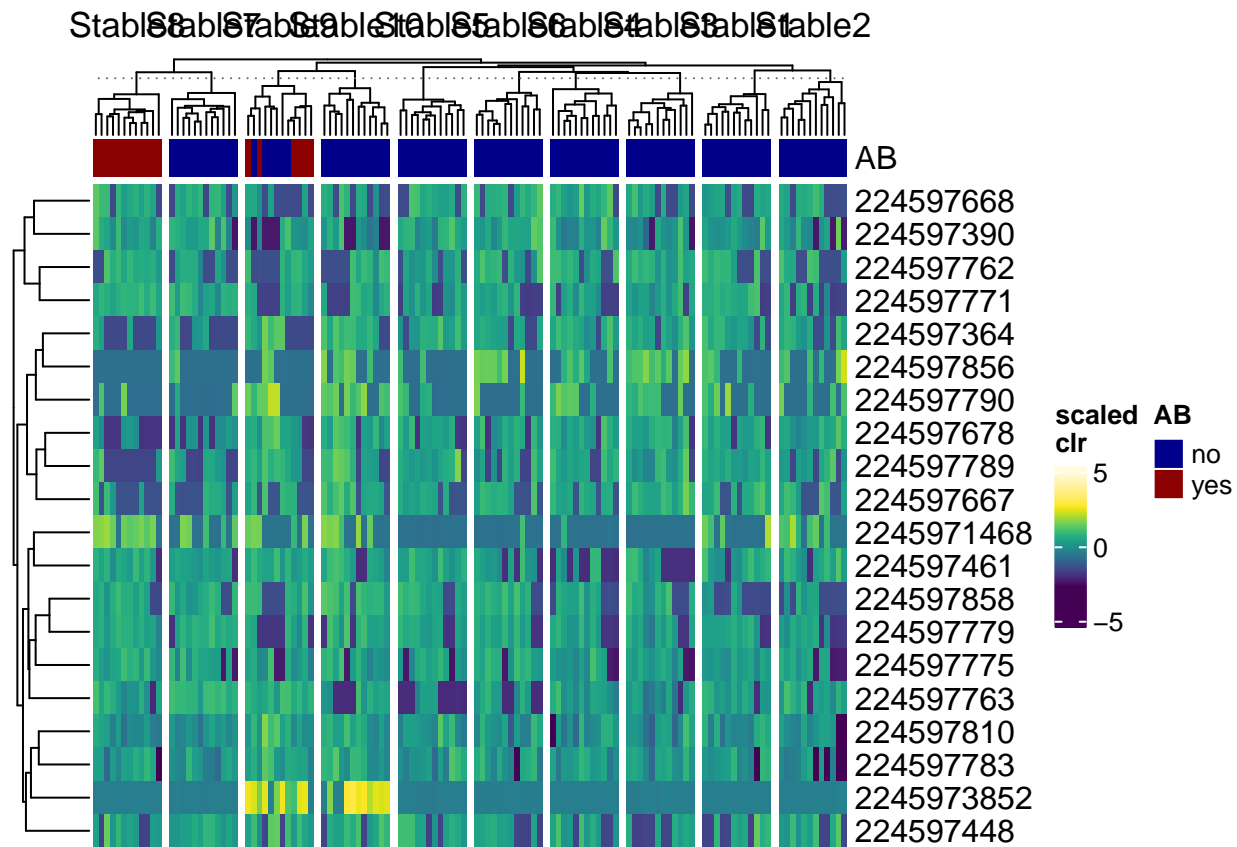
heatmaps on ASV level

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse <- transformCounts(tse, method = "relabundance")
tse <- transformCounts(tse, MARGIN = "samples", method = "clr", assay.type = "counts", pseudocount=1) #
tse <- transformCounts(tse, assay.type = "clr",
                       MARGIN = "features",
                       method = "z", name = "clr_z")

top_taxa <- getTopTaxa(tse, top = 20)
tse <- tse[top_taxa, ]

# ASV heatmap AB
tse@metadata$anno_colors$AB = c(yes = "darkred",no = "darkblue")

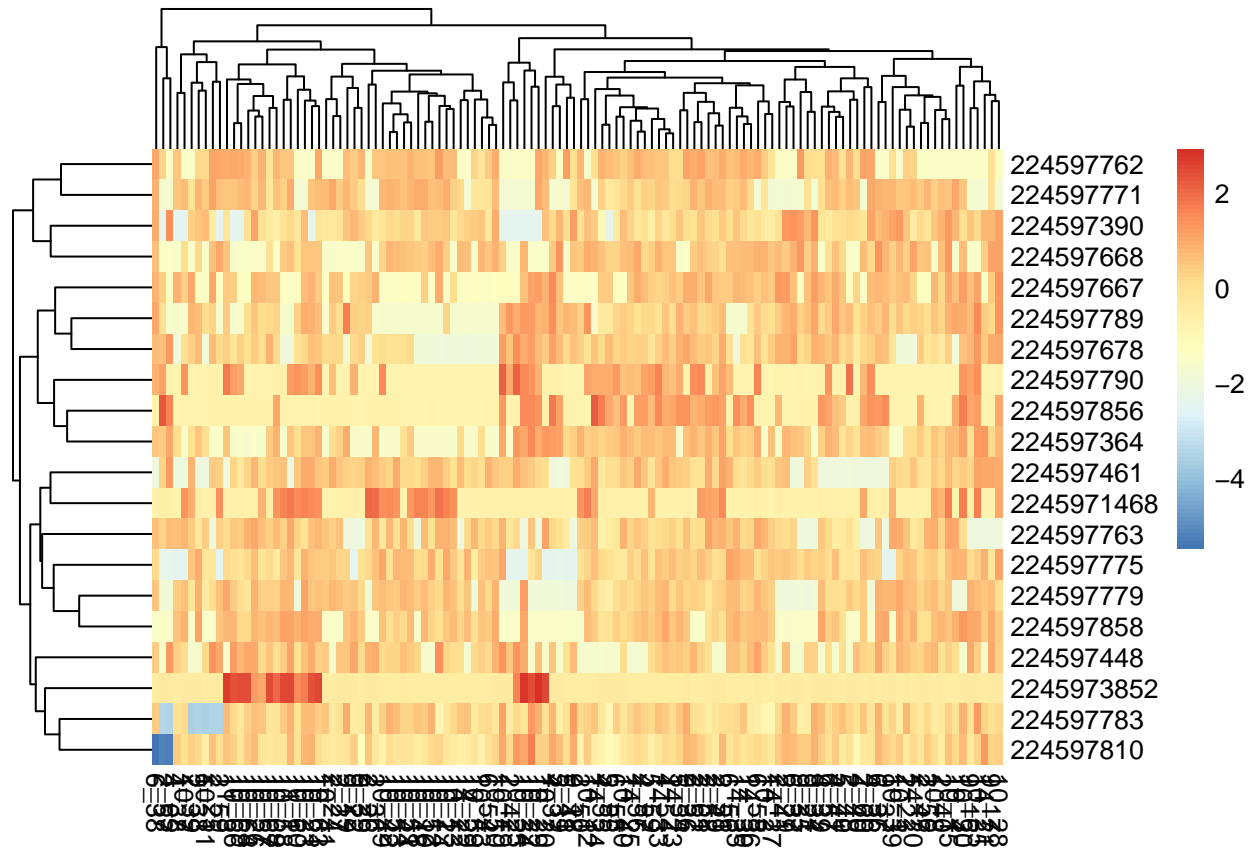
sechm(tse,
      features = rownames(tse),
      assayName = "clr",
      do.scale = TRUE,
      top_annotation = c("AB"),
      gaps_at = "Stables",
      hmcols = viridis(256),
      cluster_cols = TRUE, cluster_rows = TRUE)
```

ASV heatmap

```
mat <- assay(tse, "clr_z")
```

```
pheatmap(mat)
```



```
# ASV heatmap hierarchal clustering with AB

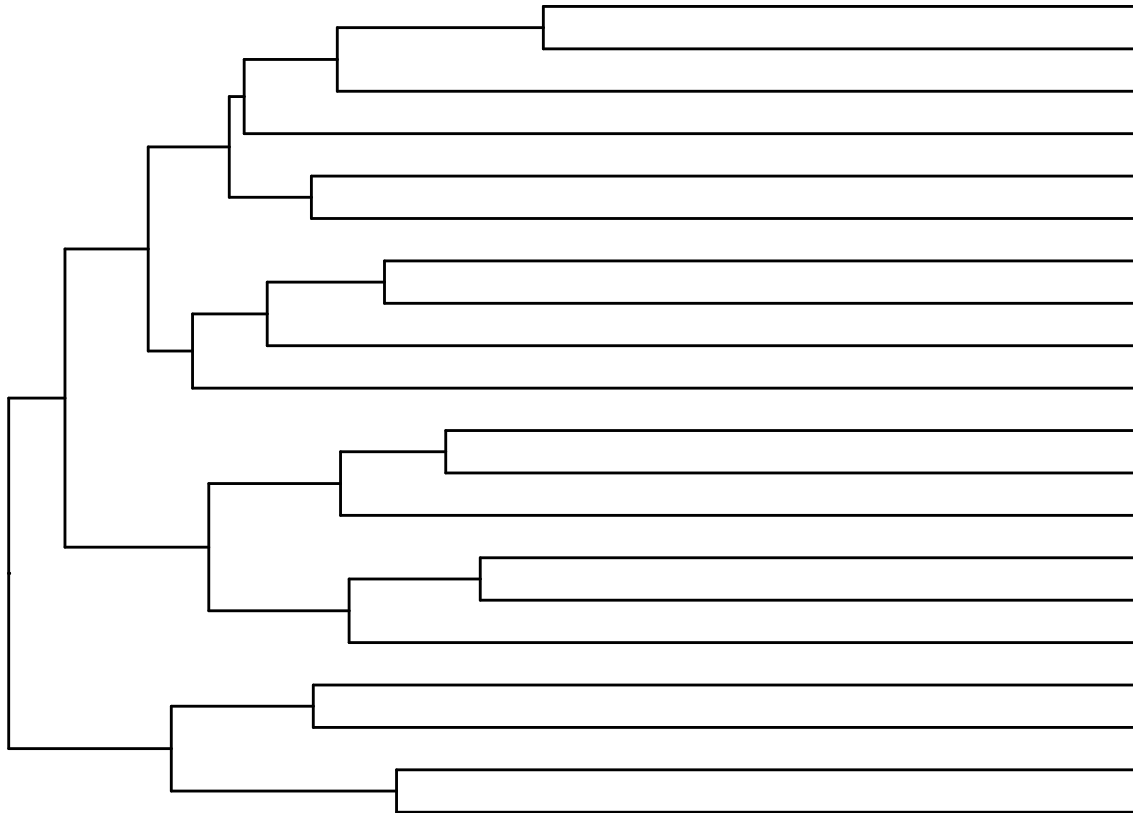
# Clustering both samples and features hierarchically

taxa_hclust <- hclust(dist(mat), method = "complete")

# Creates a phylogenetic tree
taxa_tree <- as.phylo(taxa_hclust)

# Plot taxa tree
taxa_tree <- ggtree(taxa_tree) +
  theme(plot.margin=margin(0,0,0,0)) # removes margins

taxa_tree # based on this tree, we'll create two clusters
```



```

# Get order of taxa in plot
taxa_ordered <- get_taxa_name(taxa_tree)

taxa_clusters <- cutree(tree = taxa_hclust, k = 2) # 2 clusters based on methods in Clustering.R script

# Converts into data frame
taxa_clusters <- data.frame(clusters = taxa_clusters)
taxa_clusters$clusters <- factor(taxa_clusters$clusters)

# Order data so that it's same as in phylo tree
taxa_clusters <- taxa_clusters[taxa_ordered, , drop = FALSE]

# Prints taxa and their clusters
taxa_clusters

```

```

##           clusters
## 224597858         1
## 224597779         1
## 224597775         1
## 224597763         1
## 2245971468        1
## 224597461         1
## 224597810         1
## 224597783         1
## 2245973852        1
## 224597448         1

```

```
## 224597364      1
## 224597856      1
## 224597790      1
## 224597678      1
## 224597789      1
## 224597667      1
## 224597668      2
## 224597390      2
## 224597771      2
## 224597762      2
```

```
# Prints taxa and their clusters
rowData(tse_phylum)$clusters
```

```
## [1] 1 2 1 2 2 1 1
## Levels: 1 2
```

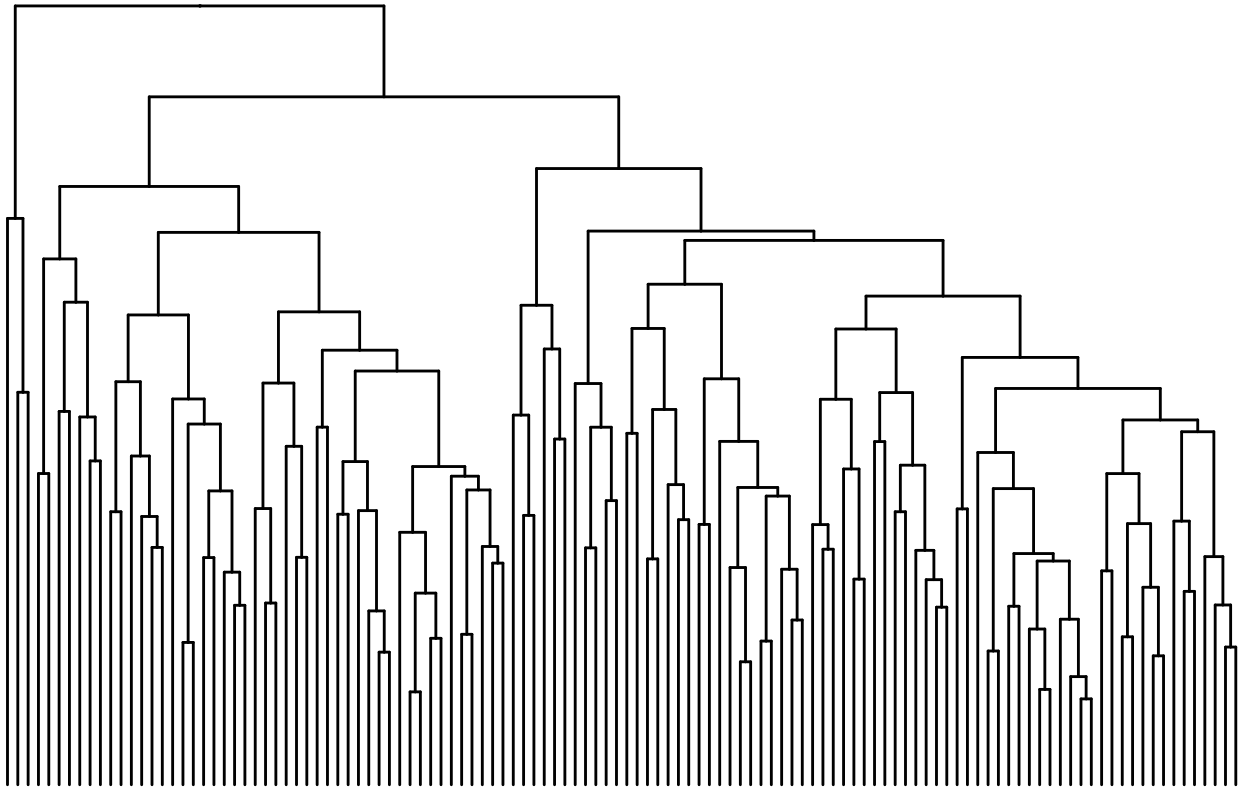
```
sample_hclust <- hclust(dist(t(mat)), method = "complete")

# Creates a phylogenetic tree
sample_tree <- as.phylo(sample_hclust)

# Plot sample tree
sample_tree <- ggtree(sample_tree) + layout_dendrogram() +
  theme(plot.margin=margin(0,0,0,0)) # removes margins

# Get order of samples in plot
samples_ordered <- rev(get_taxa_name(sample_tree))

# to view the tree, run
sample_tree
```



```
# Creates clusters
sample_clusters <- factor(cutree(tree = sample_hclust, k = 2))

# Converts into data frame
sample_data <- data.frame(clusters = sample_clusters)

# Order data so that it's same as in phylo tree
sample_data <- sample_data[samples_ordered, , drop = FALSE]

# Order data based on
tse_phylum <- tse_phylum[ , rownames(sample_data)]

# Add sample type data
sample_data$sample_types <- colData(tse_phylum)$AB

sample_data
```

```
##      clusters sample_types
## 6_38         2          no
## 2_57         2          no
## 4_65         2          no
## 4_38         1          no
## 10_7         1          no
## 2_25         1          no
## 2_59         1          no
## 2_39         1          no
```

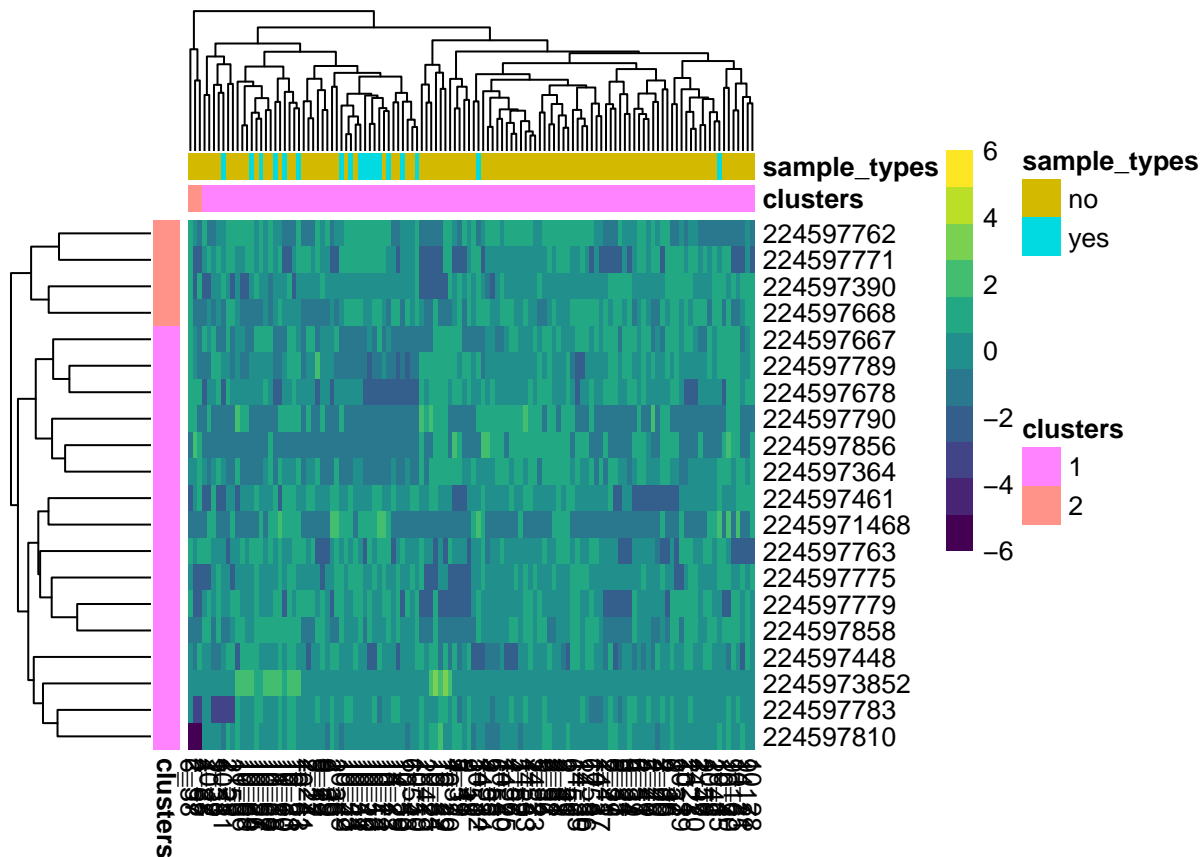
## 9_38	1	no
## 10_11	1	yes
## 10_58	1	yes
## 11_1	1	no
## 10_60	1	yes
## 10_33	1	no
## 10_21	1	no
## 10_63	1	yes
## 11_3	1	no
## 10_66	1	no
## 10_69	1	no
## 10_67	1	no
## 10_68	1	no
## 10_64	1	yes
## 10_26	1	no
## 10_57	1	yes
## 10_41	1	no
## 2_24	1	no
## 2_27	1	no
## 9_19	1	no
## 9_22	1	no
## 9_39	1	no
## 2_26	1	no
## 2_36	1	no
## 14_22	1	no
## 14_29	1	no
## 10_59	1	yes
## 10_43	1	no
## 6_54	1	no
## 10_50	1	yes
## 10_49	1	yes
## 10_42	1	no
## 10_53	1	yes
## 10_44	1	no
## 10_51	1	yes
## 10_48	1	yes
## 10_10	1	yes
## 10_52	1	yes
## 10_14	1	yes
## 10_2	1	no
## 10_13	1	yes
## 10_19	1	no
## 2_42	1	no
## 10_25	1	no
## 10_34	1	no
## 10_22	1	no
## 10_29	1	no
## 10_20	1	no
## 4_37	1	no
## 5_39	1	no
## 5_41	1	no
## 9_18	1	no
## 10_4	1	no
## 10_30	1	no

## 9_16	1	no
## 10_35	1	no
## 14_21	1	no
## 9_17	1	no
## 10_28	1	no
## 6_36	1	no
## 9_37	1	no
## 10_39	1	no
## 10_1	1	no
## 2_23	1	no
## 14_20	1	no
## 2_47	1	no
## 2_56	1	no
## 10_8	1	no
## 2_40	1	no
## 10_15	1	yes
## 2_41	1	no
## 2_29	1	no
## 6_37	1	no
## 9_35	1	no
## 9_21	1	no
## 9_34	1	no
## 2_60	1	no
## 4_36	1	no
## 2_48	1	no
## 4_40	1	no
## 4_39	1	no
## 6_57	1	no
## 4_41	1	no
## 5_40	1	no
## 2_50	1	no
## 10_12	1	yes
## 14_34	1	no
## 4_55	1	no
## 5_55	1	no
## 6_55	1	no
## 10_40	1	no
## 14_25	1	no
## 4_56	1	no
## 4_57	1	no
## 5_59	1	no
## 14_33	1	no
## 4_54	1	no
## 14_23	1	no
## 5_54	1	no
## 9_36	1	no
## 2_52	1	no
## 2_61	1	no
## 2_58	1	no
## 2_49	1	no
## 2_51	1	no
## 6_58	1	no
## 10_3	1	no
## 14_27	1	no

```
## 6_56      1      no
## 14_35     1      no
## 14_30     1      no
## 14_36     1      no
```

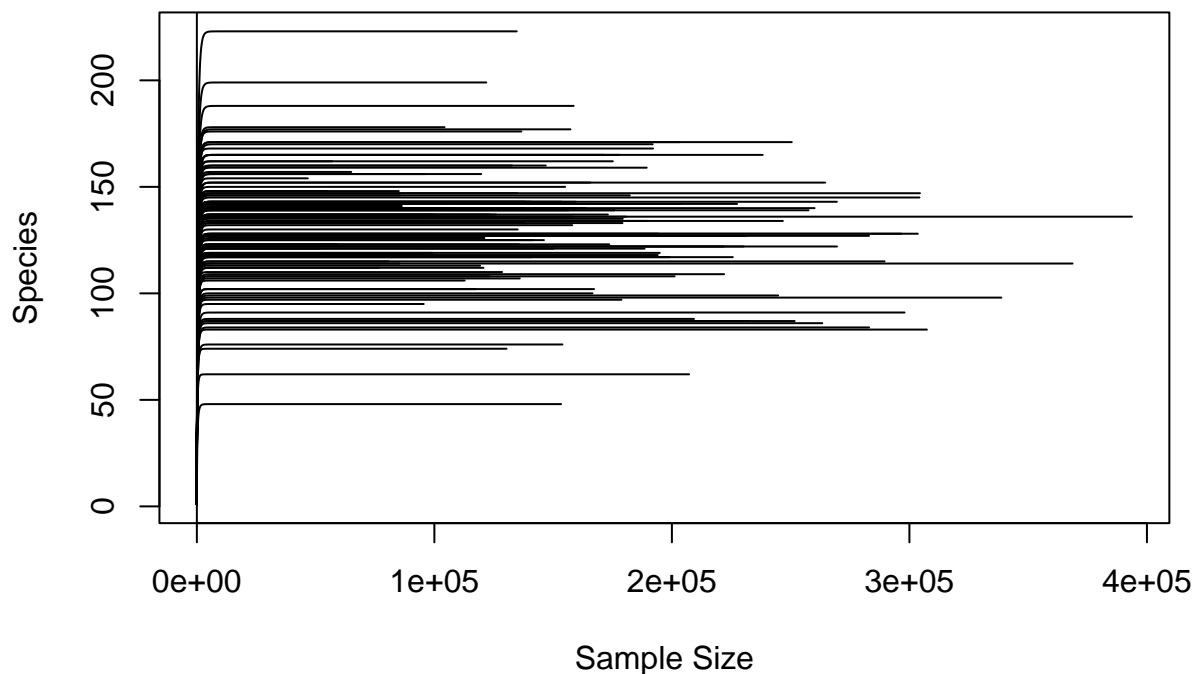
```
breaks <- seq(-ceiling(max(abs(mat))), ceiling(max(abs(mat))),
              length.out = ifelse( max(abs(mat))>5, 2*ceiling(max(abs(mat))), 10 ) )

pheatmap(mat, annotation_row = taxa_clusters,
          annotation_col = sample_data,
          breaks = breaks,
          color = colorRampPalette(viridis(256))(length(breaks)-1))
```



Alpha diversity

```
otu_tab <- t(abundances(subset16S))
# rarefaction curve
vegan::rarecurve(otu_tab,
                 step = 50, label = FALSE,
                 sample = min(rowSums(otu_tab),
                                col = "blue", cex = 0.6))
```

```
# samples plateau so sufficient sequencing depth
```

```
summary(goods(otu_tab)) # there are no singletons in this data, already filtered out, means that richness
```

```
##      no.sing      no.seqs      goods
## Min.   :0      Min.   : 46731  Min.   :100
## 1st Qu.:0      1st Qu.:125421  1st Qu.:100
## Median :0      Median :161649  Median :100
## Mean   :0      Mean   :173742  Mean   :100
## 3rd Qu.:0      3rd Qu.:212488  3rd Qu.:100
## Max.   :0      Max.   :393697  Max.   :100
```

```
#rarefy to equal library size or not?
```

```
lib.div <- microbiome::alpha(subset16S, index = "all")
lib.div2 <- richness(subset16S)
lib.div$ReadsPerSample <- sample_sums(subset16S)
lib.div$Observed <- lib.div2$observed
colnames(lib.div)
```

```
## [1] "observed"          "chao1"
## [3] "diversity_inverse_simpson" "diversity_gini_simpson"
## [5] "diversity_shannon"    "diversity_fisher"
## [7] "diversity_coverage"   "evenness_camargo"
## [9] "evenness_pielou"      "evenness_simpson"
## [11] "evenness_evar"        "evenness_bulla"
```

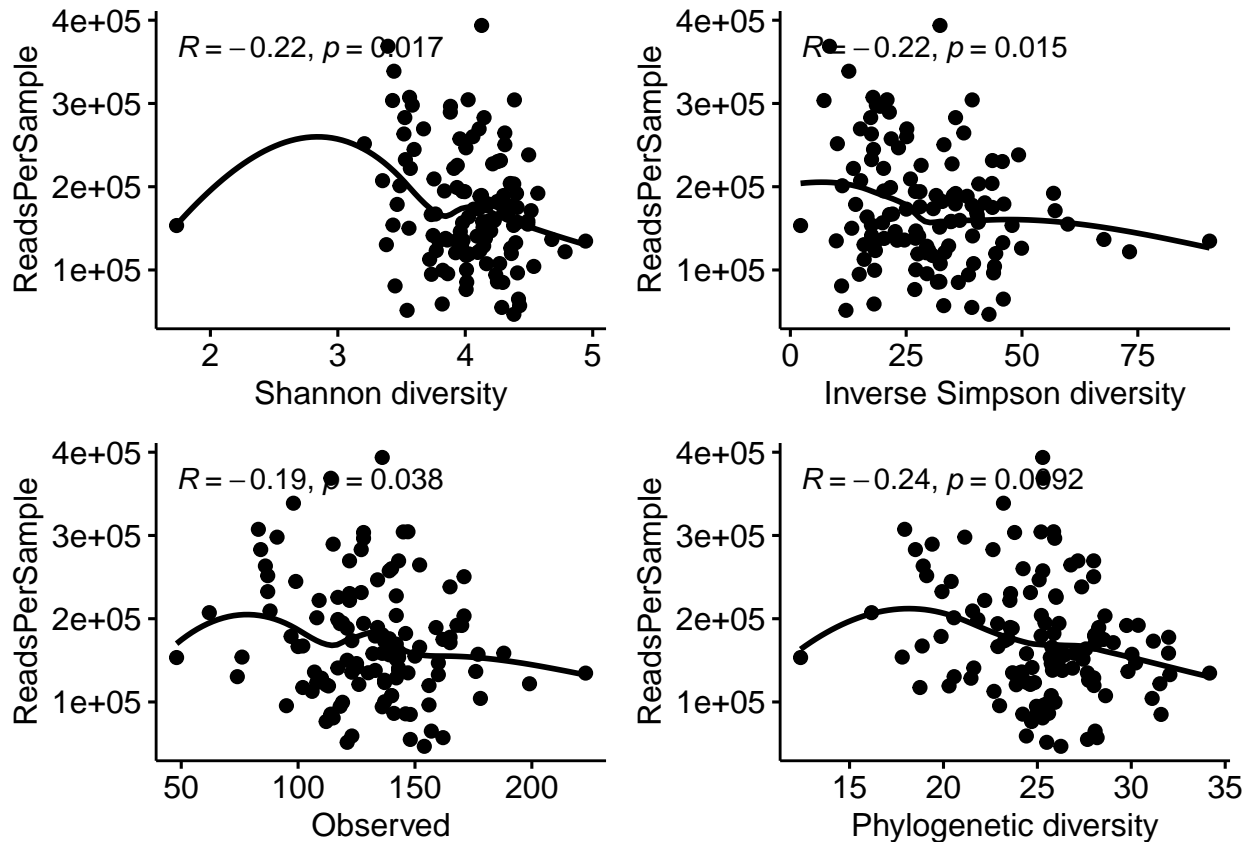
```
## [13] "dominance_dbp"           "dominance_dmn"
## [15] "dominance_absolute"      "dominance_relative"
## [17] "dominance_simpson"       "dominance_core_abundance"
## [19] "dominance_gini"         "rarity_log_modulo_skewness"
## [21] "rarity_low_abundance"    "rarity_rare_abundance"
## [23] "ReadsPerSample"         "Observed"

p1 = ggscatter(lib.div, "diversity_shannon", "ReadsPerSample", xlab = "Shannon diversity", add = "loess",
  stat_cor(method = "pearson"))
p2 = ggscatter(lib.div, "diversity_inverse_simpson", "ReadsPerSample", xlab = "Inverse Simpson diversity", add = "loess",
  stat_cor(method = "pearson"))
p3 = ggscatter(lib.div, "observed", "ReadsPerSample", xlab = "Observed", add = "loess") +
  stat_cor(method = "pearson")

df.pd <- pd(t(as.data.frame(subset16S@otu_table)), subset16S@phy_tree, include.root=T) # transposing for phylogenetic diversity
lib.div$Phylogenetic_Diversity <- df.pd$PD

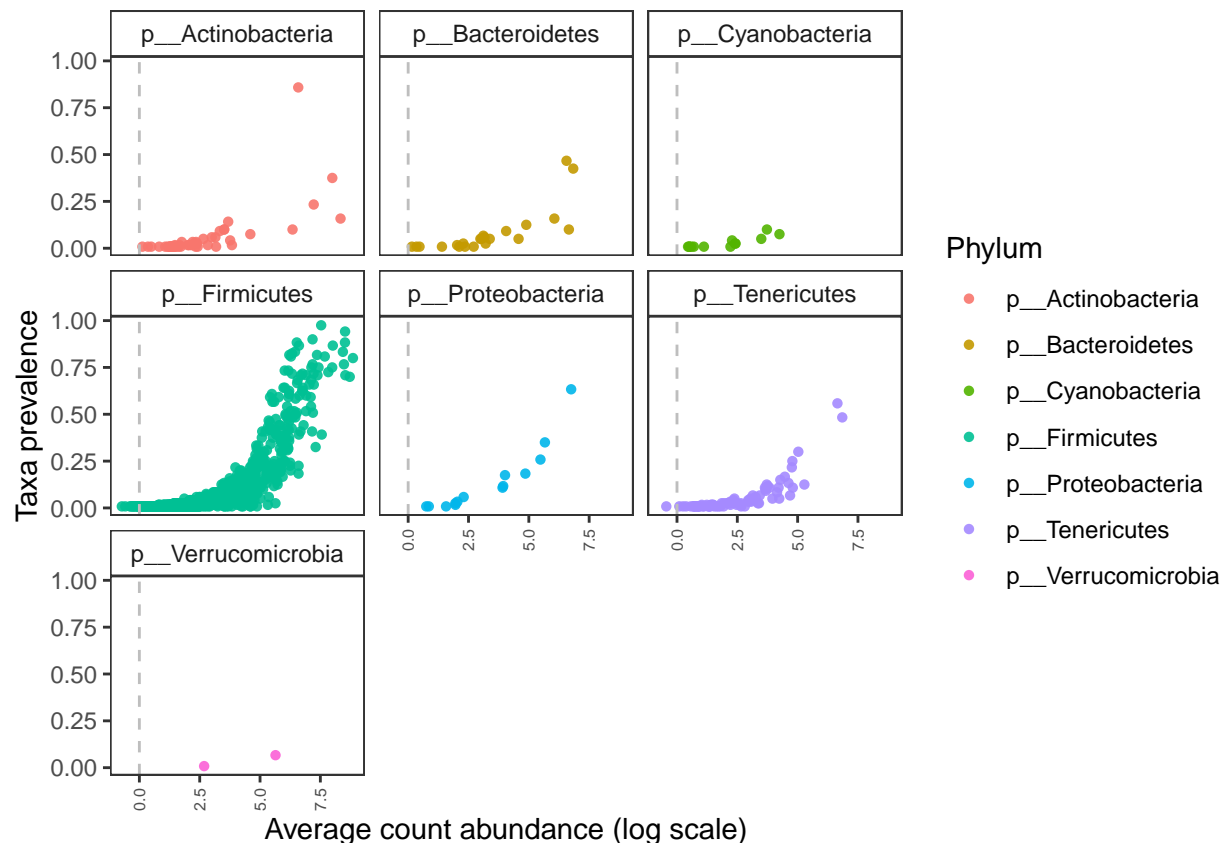
p4 = ggscatter(lib.div, "Phylogenetic_Diversity", "ReadsPerSample", xlab = "Phylogenetic diversity", add = "loess",
  stat_cor(method = "pearson"))

ggarrange(p1, p2, p3, p4, ncol = 2, nrow = 2)
```



removal of samples with lower sequencing depth not necessary for 16S dataset

```
plot_taxa_prevalence(subset16S, "Phylum") # taxa prevalence plot
```



Diversity metrics boxplots

```
hmp.div <- microbiome::alpha(subset16S, index = "all")
hmp.meta <- meta(subset16S)
hmp.meta$sam_name <- rownames(hmp.meta)
hmp.div$sam_name <- rownames(hmp.div)
div.df <- merge(hmp.div, hmp.meta, by = "sam_name")
colnames(div.df)
```

```
## [1] "sam_name" "observed"
## [3] "chao1" "diversity_inverse_simpson"
## [5] "diversity_gini_simpson" "diversity_shannon"
## [7] "diversity_fisher" "diversity_coverage"
## [9] "evenness_camargo" "evenness_pielou"
## [11] "evenness_simpson" "evenness_evar"
## [13] "evenness_bulla" "dominance_dbp"
## [15] "dominance_dmn" "dominance_absolute"
## [17] "dominance_relative" "dominance_simpson"
## [19] "dominance_core_abundance" "dominance_gini"
## [21] "rarity_log_modulo_skewness" "rarity_low_abundance"
## [23] "rarity_rare_abundance" "LibraryNumber"
## [25] "Sample_Unique" "LibraryName"
## [27] "Farm" "Farm2"
## [29] "Stable" "FarmRoundStable"
## [31] "Days" "Age"
## [33] "Sname" "WeightAnimal"
## [35] "Gender" "AgeParentStock"
```

```
## [37] "Hatchery"           "Researcher"
## [39] "AB"                 "Abday"
## [41] "FlockSize"          "FeedF"
## [43] "FeedType"           "FeedProducent"
## [45] "Cox"                "OPG"
## [47] "Cluster"            "LitterType"
## [49] "Metagenomics"       "Stables"
```

```
#based on microbial agent
```

```
div.df2 <- div.df[, c("Cox", "diversity_inverse_simpson", "diversity_gini_simpson", "diversity_shannon",
colnames(div.df2) <- c("Cox", "Inverse Simpson", "Gini-Simpson", "Shannon", "Observed", "Coverage", "Pi
```

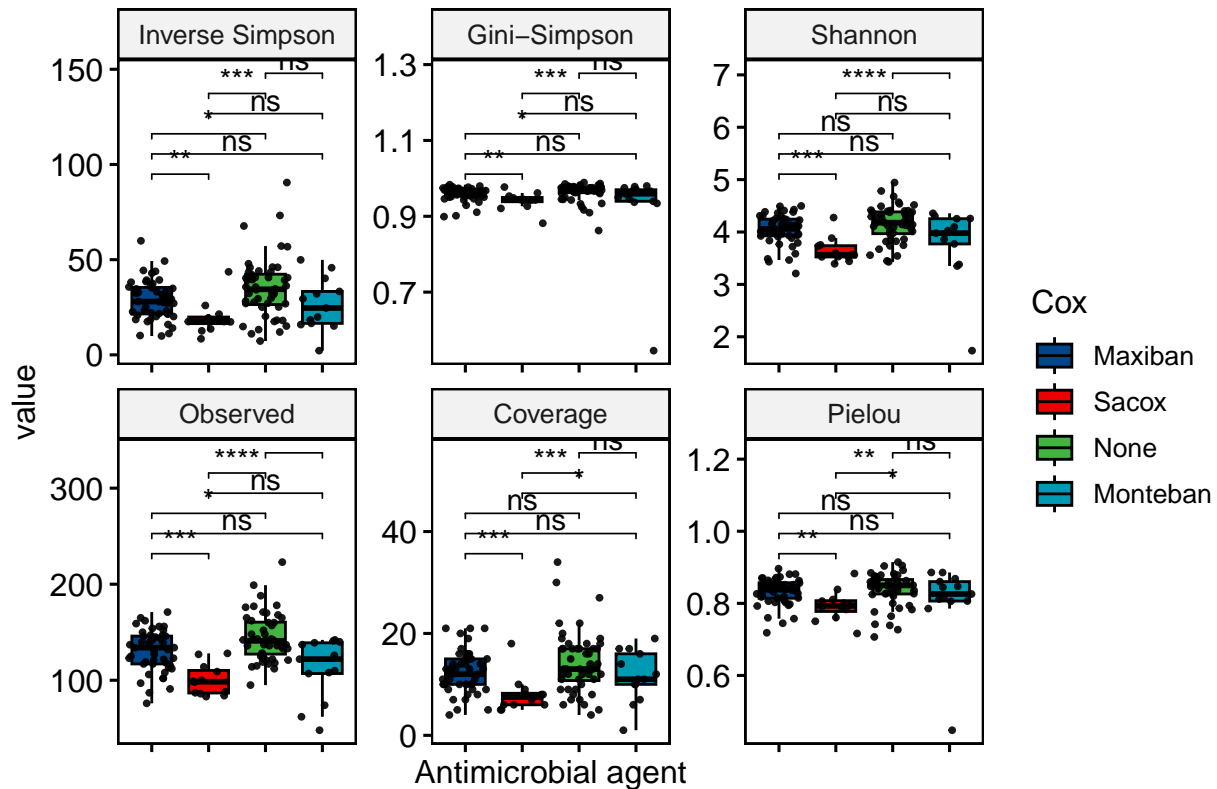
```
div_df_melt <- reshape2::melt(div.df2)
```

```
lev = c("Maxiban", "Sacox", "Monteban", "None")
```

```
L.pairs <- combn(seq_along(lev), 2, simplify = FALSE, FUN = function(i) lev[i])
```

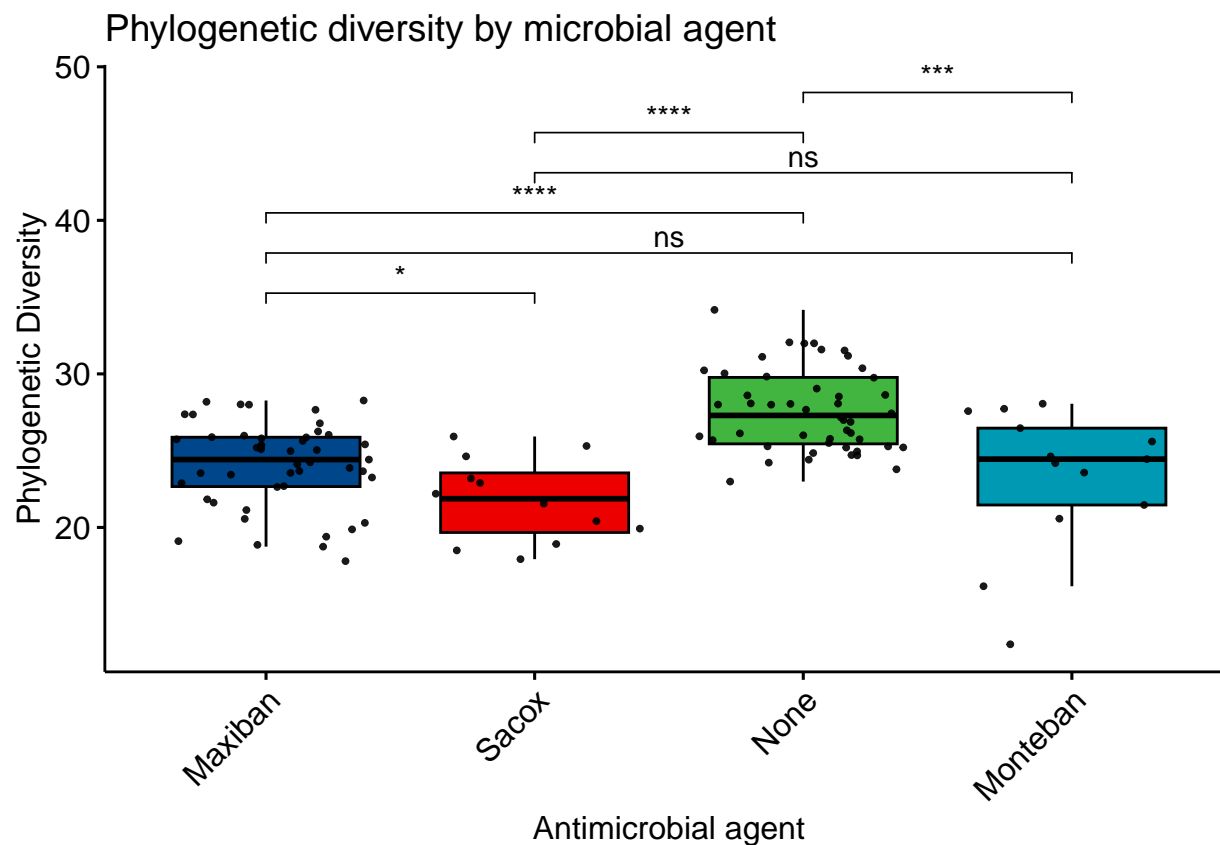
```
ggboxplot(div_df_melt, x = "Cox", y = "value",
  fill = "Cox",
  palette = "lancet",
  legend= "right",
  facet.by = "variable",
  scales = "free",
  xlab = "Antimicrobial agent",
  title = "Alpha diversity metrics by microbial agent",
  outlier.shape = NA) +
  rremove("x.text") + stat_compare_means(
  comparisons = L.pairs,
  label = "p.signif"
) + geom_jitter(size = 0.7, alpha = 0.9)
```

Alpha diversity metrics by microbial agent



```
df.pd <- pd(t(as.data.frame(subset16S@otu_table)), subset16S@phy_tree,include.root=T) # transposing for
hmp.meta$Phylogenetic_Diversity <- df.pd$PD
```

```
ggboxplot(hmp.meta,
  x = "Cox",
  y = "Phylogenetic_Diversity",
  fill = "Cox",
  order = c("Maxiban","Sacox","None","Monteban"),
  palette = "lancet",
  ylab = "Phylogenetic Diversity",
  xlab = "Antimicrobial agent",
  legend = "right",
  title = "Phylogenetic diversity by microbial agent",
  outlier.shape = NA) + rotate_x_text() +
  theme(legend.position="none", axis.text.x=element_text(angle=45,hjust=1,vjust=1,size=12)) +
  stat_compare_means(
    comparisons = L.pairs,
    label = "p.signif"
  ) + geom_jitter(size = 0.7, alpha = 0.9)
```



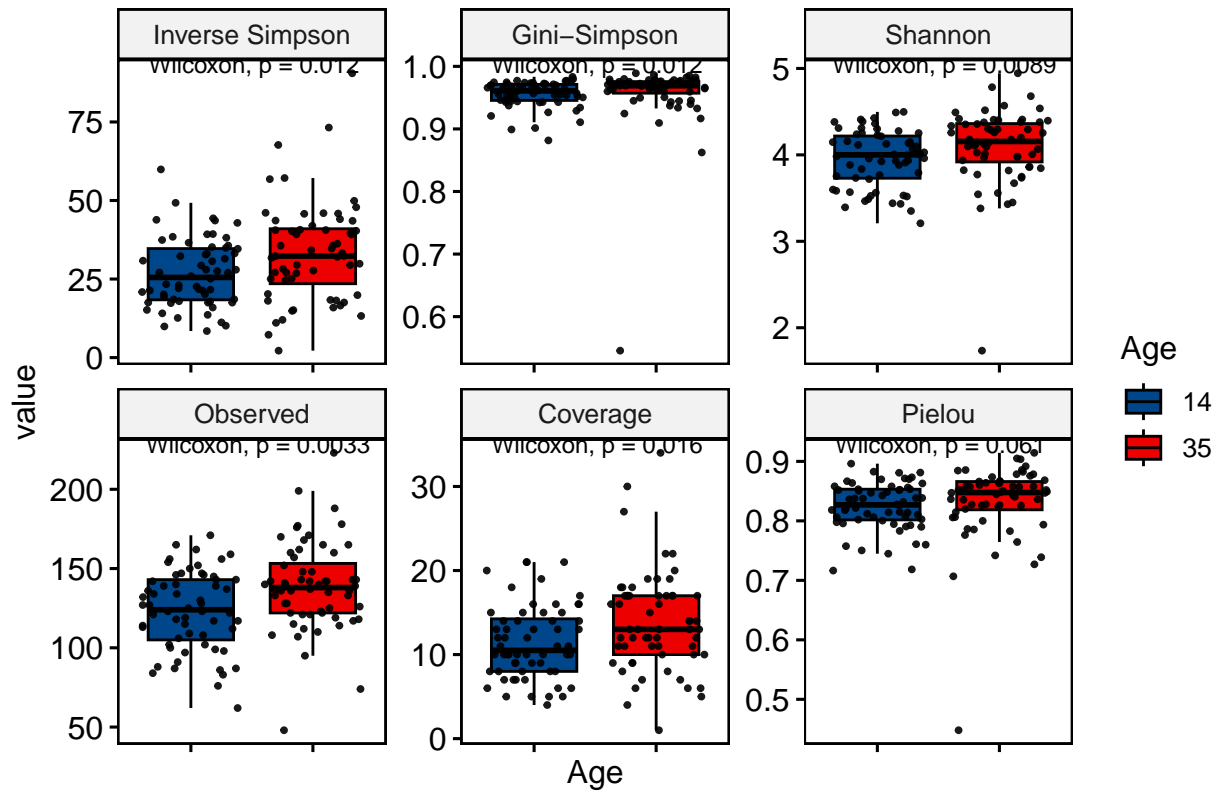
```
# age / days
```

```
div.df2 <- div.df[, c("Age", "diversity_inverse_simpson", "diversity_gini_simpson", "diversity_shannon",
colnames(div.df2) <- c("Age", "Inverse Simpson", "Gini-Simpson", "Shannon", "Observed", "Coverage", "Pi
```

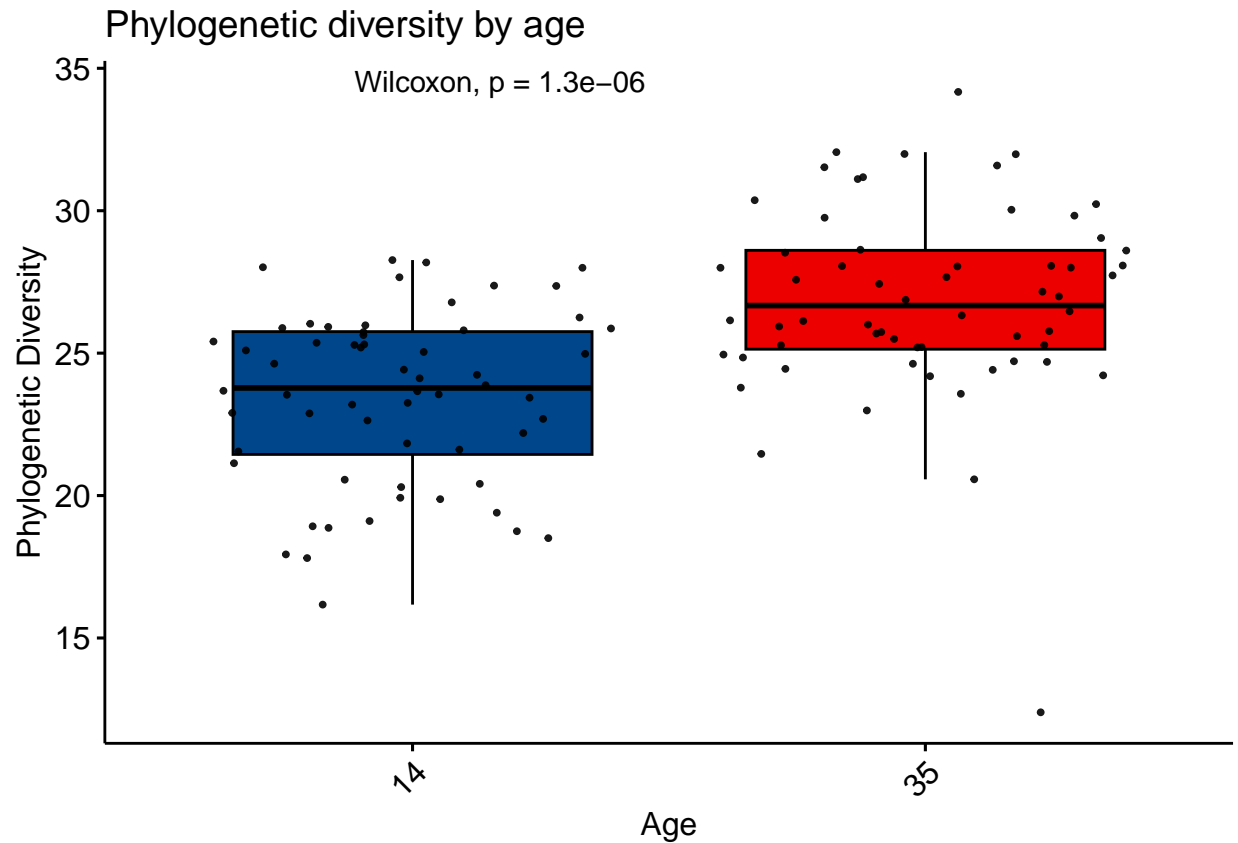
```
div.df2$Age = as.factor(div.df2$Age)
div_df_melt <- reshape2::melt(div.df2)
```

```
ggboxplot(div_df_melt, x = "Age", y = "value",
  fill = "Age",
  palette = "lancet",
  legend= "right",
  facet.by = "variable",
  scales = "free",
  title = "Alpha diversity metrics by age",
  outlier.shape = NA) +
  rremove("x.text") + stat_compare_means(method = "wilcox.test", size = 3.1) + geom_jitter(size = 0.7, a
```

Alpha diversity metrics by age



```
ggboxplot(hmp.meta,
  x = "Age",
  y = "Phylogenetic_Diversity",
  fill = "Age",
  palette = "lancet",
  ylab = "Phylogenetic Diversity",
  xlab = "Age",
  legend = "right",
  title = "Phylogenetic diversity by age",
  outlier.shape = NA) + rotate_x_text() +
  theme(legend.position="none", axis.text.x=element_text(angle=45,hjust=1,vjust=1,size=12)) +
  stat_compare_means(paired = TRUE) + geom_jitter(size = 0.7, alpha = 0.9)
```



```
# farms / company
```

```
div.df2 <- div.df[, c("Farm2", "diversity_inverse_simpson", "diversity_gini_simpson", "diversity_shannon",
colnames(div.df2) <- c("Farm", "Inverse Simpson", "Gini-Simpson", "Shannon", "Observed", "Coverage", "P")
```

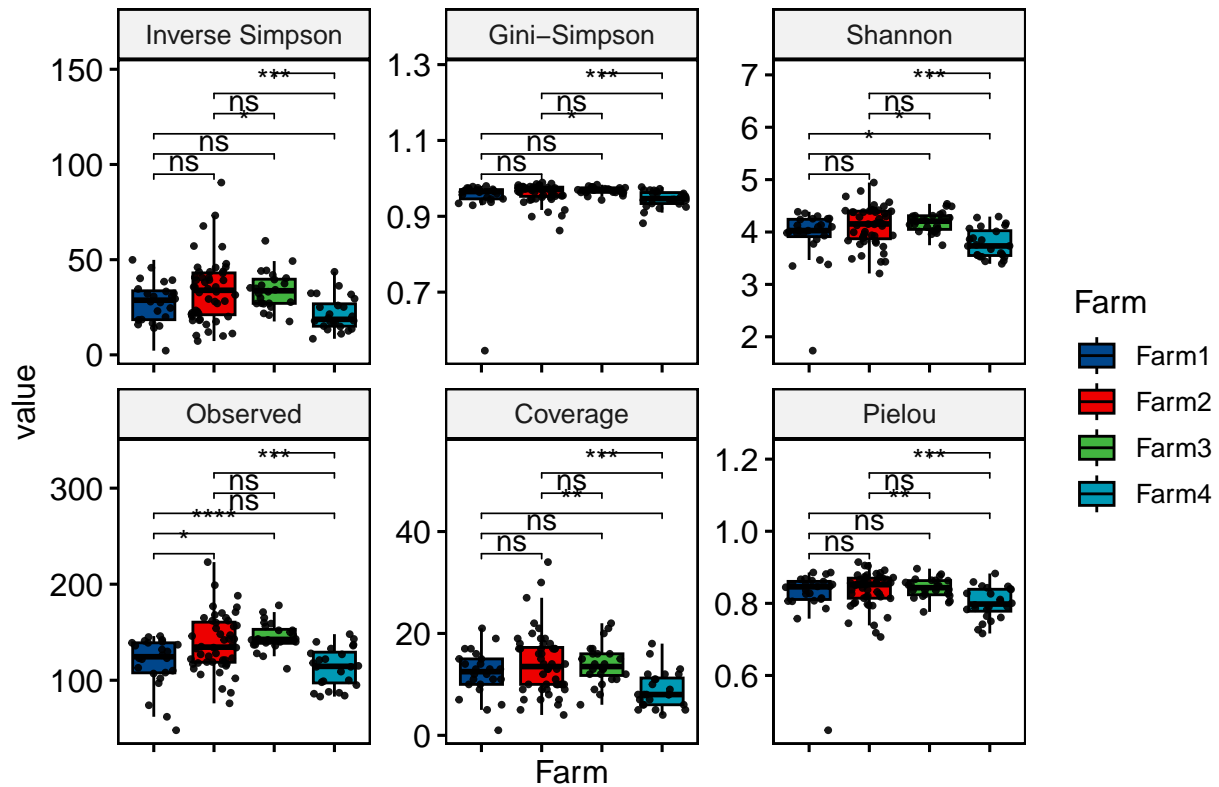
```
div_df_melt <- reshape2::melt(div.df2)
```

```
lev = c("Farm1", "Farm2", "Farm3", "Farm4")
```

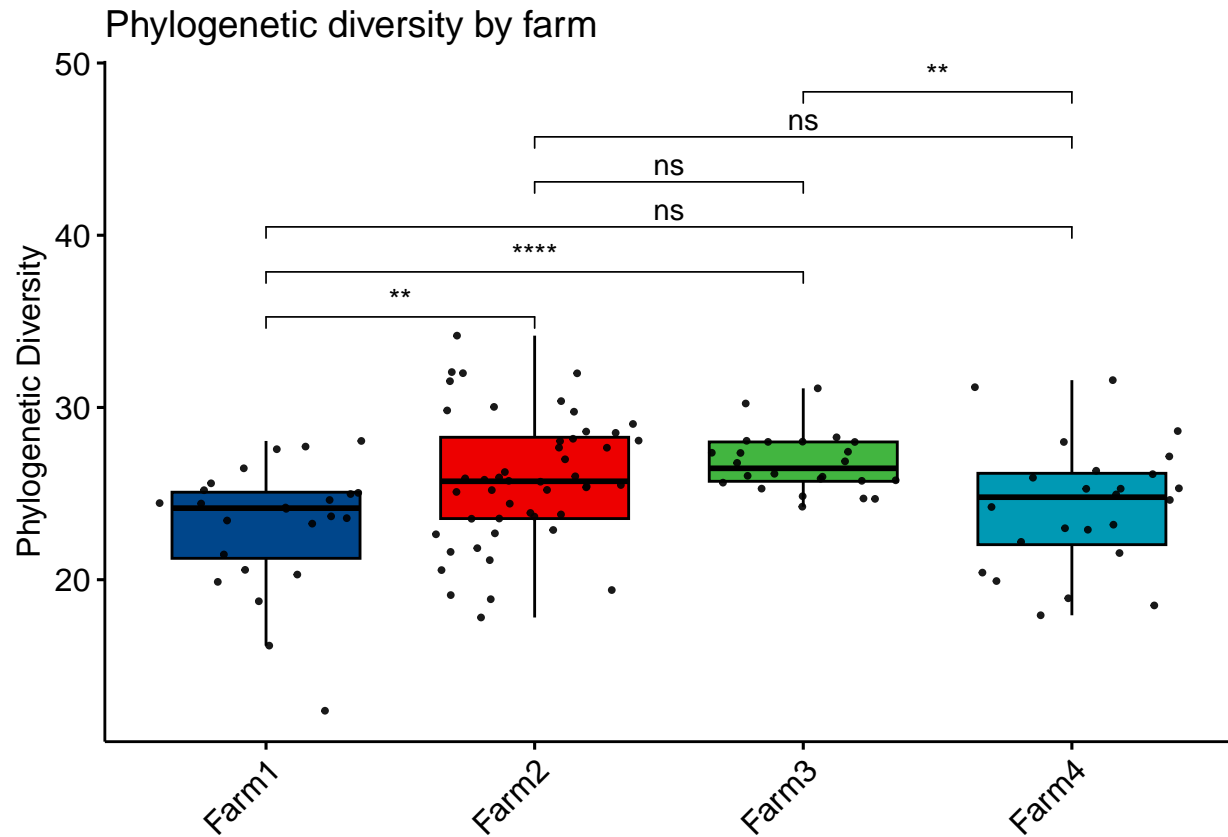
```
L.pairs <- combn(seq_along(lev), 2, simplify = FALSE, FUN = function(i) lev[i])
```

```
ggboxplot(div_df_melt, x = "Farm", y = "value",
  fill = "Farm",
  palette = "lancet",
  legend = "right",
  facet.by = "variable",
  scales = "free",
  order = lev,
  title = "Alpha diversity metrics by farm",
  outlier.shape = NA) + rotate_x_text() + rremove("x.text") + stat_compare_means(method = "wilcoxon",
  comparisons = 1,
  label = "p.sig")
) + geom_jitter(size = 0.7, alpha = 0.9)
```


Alpha diversity metrics by farm



```
ggboxplot(hmp.meta,
  x = "Farm2",
  y = "Phylogenetic_Diversity",
  fill = "Farm2",
  palette = "lancet",
  ylab = "Phylogenetic Diversity",
  xlab = "Farm",
  legend = "right",
  title = "Phylogenetic diversity by farm",
  outlier.shape = NA) + rotate_x_text() +
  theme(legend.position="none", axis.text.x=element_text(angle=45,hjust=1,vjust=1,size=12), axis.title
  stat_compare_means(
    comparisons = L.pairs,
    label = "p.signif"
  ) + geom_jitter(size = 0.7, alpha = 0.9)
```



```
# stable

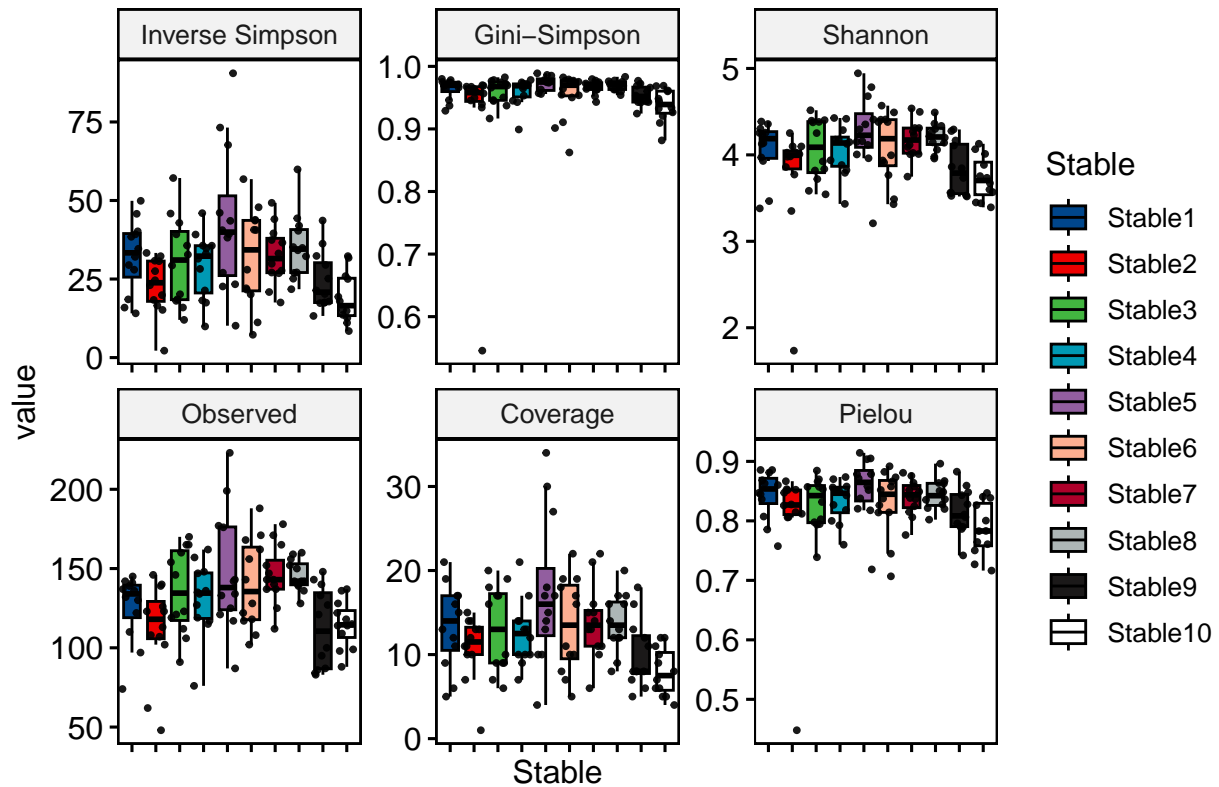
div.df2 <- div.df[, c("Stables", "diversity_inverse_simpson", "diversity_gini_simpson", "diversity_shannon", "diversity_observed", "diversity_coverage")]
colnames(div.df2) <- c("Stable", "Inverse Simpson", "Gini-Simpson", "Shannon", "Observed", "Coverage", "Coverage")

div_df_melt <- reshape2::melt(div.df2)

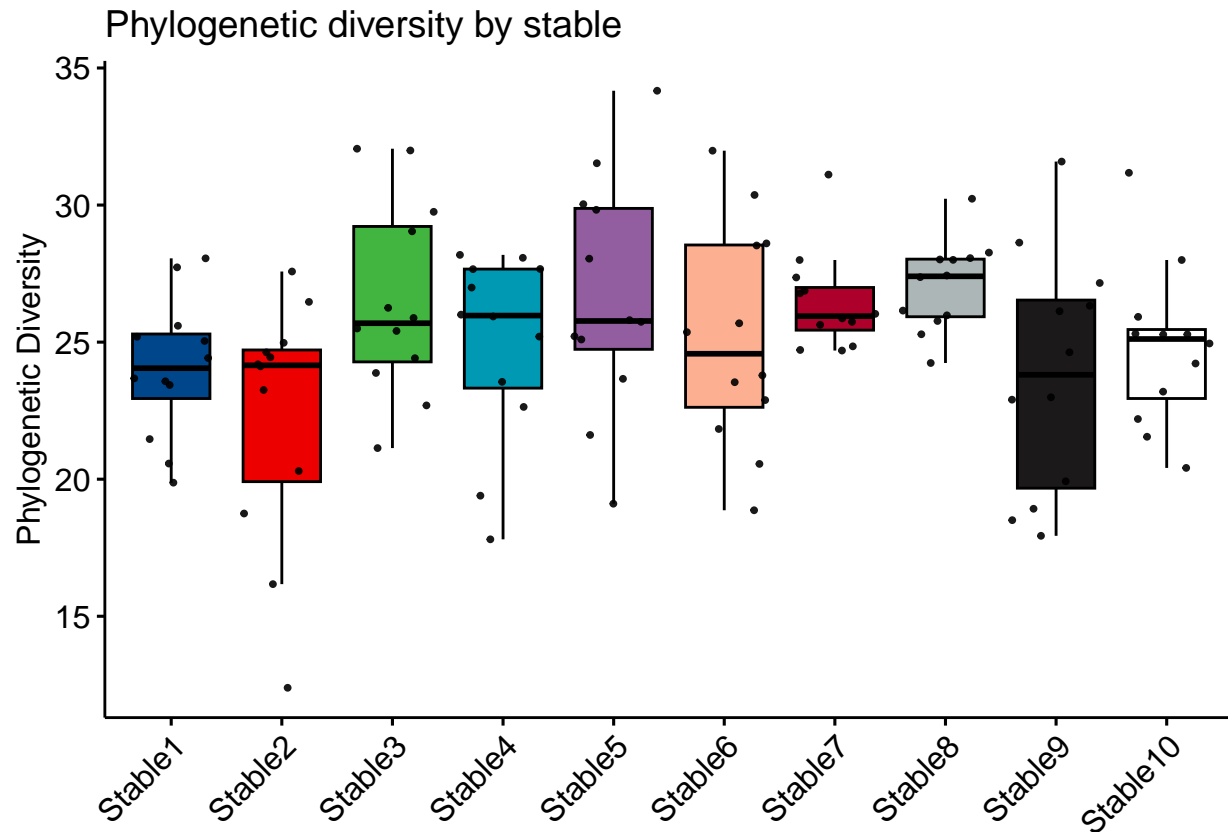
lev = c("Stable1", "Stable2", "Stable3", "Stable4", "Stable5", "Stable6", "Stable7", "Stable8", "Stable9", "Stable10")
L.pairs <- combn(seq_along(lev), 2, simplify = FALSE, FUN = function(i) lev[i])

ggboxplot(div_df_melt, x = "Stable", y = "value",
  fill = "Stable",
  palette = "lancet",
  legend = "right",
  facet.by = "variable",
  scales = "free",
  order = lev,
  title = "Alpha diversity metrics by stable",
  outlier.shape = NA) + rotate_x_text() + rremove("x.text") + geom_jitter(size = 0.7, alpha = 0.5)
```

Alpha diversity metrics by stable



```
ggboxplot(hmp.meta,
  x = "Stables",
  y = "Phylogenetic_Diversity",
  fill = "Stables",
  palette = "lancet",
  ylab = "Phylogenetic Diversity",
  xlab = "Farm",
  legend = "right",
  title = "Phylogenetic diversity by stable",
  outlier.shape = NA) + rotate_x_text() +
  theme(legend.position="none", axis.text.x=element_text(angle=45,hjust=1,vjust=1,size=12),
    axis.title.x = element_blank()) + geom_jitter(size = 0.7, alpha = 0.9)
```



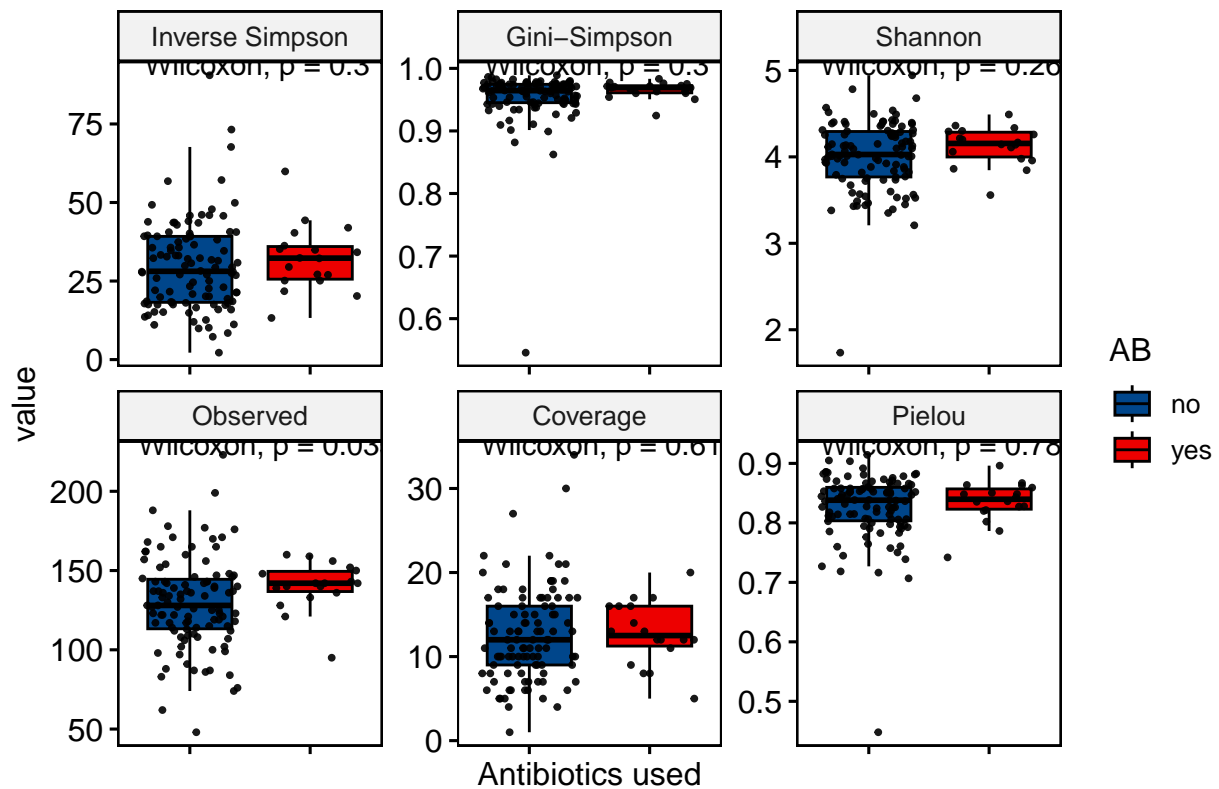
based on AB

```
div.df2 <- div.df[, c("AB", "diversity_inverse_simpson", "diversity_gini_simpson", "diversity_shannon",
colnames(div.df2) <- c("AB", "Inverse Simpson", "Gini-Simpson", "Shannon", "Observed", "Coverage", "Pie
```

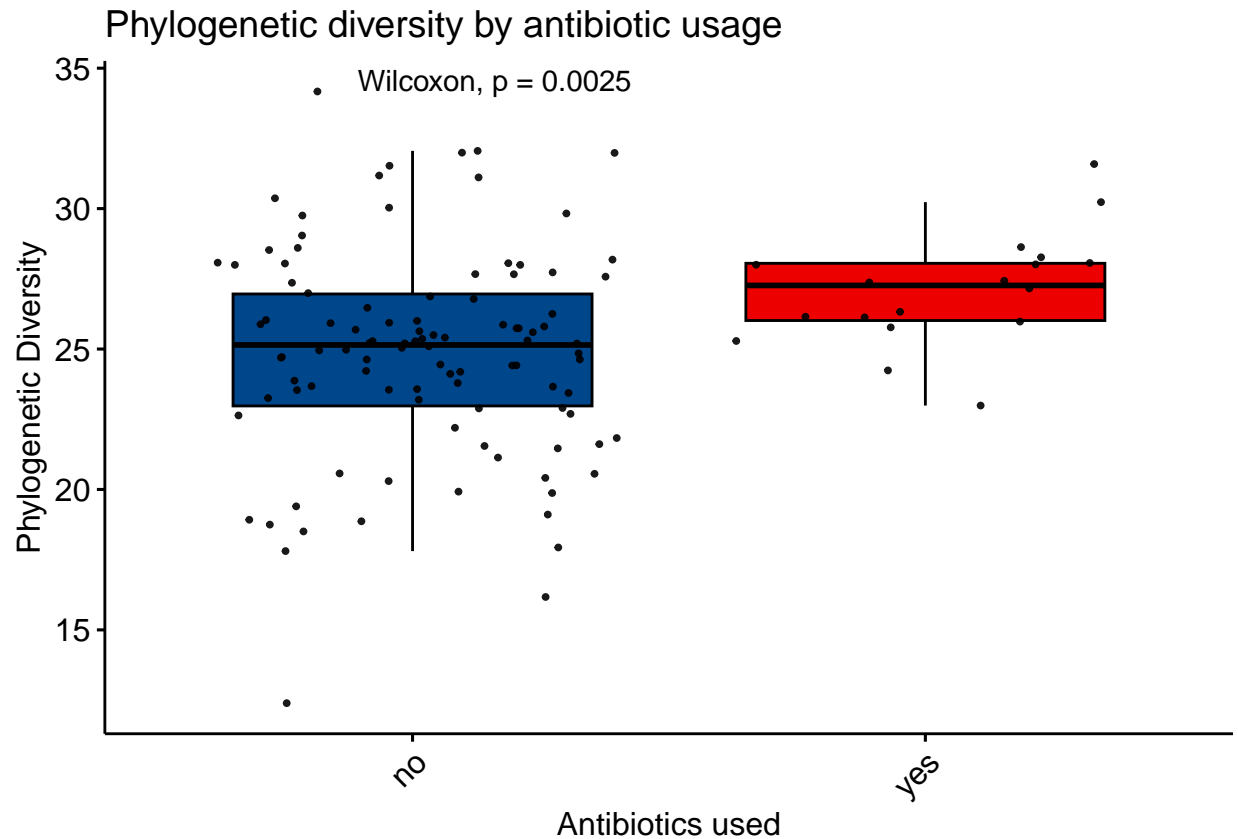
```
div_df_melt <- reshape2::melt(div.df2)
```

```
ggboxplot(div_df_melt, x = "AB", y = "value",
  fill = "AB",
  palette = "lancet",
  legend = "right",
  facet.by = "variable",
  scales = "free",
  xlab = "Antibiotics used",
  title = "Alpha diversity metrics by antibiotic usage",
  outlier.shape = NA) +
  rremove("x.text") + stat_compare_means() + geom_jitter(size = 0.7, alpha = 0.9)
```

Alpha diversity metrics by antibiotic usage



```
ggboxplot(hmp.meta,
  x = "AB",
  y = "Phylogenetic_Diversity",
  fill = "AB",
  palette = "lancet",
  ylab = "Phylogenetic Diversity",
  xlab = "Antibiotics used",
  legend = "right",
  title = "Phylogenetic diversity by antibiotic usage",
  outlier.shape = NA) + rotate_x_text() +
  theme(legend.position="none", axis.text.x=element_text(angle=45,hjust=1,vjust=1,size=12)) +
  stat_compare_means() + geom_jitter(size = 0.7, alpha = 0.9)
```



```
# based on stable and age
```

```
div.df2 <- div.df[, c("Stables", "Age", "diversity_shannon")]
colnames(div.df2) <- c("Stable", "Age", "Shannon")
```

```
div_df_melt <- reshape2::melt(div.df2)
```

```
lev = c("Stable1", "Stable2", "Stable3", "Stable4", "Stable5", "Stable6", "Stable7", "Stable8", "Stable9", "Stable10")
```

```
ggboxplot(div_df_melt, x = "Stable", y = "value",
```

```
  fill = "Age",
```

```
  palette = "lancet",
```

```
  legend = "right",
```

```
  facet.by = "variable",
```

```
  scales = "free",
```

```
  order = lev,
```

```
  title = "Shannon diversity by stable and age",
```

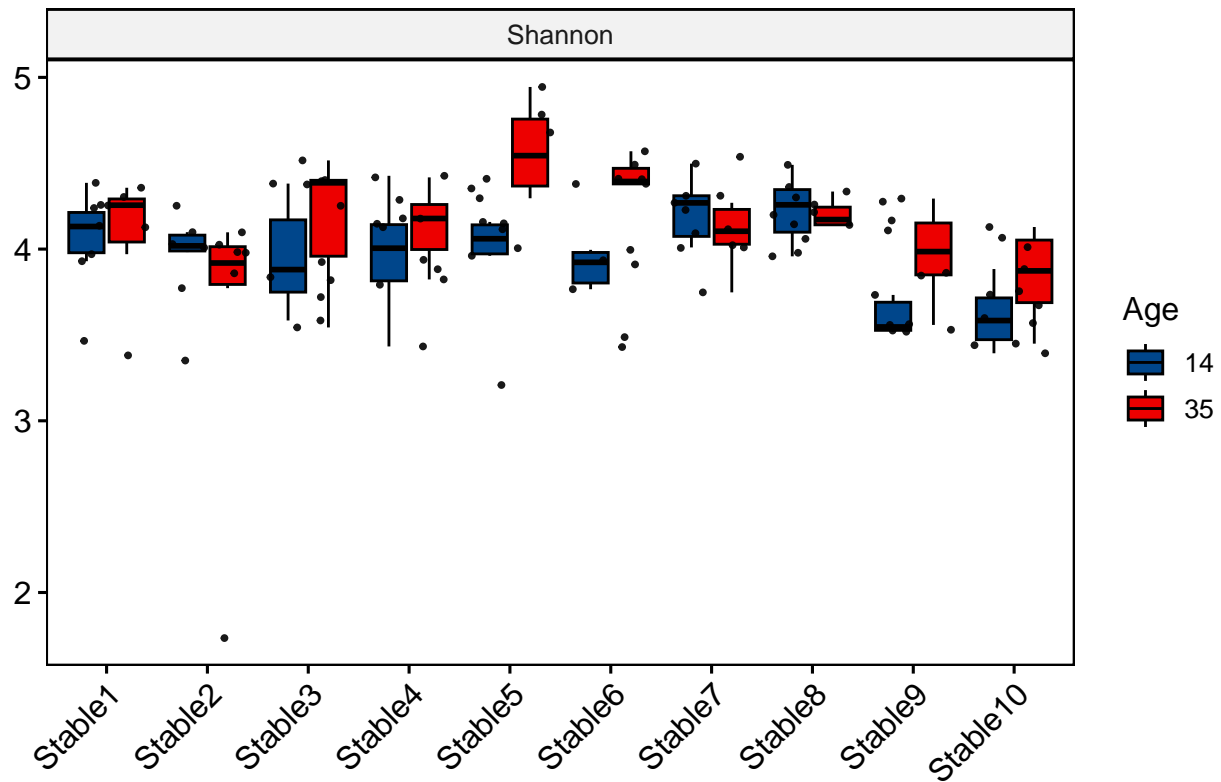
```
  xlab = FALSE,
```

```
  ylab = FALSE,
```

```
  outlier.shape = NA) + rotate_x_text() +
```

```
  theme(axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1, size = 12)) + geom_jitter(size = 0.7, alpha = 0.5)
```

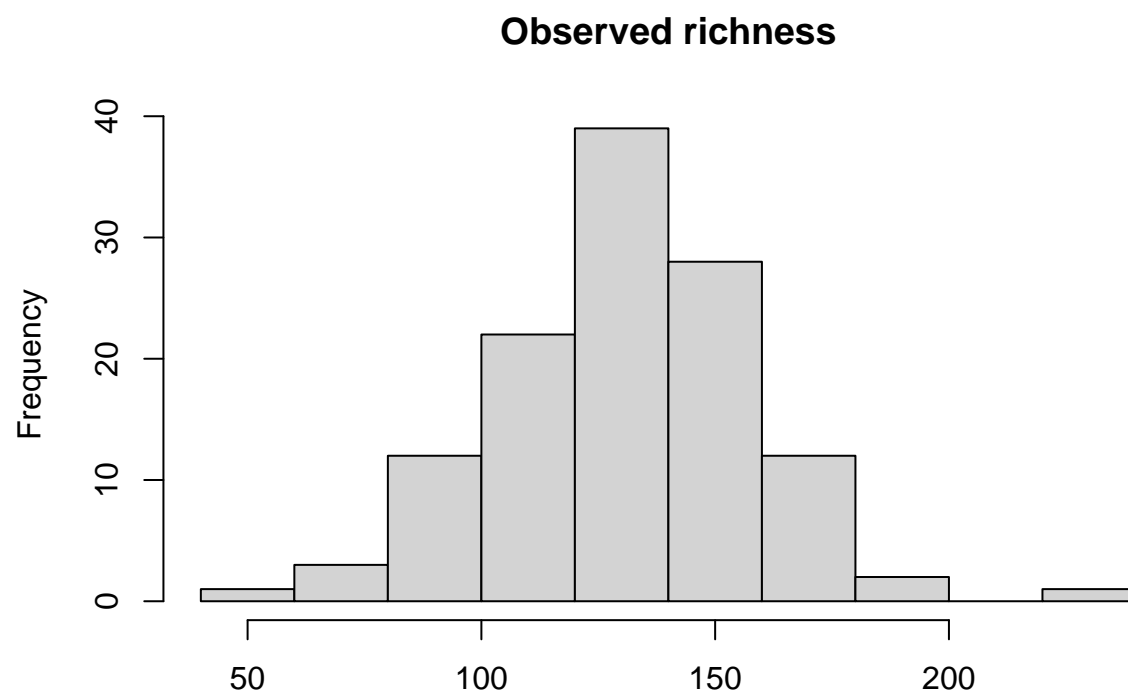
Shannon diversity by stable and age



Looking at significance

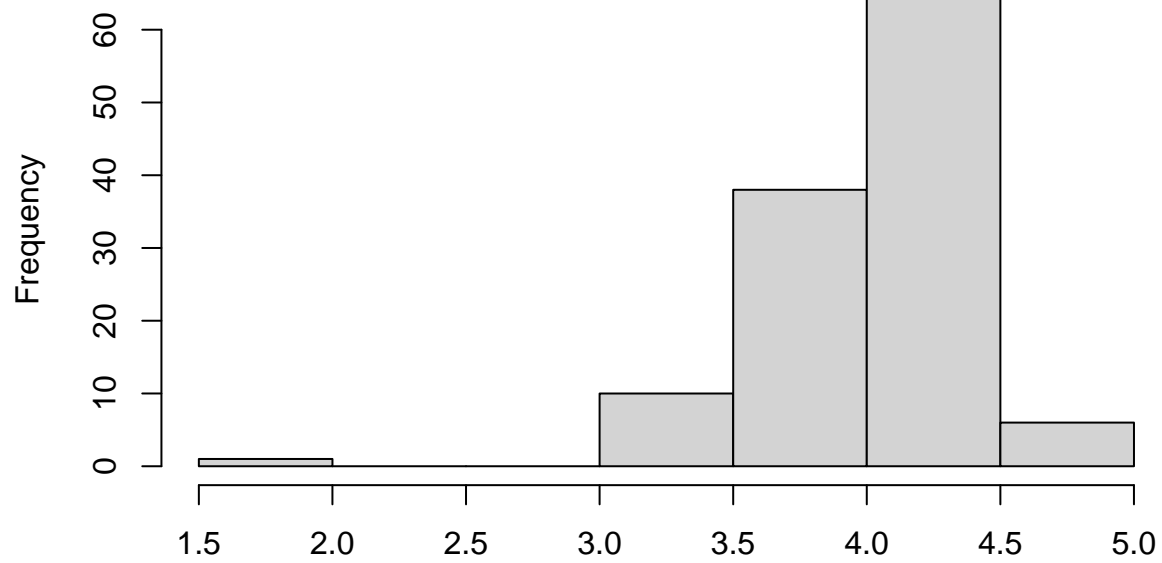
Checking for normality

```
hist(lib.div$observed, main="Observed richness", xlab="")
```

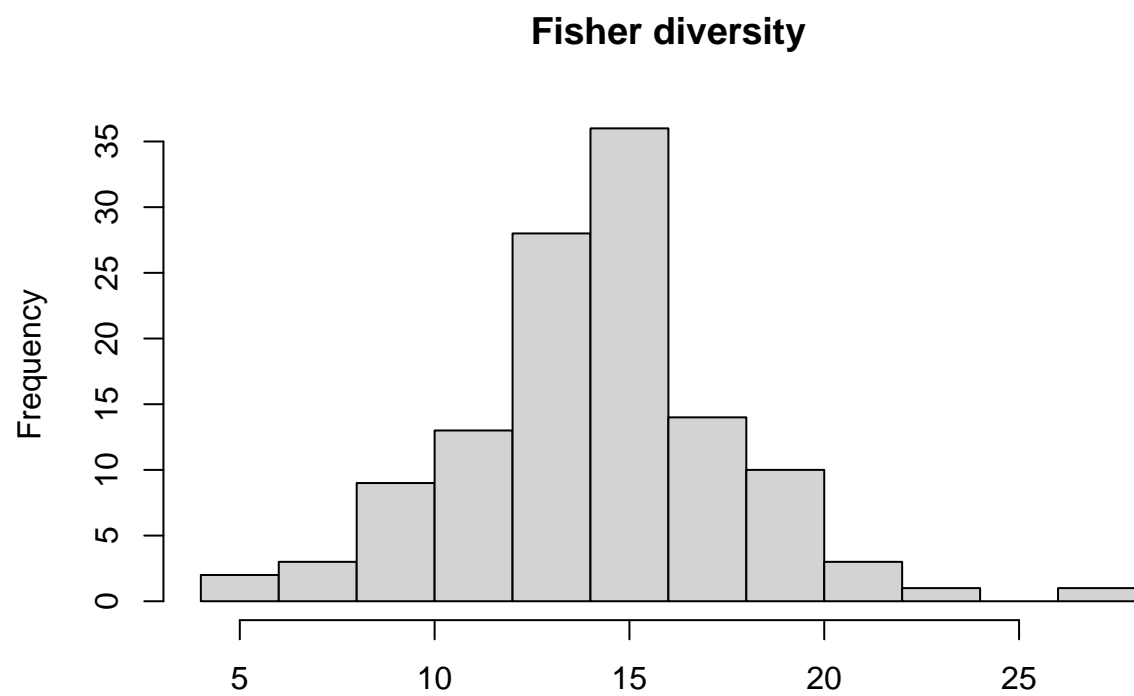


```
hist(lib.div$diversity_shannon, main="Shannon diversity", xlab="")
```


Shannon diversity

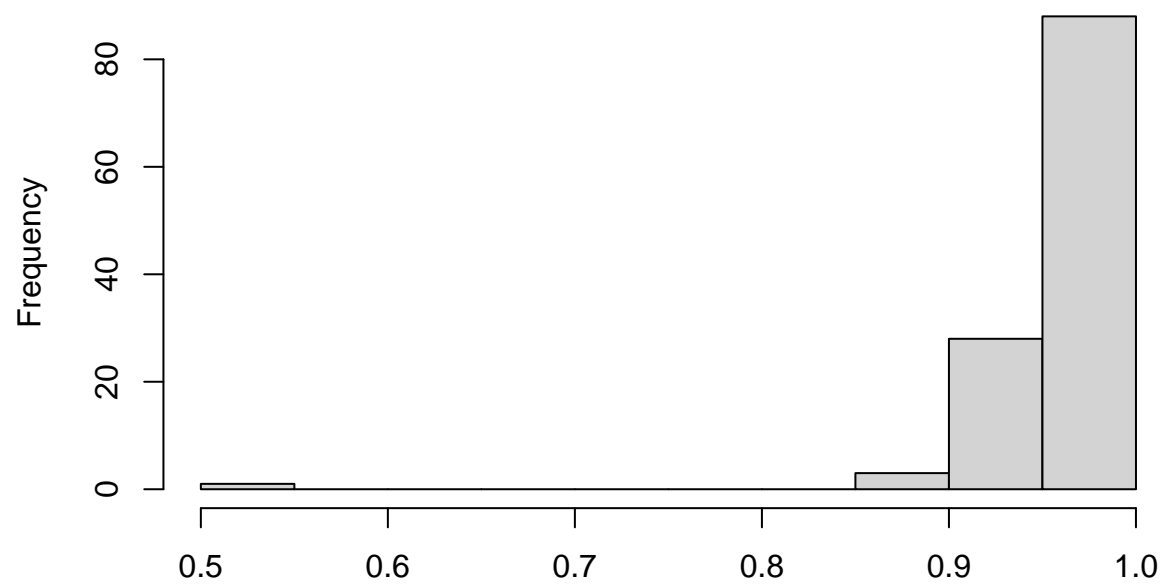


```
hist(lib.div$diversity_fisher, main="Fisher diversity", xlab="")
```



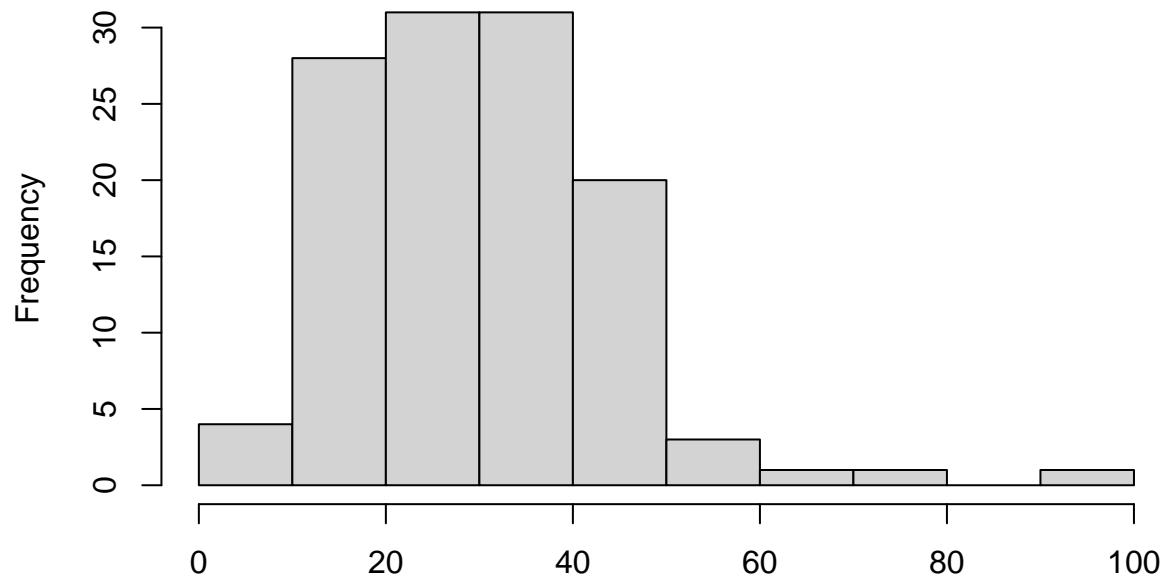
```
hist(lib.div$diversity_gini_simpson, main="Gini-Simpson diversity", xlab="")
```

Gini–Simpson diversity



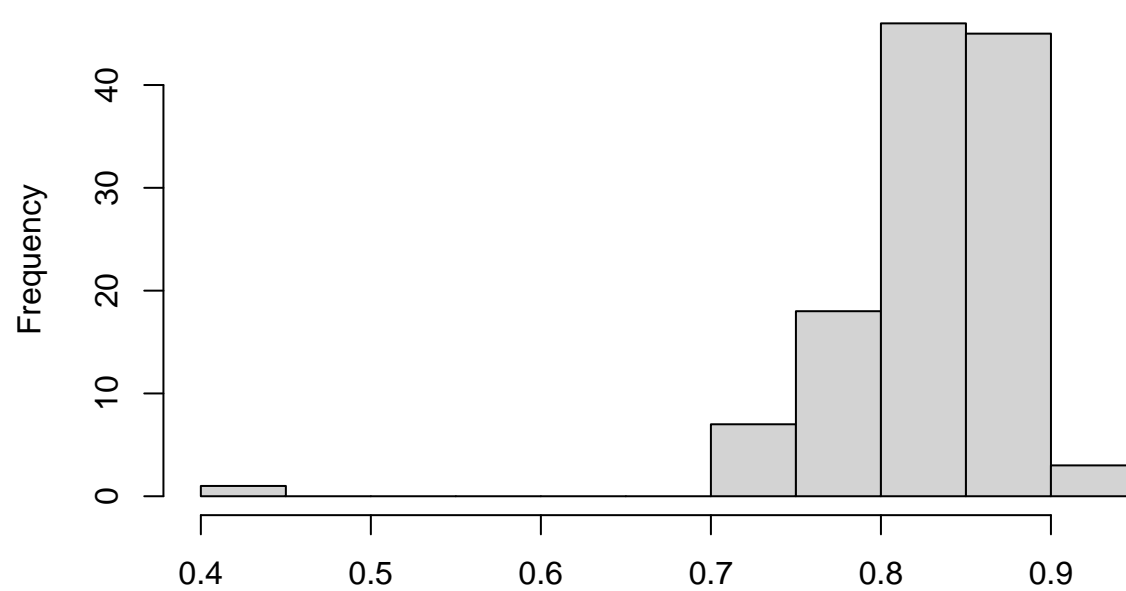
```
hist(lib.div$diversity_inverse_simpson, main="Inverse Simpson evenness", xlab="")
```

Inverse Simpson evenness

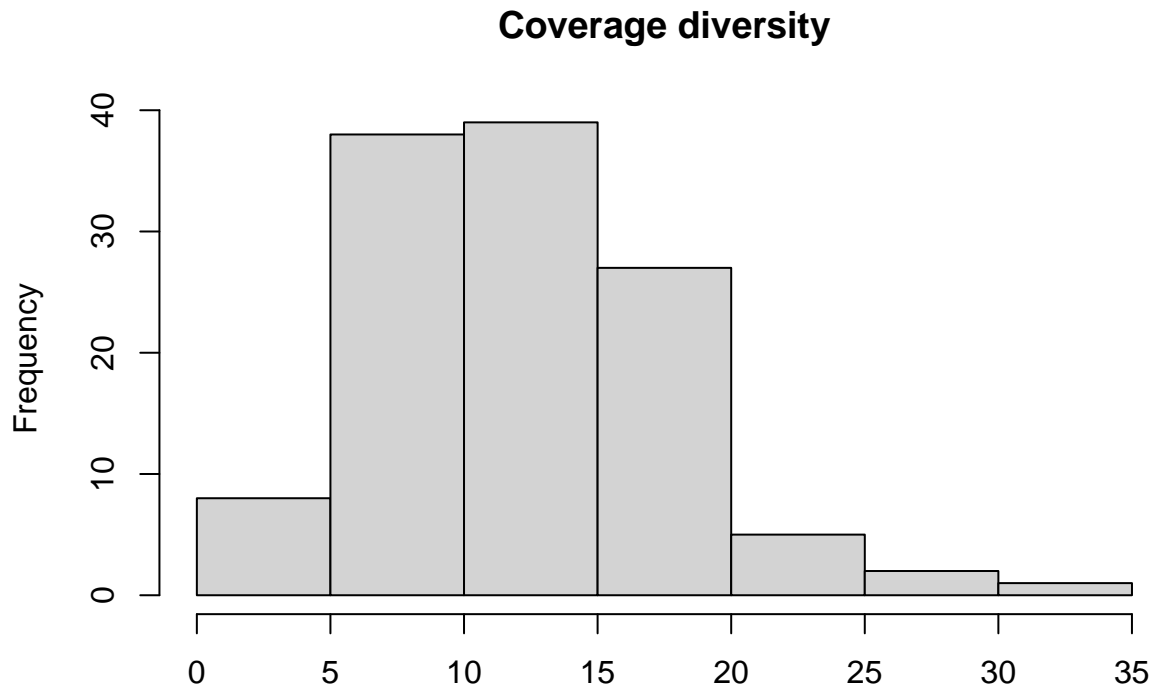


```
hist(lib.div$evenness_pielou, main="Pielou evenness", xlab="")
```

Pielou evenness



```
hist(lib.div$diversity_coverage, main="Coverage diversity", xlab="")
```



If data is normally distributed we can use ANOVA / t-tests, if not we will use Kruskal-Wallis tests. In this case, the data seems roughly normally distributed for some metrics, we can use Shapiro-Wilk tests to test for normality for individual measures

```
shapiro.test(lib.div$observed) # test deems it normally distributed  $p > 0,05$ 
```

```
##
## Shapiro-Wilk normality test
##
## data: lib.div$observed
## W = 0.98871, p-value = 0.4266
```

```
shapiro.test(lib.div$diversity_shannon) # test deems this measure not normally distributed  $p < 0,05$ 
```

```
##
## Shapiro-Wilk normality test
##
## data: lib.div$diversity_shannon
## W = 0.89676, p-value = 1.344e-07
```

```
shapiro.test(lib.div$diversity_fisher) # test deems this measure normally distributed  $p > 0,05$ 
```

```
##
## Shapiro-Wilk normality test
##
```

```

## data: lib.div$diversity_fisher
## W = 0.98911, p-value = 0.4585

shapiro.test(lib.div$diversity_gini_simpson) # test deems this measure not normally distributed p<0,05

##
## Shapiro-Wilk normality test
##
## data: lib.div$diversity_gini_simpson
## W = 0.4334, p-value < 2.2e-16

shapiro.test(lib.div$diversity_inverse_simpson) # test deems this measure not normally distributed p<0,05

##
## Shapiro-Wilk normality test
##
## data: lib.div$diversity_inverse_simpson
## W = 0.9455, p-value = 0.0001025

shapiro.test(lib.div$evenness_pielou) # test deems this measure not normally distributed p<0,05

##
## Shapiro-Wilk normality test
##
## data: lib.div$evenness_pielou
## W = 0.79225, p-value = 9.689e-12

shapiro.test(lib.div$diversity_coverage) # test deems this measure not normally distributed p<0,05

##
## Shapiro-Wilk normality test
##
## data: lib.div$diversity_coverage
## W = 0.95673, p-value = 0.000697

shapiro.test(lib.div$Phylogenetic_Diversity) # test deems this measure normally distributed p>0,05

##
## Shapiro-Wilk normality test
##
## data: lib.div$Phylogenetic_Diversity
## W = 0.9791, p-value = 0.0587

# Based on shapiro-wilk tests we will assume normality for some measures
# The variables that we are interested in are the Age, which Farm the samples are from, and whether ant

# We will run ANOVAs for the normally distributed variables

# Age

# Normally distributed with only 2 levels, so we can use t-tests :

t.test(lib.div$observed ~ sample_data(subset16S)$Age) # significant

```

```
##
## Welch Two Sample t-test
##
## data: lib.div$observed by sample_data(subset16S)$Age
## t = -3.2512, df = 116.43, p-value = 0.001503
## alternative hypothesis: true difference in means between group 14 and group 35 is not equal to 0
## 95 percent confidence interval:
## -25.371419 -6.161914
## sample estimates:
## mean in group 14 mean in group 35
## 123.1833 138.9500
```

```
t.test(lib.div$diversity_fisher ~ sample_data(subset16S)$Age) # significant
```

```
##
## Welch Two Sample t-test
##
## data: lib.div$diversity_fisher by sample_data(subset16S)$Age
## t = -3.7527, df = 117.44, p-value = 0.0002734
## alternative hypothesis: true difference in means between group 14 and group 35 is not equal to 0
## 95 percent confidence interval:
## -3.491963 -1.079515
## sample estimates:
## mean in group 14 mean in group 35
## 13.01596 15.30170
```

```
t.test(lib.div$Phylogenetic_Diversity ~ sample_data(subset16S)$Age) # significant
```

```
##
## Welch Two Sample t-test
##
## data: lib.div$Phylogenetic_Diversity by sample_data(subset16S)$Age
## t = -5.8275, df = 116.62, p-value = 5.097e-08
## alternative hypothesis: true difference in means between group 14 and group 35 is not equal to 0
## 95 percent confidence interval:
## -4.565813 -2.249550
## sample estimates:
## mean in group 14 mean in group 35
## 23.46607 26.87375
```

```
# Non-normally distributed
```

```
wilcox.test(lib.div$diversity_shannon ~ sample_data(subset16S)$Age) # shannon diversity seems to signif
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: lib.div$diversity_shannon by sample_data(subset16S)$Age
## W = 1301, p-value = 0.008885
## alternative hypothesis: true location shift is not equal to 0
```



```
wilcox.test(lib.div$diversity_gini_simpson ~ sample_data(subset16S)$Age) # significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$diversity_gini_simpson by sample_data(subset16S)$Age  
## W = 1323, p-value = 0.01239  
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$diversity_inverse_simpson ~ sample_data(subset16S)$Age) # significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$diversity_inverse_simpson by sample_data(subset16S)$Age  
## W = 1323, p-value = 0.01239  
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$evenness_pielou ~ sample_data(subset16S)$Age) # not significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$evenness_pielou by sample_data(subset16S)$Age  
## W = 1443, p-value = 0.06133  
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$diversity_coverage ~ sample_data(subset16S)$Age) # significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$diversity_coverage by sample_data(subset16S)$Age  
## W = 1340.5, p-value = 0.01577  
## alternative hypothesis: true location shift is not equal to 0
```

```
# For age, the groups seems significantly different in all metrics except pielou evenness.
```

```
# Antibiotics
```

```
t.test(lib.div$observed ~ sample_data(subset16S)$AB) # significant
```

```
##  
## Welch Two Sample t-test  
##  
## data: lib.div$observed by sample_data(subset16S)$AB  
## t = -2.3721, df = 42.794, p-value = 0.02225  
## alternative hypothesis: true difference in means between group no and group yes is not equal to 0  
## 95 percent confidence interval:  
## -20.17172 -1.63220
```

```
## sample estimates:
## mean in group no mean in group yes
##      129.4314      140.3333
```

```
t.test(lib.div$diversity_fisher ~ sample_data(subset16S)$AB) # significant
```

```
##
## Welch Two Sample t-test
##
## data: lib.div$diversity_fisher by sample_data(subset16S)$AB
## t = -2.0857, df = 44.774, p-value = 0.04273
## alternative hypothesis: true difference in means between group no and group yes is not equal to 0
## 95 percent confidence interval:
## -2.3593692 -0.0410508
## sample estimates:
## mean in group no mean in group yes
##      13.97879      15.17900
```

```
t.test(lib.div$Phylogenetic_Diversity ~ sample_data(subset16S)$AB) # significant
```

```
##
## Welch Two Sample t-test
##
## data: lib.div$Phylogenetic_Diversity by sample_data(subset16S)$AB
## t = -3.7313, df = 40.877, p-value = 0.0005798
## alternative hypothesis: true difference in means between group no and group yes is not equal to 0
## 95 percent confidence interval:
## -3.481642 -1.036191
## sample estimates:
## mean in group no mean in group yes
##      24.83107      27.08999
```

```
# Non-normally distributed
```

```
wilcox.test(lib.div$diversity_shannon ~ sample_data(subset16S)$AB) # shannon diversity does not seem to
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: lib.div$diversity_shannon by sample_data(subset16S)$AB
## W = 765, p-value = 0.2624
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$diversity_gini_simpson ~ sample_data(subset16S)$AB) # not significant
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: lib.div$diversity_gini_simpson by sample_data(subset16S)$AB
## W = 776, p-value = 0.2984
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$diversity_inverse_simpson ~ sample_data(subset16S)$AB) # not significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$diversity_inverse_simpson by sample_data(subset16S)$AB  
## W = 776, p-value = 0.2984  
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$evenness_pielou ~ sample_data(subset16S)$AB) # not significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$evenness_pielou by sample_data(subset16S)$AB  
## W = 879, p-value = 0.7772  
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(lib.div$diversity_coverage ~ sample_data(subset16S)$AB) # not significant
```

```
##  
## Wilcoxon rank sum test with continuity correction  
##  
## data: lib.div$diversity_coverage by sample_data(subset16S)$AB  
## W = 848, p-value = 0.6088  
## alternative hypothesis: true location shift is not equal to 0
```

```
# used the following functions to get means and sd per variable and alpha diversity metric  
#lib.div.ab = lib.div  
#lib.div.ab$AB = sample_data(subset16S)$AB  
#aggregate(lib.div.ab$observed, list(lib.div.ab$AB), FUN=mean)  
#aggregate(lib.div.ab$observed, list(lib.div.ab$AB), FUN=sd)
```

```
# AB does not seem to significantly differ in their alpha diversities except for observed, PD and fisher
```

```
# Farm has more than 2 levels, so we will use ANOVAs for normally distributed metrics
```

```
aov.observed.farm = aov(lib.div$observed ~ sample_data(subset16S)$Farm2)  
summary(aov.observed.farm)
```

```
##  
##               Df Sum Sq Mean Sq F value    Pr(>F)  
## sample_data(subset16S)$Farm2    3  19238    6413   10.41 4.07e-06 ***  
## Residuals                   116  71473     616  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov.observed.farm) # only not significant between 1 and 4 and 3 and 2
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lib.div$observed ~ sample_data(subset16S)$Farm2)
##
## $'sample_data(subset16S)$Farm2'
##      diff      lwr      upr      p adj
## Farm2-Farm1 20.4375  4.261634 36.613366 0.0070916
## Farm3-Farm1 27.2500  8.571718 45.928282 0.0012984
## Farm4-Farm1 -4.8750 -23.553282 13.803282 0.9043806
## Farm3-Farm2  6.8125 -9.363366 22.988366 0.6915866
## Farm4-Farm2 -25.3125 -41.488366 -9.136634 0.0004785
## Farm4-Farm3 -32.1250 -50.803282 -13.446718 0.0001014
```

```
aov.fisher.farm = aov(lib.div$diversity_fisher ~ sample_data(subset16S)$Farm2)
summary(aov.fisher.farm)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## sample_data(subset16S)$Farm2  3  294.8   98.27    9.7 9.2e-06 ***
## Residuals              116 1175.2   10.13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov.fisher.farm) # only not significant between 1 and 4 and 3 and 2
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lib.div$diversity_fisher ~ sample_data(subset16S)$Farm2)
##
## $'sample_data(subset16S)$Farm2'
##      diff      lwr      upr      p adj
## Farm2-Farm1 2.4636185 0.3893902 4.537847 0.0129624
## Farm3-Farm1 3.0034387 0.6083262 5.398551 0.0076605
## Farm4-Farm1 -0.9432080 -3.3383205 1.451905 0.7342758
## Farm3-Farm2 0.5398202 -1.5344081 2.614048 0.9051069
## Farm4-Farm2 -3.4068265 -5.4810547 -1.332598 0.0002229
## Farm4-Farm3 -3.9466467 -6.3417592 -1.551534 0.0002113
```

```
# Non-normally distributed
```

```
kruskal.test(lib.div$diversity_shannon ~ sample_data(subset16S)$Farm2) # shannon diversity seems to sig
```

```
##
## Kruskal-Wallis rank sum test
##
## data: lib.div$diversity_shannon by sample_data(subset16S)$Farm2
## Kruskal-Wallis chi-squared = 21.53, df = 3, p-value = 8.171e-05
```

```
pairwise.wilcox.test(lib.div$diversity_shannon, sample_data(subset16S)$Farm2, p.adjust.method="fdr") #
```

```

##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$diversity_shannon and sample_data(subset16S)$Farm2
##
##      Farm1  Farm2  Farm3
## Farm2 0.14005 -      -
## Farm3 0.03874 0.57297 -
## Farm4 0.04790 0.00047 2e-05
##
## P value adjustment method: fdr

kruskal.test(lib.div$diversity_gini_simpson ~ sample_data(subset16S)$Farm2) # significant

##
## Kruskal-Wallis rank sum test
##
## data: lib.div$diversity_gini_simpson by sample_data(subset16S)$Farm2
## Kruskal-Wallis chi-squared = 17.336, df = 3, p-value = 0.0006028

pairwise.wilcox.test(lib.div$diversity_gini_simpson, sample_data(subset16S)$Farm2, p.adjust.method="fdr")

##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$diversity_gini_simpson and sample_data(subset16S)$Farm2
##
##      Farm1  Farm2  Farm3
## Farm2 0.15047 -      -
## Farm3 0.09692 0.78047 -
## Farm4 0.08732 0.00256 0.00015
##
## P value adjustment method: fdr

kruskal.test(lib.div$diversity_inverse_simpson ~ sample_data(subset16S)$Farm2) # not significant

##
## Kruskal-Wallis rank sum test
##
## data: lib.div$diversity_inverse_simpson by sample_data(subset16S)$Farm2
## Kruskal-Wallis chi-squared = 17.336, df = 3, p-value = 0.0006028

pairwise.wilcox.test(lib.div$diversity_inverse_simpson, sample_data(subset16S)$Farm2, p.adjust.method="fdr")

##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$diversity_inverse_simpson and sample_data(subset16S)$Farm2
##
##      Farm1  Farm2  Farm3
## Farm2 0.15047 -      -

```

```

## Farm3 0.09692 0.78047 -
## Farm4 0.08732 0.00256 0.00015
##
## P value adjustment method: fdr

kruskal.test(lib.div$evenness_pielou ~ sample_data(subset16S)$Farm2) # significant

##
## Kruskal-Wallis rank sum test
##
## data: lib.div$evenness_pielou by sample_data(subset16S)$Farm2
## Kruskal-Wallis chi-squared = 14.059, df = 3, p-value = 0.002826

pairwise.wilcox.test(lib.div$evenness_pielou, sample_data(subset16S)$Farm2, p.adjust.method="fdr") # di

##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$evenness_pielou and sample_data(subset16S)$Farm2
##
##      Farm1  Farm2  Farm3
## Farm2 0.8283 -      -
## Farm3 0.8283 0.9197 -
## Farm4 0.0103 0.0028 0.0028
##
## P value adjustment method: fdr

# agent also has more than 2 levels, so we will use ANOVAs for normally distributed metrics

aov.observed.agent = aov(lib.div$observed ~ sample_data(subset16S)$Cox)
summary(aov.observed.agent)

##              Df Sum Sq Mean Sq F value    Pr(>F)
## sample_data(subset16S)$Cox    3   24998      8333   14.71 3.52e-08 ***
## Residuals              116   65714       566
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

TukeyHSD(aov.observed.agent) # only not significant between sacox and monteban

## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lib.div$observed ~ sample_data(subset16S)$Cox)
##
## $'sample_data(subset16S)$Cox'
##              diff              lwr              upr              p adj
## Monteban-Maxiban -18.34043 -37.782360    1.101509 0.0719535
## None-Maxiban      14.30541    1.573992   27.036824 0.0210505
## Sacox-Maxiban     -30.09043 -50.156892  -10.023959 0.0008908
## None-Monteban      32.64583   13.247828   52.043839 0.0001481
## Sacox-Monteban    -11.75000  -36.586601   13.086601 0.6071257
## Sacox-None        -44.39583  -64.419741  -24.371926 0.0000004

```

```
aov.fisher.agent = aov(lib.div$diversity_fisher ~ sample_data(subset16S)$Cox)
summary(aov.fisher.agent)
```

```
##              Df Sum Sq Mean Sq F value    Pr(>F)
## sample_data(subset16S)$Cox    3  443.3   147.75    16.69 4.42e-09 ***
## Residuals              116 1026.8     8.85
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov.fisher.agent) # only not significant maxiban & monteban and sacox & monteban
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lib.div$diversity_fisher ~ sample_data(subset16S)$Cox)
##
## $'sample_data(subset16S)$Cox'
##              diff              lwr              upr              p adj
## Monteban-Maxiban -1.947259 -4.3775075  0.4829893  0.1629193
## None-Maxiban      2.025225  0.4337933  3.6166559  0.0065804
## Sacox-Maxiban     -4.096756 -6.6050714 -1.5884411  0.0002443
## None-Monteban      3.972484  1.5477264  6.3972410  0.0002324
## Sacox-Monteban    -2.149497 -5.2540807  0.9550864  0.2763516
## Sacox-None        -6.121981 -8.6249761 -3.6189855  0.0000000
```

```
# Non-normally distributed
```

```
kruskal.test(lib.div$diversity_shannon ~ sample_data(subset16S)$Cox) # shannon diversity seems to signi
```

```
##
## Kruskal-Wallis rank sum test
##
## data: lib.div$diversity_shannon by sample_data(subset16S)$Cox
## Kruskal-Wallis chi-squared = 20.995, df = 3, p-value = 0.0001056
```

```
pairwise.wilcox.test(lib.div$diversity_shannon, sample_data(subset16S)$Cox, p.adjust.method="fdr") # di
```

```
##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$diversity_shannon and sample_data(subset16S)$Cox
##
##           Maxiban Monteban None
## Monteban 0.31038 -          -
## None      0.09625 0.06237 -
## Sacox      0.00031 0.10411 8.1e-05
##
## P value adjustment method: fdr
```

```
kruskal.test(lib.div$diversity_gini_simpson ~ sample_data(subset16S)$Cox) # significant
```

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: lib.div$diversity_gini_simpson by sample_data(subset16S)$Cox  
## Kruskal-Wallis chi-squared = 15.296, df = 3, p-value = 0.00158
```

```
pairwise.wilcox.test(lib.div$diversity_gini_simpson, sample_data(subset16S)$Cox, p.adjust.method="fdr")
```

```
##  
## Pairwise comparisons using Wilcoxon rank sum exact test  
##  
## data: lib.div$diversity_gini_simpson and sample_data(subset16S)$Cox  
##  
##           Maxiban Monteban None  
## Monteban 0.4557  -          -  
## None      0.0906  0.1557  -  
## Sacox     0.0040  0.2020  0.0035  
##  
## P value adjustment method: fdr
```

```
kruskal.test(lib.div$diversity_inverse_simpson ~ sample_data(subset16S)$Cox) # not significant
```

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: lib.div$diversity_inverse_simpson by sample_data(subset16S)$Cox  
## Kruskal-Wallis chi-squared = 15.296, df = 3, p-value = 0.00158
```

```
pairwise.wilcox.test(lib.div$diversity_inverse_simpson, sample_data(subset16S)$Cox, p.adjust.method="fdr")
```

```
##  
## Pairwise comparisons using Wilcoxon rank sum exact test  
##  
## data: lib.div$diversity_inverse_simpson and sample_data(subset16S)$Cox  
##  
##           Maxiban Monteban None  
## Monteban 0.4557  -          -  
## None      0.0906  0.1557  -  
## Sacox     0.0040  0.2020  0.0035  
##  
## P value adjustment method: fdr
```

```
kruskal.test(lib.div$evenness_pielou ~ sample_data(subset16S)$Cox) # significant
```

```
##  
## Kruskal-Wallis rank sum test  
##  
## data: lib.div$evenness_pielou by sample_data(subset16S)$Cox  
## Kruskal-Wallis chi-squared = 10.984, df = 3, p-value = 0.01181
```



```
pairwise.wilcox.test(lib.div$evenness_pielou, sample_data(subset16S)$Cox, p.adjust.method="fdr") # same
```

```
##
## Pairwise comparisons using Wilcoxon rank sum exact test
##
## data: lib.div$evenness_pielou and sample_data(subset16S)$Cox
##
##           Maxiban Monteban None
## Monteban 0.750    -          -
## None      0.377    0.454    -
## Sacox     0.010    0.051    0.010
##
## P value adjustment method: fdr
```

Beta diversity

```
estimate_richness(subset16S) # no singletons
```

	Observed	Chao1	se.chao1	ACE	se.ACE	Shannon	Simpson	InvSimpson
## X2_23	132	132	0	NaN	NaN	4.136003	0.9711196	34.625599
## X2_24	139	139	0	NaN	NaN	4.126936	0.9642492	27.971432
## X2_25	97	97	0	NaN	NaN	3.465461	0.9290117	14.086821
## X2_26	130	130	0	NaN	NaN	3.929347	0.9460238	18.526673
## X2_27	136	136	0	NaN	NaN	4.239237	0.9739942	38.452916
## X2_29	145	145	0	NaN	NaN	4.385673	0.9745243	39.253112
## X2_36	123	123	0	NaN	NaN	4.098432	0.9675785	30.843755
## X2_39	123	123	0	NaN	NaN	3.982521	0.9565922	23.037346
## X2_40	146	146	0	NaN	NaN	4.252383	0.9690197	32.278559
## X2_41	102	102	0	NaN	NaN	4.008183	0.9674078	30.682184
## X2_42	113	113	0	NaN	NaN	4.029328	0.9636649	27.521614
## X2_47	141	141	0	NaN	NaN	4.257202	0.9688112	32.062832
## X2_48	74	74	0	NaN	NaN	3.380875	0.9371476	15.910281
## X2_49	110	110	0	NaN	NaN	3.970268	0.9660476	29.453042
## X2_50	137	137	0	NaN	NaN	4.357221	0.9799604	49.901207
## X2_51	122	122	0	NaN	NaN	4.253943	0.9781493	45.765045
## X2_52	142	142	0	NaN	NaN	4.303497	0.9751186	40.190629
## X2_56	126	126	0	NaN	NaN	4.097594	0.9699867	33.318612
## X2_57	48	48	0	NaN	NaN	1.734529	0.5455967	2.200688
## X2_58	108	108	0	NaN	NaN	3.772379	0.9455148	18.353603
## X2_59	107	107	0	NaN	NaN	3.858977	0.9593703	24.612528
## X2_60	139	139	0	NaN	NaN	3.978746	0.9497115	19.885269
## X2_61	140	140	0	NaN	NaN	4.025627	0.9395879	16.552984
## X4_36	119	119	0	NaN	NaN	3.836459	0.9503957	20.159535
## X4_37	91	91	0	NaN	NaN	3.584282	0.9461311	18.563597
## X4_38	146	146	0	NaN	NaN	4.252073	0.9694887	32.774785
## X4_39	106	106	0	NaN	NaN	3.720320	0.9372538	15.937227
## X4_40	154	154	0	NaN	NaN	4.381358	0.9766794	42.880526
## X4_41	112	112	0	NaN	NaN	3.924462	0.9658913	29.318027
## X4_54	170	170	0	NaN	NaN	4.402081	0.9719656	35.670497
## X4_55	121	121	0	NaN	NaN	3.543604	0.9166889	12.003196
## X4_56	165	165	0	NaN	NaN	4.376416	0.9745189	39.244723

## X4_57	160	160	0	NaN	NaN	4.395525	0.9781892	45.848768
## X4_65	62	62	0	NaN	NaN	3.350815	0.9340956	15.173499
## X5_39	76	76	0	NaN	NaN	3.432905	0.9428191	17.488352
## X5_40	162	162	0	NaN	NaN	4.426712	0.9697948	33.106899
## X5_41	147	147	0	NaN	NaN	3.792368	0.8991109	9.911873
## X5_54	165	165	0	NaN	NaN	4.516410	0.9825044	57.157274
## X5_55	123	123	0	NaN	NaN	3.819140	0.9446352	18.062019
## X5_59	157	157	0	NaN	NaN	4.417525	0.9782484	45.973571
## X6_36	127	127	0	NaN	NaN	4.147429	0.9719594	35.662588
## X6_37	115	115	0	NaN	NaN	3.882798	0.9531995	21.367304
## X6_38	134	134	0	NaN	NaN	4.127256	0.9682224	31.468693
## X6_54	135	135	0	NaN	NaN	4.178638	0.9719210	35.613788
## X6_55	148	148	0	NaN	NaN	4.286540	0.9744719	39.172460
## X6_56	136	136	0	NaN	NaN	4.177493	0.9711918	34.712361
## X6_57	119	119	0	NaN	NaN	3.823249	0.9450037	18.183044
## X6_58	117	117	0	NaN	NaN	3.937373	0.9645764	28.229778
## X9_16	125	125	0	NaN	NaN	3.961053	0.9558198	22.634587
## X9_17	121	121	0	NaN	NaN	4.115315	0.9737945	38.159910
## X9_18	134	134	0	NaN	NaN	4.005118	0.9571588	23.342017
## X9_19	87	87	0	NaN	NaN	3.208366	0.9014346	10.145551
## X9_21	143	143	0	NaN	NaN	4.158081	0.9629915	27.020836
## X9_22	117	117	0	NaN	NaN	4.149825	0.9745724	39.327320
## X9_34	122	122	0	NaN	NaN	3.910564	0.9502966	20.119340
## X9_35	108	108	0	NaN	NaN	3.487252	0.9107067	11.199048
## X9_36	117	117	0	NaN	NaN	3.935009	0.9537531	21.623091
## X9_37	118	118	0	NaN	NaN	3.995557	0.9642583	27.978554
## X9_38	102	102	0	NaN	NaN	3.766274	0.9546683	22.059594
## X9_39	156	156	0	NaN	NaN	4.409694	0.9771994	43.858458
## X10_1	147	147	0	NaN	NaN	4.022611	0.9520885	20.871823
## X10_2	171	171	0	NaN	NaN	4.310664	0.9698373	33.153575
## X10_3	143	143	0	NaN	NaN	4.227802	0.9726454	36.556920
## X10_4	152	152	0	NaN	NaN	4.310282	0.9732866	37.434357
## X10_7	137	137	0	NaN	NaN	4.009593	0.9630614	27.071976
## X10_8	165	165	0	NaN	NaN	4.497594	0.9797021	49.266232
## X10_10	159	159	0	NaN	NaN	4.300777	0.9715780	35.183966
## X10_11	150	150	0	NaN	NaN	4.490556	0.9833046	59.896830
## X10_12	142	142	0	NaN	NaN	4.214847	0.9713536	34.908339
## X10_13	156	156	0	NaN	NaN	4.361647	0.9774440	44.334088
## X10_14	139	139	0	NaN	NaN	3.957786	0.9540649	21.769848
## X10_15	140	140	0	NaN	NaN	4.059547	0.9601771	25.111174
## X10_19	84	84	0	NaN	NaN	3.525545	0.9423290	17.339745
## X10_20	87	87	0	NaN	NaN	3.530217	0.9429301	17.522359
## X10_21	127	127	0	NaN	NaN	4.276047	0.9770737	43.618080
## X10_22	86	86	0	NaN	NaN	3.518669	0.9428566	17.499831
## X10_25	83	83	0	NaN	NaN	3.562820	0.9439357	17.836654
## X10_26	100	100	0	NaN	NaN	3.732990	0.9532478	21.389377
## X10_28	88	88	0	NaN	NaN	3.754545	0.9614331	25.928982
## X10_29	114	114	0	NaN	NaN	3.393108	0.8816650	8.450584
## X10_30	109	109	0	NaN	NaN	3.568981	0.9265013	13.605679
## X10_33	128	128	0	NaN	NaN	3.883777	0.9483538	19.362526
## X10_34	98	98	0	NaN	NaN	3.440144	0.9207547	12.619050
## X10_35	99	99	0	NaN	NaN	3.598482	0.9442832	17.947893
## X10_39	125	125	0	NaN	NaN	3.747857	0.9430010	17.544153
## X10_40	112	112	0	NaN	NaN	4.006892	0.9627609	26.853519

##	X10_41	140	140	0	NaN	NaN	4.269641	0.9747131	39.546109
##	X10_42	143	143	0	NaN	NaN	4.092277	0.9638905	27.693543
##	X10_43	178	178	0	NaN	NaN	4.537584	0.9773062	44.064913
##	X10_44	137	137	0	NaN	NaN	4.115558	0.9665253	29.873330
##	X10_48	160	160	0	NaN	NaN	4.199373	0.9631082	27.106304
##	X10_49	142	142	0	NaN	NaN	4.140257	0.9689761	32.233257
##	X10_50	128	128	0	NaN	NaN	3.978572	0.9629375	26.981421
##	X10_51	152	152	0	NaN	NaN	4.335136	0.9752193	40.354031
##	X10_52	136	136	0	NaN	NaN	4.259292	0.9761764	41.975197
##	X10_53	142	142	0	NaN	NaN	4.144321	0.9707317	34.166653
##	X10_57	140	140	0	NaN	NaN	4.166506	0.9690959	32.358201
##	X10_58	121	121	0	NaN	NaN	3.558282	0.9245442	13.252790
##	X10_59	148	148	0	NaN	NaN	4.294089	0.9724177	36.255089
##	X10_60	95	95	0	NaN	NaN	3.862023	0.9660824	29.483175
##	X10_63	143	143	0	NaN	NaN	4.108655	0.9602230	25.140134
##	X10_64	133	133	0	NaN	NaN	3.845720	0.9506031	20.244171
##	X10_66	137	137	0	NaN	NaN	4.066091	0.9600511	25.031975
##	X10_67	118	118	0	NaN	NaN	3.734614	0.9327431	14.868368
##	X10_68	114	114	0	NaN	NaN	4.011428	0.9684984	31.744427
##	X10_69	115	115	0	NaN	NaN	3.449319	0.9095590	11.056929
##	X11_1	136	136	0	NaN	NaN	4.128541	0.9690378	32.297435
##	X11_3	122	122	0	NaN	NaN	3.672527	0.9338568	15.118723
##	X14_20	177	177	0	NaN	NaN	4.409254	0.9753444	40.558697
##	X14_21	142	142	0	NaN	NaN	4.353094	0.9770271	43.529539
##	X14_22	133	133	0	NaN	NaN	4.296036	0.9783014	46.086004
##	X14_23	199	199	0	NaN	NaN	4.783165	0.9863423	73.218561
##	X14_25	176	176	0	NaN	NaN	4.679120	0.9852256	67.684481
##	X14_27	223	223	0	NaN	NaN	4.944154	0.9889503	90.500150
##	X14_29	168	168	0	NaN	NaN	4.569564	0.9823959	56.804951
##	X14_30	188	188	0	NaN	NaN	4.491616	0.9753695	40.600036
##	X14_33	162	162	0	NaN	NaN	4.406904	0.9770711	43.613089
##	X14_34	128	128	0	NaN	NaN	3.429396	0.8623682	7.265762
##	X14_35	171	171	0	NaN	NaN	4.380340	0.9754211	40.685323
##	X14_36	143	143	0	NaN	NaN	4.379227	0.9791060	47.860532
##	Fisher								
##	X2_23	14.163010							
##	X2_24	14.814971							
##	X2_25	9.896331							
##	X2_26	14.190333							
##	X2_27	15.623575							
##	X2_29	14.578601							
##	X2_36	12.941416							
##	X2_39	13.328383							
##	X2_40	17.140224							
##	X2_41	10.996498							
##	X2_42	12.311257							
##	X2_47	16.462924							
##	X2_48	7.588284							
##	X2_49	11.837343							
##	X2_50	15.179815							
##	X2_51	12.413289							
##	X2_52	15.242362							
##	X2_56	13.886854							
##	X2_57	4.609881							

```
## X2_58 11.654290
## X2_59 11.399075
## X2_60 15.023335
## X2_61 15.063981
## X4_36 12.305789
## X4_37 8.716916
## X4_38 15.586719
## X4_39 11.537281
## X4_40 19.831817
## X4_41 12.171287
## X4_54 18.370424
## X4_55 14.841290
## X4_56 17.931372
## X4_57 17.960087
## X4_65 5.925936
## X5_39 7.671534
## X5_40 20.407865
## X5_41 16.292875
## X5_54 18.013456
## X5_55 14.835637
## X5_59 19.333468
## X6_36 12.683630
## X6_37 11.331777
## X6_38 14.095720
## X6_54 14.306480
## X6_55 18.506106
## X6_56 14.640803
## X6_57 13.343276
## X6_58 11.875237
## X9_16 13.450478
## X9_17 12.585239
## X9_18 13.672532
## X9_19 8.444623
## X9_21 15.750868
## X9_22 12.545120
## X9_34 12.464780
## X9_35 11.005212
## X9_36 12.046206
## X9_37 12.196534
## X9_38 10.546501
## X9_39 18.188937
## X10_1 14.801263
## X10_2 17.913932
## X10_3 15.474146
## X10_4 15.608820
## X10_7 15.627062
## X10_8 17.314410
## X10_10 17.070298
## X10_11 16.383901
## X10_12 14.722089
## X10_13 17.686567
## X10_14 14.172646
## X10_15 14.270153
## X10_19 8.022038
```

```

## X10_20 8.517057
## X10_21 12.973489
## X10_22 8.296877
## X10_25 7.848329
## X10_26 10.320954
## X10_28 8.725458
## X10_29 10.934345
## X10_30 10.995780
## X10_33 12.728324
## X10_34 9.333958
## X10_35 9.774689
## X10_39 13.503588
## X10_40 12.889365
## X10_41 15.867978
## X10_42 15.715324
## X10_43 20.904381
## X10_44 15.222496
## X10_48 17.734041
## X10_49 15.451132
## X10_50 13.353367
## X10_51 16.494365
## X10_52 14.412306
## X10_53 15.760869
## X10_57 15.870862
## X10_58 12.927447
## X10_59 17.425828
## X10_60 10.411951
## X10_63 14.552490
## X10_64 14.521762
## X10_66 14.605380
## X10_67 13.300472
## X10_68 12.965425
## X10_69 13.186276
## X11_1 13.199825
## X11_3 12.195960
## X14_20 19.698534
## X14_21 14.910568
## X14_22 14.070685
## X14_23 23.233005
## X14_25 19.924708
## X14_27 26.083193
## X14_29 18.125905
## X14_30 21.059405
## X14_33 17.598106
## X14_34 12.695835
## X14_35 18.362334
## X14_36 15.547467

```

```

dist = "bray"
ord_meths = c("DCA", "CCA", "RDA", "NMDS", "MDS", "PCoA", "DPCoA")
plist = llply(as.list(ord_meths), function(i, physeq, dist){
  ordi = ordinate(subset16S, method=i, distance=dist)
  plot_ordination(subset16S, ordi, "samples", color="Age", shape = "AB")
}, subset16S, dist)

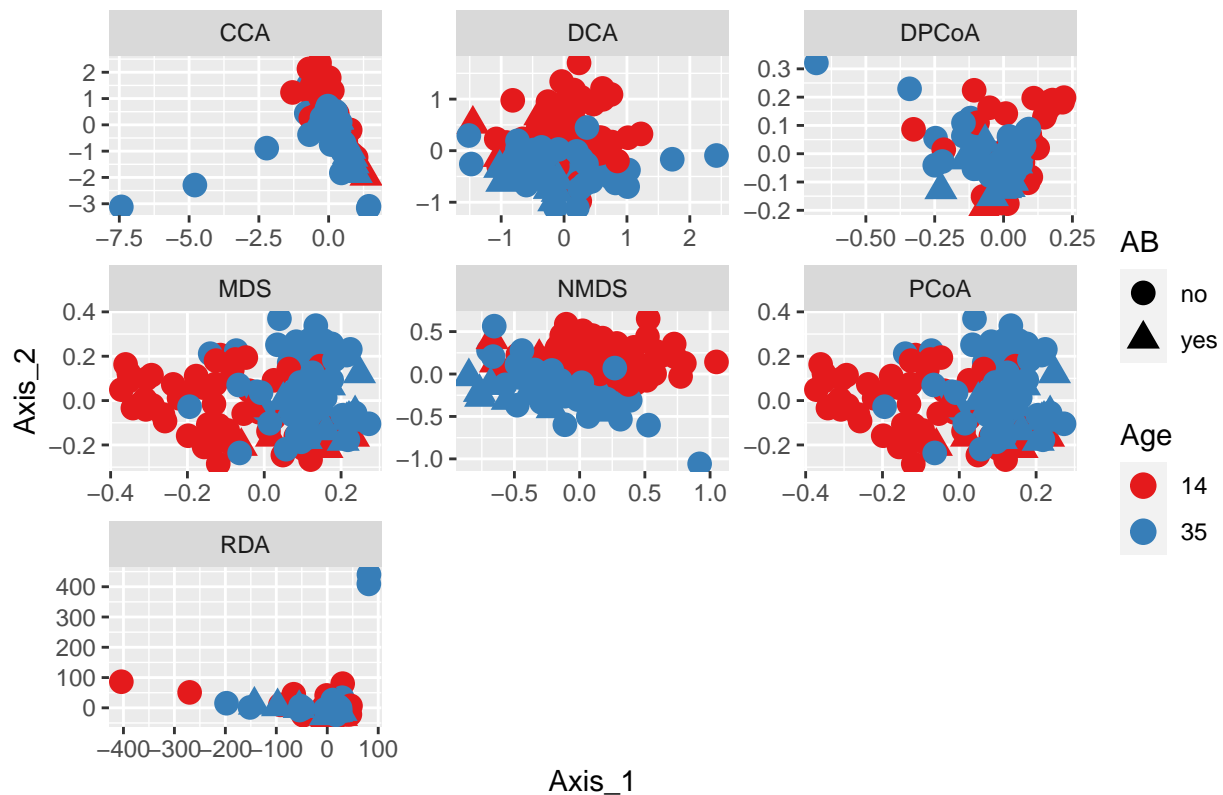
```

```
## Square root transformation
## Wisconsin double standardization
## Run 0 stress 0.2084961
## Run 1 stress 0.2317321
## Run 2 stress 0.2126819
## Run 3 stress 0.2337359
## Run 4 stress 0.2085024
## ... Procrustes: rmse 0.0007735775  max resid 0.00620484
## ... Similar to previous best
## Run 5 stress 0.211993
## Run 6 stress 0.2095192
## Run 7 stress 0.2208887
## Run 8 stress 0.2247976
## Run 9 stress 0.2159559
## Run 10 stress 0.2188857
## Run 11 stress 0.2295143
## Run 12 stress 0.2095333
## Run 13 stress 0.4136506
## Run 14 stress 0.2308722
## Run 15 stress 0.226104
## Run 16 stress 0.2219128
## Run 17 stress 0.2119357
## Run 18 stress 0.2252078
## Run 19 stress 0.2127853
## Run 20 stress 0.2095192
## *** Best solution repeated 1 times
```

```
names(plist) <- ord_meths

pdataframe = ldply(plist, function(x){
  df = x$data[, 1:2]
  colnames(df) = c("Axis_1", "Axis_2")
  return(cbind(df, x$data))
})
names(pdataframe)[1] = "method"
ggplot(pdataframe, aes(Axis_1, Axis_2, color=Age, shape=AB)) +
  geom_point(size=4) +
  facet_wrap(~method, scales="free") +
  scale_fill_brewer(type="qual", palette="Set1") +
  scale_colour_brewer(type="qual", palette="Set1") +
  ggtitle("Different ordination methods for 16S data (Bray-Curtis)")
```

Different ordination methods for 16S data (Bray–Curtis)



```
# PCoAs for different methods
```

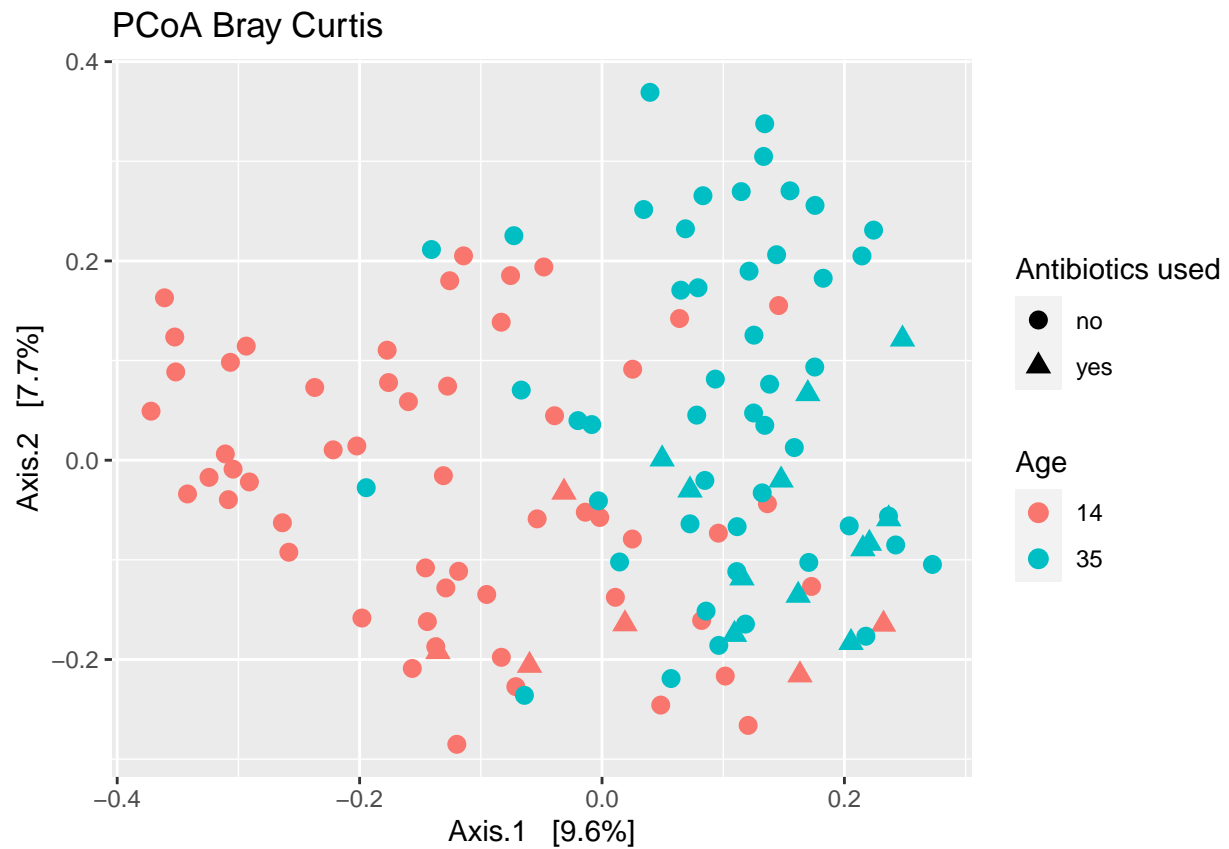
```
# functionize plotting pcoa
```

```
plot_pcoa_ordination <- function(data, pcoa, var, title) {
  p <- plot_ordination(data, pcoa, color = var, shape = "AB") +
    geom_point(size = 3) +
    labs(title = title, color = var, shape = "Antibiotics used")

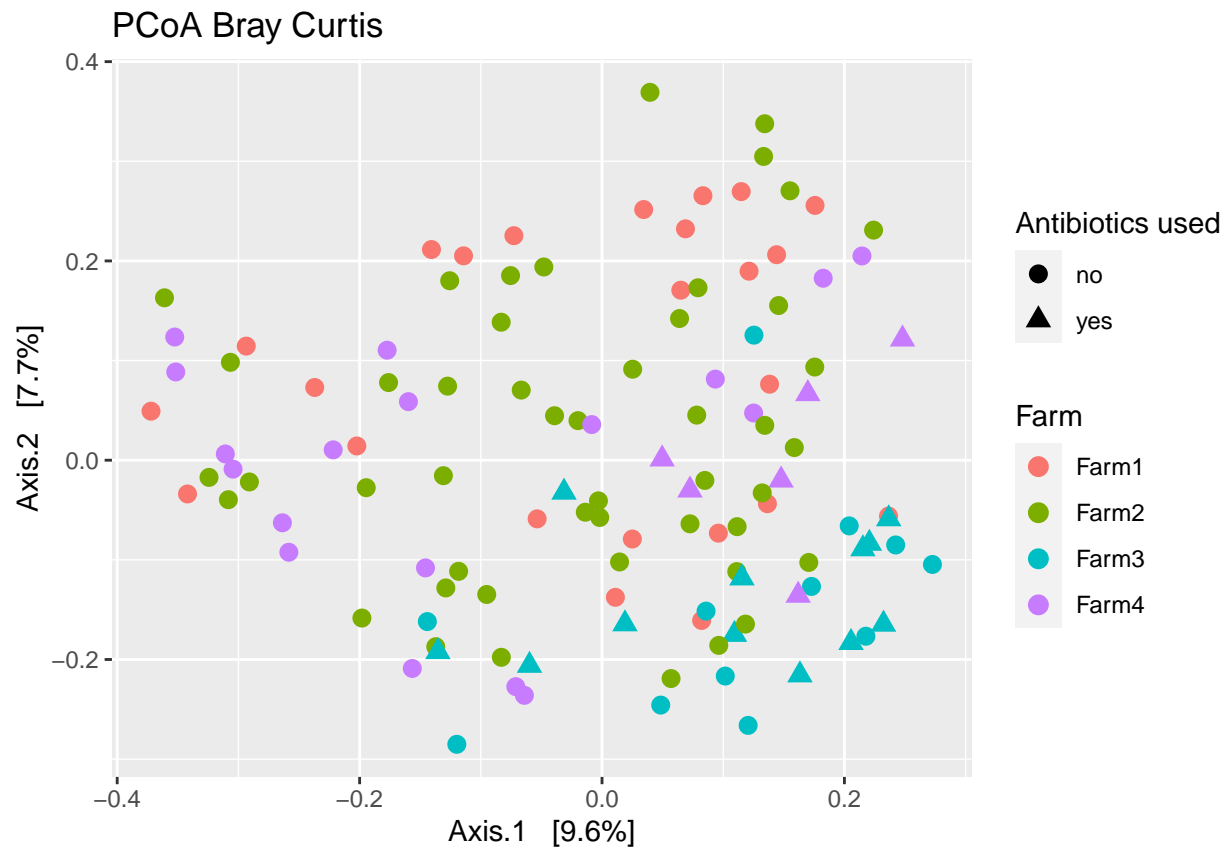
  return(p)
}
```

```
pcoa_bc = ordinate(subset16S, "PCoA", "bray")
pcoa_unifrac = ordinate(subset16S, "PCoA", "unifrac")
pcoa_wunifrac = ordinate(subset16S, "PCoA", "wunifrac")
pcoa_jsd = ordinate(subset16S, "PCoA", "jsd")
pcoa_jaccard = ordinate(subset16S, "PCoA", "jaccard", binary=TRUE)
```

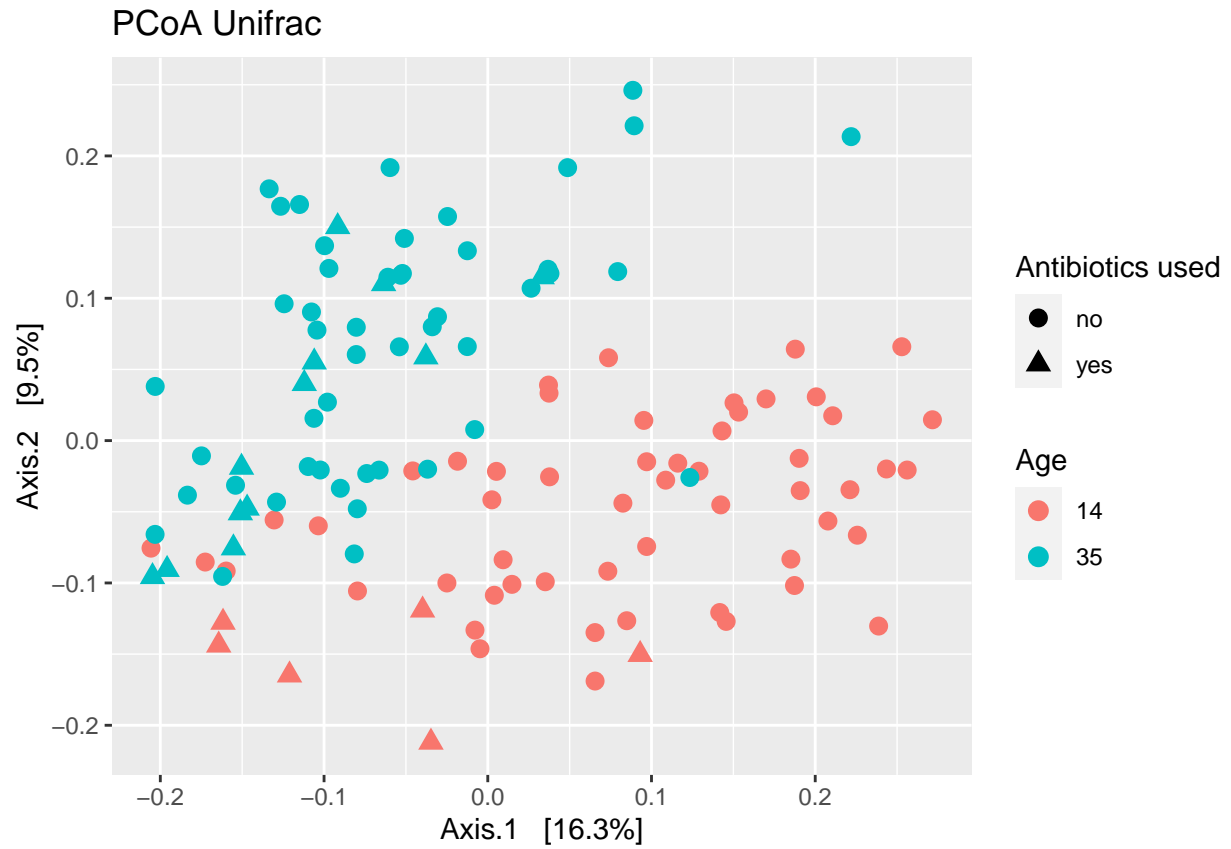
```
plot_pcoa_ordination(subset16S, pcoa_bc, "Age", "PCoA Bray Curtis")
```



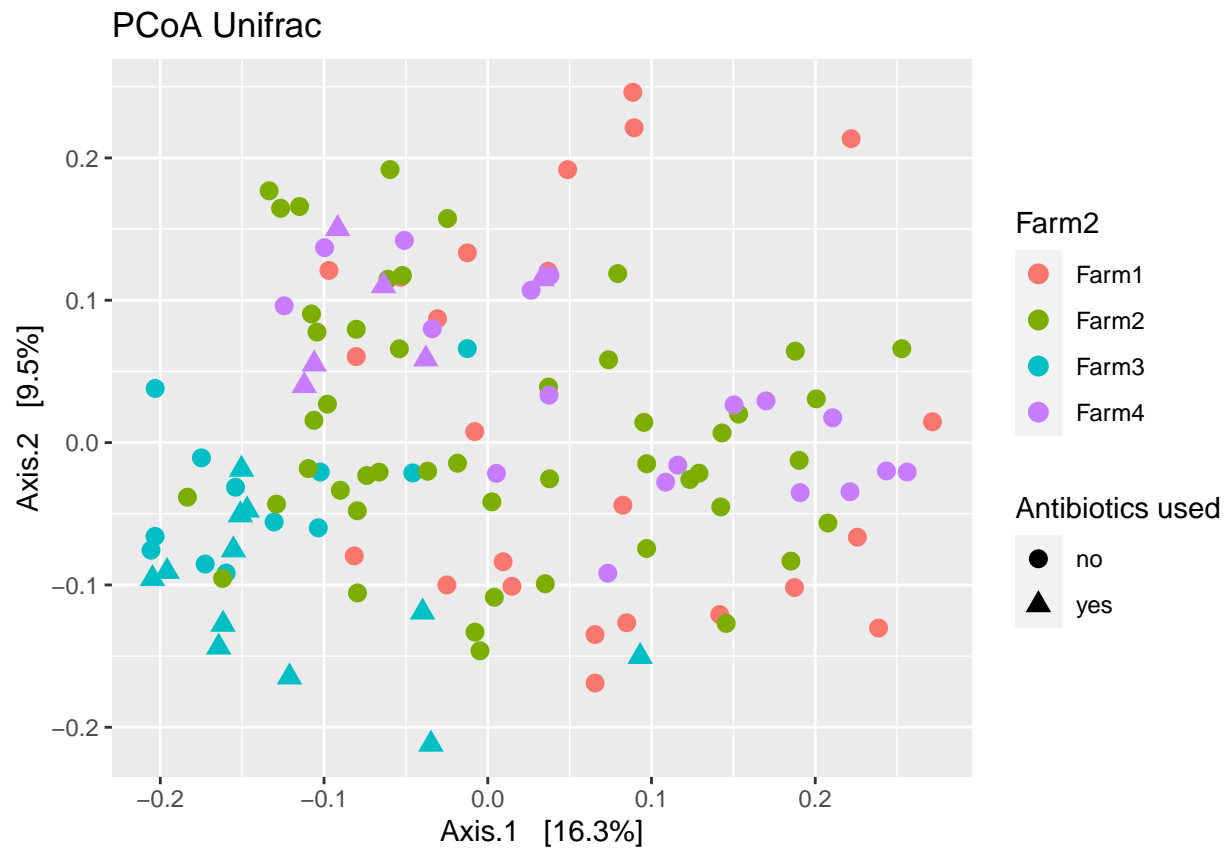
```
# proper order of legend:
plot_ordination(subset16S, pcoa_bc, color = "Farm2", shape = "AB") +
  geom_point(size = 3) +
  labs(title = "PCoA Bray Curtis", color = "Farm", shape = "Antibiotics used")
```

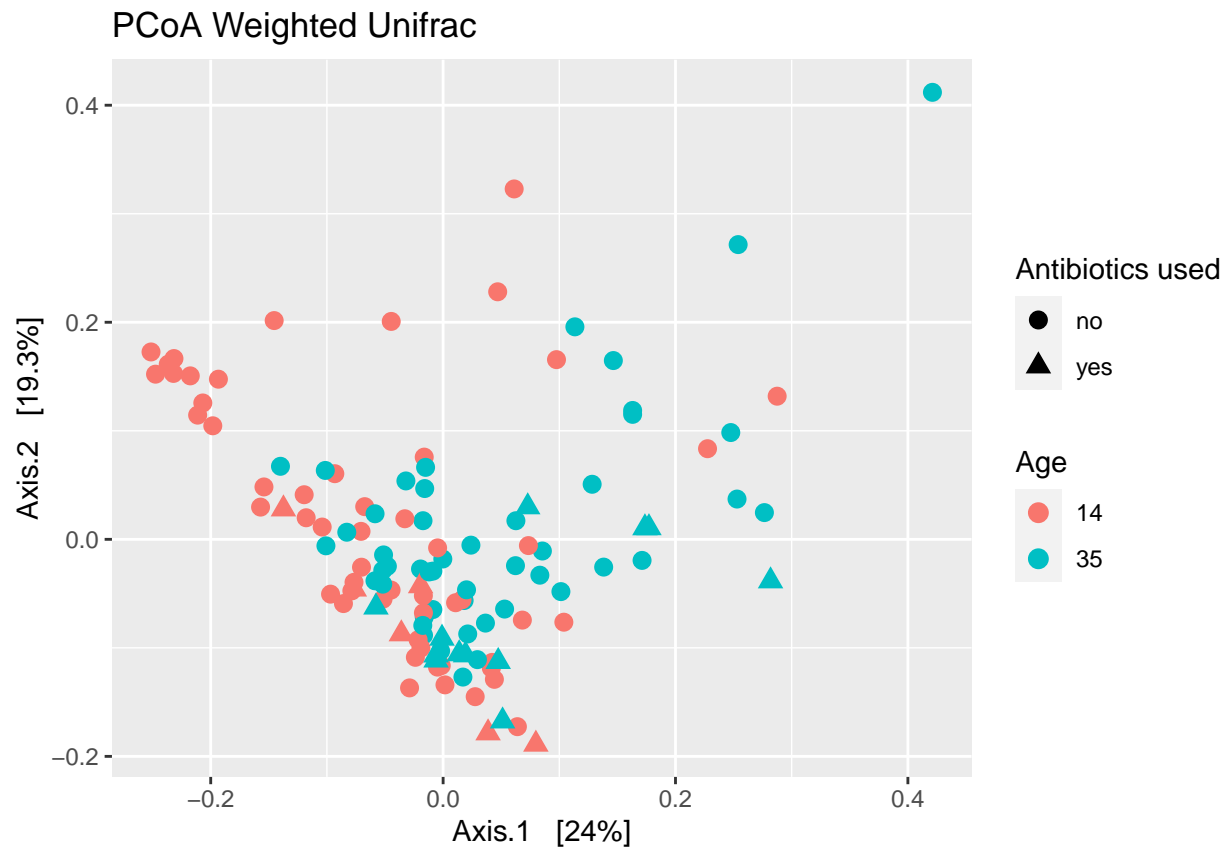
```
plot_pcoa_ordination(subset16S, pcoa_unifrac, "Age", "PCoA Unifrac")
```



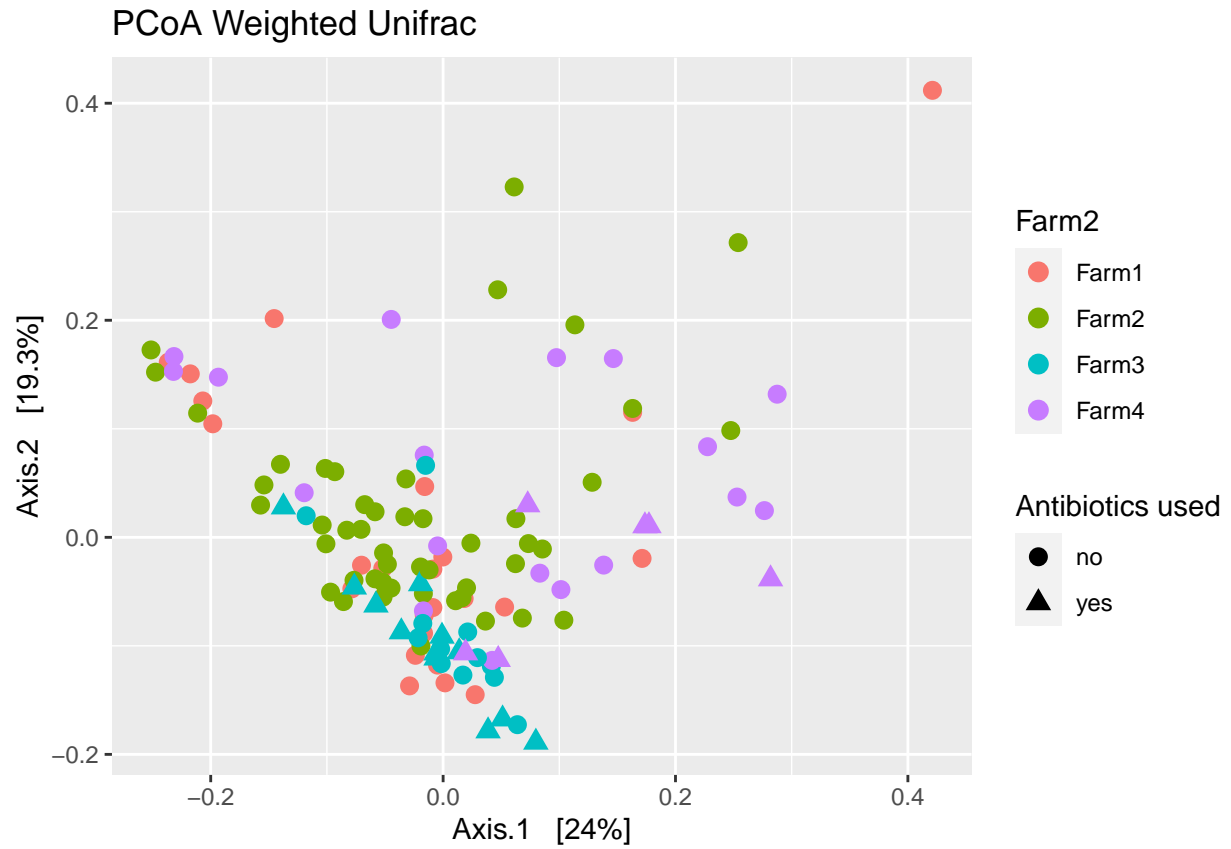
```
plot_pcoa_ordination(subset16S, pcoa_unifrac, "Farm2", "PCoA Unifrac")
```



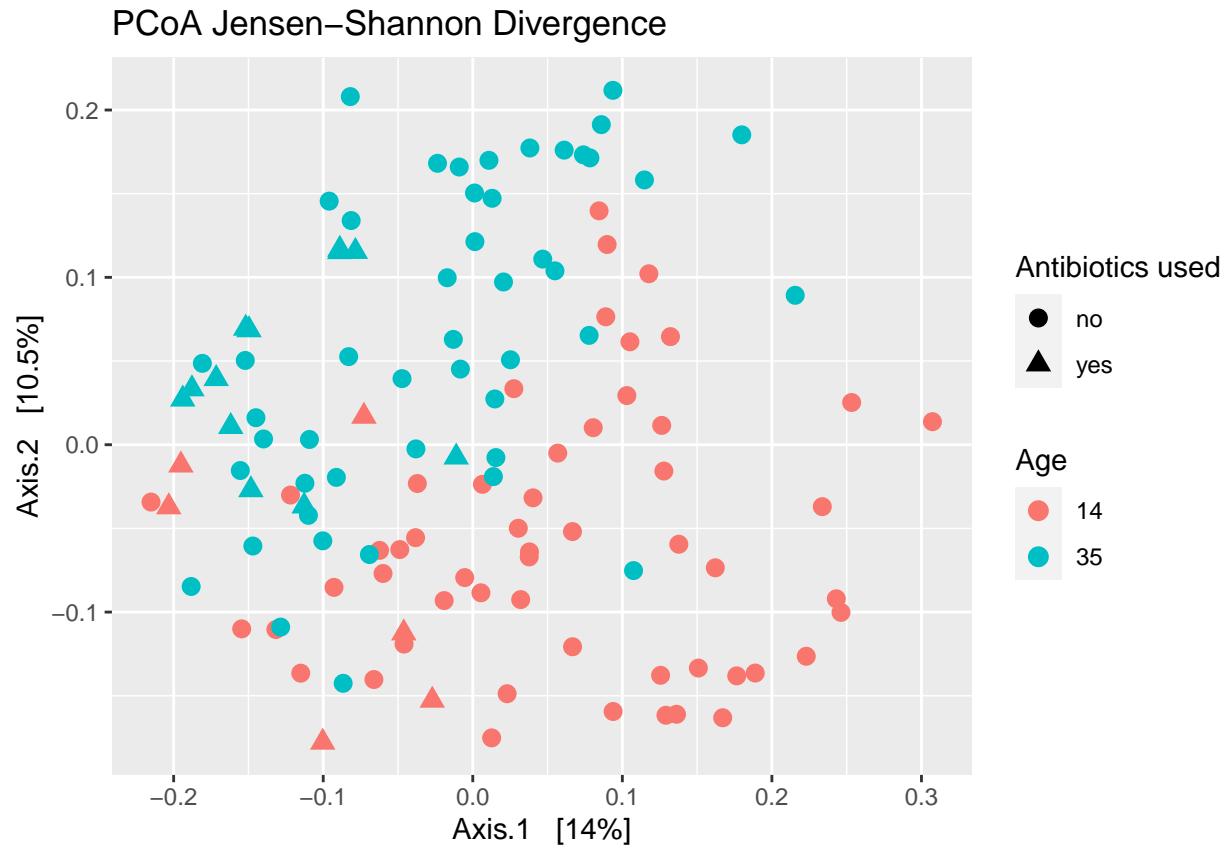
```
plot_pcoa_ordination(subset16S, pcoa_wunifrac, "Age", "PCoA Weighted Unifrac")
```



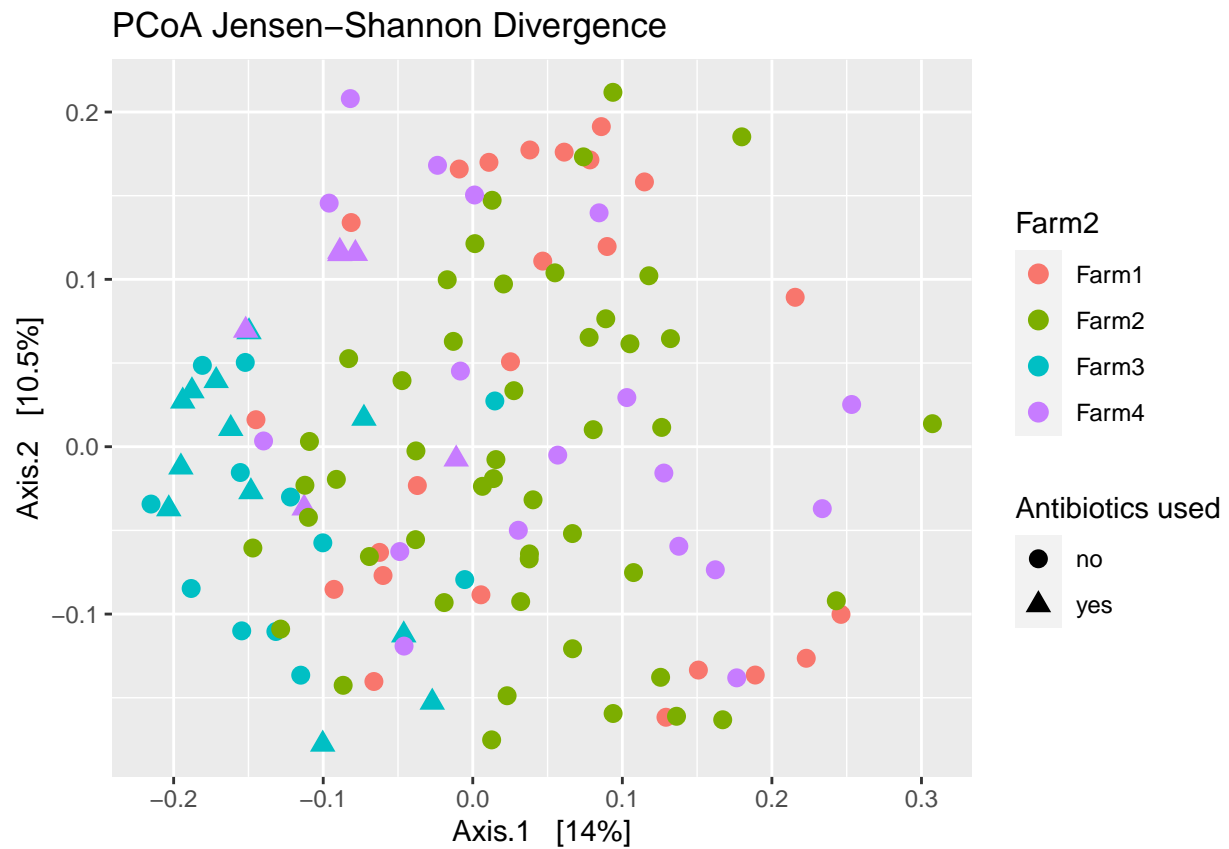
```
plot_pcoa_ordination(subset16S, pcoa_wunifrac, "Farm2", "PCoA Weighted Unifrac")
```



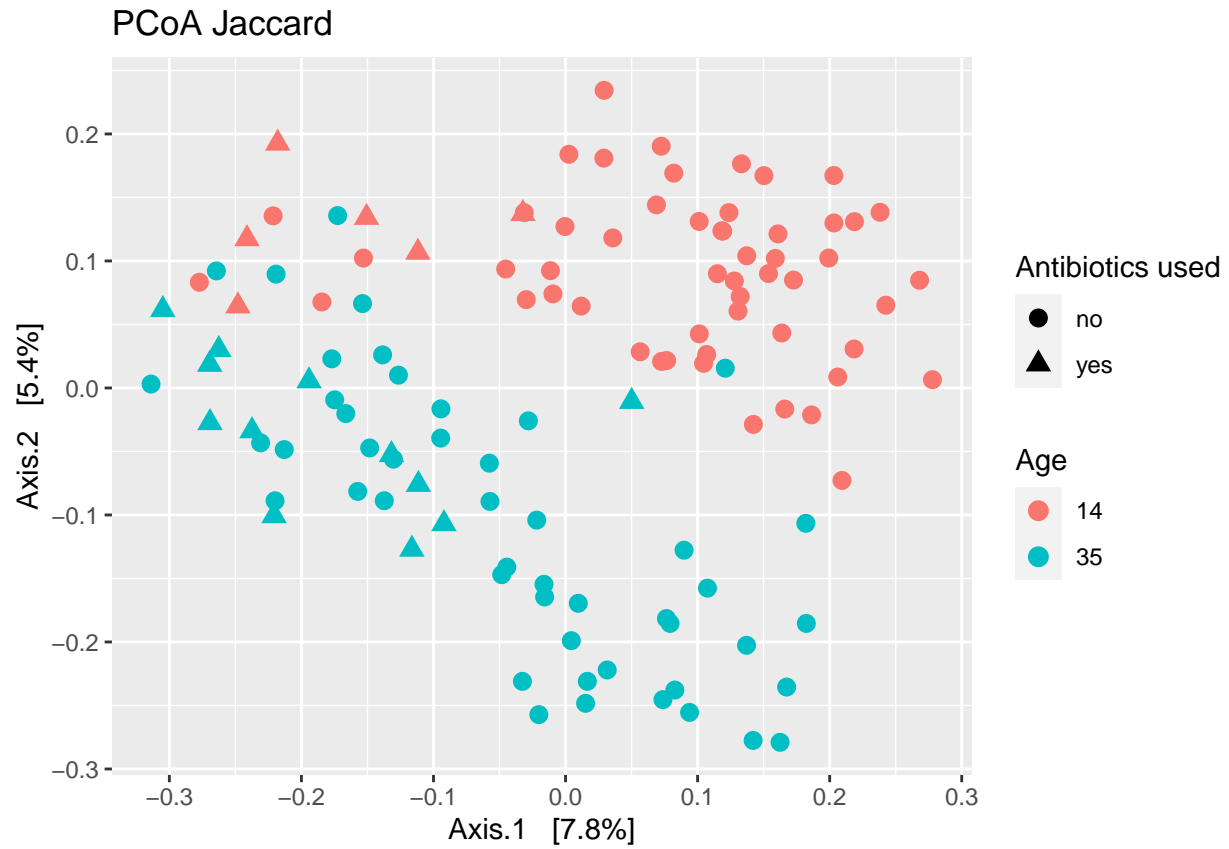
```
plot_pcoa_ordination(subset16S, pcoa_jsd, "Age", "PCoA Jensen-Shannon Divergence")
```



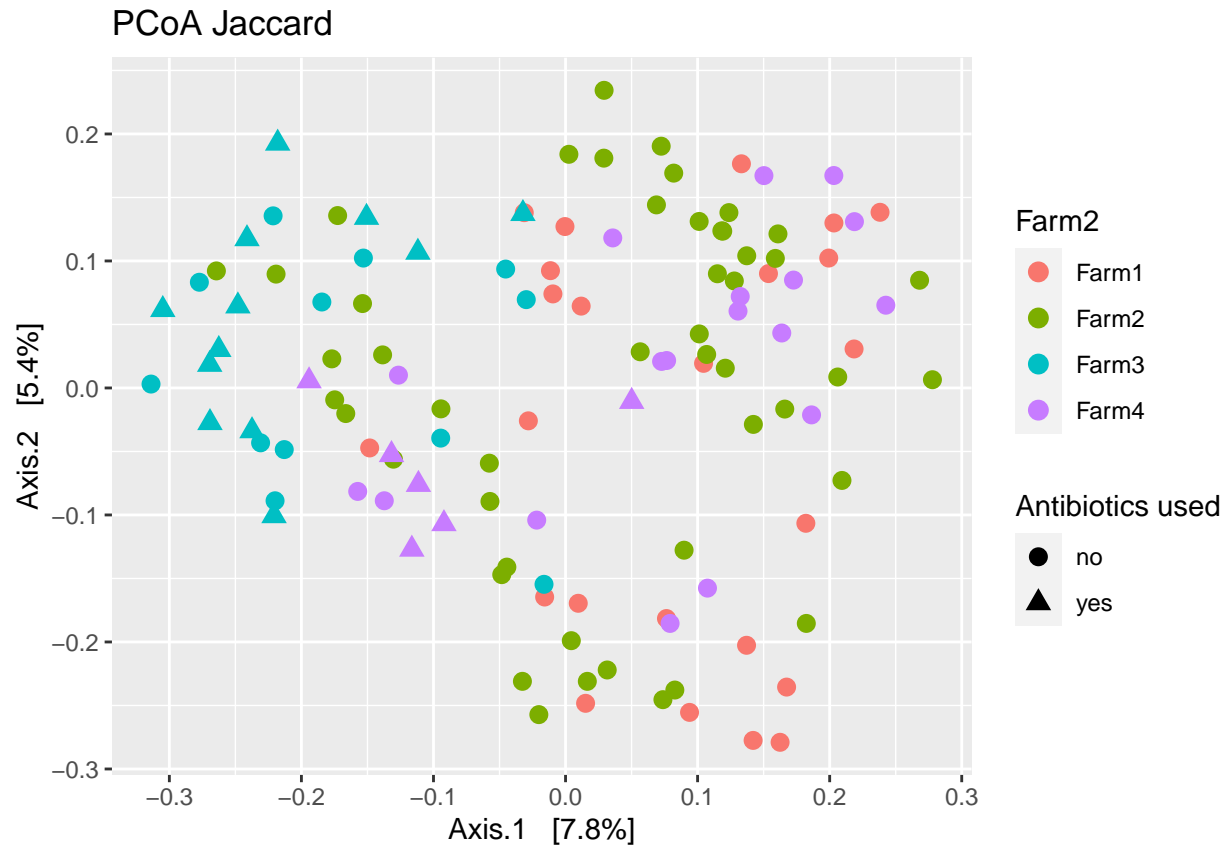
```
plot_pcoa_ordination(subset16S, pcoa_jsd, "Farm2", "PCoA Jensen-Shannon Divergence")
```



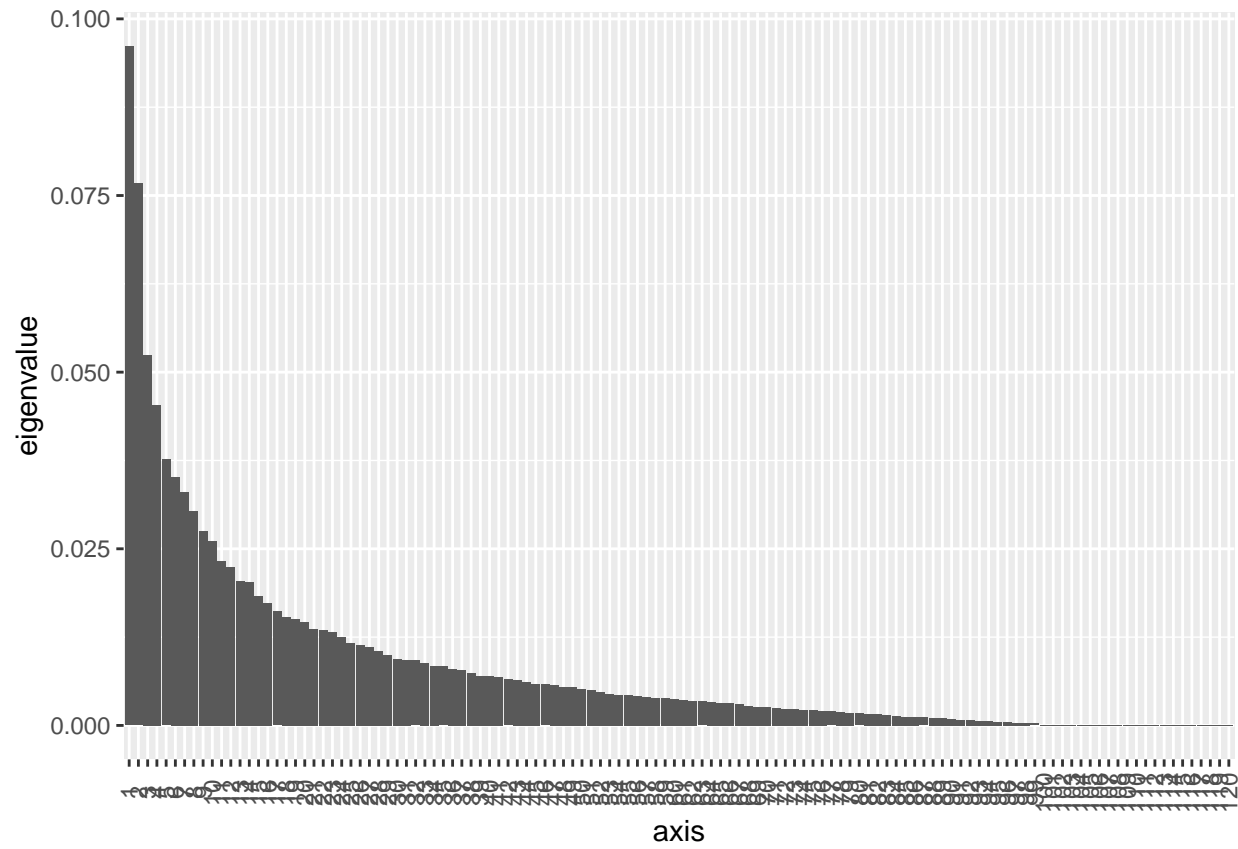
```
plot_pcoa_ordination(subset16S, pcoa_jaccard, "Age", "PCoA Jaccard")
```



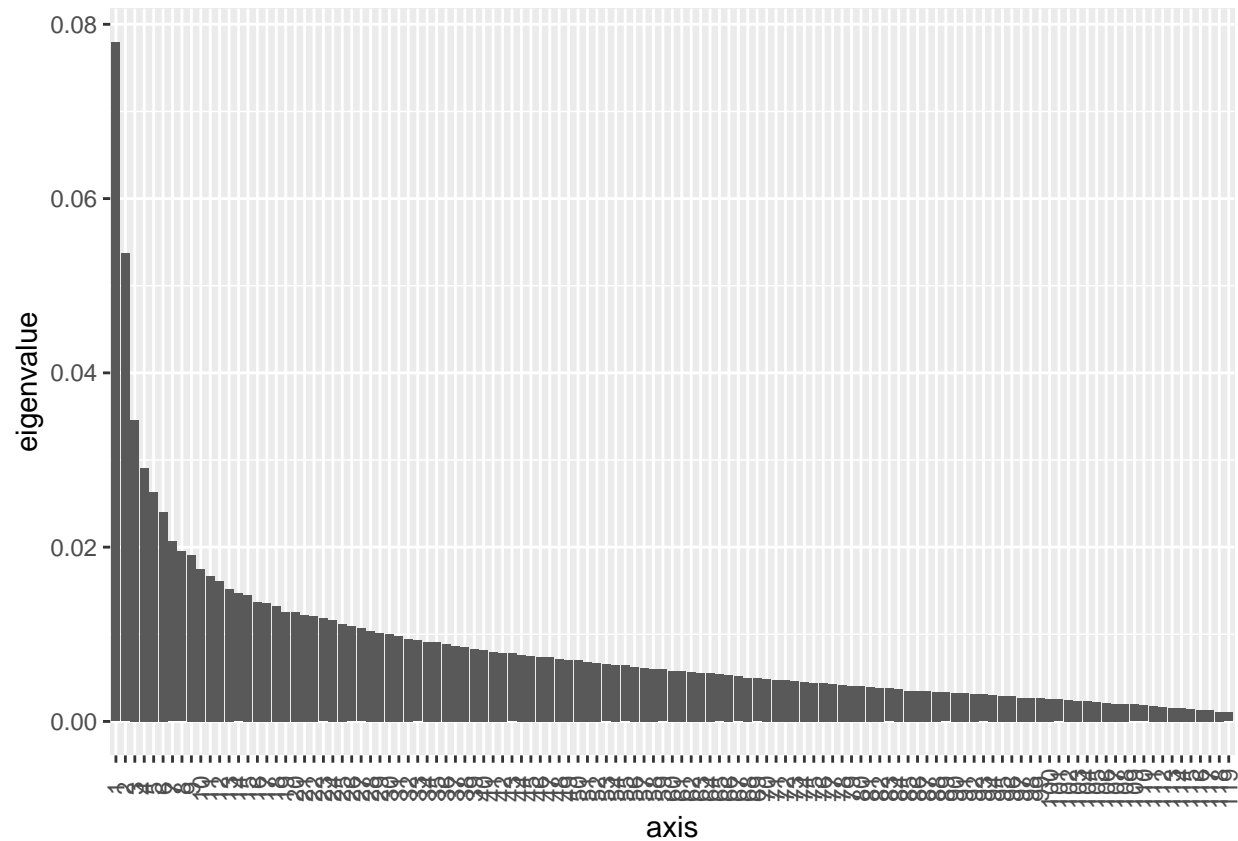
```
plot_pcoa_ordination(subset16S, pcoa_jaccard, "Farm2", "PCoA Jaccard")
```

```
#scree plots can be made for any of the PCoAs, those that explain less than 10% of variance on first a  
plot_scee(pcoa_bc)
```



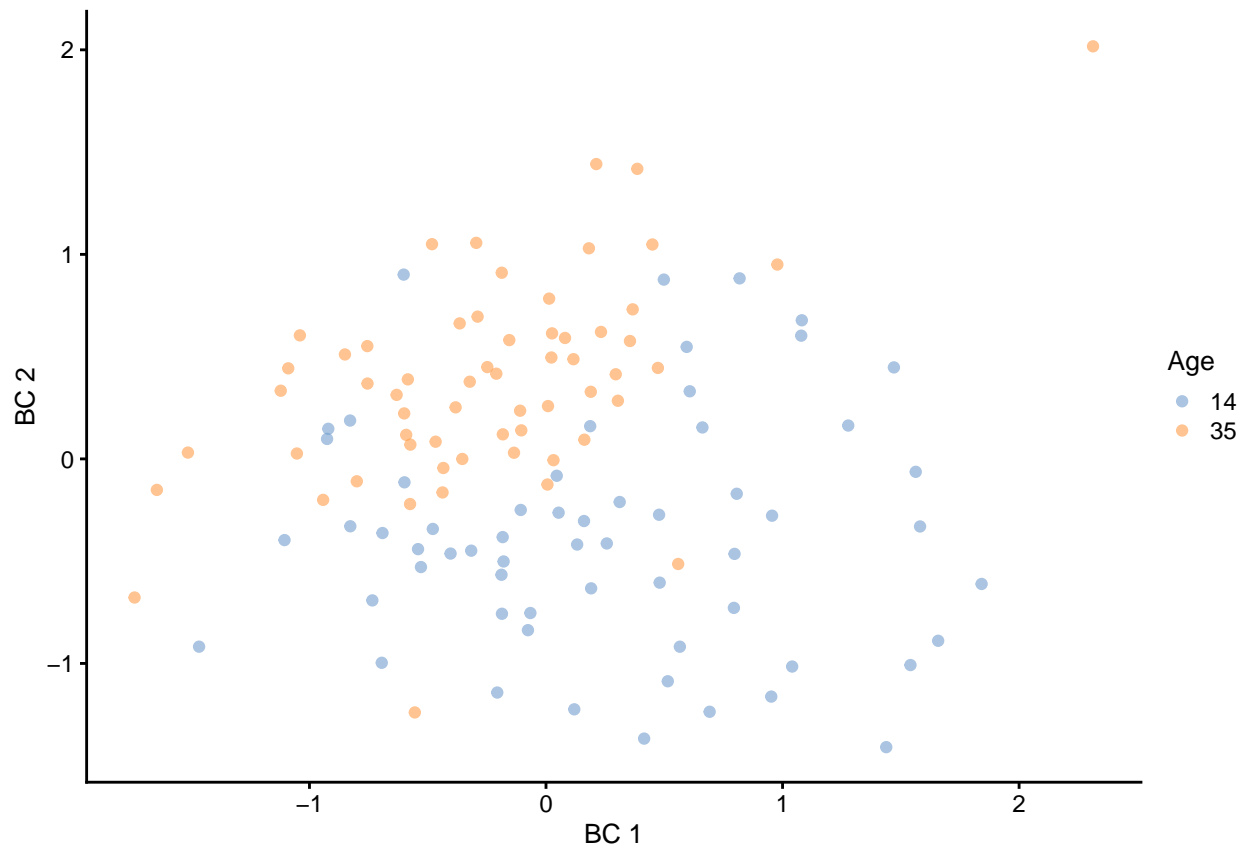
```
plot_scee(pcoa_jaccard)
```



```
# NMDS
```

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse %<>% transformCounts( method = "relabundance")
tse %<>% runNMDS(FUN = vegan::vegdist, name = "BC", nmfsFUN = "monoMDS",
                 exprs_values = "relabundance",
                 keep_dist = TRUE)

tse %>% plotReducedDim("BC", colour_by = "Age")
```



PERMANOVAs

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse <- transformCounts(tse, method = "relabundance")

adonis2(t(assay(tse, "relabundance")) ~ AB, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ AB, data = colData(tse), permutations = 9999)
##          Df SumOfSqs      R2      F Pr(>F)
## AB         1    0.867 0.02591 3.139 1e-04 ***
## Residual 118   32.594 0.97409
## Total     119   33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(t(assay(tse, "relabundance")) ~ Cox, data = colData(tse), permutations = 9999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
```

```

## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Cox, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Cox         3      3.424 0.10233 4.4078 1e-04 ***
## Residual    116     30.037 0.89767
## Total       119     33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ Researcher, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Researcher, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher   4      1.942 0.05803 1.7711 1e-04 ***
## Residual     115     31.520 0.94197
## Total        119     33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ FeedProducent, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ FeedProducent, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## FeedProducent  2      2.262 0.0676 4.241 1e-04 ***
## Residual      117     31.200 0.9324
## Total         119     33.462 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ LitterType, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ LitterType, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType    2      2.054 0.06137 3.825 1e-04 ***
## Residual      117     31.408 0.93863
## Total         119     33.462 1.00000

```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ FeedType, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ FeedType, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## FeedType   1      1.828 0.05464 6.8206 1e-04 ***
## Residual 118     31.633 0.94536
## Total     119     33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ Gender, data = colData(tse), permutations = 9999) # NOT signifi

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Gender, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1      0.247 0.00739 0.8784 0.6816
## Residual 118     33.214 0.99261
## Total     119     33.462 1.00000

adonis2(t(assay(tse, "relabundance")) ~ Stables, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Stables, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Stables     9      5.606 0.16753 2.4596 1e-04 ***
## Residual 110     27.856 0.83247
## Total     119     33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse, "relabundance")) ~ FlockSize, data = colData(tse), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
```

```
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ FlockSize, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## FlockSize   5    4.167 0.12453 3.2432 1e-04 ***
## Residual  114   29.295 0.87547
## Total     119   33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(t(assay(tse, "relabundance")) ~ Farm2, data = colData(tse), permutations = 9999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Farm2, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2       3    3.452 0.10315 4.4474 1e-04 ***
## Residual  116   30.010 0.89685
## Total     119   33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(t(assay(tse, "relabundance")) ~ AgeParentStock, data = colData(tse), permutations = 9999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ AgeParentStock, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## AgeParentStock  4    3.980 0.11895 3.8817 1e-04 ***
## Residual       115   29.481 0.88105
## Total          119   33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(t(assay(tse, "relabundance")) ~ Age, data = colData(tse), permutations = 9999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse, "relabundance")) ~ Age, data = colData(tse), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Age         1    1.828 0.05464 6.8206 1e-04 ***
## Residual  118   31.633 0.94536
## Total     119   33.462 1.00000
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# variances: AB: 0.026, Cox: 0.102, Researcher: 0.06, FP : 0.067, LitterType: 0.061, FT :0.055, Gender:
# Stable: 0.167, FS: 0.1245, Farm 0.103, APS : 0.118, Age: 0.054
# Order: Stable>FS>APS>Farm>Cox>FP>LT>Researcher>FT>Age>AB>Gender

# Mixed models ( out of scope)
#adonis2(t(assay(tse, "relabundance")) ~ Stables * AB, data = colData(tse), permutations = 9999)

# basically, composition seems to be different over every single variable, except for gender

# on genus level
tse_genus <- agglomerateByRank(tse, "Genus")
tse_genus <- transformCounts(tse_genus, method = "relabundance")

adonis2(t(assay(tse_genus, "relabundance")) ~ AB, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ AB, data = colData(tse_genus), permutations =
##           Df SumOfSqs      R2      F Pr(>F)
## AB           1   0.4471 0.03209 3.9117 4e-04 ***
## Residual 118   13.4862 0.96791
## Total      119   13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ Cox, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ Cox, data = colData(tse_genus), permutations
##           Df SumOfSqs      R2      F Pr(>F)
## Cox           3    1.505 0.10801 4.6823 1e-04 ***
## Residual 116   12.428 0.89199
## Total      119   13.933 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ Researcher, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
```



```

## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ Researcher, data = colData(tse_genus), permu
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher   4   1.0471 0.07515 2.3362 2e-04 ***
## Residual    115  12.8862 0.92485
## Total       119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ FeedProducent, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ FeedProducent, data = colData(tse_genus), per
##           Df SumOfSqs      R2      F Pr(>F)
## FeedProducent   2   0.9176 0.06586 4.1241 1e-04 ***
## Residual       117  13.0157 0.93414
## Total          119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ LitterType, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ LitterType, data = colData(tse_genus), permu
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType     2   0.6996 0.05021 3.0926 1e-04 ***
## Residual      117  13.2337 0.94979
## Total         119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ FeedType, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ FeedType, data = colData(tse_genus), permu
##           Df SumOfSqs      R2      F Pr(>F)
## FeedType      1   0.9844 0.07065 8.9705 1e-04 ***
## Residual     118  12.9489 0.92935
## Total        119  13.9333 1.00000

```

```

## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ Gender, data = colData(tse_genus), permutations = 9999) #

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ Gender, data = colData(tse_genus), permutati
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1   0.1175 0.00843 1.0033 0.4282
## Residual 118  13.8158 0.99157
## Total      119  13.9333 1.00000

adonis2(t(assay(tse_genus, "relabundance")) ~ Stables, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ Stables, data = colData(tse_genus), permutat
##           Df SumOfSqs      R2      F Pr(>F)
## Stables     9   2.3359 0.16765 2.4618 1e-04 ***
## Residual 110  11.5974 0.83235
## Total      119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ FlockSize, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ FlockSize, data = colData(tse_genus), permut
##           Df SumOfSqs      R2      F Pr(>F)
## FlockSize   5   1.7048 0.12235 3.1785 1e-04 ***
## Residual  114  12.2285 0.87765
## Total      119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

adonis2(t(assay(tse_genus, "relabundance")) ~ Farm2, data = colData(tse_genus), permutations = 9999)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free

```

```
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ Farm2, data = colData(tse_genus), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2      3   1.3884 0.09965 4.2795 1e-04 ***
## Residual 116  12.5449 0.90035
## Total    119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(t(assay(tse_genus, "relabundance")) ~ AgeParentStock, data = colData(tse_genus), permutations = 9999)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 9999
##
## adonis2(formula = t(assay(tse_genus, "relabundance")) ~ AgeParentStock, data = colData(tse_genus), permutations = 9999)
##           Df SumOfSqs      R2      F Pr(>F)
## AgeParentStock  4   1.5725 0.11286 3.6576 1e-04 ***
## Residual      115  12.3608 0.88714
## Total        119  13.9333 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

same trends on genus level (and on phylum level, though p values become higher)

for different ordination methods

```
ps1.rel <- microbiome::transform(subset16S, "compositional")
metadf <- data.frame(sample_data(ps1.rel))

# alternative calculations
#otu <- abundances(ps1.rel)
#meta <- meta(ps1.rel)
#adonis2(t(otu) ~ Age, data = meta, permutations=9999, method = "bray")

#permanova = adonis(t(otu) ~ Age, data = meta, permutations=9999, method = "bray")
#permanova$aov.tab

unifrac.dist <- UniFrac(ps1.rel)

adonis2(unifrac.dist ~ Age, data = metadf)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Age, data = metadf)
```

```
##           Df SumOfSqs      R2      F Pr(>F)
## Age         1   1.0901 0.09194 11.947  0.001 ***
## Residual 118  10.7664 0.90806
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ AB, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ AB, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## AB          1   0.4532 0.03822  4.6893  0.001 ***
## Residual 118  11.4033 0.96178
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ Farm2, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Farm2, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2       3   1.5651 0.13201  5.8806  0.001 ***
## Residual 116  10.2914 0.86799
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ Cox, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Cox, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Cox         3   1.8178 0.15331  7.0015  0.001 ***
## Residual 116  10.0388 0.84669
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ Researcher, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Researcher, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher  4   0.8538 0.07201 2.231  0.001 ***
## Residual   115  11.0027 0.92799
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ LitterType, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ LitterType, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType  2   0.7815 0.06591 4.1281  0.001 ***
## Residual   117  11.0750 0.93409
## Total      119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(unifrac.dist ~ Gender, data = metadf) # not sign
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Gender, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1   0.0947 0.00799 0.9502  0.518
## Residual   118  11.7618 0.99201
## Total      119  11.8565 1.00000
```

```
adonis2(unifrac.dist ~ Stables, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = unifrac.dist ~ Stables, data = metadf)
```

```
##           Df SumOfSqs      R2      F Pr(>F)
## Stables    9   2.5652 0.21636 3.3744 0.001 ***
## Residual 110   9.2913 0.78364
## Total     119  11.8565 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

same patterns arise

```
wunifrac.dist <- UniFrac(ps1.rel,
                        weighted = TRUE)

adonis2(wunifrac.dist ~ Age, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Age, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Age         1   0.4772 0.06566 8.2918 0.001 ***
## Residual 118   6.7911 0.93434
## Total     119   7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ AB, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ AB, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## AB          1   0.2231 0.03069 3.7362 0.002 **
## Residual 118   7.0452 0.96931
## Total     119   7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ Farm2, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Farm2, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2       3   0.7339 0.10098 4.343 0.001 ***
```

```
## Residual 116    6.5344 0.89902
## Total      119    7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ Cox, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Cox, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Cox         3   0.7493 0.1031 4.4446  0.001 ***
## Residual 116   6.5190 0.8969
## Total      119   7.2683 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ Researcher, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Researcher, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher  4   0.5650 0.07773 2.4232  0.001 ***
## Residual   115   6.7033 0.92227
## Total      119   7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ LitterType, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ LitterType, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType  2   0.3604 0.04959 3.0524  0.001 ***
## Residual   117   6.9079 0.95041
## Total      119   7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(wunifrac.dist ~ Gender, data = metadf) # not sign
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Gender, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1  0.0359 0.00493 0.5849  0.84
## Residual 118  7.2325 0.99507
## Total     119  7.2683 1.00000
```

```
adonis2(wunifrac.dist ~ Stables, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = wunifrac.dist ~ Stables, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Stables      9  1.2663 0.17422 2.5785 0.001 ***
## Residual 110  6.0021 0.82578
## Total     119  7.2683 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# same patterns
```

```
jsd.dist <- phyloseq::distance(ps1.rel, "jsd")
```

```
adonis2(jsd.dist ~ Age, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Age, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Age          1  0.8836 0.07447 9.4942 0.001 ***
## Residual 118 10.9818 0.92553
## Total     119 11.8654 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ AB, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
```



```
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ AB, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## AB           1   0.4364 0.03678 4.5059  0.001 ***
## Residual 118  11.4290 0.96322
## Total      119  11.8654 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ Farm2, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Farm2, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2        3   1.6671 0.1405 6.3206  0.001 ***
## Residual 116  10.1983 0.8595
## Total      119  11.8654 1.0000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ Cox, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Cox, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Cox         3   1.6355 0.13783 6.1817  0.001 ***
## Residual 116  10.2299 0.86217
## Total      119  11.8654 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ Researcher, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Researcher, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher   4   0.8256 0.06958 2.1499  0.001 ***
## Residual    115  11.0398 0.93042
```

```
## Total      119  11.8654 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ LitterType, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ LitterType, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType  2    0.970 0.08175 5.208  0.001 ***
## Residual   117   10.895 0.91825
## Total      119   11.865 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jsd.dist ~ Gender, data = metadf) # not sign
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Gender, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1   0.0978 0.00824 0.9809  0.478
## Residual  118   11.7676 0.99176
## Total     119   11.8654 1.00000
```

```
adonis2(jsd.dist ~ Stables, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jsd.dist ~ Stables, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Stables     9   2.5398 0.21405 3.3287  0.001 ***
## Residual  110   9.3256 0.78595
## Total     119   11.8654 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# same is true for JSD
```

```
bray.dist <- phyloseq::distance(ps1.rel, "bray")
```

```
adonis2(bray.dist ~ Age, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Age, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Age         1      1.828 0.05464 6.8206  0.001 ***
## Residual 118      31.633 0.94536
## Total      119      33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ AB, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ AB, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## AB          1      0.867 0.02591 3.139  0.001 ***
## Residual 118      32.594 0.97409
## Total      119      33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ Farm2, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Farm2, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2       3      3.452 0.10315 4.4474  0.001 ***
## Residual 116      30.010 0.89685
## Total      119      33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ Cox, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Cox, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
```

```
## Cox      3      3.424 0.10233 4.4078 0.001 ***
## Residual 116    30.037 0.89767
## Total    119    33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ Researcher, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Researcher, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher  4      1.942 0.05803 1.7711 0.001 ***
## Residual    115    31.520 0.94197
## Total       119    33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ LitterType, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ LitterType, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType  2      2.054 0.06137 3.825 0.001 ***
## Residual    117    31.408 0.93863
## Total       119    33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(bray.dist ~ Gender, data = metadf) # not sign
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Gender, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1      0.247 0.00739 0.8784 0.694
## Residual    118    33.214 0.99261
## Total       119    33.462 1.00000
```

```
adonis2(bray.dist ~ Stables, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray.dist ~ Stables, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Stables    9      5.606 0.16753 2.4596 0.001 ***
## Residual 110      27.856 0.83247
## Total     119      33.462 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

and BC

```
jaccard.dist <- phyloseq::distance(ps1.rel, "jaccard")
adonis2(jaccard.dist ~ Age, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Age, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Age         1      1.594 0.03685 4.5146 0.001 ***
## Residual 118      41.670 0.96315
## Total     119      43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ AB, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ AB, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## AB         1      0.813 0.01879 2.2592 0.001 ***
## Residual 118      42.451 0.98121
## Total     119      43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ Farm2, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
```

```
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Farm2, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Farm2      3      3.226 0.07456 3.1151 0.001 ***
## Residual 116      40.038 0.92544
## Total     119      43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ Cox, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Cox, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Cox        3      3.172 0.07331 3.0589 0.001 ***
## Residual 116      40.092 0.92669
## Total     119      43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ Researcher, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Researcher, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Researcher  4      2.091 0.04833 1.4601 0.001 ***
## Residual   115      41.173 0.95167
## Total      119      43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ LitterType, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ LitterType, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## LitterType  2      1.950 0.04508 2.7616 0.001 ***
## Residual   117      41.314 0.95492
## Total      119      43.264 1.00000
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
adonis2(jaccard.dist ~ Gender, data = metadf) # not sign
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Gender, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Gender      1    0.332 0.00766 0.9111  0.701
## Residual 118   42.932 0.99234
## Total     119   43.264 1.00000
```

```
adonis2(jaccard.dist ~ Stables, data = metadf)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = jaccard.dist ~ Stables, data = metadf)
##           Df SumOfSqs      R2      F Pr(>F)
## Stables      9    5.818 0.13447 1.8989  0.001 ***
## Residual 110   37.446 0.86553
## Total     119   43.264 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# as well as jaccard
```

PERMANOVA plots - Age

```
permanova_age <- adonis(t(assay(tse, "relabundance")) ~ Age, data = colData(tse), permutations = 9999)

coef <- coefficients(permanova_age)["Age1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))], 20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef))))

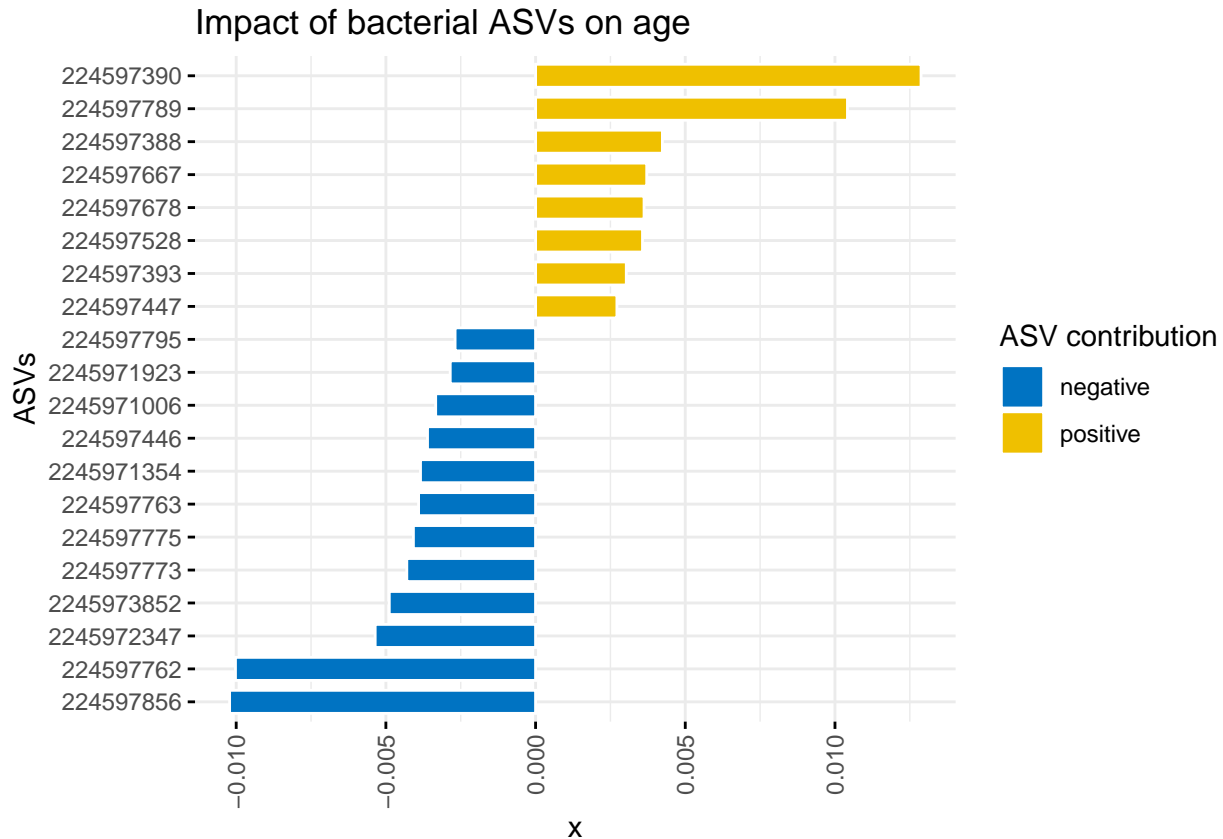
df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

ggbarplot(df, x = "y", y = "x",
           fill = "contr",           # change fill color by mpg_level
           color = "white",         # Set bar border colors to white
           palette = "jco",         # jco journal color palett. see ?ggpar
           sort.val = "asc",         # Sort the value in ascending order)
```

```

sort.by.groups = FALSE,      # Don't sort inside each group
x.text.angle = 90,           # Rotate vertically x axis texts
xlab = "ASVs",
legend.title = "ASV contribution",
title = "Impact of bacterial ASVs on age",
rotate = TRUE,
ggtheme = theme_minimal()

```



Antibiotic treatment

```

permanova_AB <- adonis(t(assay(tse, "relabundance")) ~ AB, data = colData(tse), permutations = 9999)

coef <- coefficients(permanova_AB)["AB1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))], 20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

ggbarplot(df, x = "y", y = "x",
           fill = "contr",      # change fill color by mpg_level
           color = "white",     # Set bar border colors to white
           palette = "jco",     # jco journal color palett. see ?ggpar

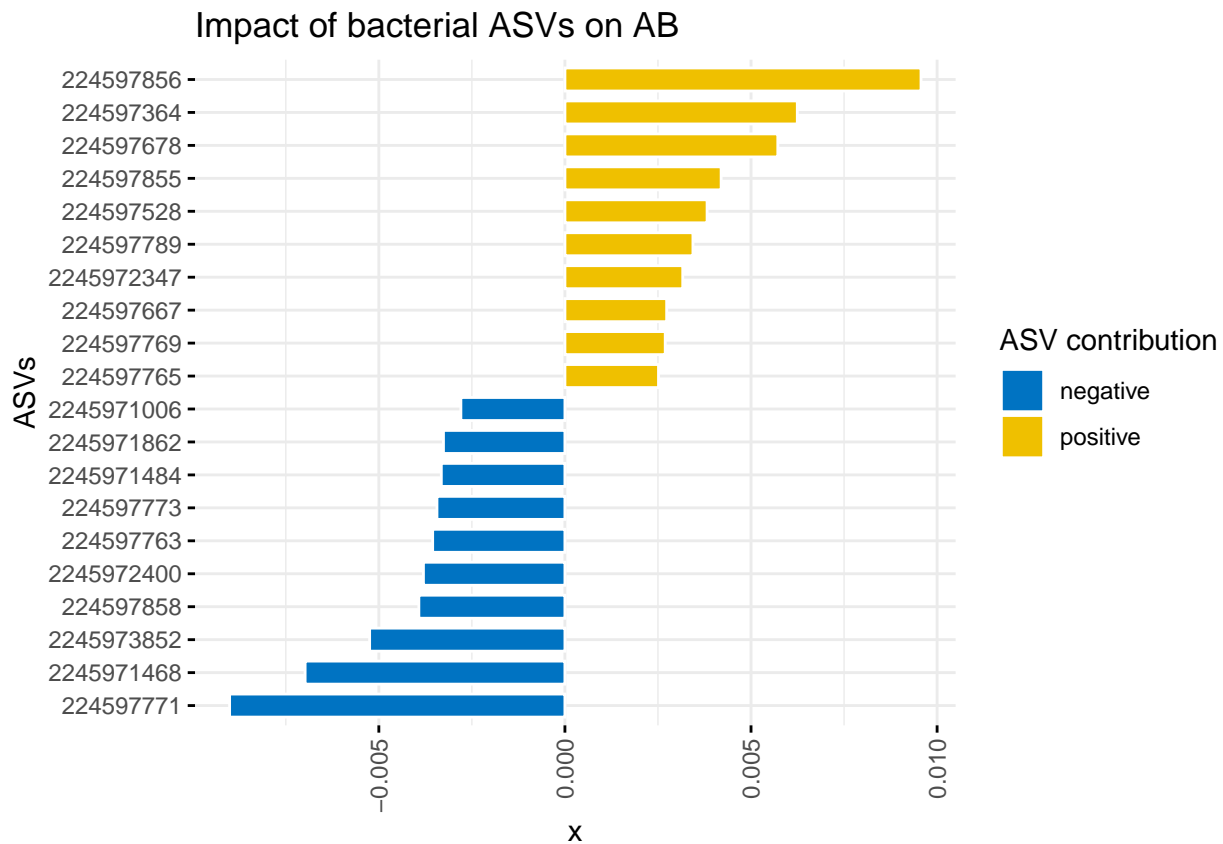
```



```

sort.val = "asc",          # Sort the value in ascending order
sort.by.groups = FALSE,   # Don't sort inside each group
x.text.angle = 90,        # Rotate vertically x axis texts
xlab = "ASVs",
legend.title = "ASV contribution",
title = "Impact of bacterial ASVs on AB",
rotate = TRUE,
ggtheme = theme_minimal()

```



Stable

```

permanova_stable <- adonis(t(assay(tse, "relabundance")) ~ Stables, data = colData(tse), permutations =

coef <- coefficients(permanova_stable)["Stables1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef)))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

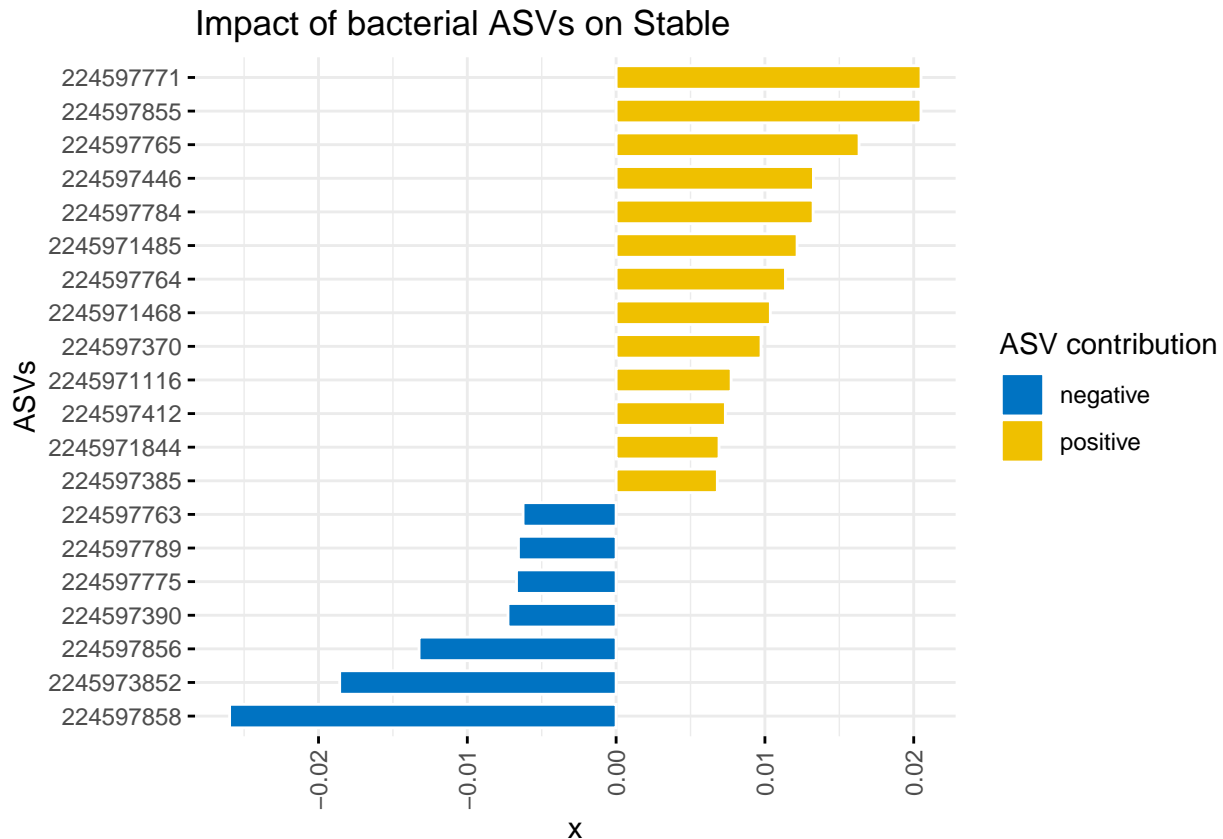
ggbarplot(df, x = "y", y = "x",
          fill = "contr",          # change fill color by mpg_level
          color = "white",        # Set bar border colors to white

```

```

palette = "jco",           # jco journal color palett. see ?ggpar
sort.val = "asc",          # Sort the value in ascending order
sort.by.groups = FALSE,   # Don't sort inside each group
x.text.angle = 90,         # Rotate vertically x axis texts
xlab = "ASVs",
legend.title = "ASV contribution",
title = "Impact of bacterial ASVs on Stable",
rotate = TRUE,
ggtheme = theme_minimal()

```



Farm

```

permanova_farm <- adonis(t(assay(tse, "relabundance")) ~ Farm2, data = colData(tse), permutations = 999)

coef <- coefficients(permanova_farm)["Farm21",]
top.coef <- sort(head(coef[rev(order(abs(coef)))], 20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

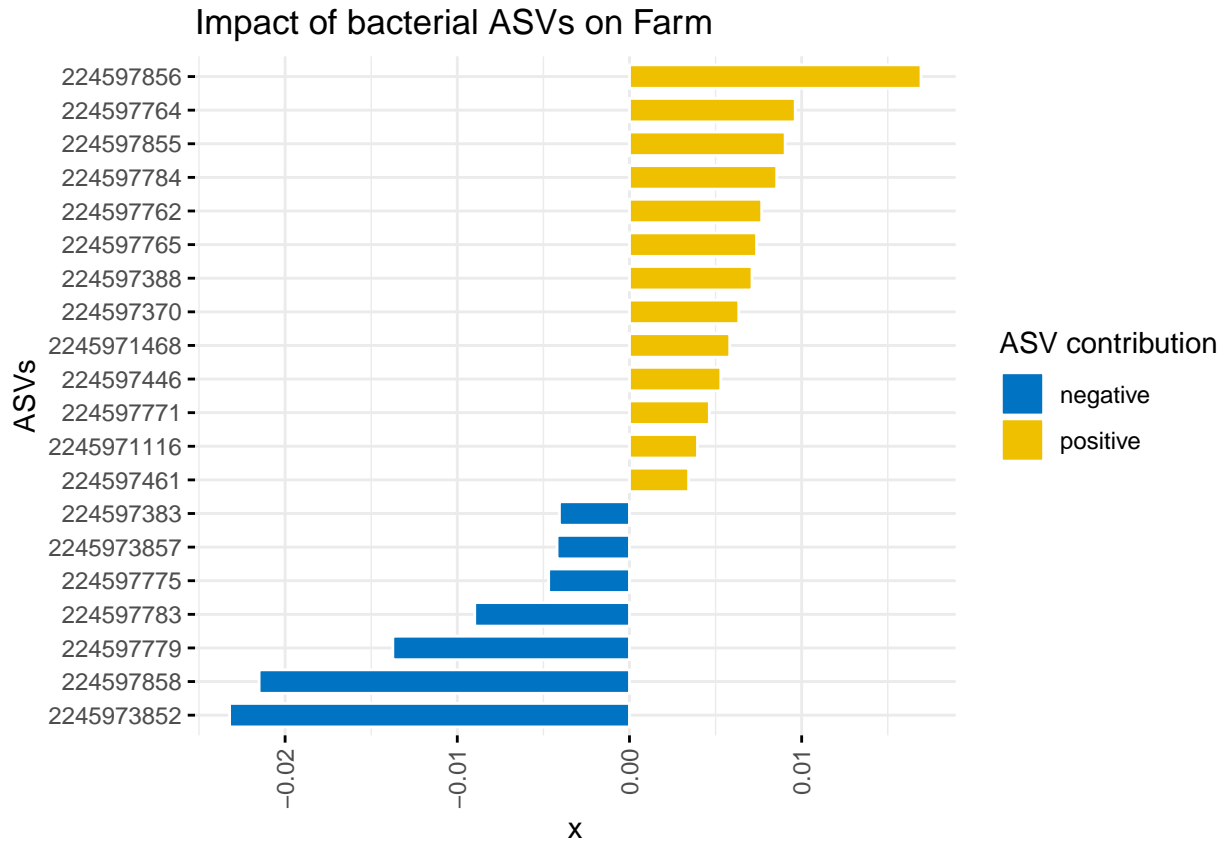
ggbarplot(df, x = "y", y = "x",
           fill = "contr",           # change fill color by mpg_level

```

```

color = "white",          # Set bar border colors to white
palette = "jco",          # jco journal color palett. see ?ggpar
sort.val = "asc",         # Sort the value in ascending order
sort.by.groups = FALSE,  # Don't sort inside each group
x.text.angle = 90,        # Rotate vertically x axis texts
xlab = "ASVs",
legend.title = "ASV contribution",
title = "Impact of bacterial ASVs on Farm",
rotate = TRUE,
ggtheme = theme_minimal()

```



Agent

```

permanova_agent <- adonis(t(assay(tse, "relabundance"))) ~ Cox, data = colData(tse), permutations = 9999

coef <- coefficients(permanova_agent)["Cox1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

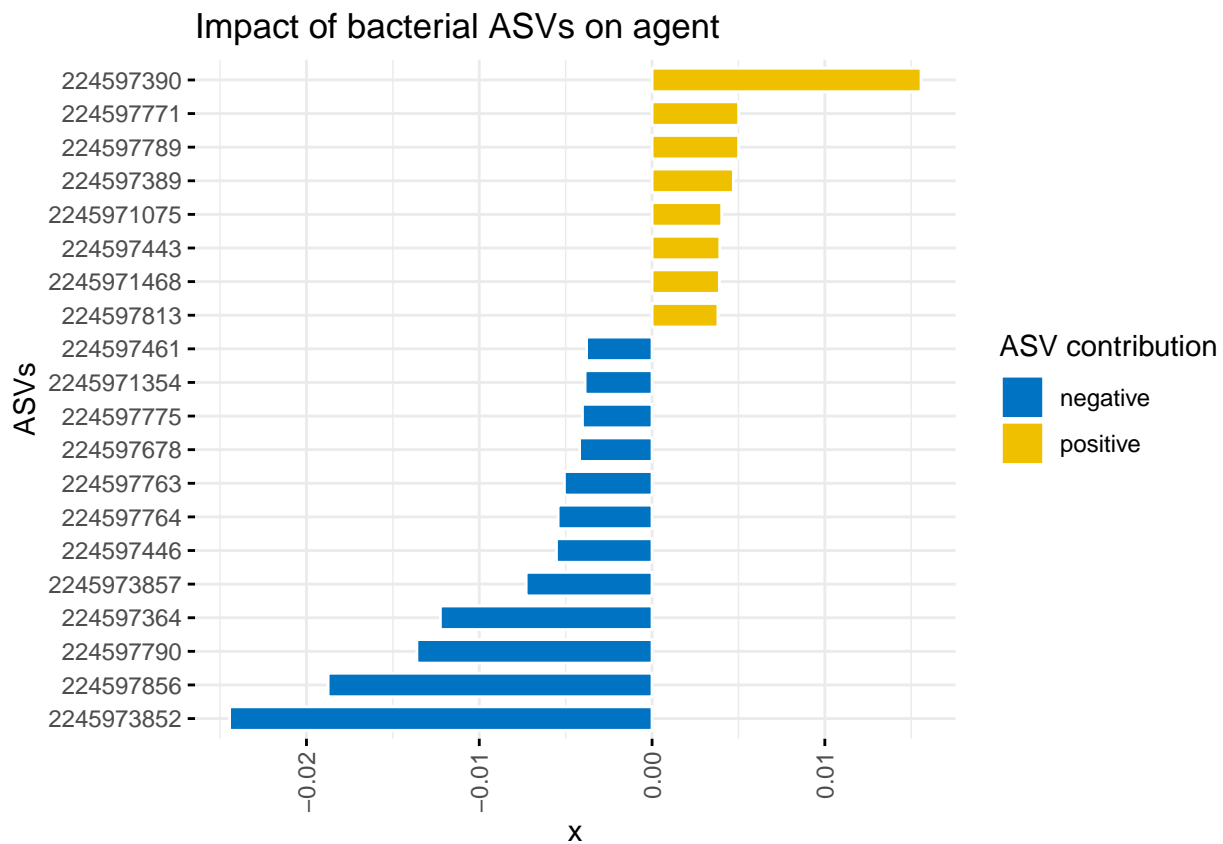
ggbarplot(df, x = "y", y = "x",

```

```

fill = "contr",          # change fill color by mpg_level
color = "white",         # Set bar border colors to white
palette = "jco",         # jco journal color palett. see ?ggpar
sort.val = "asc",        # Sort the value in ascending order
sort.by.groups = FALSE,  # Don't sort inside each group
x.text.angle = 90,       # Rotate vertically x axis texts
xlab = "ASVs",
legend.title = "ASV contribution",
title = "Impact of bacterial ASVs on agent",
rotate = TRUE,
ggtheme = theme_minimal()

```



Same plots but for genera - Age

```

permanova_age <- adonis(t(assay(tse_genus, "relabundance"))) ~ Age, data = colData(tse_genus), permutations = 1000

coef <- coefficients(permanova_age)["Age1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))], 20))

df = data.frame(x = top.coef,
               y = factor(names(top.coef),
                           unique(names(top.coef))))

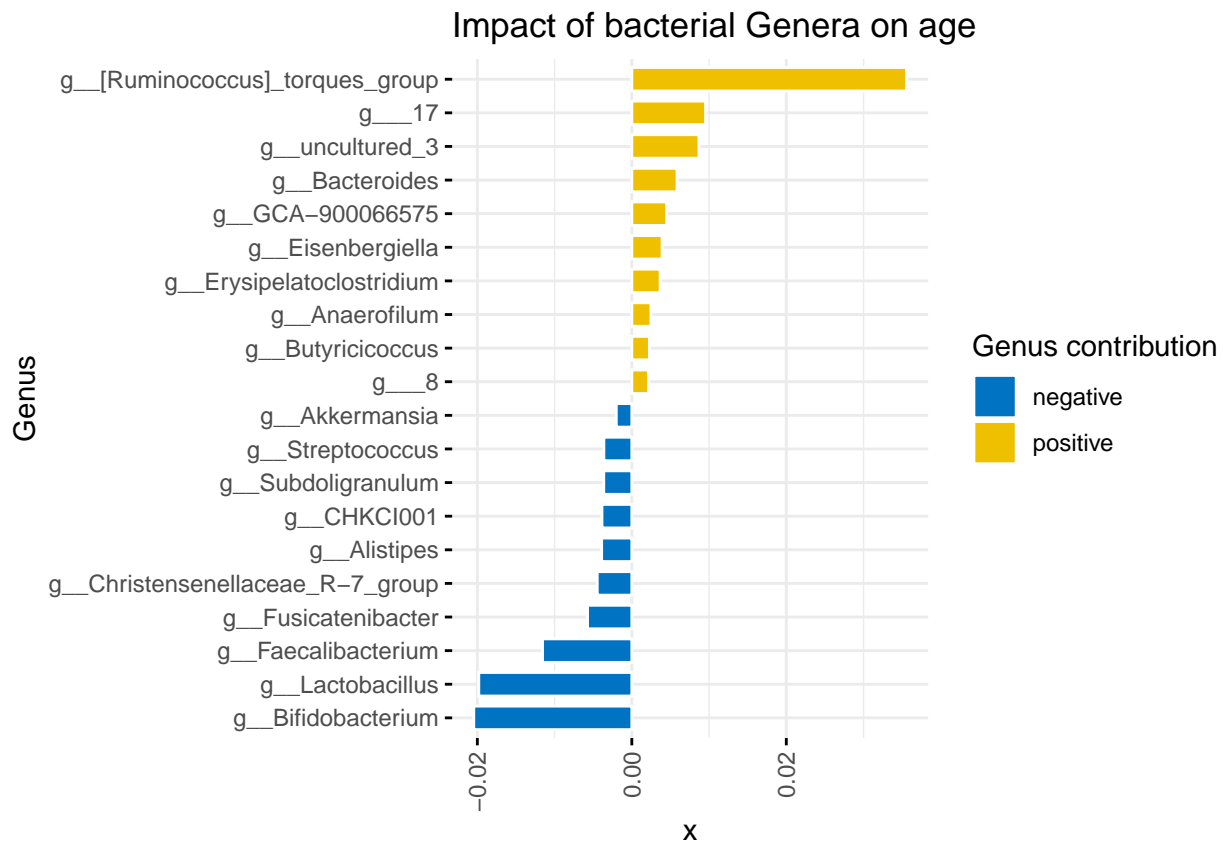
df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

```

```

ggbarplot(df, x = "y", y = "x",
  fill = "contr",          # change fill color by mpg_level
  color = "white",         # Set bar border colors to white
  palette = "jco",         # jco journal color palett. see ?ggpar
  sort.val = "asc",        # Sort the value in ascending order
  sort.by.groups = FALSE,  # Don't sort inside each group
  x.text.angle = 90,       # Rotate vertically x axis texts
  xlab = "Genus",
  legend.title = "Genus contribution",
  title = "Impact of bacterial Genera on age",
  rotate = TRUE,
  ggtheme = theme_minimal())

```



Antibiotic treatment

```

permanova_AB <- adonis(t(assay(tse_genus, "relabundance")) ~ AB, data = colData(tse_genus), permutations = 1000)

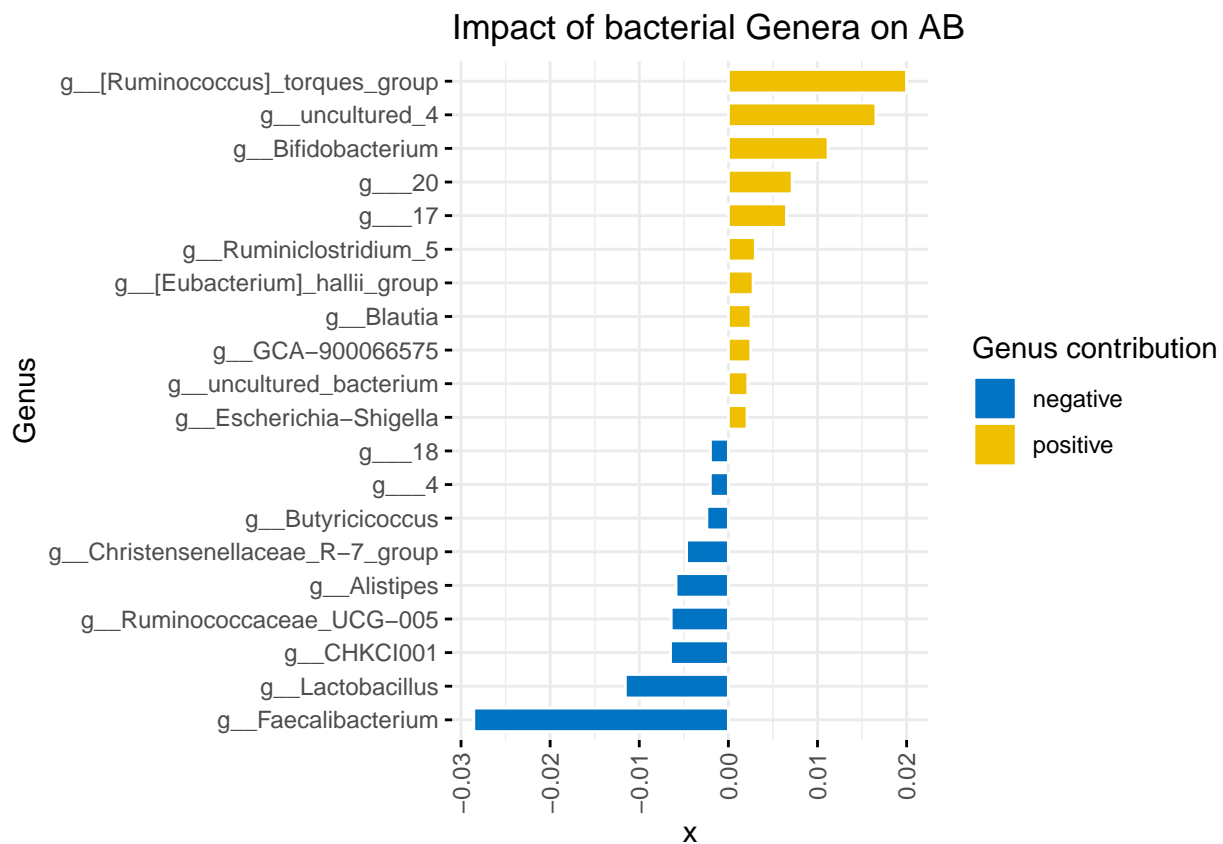
coef <- coefficients(permanova_AB)["AB1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))], 20))

df = data.frame(x = top.coef,
  y = factor(names(top.coef),
    unique(names(top.coef))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
  levels = c("negative", "positive"))

```

```
ggbarplot(df, x = "y", y = "x",
          fill = "contr",          # change fill color by mpg_level
          color = "white",         # Set bar border colors to white
          palette = "jco",         # jco journal color palett. see ?ggpar
          sort.val = "asc",        # Sort the value in ascending order
          sort.by.groups = FALSE,  # Don't sort inside each group
          x.text.angle = 90,       # Rotate vertically x axis texts
          xlab = "Genus",
          legend.title = "Genus contribution",
          title = "Impact of bacterial Genera on AB",
          rotate = TRUE,
          ggtheme = theme_minimal())
```



Stable

```
permanova_stable <- adonis(t(assay(tse_genus, "relabundance")) ~ Stables, data = colData(tse_genus), per
```

```
coef <- coefficients(permanova_stable)["Stables1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

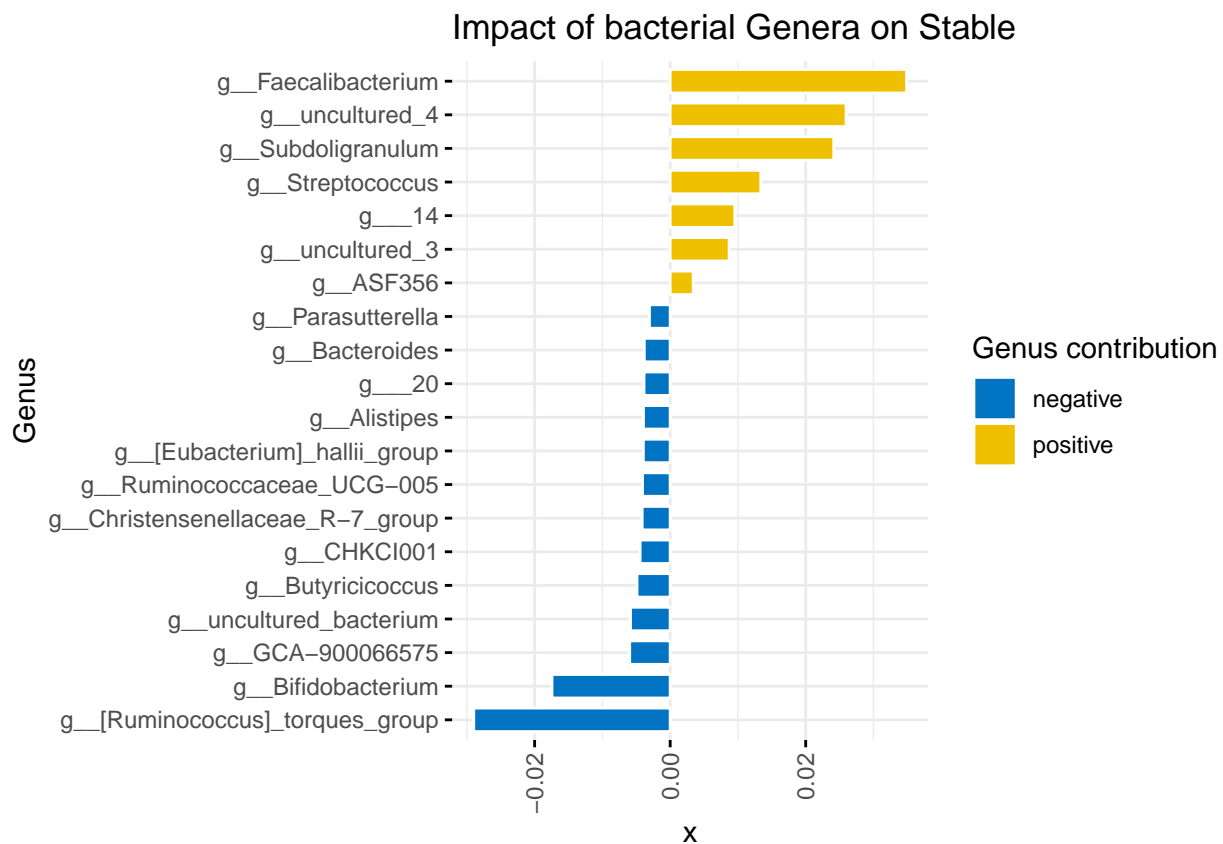
df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef)))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))
```

```

ggbarplot(df, x = "y", y = "x",
          fill = "contr",          # change fill color by mpg_level
          color = "white",         # Set bar border colors to white
          palette = "jco",         # jco journal color palett. see ?ggpar
          sort.val = "asc",        # Sort the value in ascending order
          sort.by.groups = FALSE,  # Don't sort inside each group
          x.text.angle = 90,       # Rotate vertically x axis texts
          xlab = "Genus",
          legend.title = "Genus contribution",
          title = "Impact of bacterial Genera on Stable",
          rotate = TRUE,
          ggtheme = theme_minimal())

```



Farm

```

permanova_farm <- adonis(t(assay(tse_genus, "relabundance")) ~ Farm2, data = colData(tse_genus), permu
coef <- coefficients(permanova_farm)["Farm21",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

df = data.frame(x = top.coef,
                y = factor(names(top.coef),
                           unique(names(top.coef))))

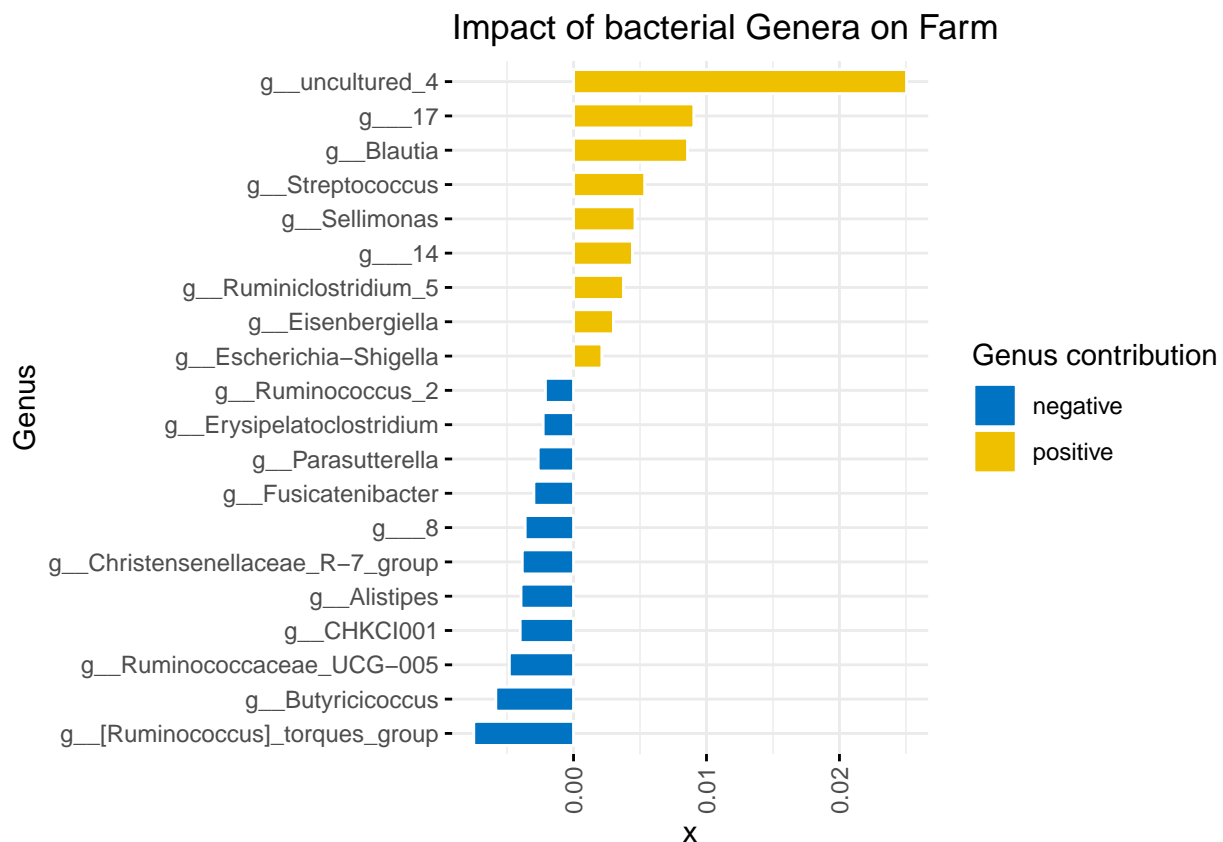
df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
                  levels = c("negative", "positive"))

```

```

ggbarplot(df, x = "y", y = "x",
  fill = "contr",          # change fill color by mpg_level
  color = "white",         # Set bar border colors to white
  palette = "jco",         # jco journal color palett. see ?ggpar
  sort.val = "asc",        # Sort the value in ascending order
  sort.by.groups = FALSE,  # Don't sort inside each group
  x.text.angle = 90,       # Rotate vertically x axis texts
  xlab = "Genus",
  legend.title = "Genus contribution",
  title = "Impact of bacterial Genera on Farm",
  rotate = TRUE,
  ggtheme = theme_minimal())

```



Agent

```

permanova_agent <- adonis(t(assay(tse_genus, "relabundance"))) ~ Cox, data = colData(tse_genus), permuta

coef <- coefficients(permanova_agent)["Cox1",]
top.coef <- sort(head(coef[rev(order(abs(coef)))],20))

df = data.frame(x = top.coef,
  y = factor(names(top.coef),
    unique(names(top.coef))))

df$contr <- factor(ifelse(df$x < 0, "negative", "positive"),
  levels = c("negative", "positive"))

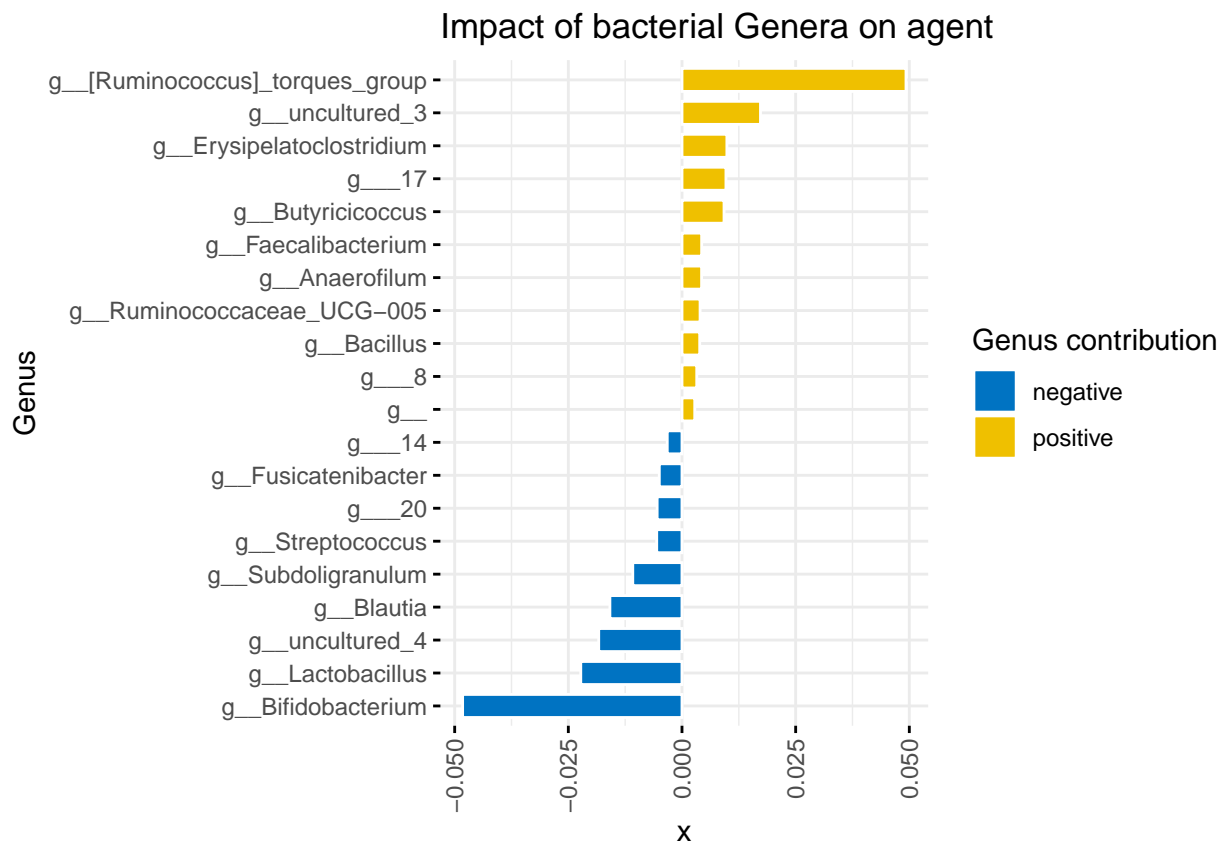
```



```

ggbarplot(df, x = "y", y = "x",
          fill = "contr",           # change fill color by mpg_level
          color = "white",         # Set bar border colors to white
          palette = "jco",         # jco journal color palett. see ?ggpar
          sort.val = "asc",        # Sort the value in ascending order
          sort.by.groups = FALSE,  # Don't sort inside each group
          x.text.angle = 90,       # Rotate vertically x axis texts
          xlab = "Genus",
          legend.title = "Genus contribution",
          title = "Impact of bacterial Genera on agent",
          rotate = TRUE,
          ggtheme = theme_minimal())

```



checking homogeneity condition - bray-curtis ANOVAs are performed on betadispers of our rel abund data to test whether groups are more variable than others

```

# Bray
ps.rel = microbiome::transform(subset16S, "compositional")
meta = meta(ps.rel)
anova(betadisper(vegdist(t(abundances(ps.rel)))), meta$Age))

```

```

## Analysis of Variance Table
##
## Response: Distances
##      Df  Sum Sq  Mean Sq F value    Pr(>F)

```

```
## Groups      1 0.04882 0.048824 12.036 0.0007295 ***
## Residuals 118 0.47868 0.004057
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$AB))
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups      1 0.03423 0.034229  8.4594 0.00434 **
## Residuals 118 0.47746 0.004046
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$Farm2))
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups      3 0.05386 0.0179546  3.488 0.01805 *
## Residuals 116 0.59712 0.0051476
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$Stables))
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups      9 0.10294 0.0114374  2.2578 0.02333 *
## Residuals 110 0.55723 0.0050658
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$Cox))
```

```
## Analysis of Variance Table
##
## Response: Distances
##      Df Sum Sq Mean Sq F value Pr(>F)
## Groups      3 0.05437 0.0181226  3.5909 0.01585 *
## Residuals 116 0.58544 0.0050469
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$Researcher))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      4 0.11864 0.0296606  6.4411 0.0001031 ***
## Residuals 115 0.52956 0.0046049
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$LitterType)) # homogeneous
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      2 0.00394 0.0019691  0.3935 0.6756
## Residuals 117 0.58548 0.0050041
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$Gender)) # homogeneous
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      1 0.00107 0.0010715  0.2599 0.6111
## Residuals 118 0.48643 0.0041223
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$FlockSize))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      5 0.10963 0.0219269  4.6166 0.0007136 ***
## Residuals 114 0.54145 0.0047496
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$AgeParentStock)) # homogeneous
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value Pr(>F)
## Groups      4 0.04865 0.0121630  2.213 0.07188 .
## Residuals 115 0.63206 0.0054962
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$FeedProducent))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Groups      2 0.06978  0.034891   7.7777 0.0006739 ***
## Residuals 117 0.52487  0.004486
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), meta$FeedType))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Groups      1 0.04882  0.048824  12.036 0.0007295 ***
## Residuals 118 0.47868  0.004057
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Jaccard
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$Age))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Groups      1 0.023087 0.0230874  12.572 0.0005626 ***
## Residuals 118 0.216706 0.0018365
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$AB))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Groups      1 0.022032 0.0220319  12.296 0.000643 ***
## Residuals 118 0.211439 0.0017919
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$Farm2))
```

```
## Analysis of Variance Table
##
## Response: Distances
```

```
##           Df   Sum Sq   Mean Sq F value   Pr(>F)
## Groups      3 0.027311 0.0091035   3.7252 0.01337 *
## Residuals 116 0.283478 0.0024438
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$Stables))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value   Pr(>F)
## Groups      9 0.046992 0.0052213   2.1071 0.03471 *
## Residuals 110 0.272580 0.0024780
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$Researcher))
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value   Pr(>F)
## Groups      4 0.10745 0.0268626  12.64 1.474e-08 ***
## Residuals 115 0.24439 0.0021251
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$LitterType)) # homogeneous
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value   Pr(>F)
## Groups      2 0.000853 0.00042627  0.1859 0.8306
## Residuals 117 0.268245 0.00229269
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$Gender)) # homogeneous
```

```
## Analysis of Variance Table
##
## Response: Distances
##           Df   Sum Sq   Mean Sq F value   Pr(>F)
## Groups      1 0.000528 0.0005283   0.2968 0.5869
## Residuals 118 0.210023 0.0017799
```

```
anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$FlockSize))
```

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

```

## Response: Distances
##           Df    Sum Sq  Mean Sq F value    Pr(>F)
## Groups      5 0.067558 0.013512  6.0427 5.345e-05 ***
## Residuals 114 0.254905 0.002236
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$AgeParentStock)) # homogeneous

## Analysis of Variance Table
##
## Response: Distances
##           Df    Sum Sq  Mean Sq F value    Pr(>F)
## Groups      4 0.022427 0.0056068  2.1203 0.08273 .
## Residuals 115 0.304107 0.0026444
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$FeedProducent))

## Analysis of Variance Table
##
## Response: Distances
##           Df    Sum Sq  Mean Sq F value    Pr(>F)
## Groups      2 0.037045 0.0185225  8.975 0.0002365 ***
## Residuals 117 0.241463 0.0020638
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(betadisper(vegdist(t(abundances(ps.rel))), method = "jaccard"), meta$FeedType))

## Analysis of Variance Table
##
## Response: Distances
##           Df    Sum Sq  Mean Sq F value    Pr(>F)
## Groups      1 0.023087 0.0230874 12.572 0.0005626 ***
## Residuals 118 0.216706 0.0018365
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# group variances are not homogenous in most cases, so there are differences in variances between group

# Tukey tests can be performed to see if and which groups differ in relation to variance

TukeyHSD(betadisper(vegdist(t(abundances(ps.rel)))), meta$Farm2))

## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = distances ~ group, data = df)
##

```

```
## $group
##           diff           lwr           upr           p adj
## Farm2-Farm1 -0.01283746 -0.05959222  0.033917288 0.8906792
## Farm3-Farm1 -0.06128653 -0.11527427 -0.007298795 0.0193219
## Farm4-Farm1 -0.02958711 -0.08357485  0.024400627 0.4842422
## Farm3-Farm2 -0.04844907 -0.09520382 -0.001694317 0.0391860
## Farm4-Farm2 -0.01674965 -0.06350440  0.030005106 0.7867504
## Farm4-Farm3  0.03169942 -0.02228831  0.085687160 0.4227048
```

```
# different way of calculating homogeneity, permutation tests, null = no difference in dispersion between
permutest(betadisper(vegdist(t(abundances(ps.rel))), meta$Age), pairwise = TRUE)
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups      1 0.04882 0.048824 12.036   999  0.001 ***
## Residuals 118 0.47868 0.004057
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##           14      35
## 14           0.001
## 35 0.00072948
```

```
permutest(betadisper(unifrac.dist, metadf$Age), pairwise = TRUE) # looks like unifrac distances are hom
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups      1 0.002189 0.0021888 0.8904   999  0.336
## Residuals 118 0.290075 0.0024583
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##           14      35
## 14           0.343
## 35 0.3473
```

```
permutest(betadisper(unifrac.dist, metadf$AB), pairwise = TRUE) # not for AB though
```

```
##
## Permutation test for homogeneity of multivariate dispersions
```

```
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups      1 0.016272 0.0162721 7.5077   999  0.013 *
## Residuals 118 0.255751 0.0021674
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##           no  yes
## no           0.012
## yes 0.0070982
```

```
permutest(betadisper(bray.dist, metadf$Age), pairwise = TRUE) # there are differences in P value with o
```

```
##
## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##           Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups      1 0.04882 0.048824 12.036   999  0.002 **
## Residuals 118 0.47868 0.004057
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##           14   35
## 14           0.002
## 35 0.00072948
```

SIMPER - to save time, analyses are not rerun for Rmarkdown, but earlier results are loaded we'll use MT as abbreviation for metataxonomics instead of 16s since R does not like its objects starting with numbers

```
source("../Results/Scripts/Steinberger_scripts/simper_pretty.r")
source("../Results/Scripts/Steinberger_scripts/R_krusk.r")

#Age

#simpert.pretty(otu_table(subset16S), metrics = sample_data(subset16S), interesting = c("Age"), perc_cut

#MT_age = data.frame(read.csv("MT_age_clean_simper.csv"))

#kruskal.pretty(otu_table(subset16S), metrics = sample_data(subset16S), csv = MT_age, interesting = c('

KW_MT_age = data.frame(read.csv("MT_Age_krusk_simper.csv"))
KW_MT_age = KW_MT_age[KW_MT_age$fdr_krusk_p.val < 0.05,] # filter out non-significant results, based on
KW_MT_age = KW_MT_age[with(KW_MT_age, order(SIMPER, decreasing = TRUE)),]
```



```
KW_MT_age$OTU = as.factor(KW_MT_age$OTU)
```

```
KW_MT_age %>% mutate_if(is.numeric, format.pval, 2) %>% dplyr::select("SIMPER", "OTU", "fdr_krusk_p.val")  
  rowwise() %>% mutate(Combined = paste("ASV =", OTU, ", SIMPER =", SIMPER, ", p-value =", fdr_krusk_p.val))  
  dplyr::select(Combined)
```

```
## # A tibble: 3 x 1  
## # Rowwise:  
##   Combined  
##   <chr>  
## 1 ASV = 224597762 , SIMPER = 0.035 , p-value = 0.00068  
## 2 ASV = 224597390 , SIMPER = 0.028 , p-value = 0.00906  
## 3 ASV = 224597789 , SIMPER = 0.018 , p-value = 1.8e-07
```

```
#AB
```

```
#simper.pretty(otu_table(subset16S), metrics = sample_data(subset16S), interesting = c("AB"), perc_cut = 0.05)
```

```
#MT_AB = data.frame(read.csv("MT_AB_clean_simper.csv"))
```

```
#kruskal.pretty(otu_table(subset16S), metrics = sample_data(subset16S), csv = MT_AB, interesting = c('AB'))
```

```
KW_MT_AB = data.frame(read.csv("MT_AB_krusk_simper.csv"))
```

```
KW_MT_AB = KW_MT_AB[KW_MT_AB$fdr_krusk_p.val < 0.05,] # filter out non-significant results, based on fdr
```

```
KW_MT_AB = KW_MT_AB[with(KW_MT_AB, order(SIMPER, decreasing = TRUE)),]
```

```
KW_MT_AB$OTU = as.factor(KW_MT_AB$OTU)
```

```
KW_MT_AB %>% mutate_if(is.numeric, format.pval, 2) %>% dplyr::select("SIMPER", "OTU", "fdr_krusk_p.val")  
  rowwise() %>% mutate(Combined = paste("ASV =", OTU, ", SIMPER =", SIMPER, ", p-value =", fdr_krusk_p.val))  
  dplyr::select(Combined)
```

```
## # A tibble: 5 x 1  
## # Rowwise:  
##   Combined  
##   <chr>  
## 1 ASV = 224597771 , SIMPER = 0.027 , p-value = 0.0103  
## 2 ASV = 2245971468 , SIMPER = 0.015 , p-value = 2.8e-05  
## 3 ASV = 224597789 , SIMPER = 0.015 , p-value = 0.0066  
## 4 ASV = 224597856 , SIMPER = 0.013 , p-value = 0.0027  
## 5 ASV = 224597364 , SIMPER = 0.010 , p-value = 0.0026
```

```
#Farms - too many comparisons so maybe too extensive for report
```

```
#simper.pretty(otu_table(subset16S), metrics = sample_data(subset16S), interesting = c("Farm2"), perc_cut = 0.05)
```

```
#MT_Farm = data.frame(read.csv("MT_Farm_clean_simper.csv"))
```

```
#kruskal.pretty(otu_table(subset16S), metrics = sample_data(subset16S), csv = MT_Farm, interesting = c('Farm2'))
```

```
KW_MT_Farm = data.frame(read.csv("MT_Farm_krusk_simper.csv"))
```

```
KW_MT_Farm = KW_MT_Farm[KW_MT_Farm$fdr_krusk_p.val < 0.05,] # filter out non-significant results, based on fdr
```

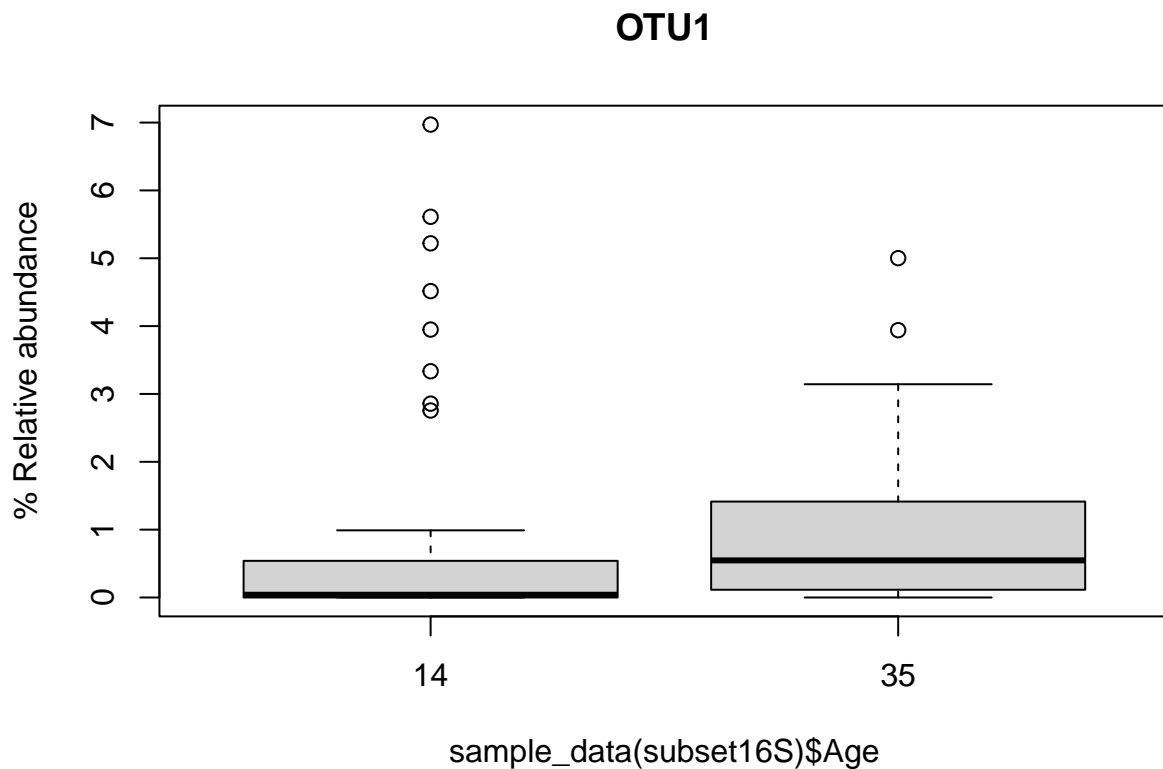
```
KW_MT_Farm = KW_MT_Farm[with(KW_MT_Farm, order(SIMPER, decreasing = TRUE)),]
```

```
KW_MT_Farm$OTU = as.factor(KW_MT_Farm$OTU)
```

```
KW_MT_Farm %>% mutate_if(is.numeric, format.pval, 2) %>% dplyr::select("Comparison", "SIMPER", "OTU", "ASV")
  rowwise() %>% mutate(Combined = paste(Comparison, "ASV =", OTU, ", SIMPER =", SIMPER, ", p-value =", p_value))
  dplyr::select(Combined)
```

```
## # A tibble: 28 x 1
## # Rowwise:
##   Combined
##   <chr>
## 1 Farm1_Farm4 ASV = 2245973852 , SIMPER = 0.067 , p-value = 2.0e-06
## 2 Farm2_Farm4 ASV = 2245973852 , SIMPER = 0.066 , p-value = 1.9e-10
## 3 Farm3_Farm4 ASV = 2245973852 , SIMPER = 0.065 , p-value = 2.0e-06
## 4 Farm1_Farm4 ASV = 224597858 , SIMPER = 0.034 , p-value = 0.00081
## 5 Farm2_Farm3 ASV = 224597779 , SIMPER = 0.033 , p-value = 0.02551
## 6 Farm1_Farm3 ASV = 224597779 , SIMPER = 0.032 , p-value = 0.00518
## 7 Farm2_Farm3 ASV = 224597763 , SIMPER = 0.031 , p-value = 0.00038
## 8 Farm2_Farm3 ASV = 224597771 , SIMPER = 0.029 , p-value = 0.00073
## 9 Farm3_Farm4 ASV = 224597771 , SIMPER = 0.025 , p-value = 0.01604
## 10 Farm1_Farm4 ASV = 224597783 , SIMPER = 0.023 , p-value = 0.01605
## # i 18 more rows
```

```
# plots to look at specific ASVs (age)
abund = otu_table(subset16S)/rowSums(otu_table(subset16S))*100
boxplot(unlist(data.frame(abund["224597762"]))) ~ sample_data(subset16S)$Age, ylab="% Relative abundance"
```



```
# specific test
kruskal.test(unlist(data.frame(otu_table(subset16S)["224597762"]), use.names = FALSE) ~ sample_data(subset16S))

##
## Kruskal-Wallis rank sum test
##
## data:  unlist(data.frame(otu_table(subset16S)["224597762"]), use.names = FALSE) by sample_data(subset16S)
## Kruskal-Wallis chi-squared = 15.191, df = 1, p-value = 9.716e-05
```

Clustering

```
# Trying out different distances, aggregation methods and indices for finding optimal number of clusters

tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)

tse <- transformCounts(tse, method = "relabundance")

assay <- t(assay(tse, "relabundance"))

diss_jaccard <- vegdist(assay, method = "jaccard")

# different aggregation methods and indices will grant different amount of clusters
NbClust(diss = diss_jaccard, distance = NULL, method = "complete", index = "mcclain")$Best.nc # two clusters

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data must be complete.

## Number_clusters      Value_Index
##           2.0000          0.0313

NbClust(diss = diss_jaccard, distance = NULL, method = "complete", index = "frey")$Best.nc # two clusters

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data must be complete.

## Number_clusters      Value_Index
##           2.0000          2.1845

NbClust(diss = diss_jaccard, distance = NULL, method = "complete", index = "cindex")$Best.nc # 15 clusters

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data must be complete.

## Number_clusters      Value_Index
##          15.000          0.638
```

```

NbClust(diss = diss_jaccard, distance = NULL, method = "complete", index = "silhouette")$Best.nc # two

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##           2.000          0.086

NbClust(diss = diss_jaccard, distance = NULL, method = "complete", index = "dunn")$Best.nc # four clust

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##           4.000          0.763

NbClust(diss = diss_jaccard, distance = NULL, method = "ward.D2", index = "silhouette")$Best.nc # 15 clu

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##          15.0000          0.0453

NbClust(diss = diss_jaccard, distance = NULL, method = "ward.D", index = "silhouette")$Best.nc # 11 clu

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##          11.0000          0.0448

NbClust(diss = diss_jaccard, distance = NULL, method = "single", index = "silhouette")$Best.nc # 15 clu

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##          15.0000          0.0827

NbClust(diss = diss_jaccard, distance = NULL, method = "average", index = "silhouette")$Best.nc # 2 clu

##
## Only frey, mcclain, cindex, sihouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##           2.000          0.086

```

```

NbClust(diss = diss_jaccard, distance = NULL, method = "mcquitty", index = "silhouette")$Best.nc # 2 clus

##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##           2.000          0.086

NbClust(diss = diss_jaccard, distance = NULL, method = "median", index = "silhouette")$Best.nc # 2 clus

##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data m

## Number_clusters      Value_Index
##           2.0000          0.1194

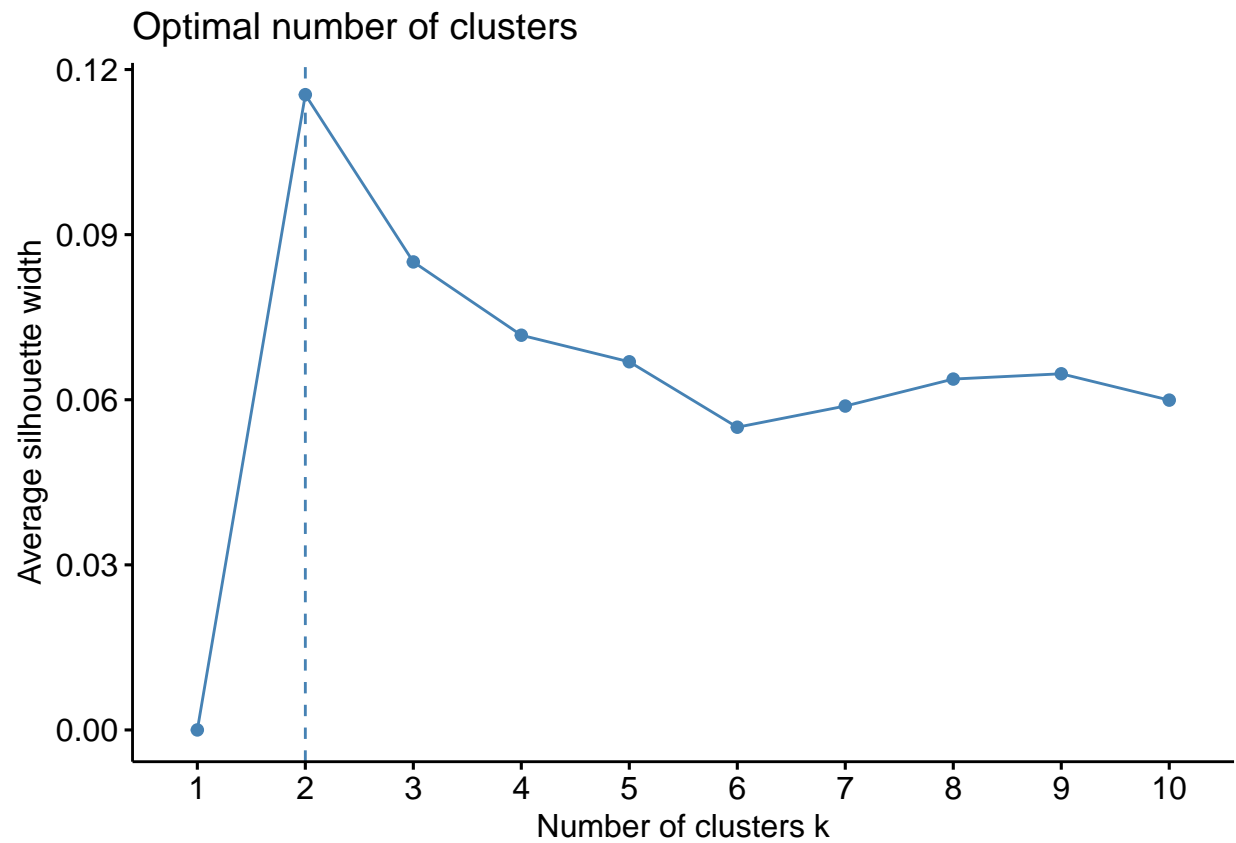
NbClust(diss = diss_jaccard, distance = NULL, method = "centroid", index = "silhouette")$Best.nc # 15 c

##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data m

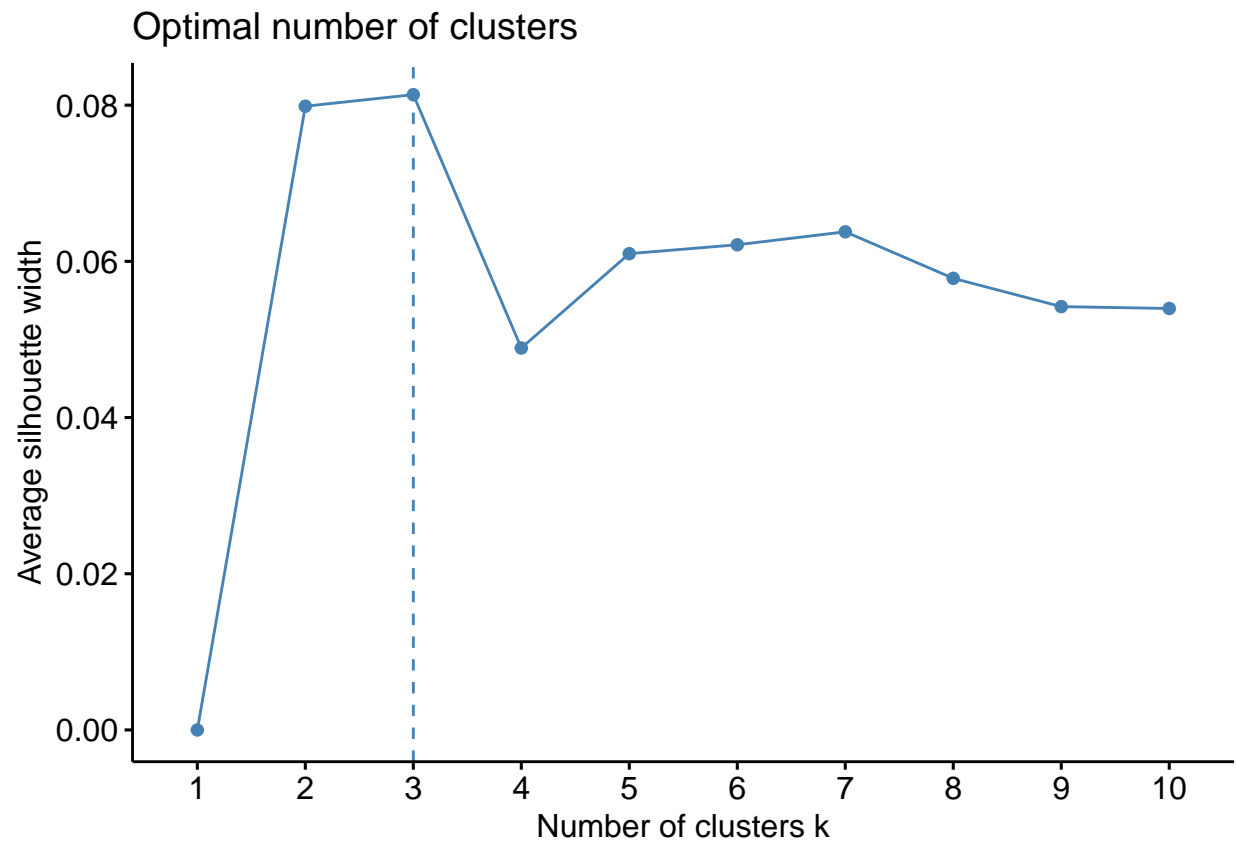
## Number_clusters      Value_Index
##           15.0000          0.1223

# silhouette (ASW), different clustering methods
diss_jaccard <- as.matrix(diss_jaccard)
fviz_nbclust(diss_jaccard, kmeans, method = "silhouette") # 2 seems optimal for k-means

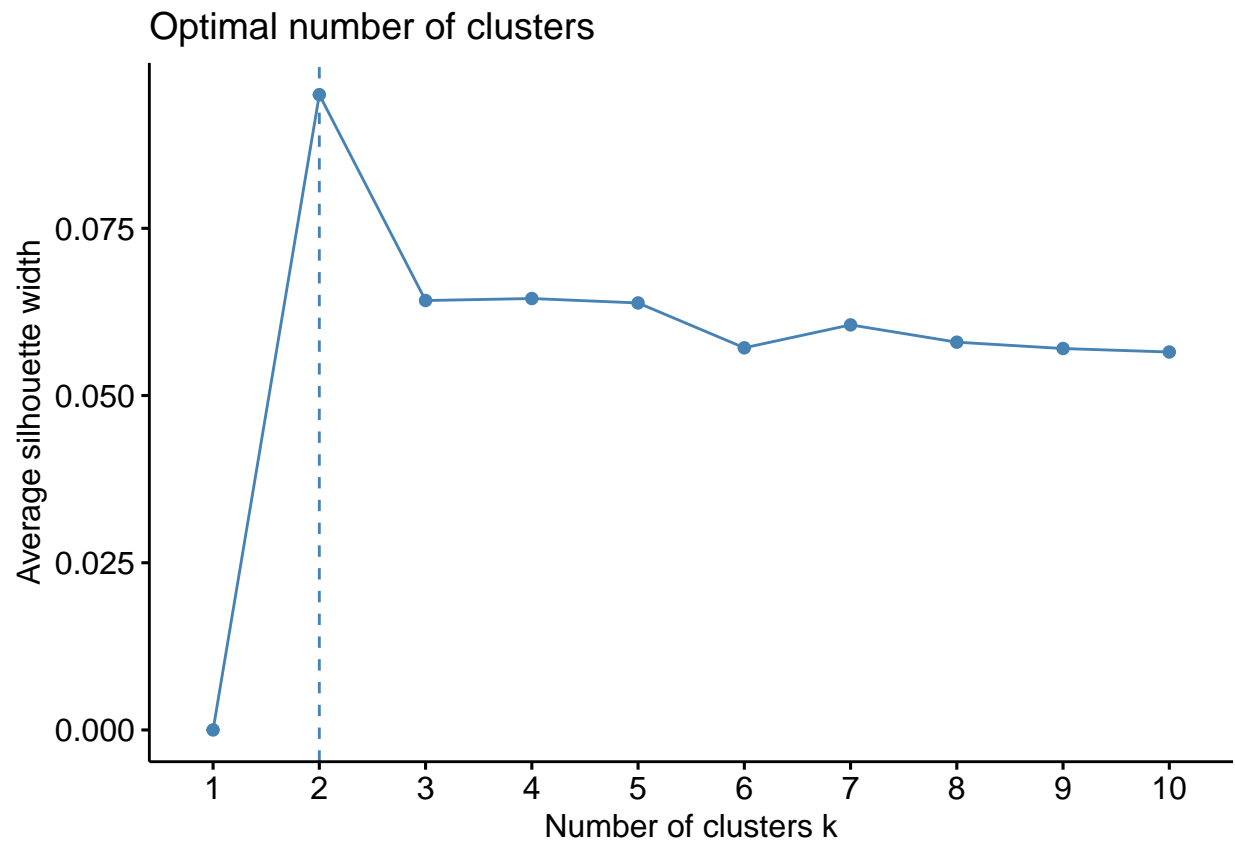
```



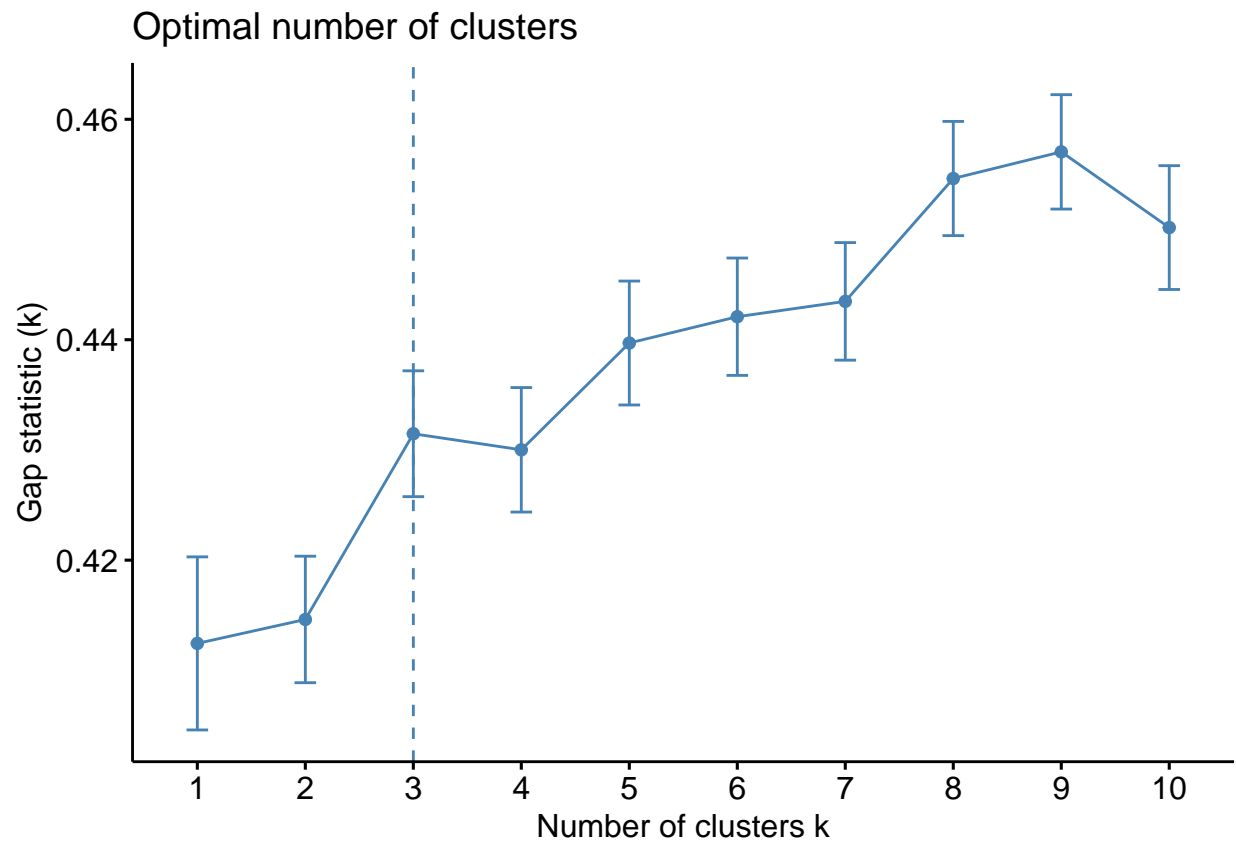
```
fviz_nbclust(diss_jaccard, cluster::pam, method = "silhouette") # 3 seems optimal for PAM
```



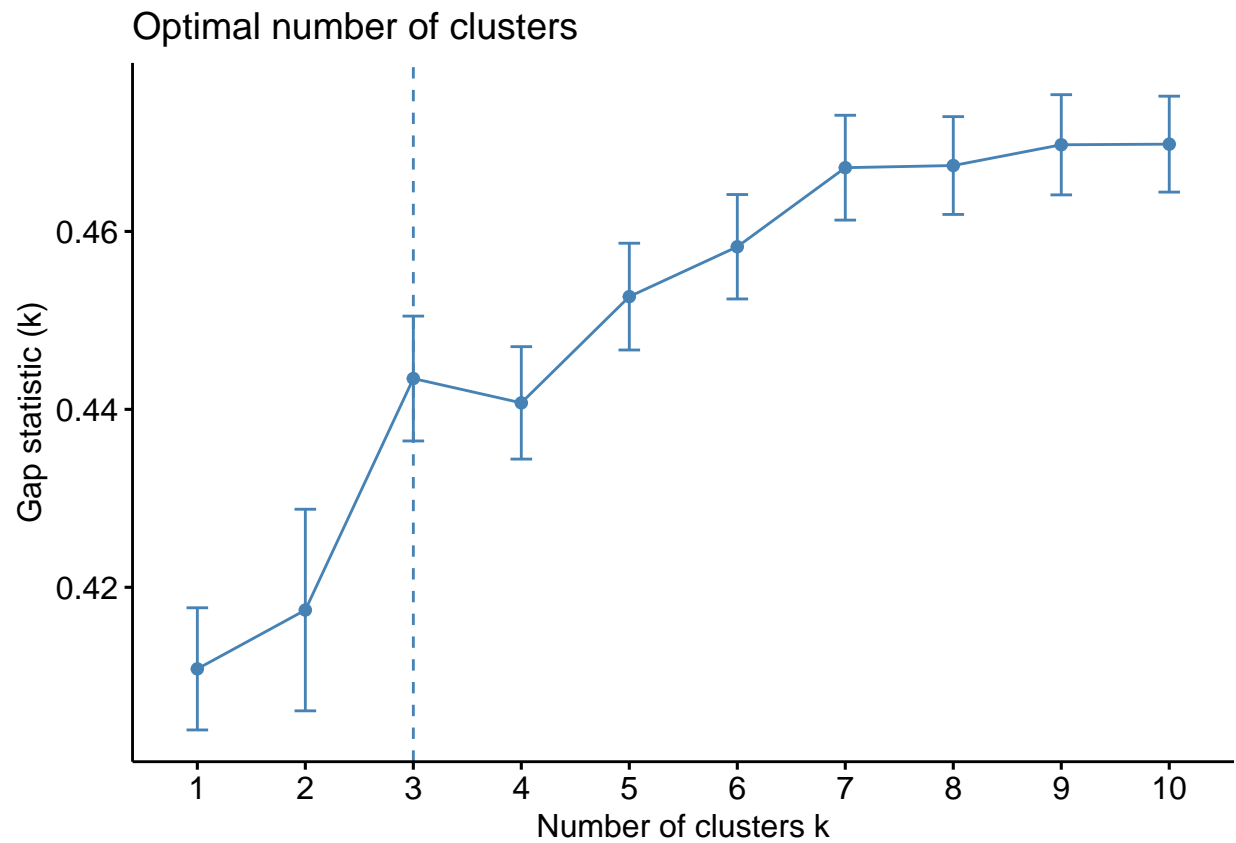
```
fviz_nbclust(diss_jaccard, hcut, method = "silhouette") # 2 seems optimal for hcut
```



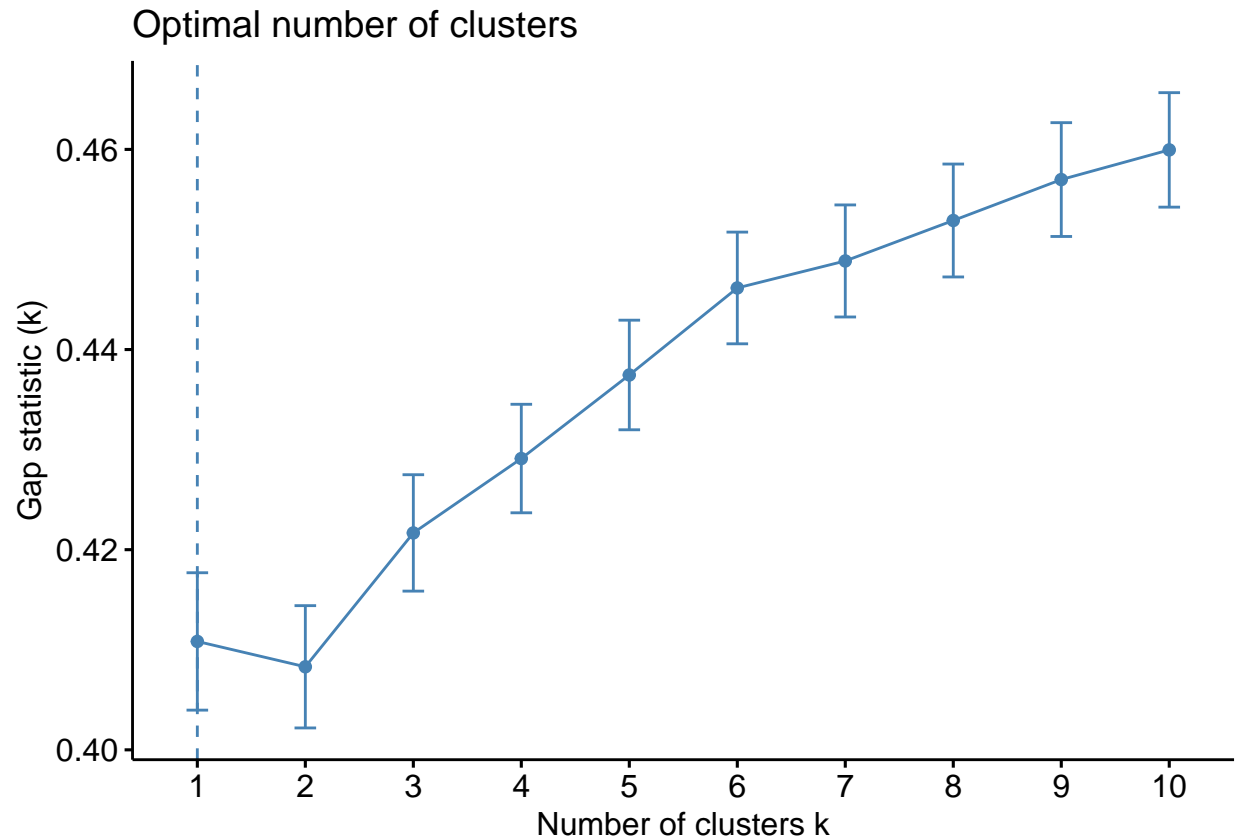
```
fviz_nbclust(diss_jaccard, kmeans, method = "gap_stat") # 3 seems optimal for k-means gap stat
```

```
fviz_nbclust(diss_jaccard, cluster::pam, method = "gap_stat") # 3 seems optimal for PAM gap stat
```



```
fviz_nbclust(diss_jaccard, hcut, method = "gap_stat") # 1 seems optimal for hcut gap stat
```



now, let's repeat this for BC

```
diss_bray <- vegdist(assay, method = "bray")
```

```
NbClust(diss = diss_bray, distance = NULL, method = "complete", index = "mcclain")$Best.nc # two clusters
```

##

Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete.

```
## Number_clusters    Value_Index
##           2.0000          0.0293
```

```
NbClust(diss = diss_bray, distance = NULL, method = "complete", index = "frey")$Best.nc # two clusters
```

##

Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete.

```
## Number_clusters    Value_Index
##           2.0000          2.4419
```

```
NbClust(diss = diss_bray, distance = NULL, method = "complete", index = "cindex")$Best.nc # 5 clusters
```

##

Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete.

```
## Number_clusters      Value_Index
##           5.0000          0.5838
```

```
NbClust(diss = diss_bray, distance = NULL, method = "complete", index = "silhouette")$Best.nc # two clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           2.0000          0.1461
```

```
NbClust(diss = diss_bray, distance = NULL, method = "complete", index = "dunn")$Best.nc # four clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           4.0000          0.6277
```

```
NbClust(diss = diss_bray, distance = NULL, method = "ward.D2", index = "silhouette")$Best.nc # 2 clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           2.0000          0.0955
```

```
NbClust(diss = diss_bray, distance = NULL, method = "ward.D", index = "silhouette")$Best.nc # 2 clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           2.0000          0.0955
```

```
NbClust(diss = diss_bray, distance = NULL, method = "single", index = "silhouette")$Best.nc # 2 clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           2.0000          0.1158
```

```
NbClust(diss = diss_bray, distance = NULL, method = "average", index = "silhouette")$Best.nc # 2 clusters
```

```
##
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be complete
```

```
## Number_clusters      Value_Index
##           2.0000          0.1461
```

```
NbClust(diss = diss_bray, distance = NULL, method = "mcquitty", index = "silhouette")$Best.nc # 2 clusters
```

```
##
```

```
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be
```

```
## Number_clusters      Value_Index  
##           2.0000          0.1461
```

```
NbClust(diss = diss_bray, distance = NULL, method = "median", index = "silhouette")$Best.nc # 2 clusters
```

```
##
```

```
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be
```

```
## Number_clusters      Value_Index  
##           2.0000          0.1158
```

```
NbClust(diss = diss_bray, distance = NULL, method = "centroid", index = "silhouette")$Best.nc # 2 clusters
```

```
##
```

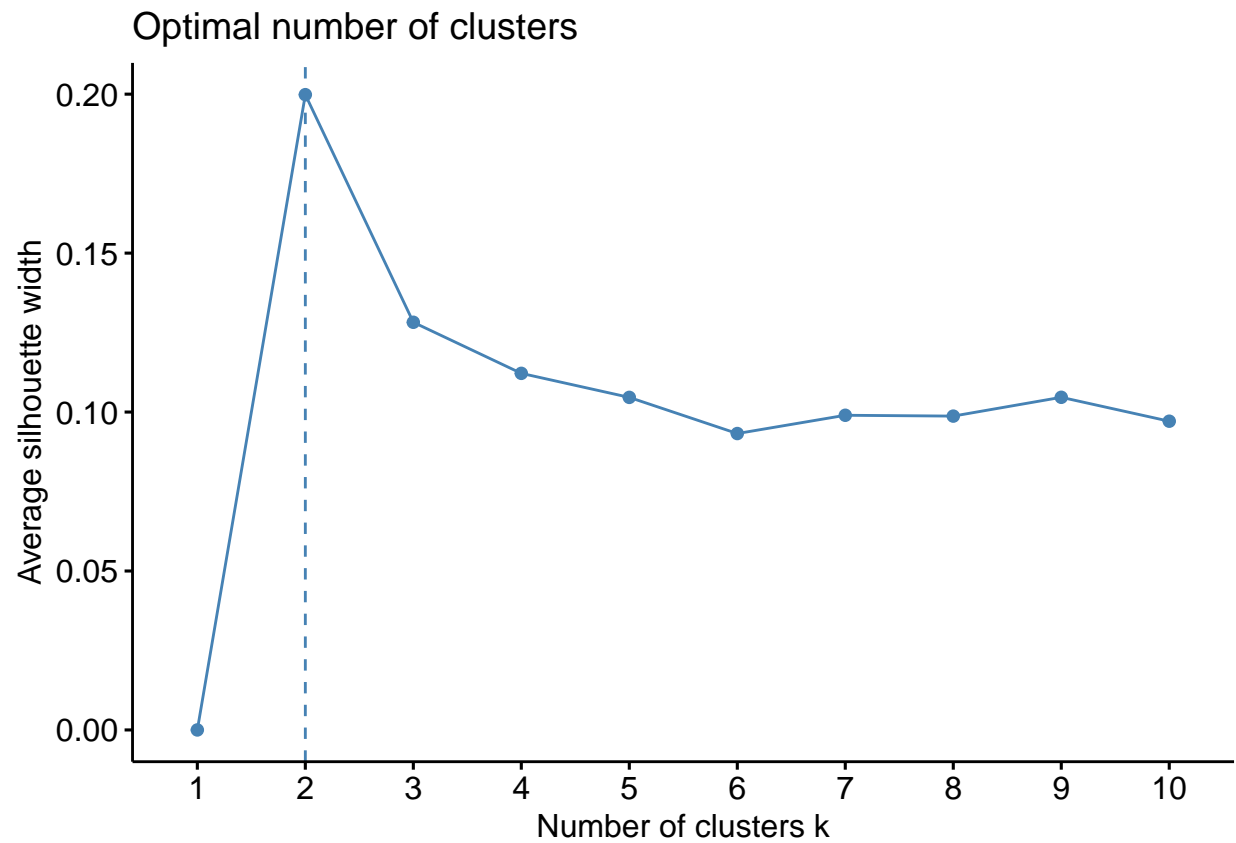
```
## Only frey, mcclain, cindex, silhouette and dunn can be computed. To compute the other indices, data must be
```

```
## Number_clusters      Value_Index  
##           2.0000          0.1972
```

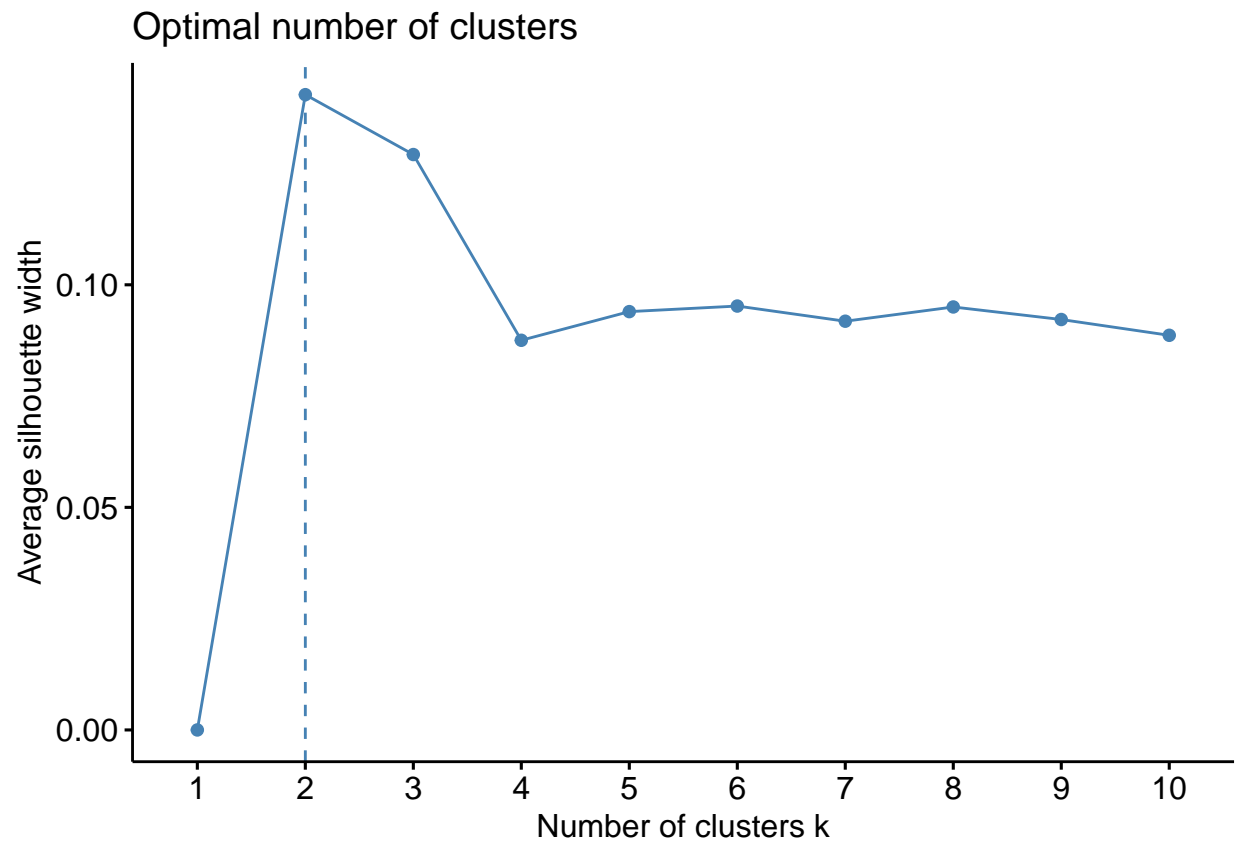
```
# silhouette (ASW)
```

```
diss_bray <- as.matrix(diss_bray)
```

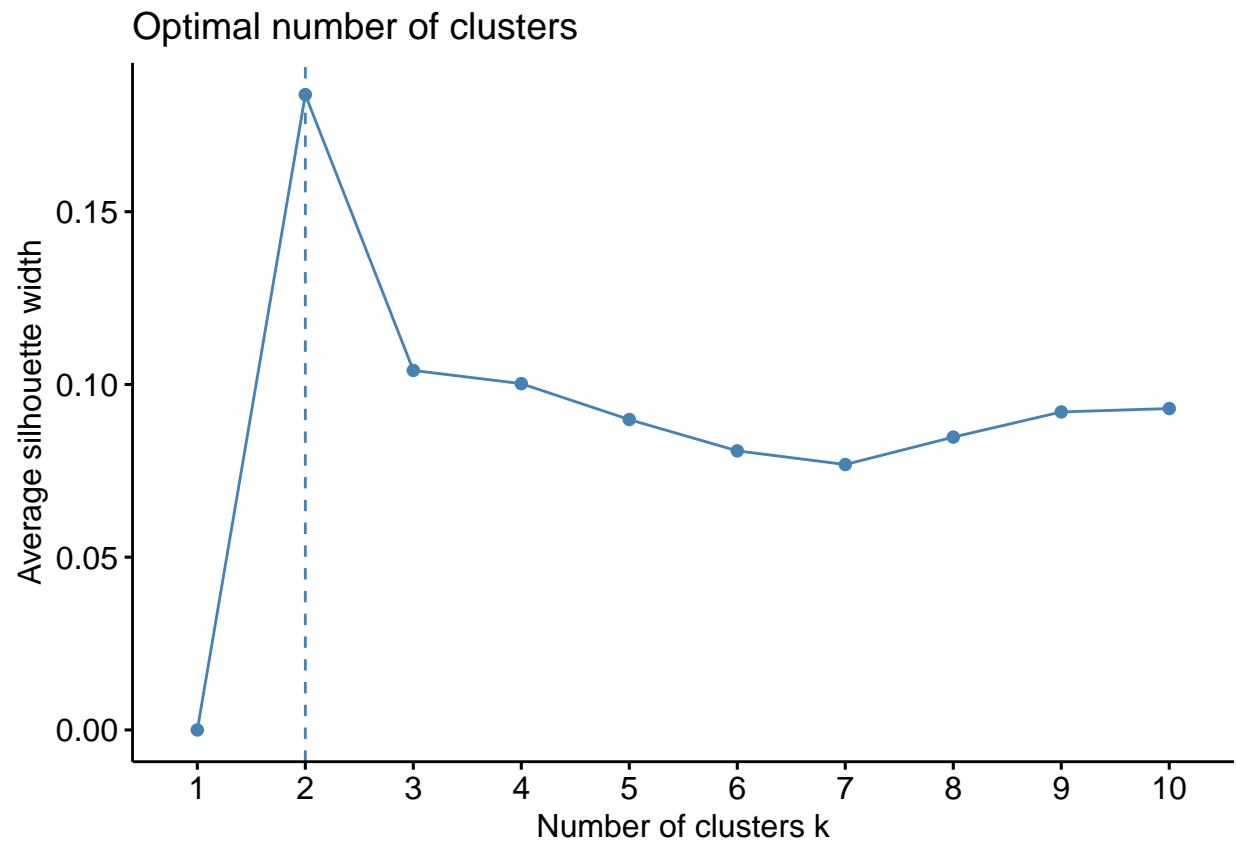
```
fviz_nbclust(diss_bray, kmeans, method = "silhouette") # 2 seems optimal
```



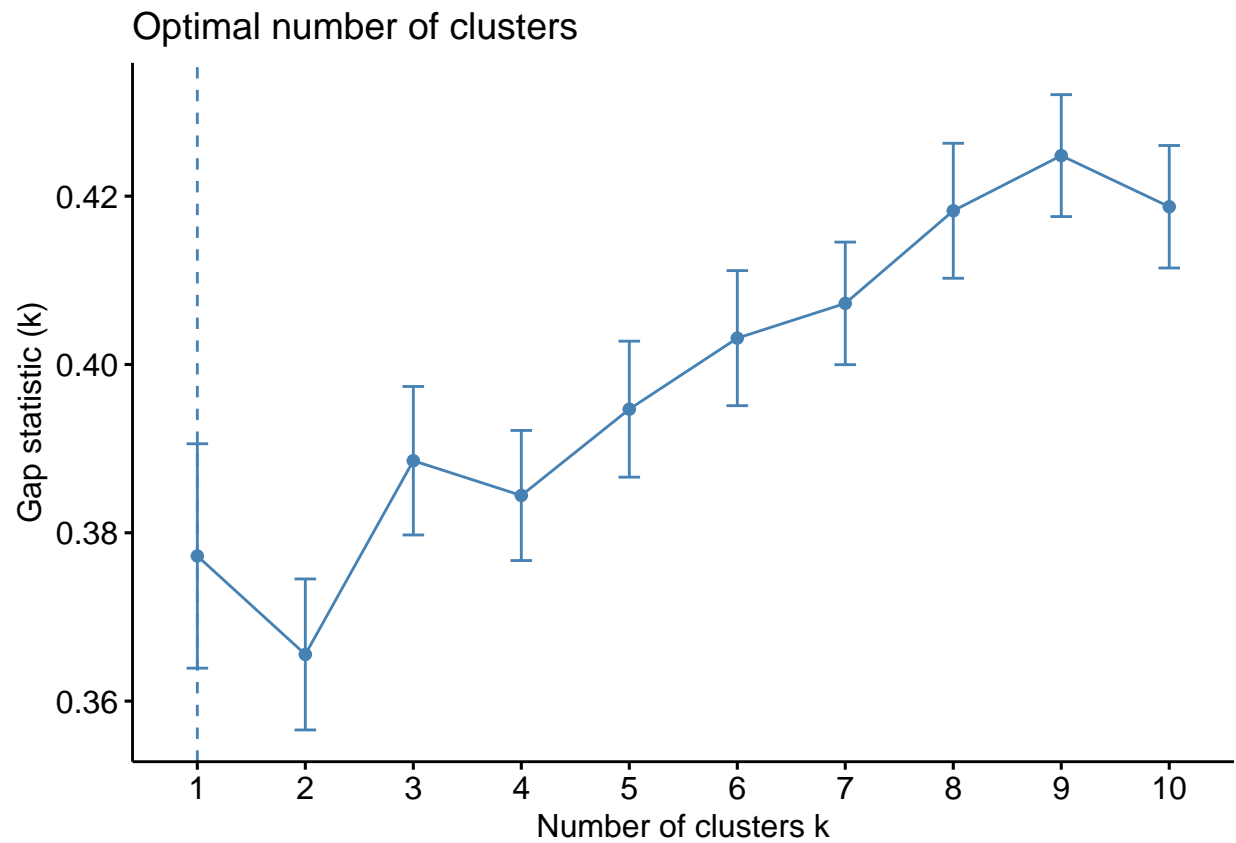
```
fviz_nbclust(diss_bray, cluster::pam, method = "silhouette") # 2 seems optimal for PAM
```



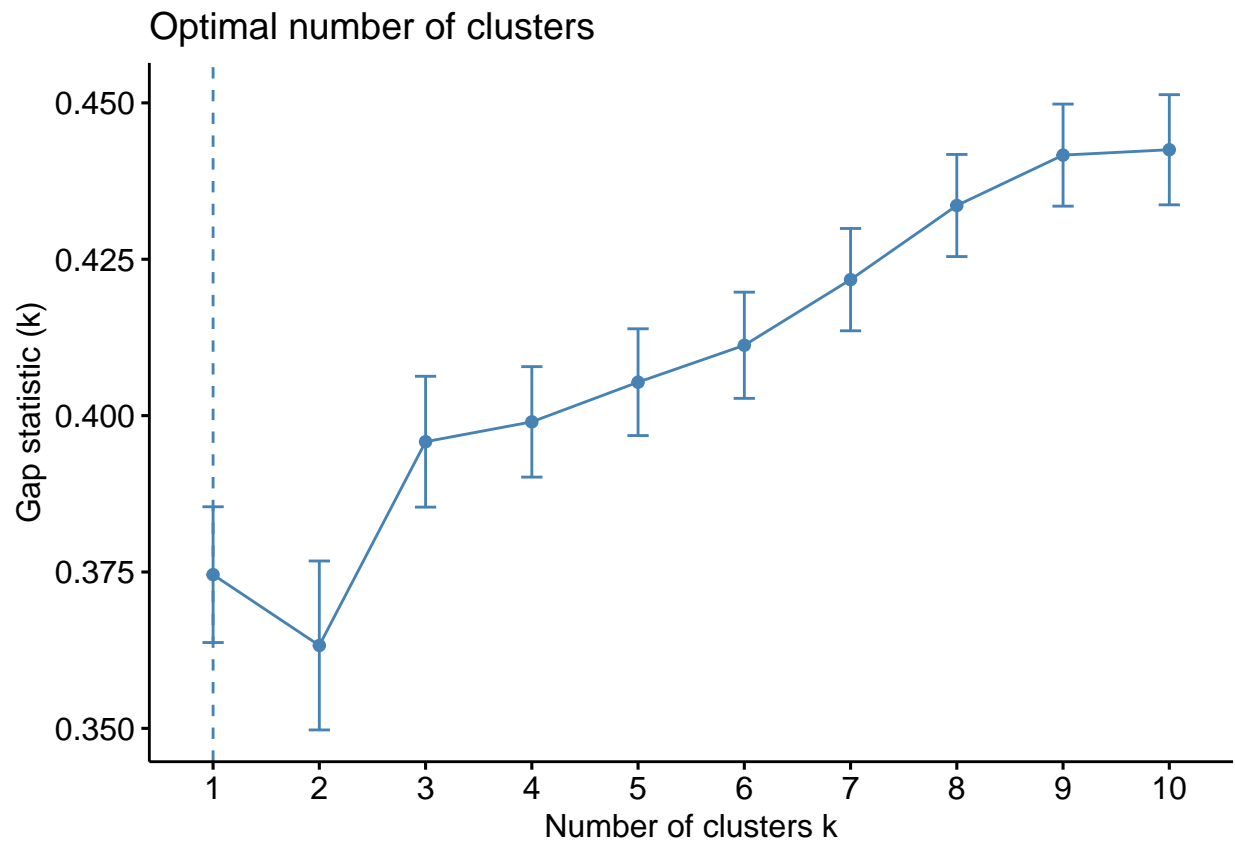
```
fviz_nbclust(diss_bray, hcut, method = "silhouette") # 2 seems optimal for hcut
```



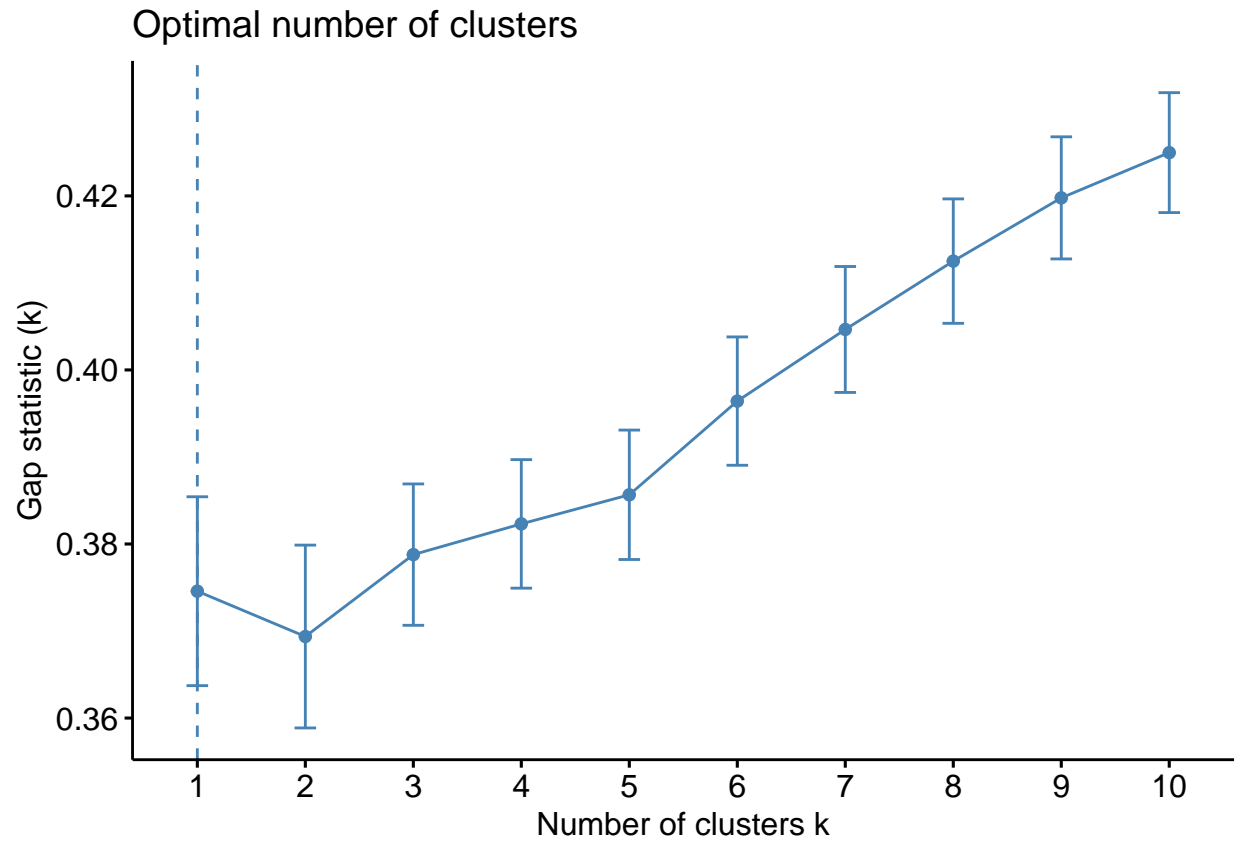
```
fviz_nbclust(diss_bray, kmeans, method = "gap_stat") # 1 seems optimal for k-means gap stat
```

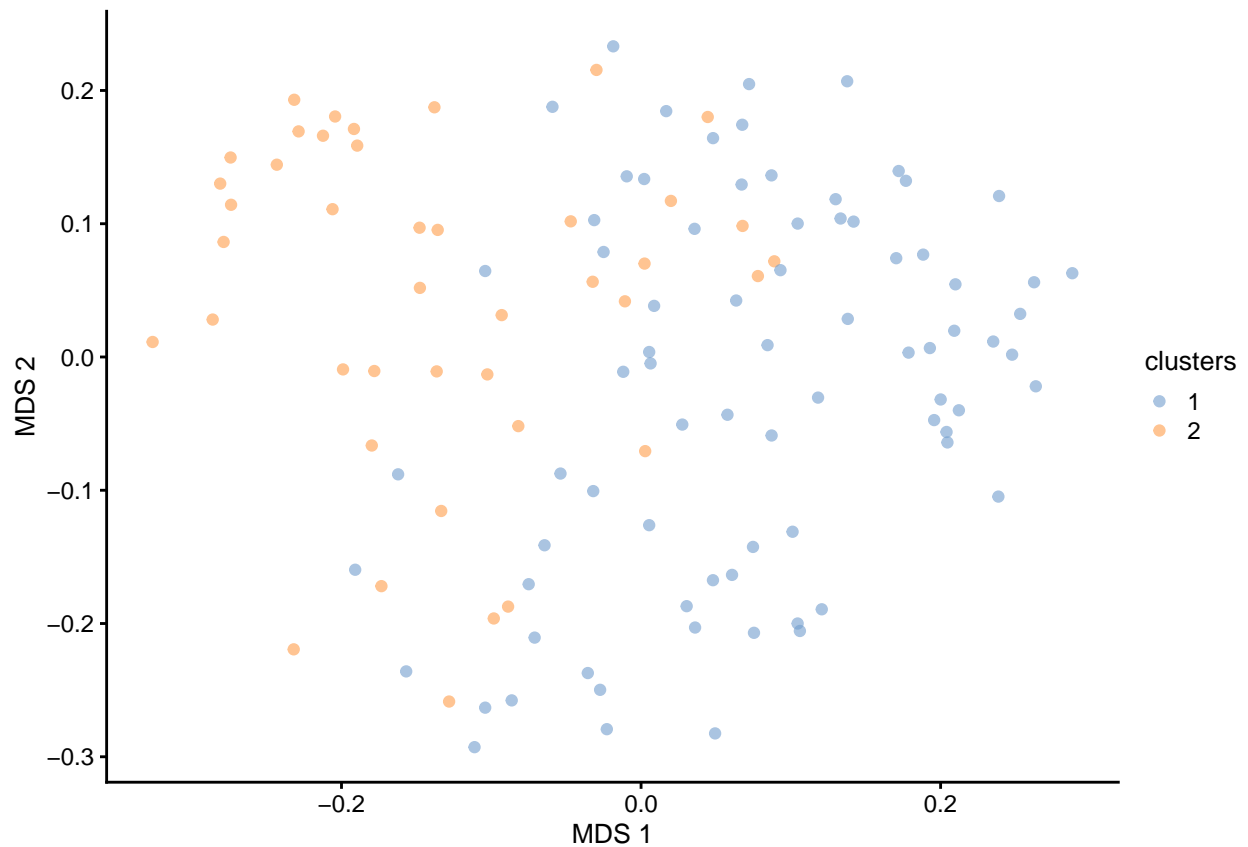
```
fviz_nbclust(diss_bray, cluster::pam, method = "gap_stat") # 1 seems optimal for PAM gap stat
```



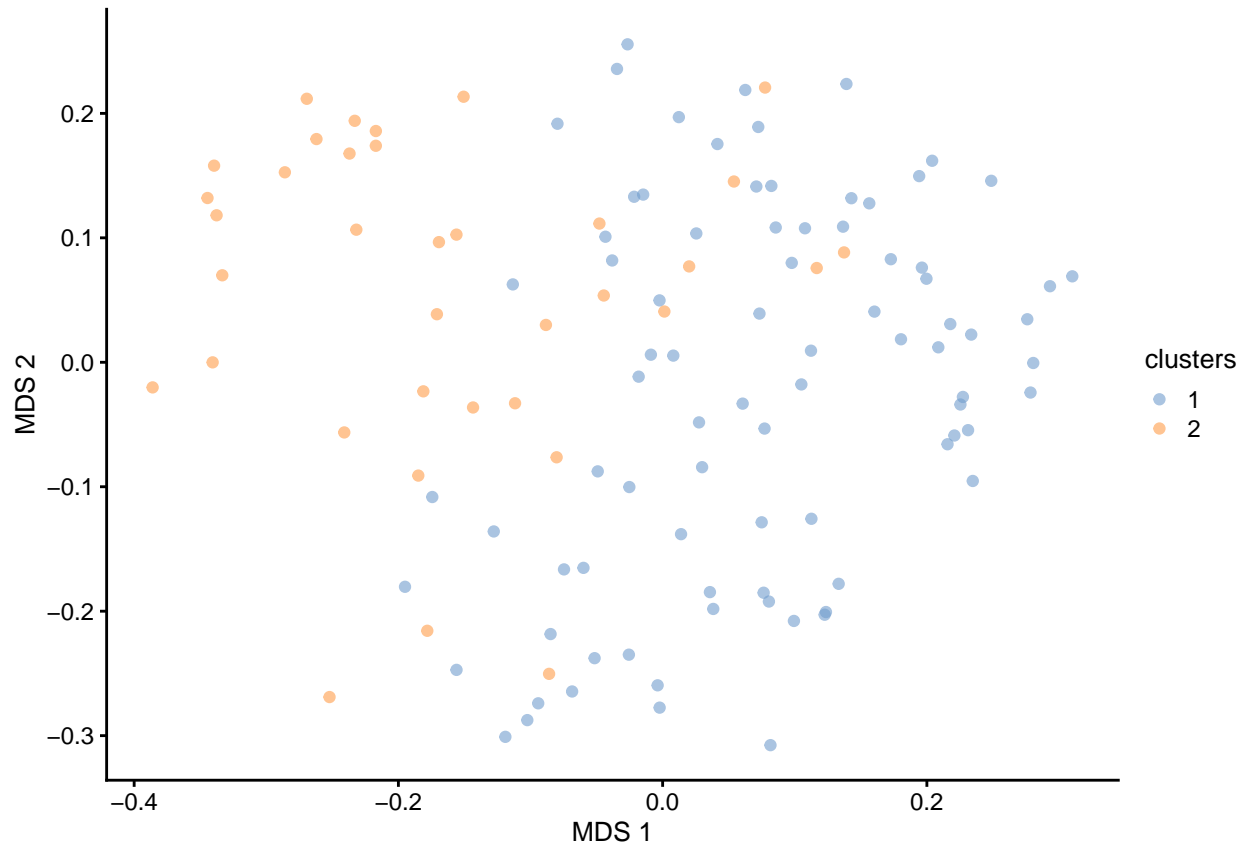
```
fviz_nbclust(diss_bray, hcut, method = "gap_stat") # 1 seems optimal for hcut gap stat
```



```
# k-means jaccard clusters
set.seed(1337)
km <- kmeans(diss_jaccard, 2, nstart = 25)
colData(tse)$clusters <- as.factor(km$cluster)
tse <- runMDS(tse, assay.type = "relabundance", FUN = vegan::vegdist, method = "jaccard")
plotReducedDim(tse, "MDS", colour_by = "clusters")
```



```
# k-means bray clusters MDS
set.seed(1337)
km <- kmeans(diss_bray, 2, nstart = 25)
colData(tse)$clusters <- as.factor(km$cluster)
tse <- runMDS(tse, assay.type = "relabundance", FUN = vegan::vegdist, method = "bray")
plotReducedDim(tse, "MDS", colour_by = "clusters")
```



```
# DMM (Laplace approximation) - ASV level
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse_dmn <- mia::runDMN(tse, name = "DMN", k = 1:7) # calculate most likely number of clusters from 1 to 7
tse_dmn
```

```
## class: TreeSummarizedExperiment
## dim: 1536 120
## metadata(1): DMN
## assays(1): counts
## rownames(1536): 2245974182 2245974034 ... 2245971310 224597986
## rowData names(6): Domain Phylum ... Family Genus
## colnames(120): 2_23 2_24 ... 14_35 14_36
## colData names(27): LibraryNumber Sample_Unique ... Metagenomics Stables
## reducedDimNames(0):
## mainExpName: NULL
## altExpNames(0):
## rowLinks: a LinkDataFrame (1536 rows)
## rowTree: 1 phylo tree(s) (1536 leaves)
## colLinks: NULL
## colTree: NULL
```

```
getDMN(tse_dmn)
```

```
## [[1]]
## class: DMN
```

```

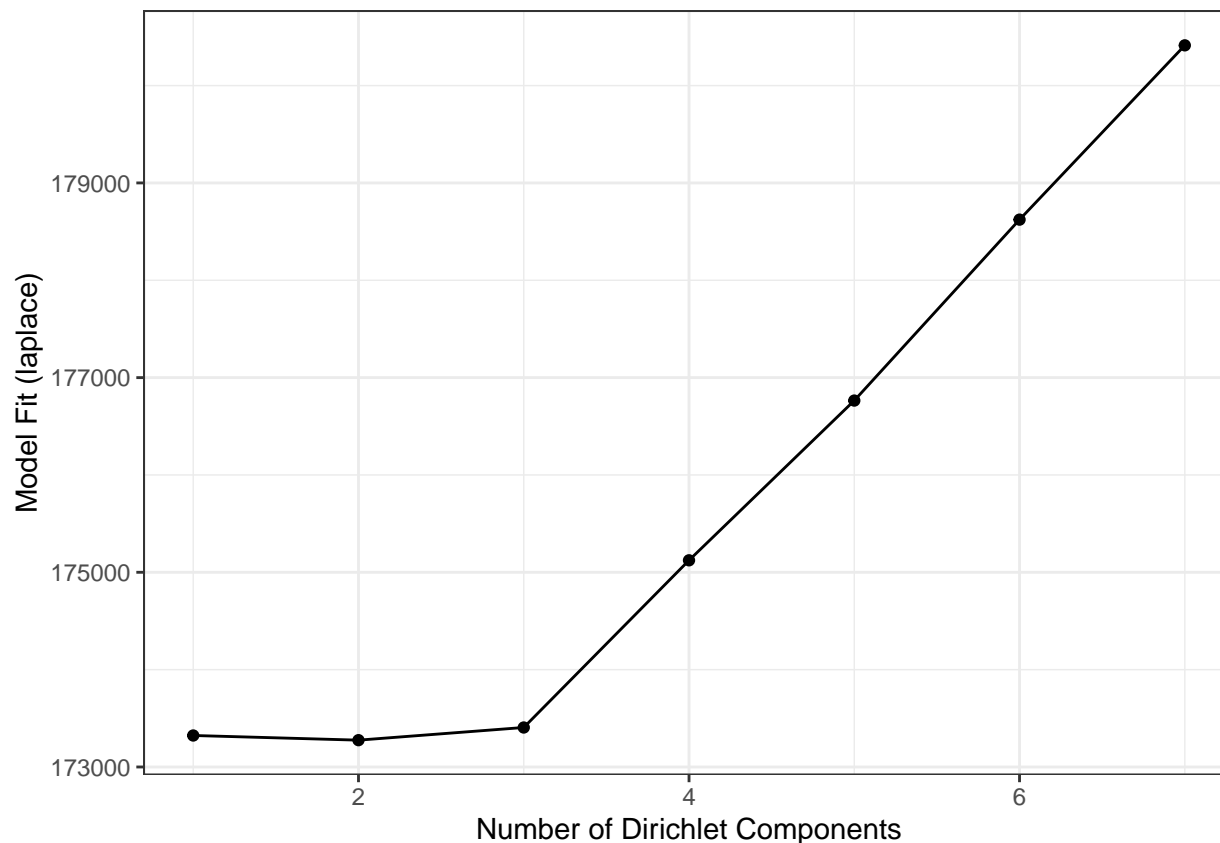
## k: 1
## samples x taxa: 120 x 1536
## Laplace: 173323.4 BIC: 177378.4 AIC: 175237.6
##
## [[2]]
## class: DMN
## k: 2
## samples x taxa: 120 x 1536
## Laplace: 173275.5 BIC: 183190 AIC: 178907.1
##
## [[3]]
## class: DMN
## k: 3
## samples x taxa: 120 x 1536
## Laplace: 173405.6 BIC: 189443.5 AIC: 183018.3
##
## [[4]]
## class: DMN
## k: 4
## samples x taxa: 120 x 1536
## Laplace: 175123.3 BIC: 197172 AIC: 188604.7
##
## [[5]]
## class: DMN
## k: 5
## samples x taxa: 120 x 1536
## Laplace: 176764.2 BIC: 204755.2 AIC: 194045.7
##
## [[6]]
## class: DMN
## k: 6
## samples x taxa: 120 x 1536
## Laplace: 178623.1 BIC: 212663.4 AIC: 199811.7
##
## [[7]]
## class: DMN
## k: 7
## samples x taxa: 120 x 1536
## Laplace: 180413.4 BIC: 220036.1 AIC: 205042.2

```

```

miaViz::plotDMNFit(tse_dmn, type = "laplace")

```



```
getBestDMNFit(tse_dmn, type = "laplace") # 2 again
```

```
## class: DMN
## k: 2
## samples x taxa: 120 x 1536
## Laplace: 173275.5 BIC: 183190 AIC: 178907.1
```

```
# genus level
```

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse_genus <- agglomerateByRank(tse, rank = "Genus", agglomerateTree = TRUE)
tse_dmn <- mia::runDMN(tse_genus, name = "DMN", k = 1:7) # calculate most likely number of clusters from
tse_dmn
```

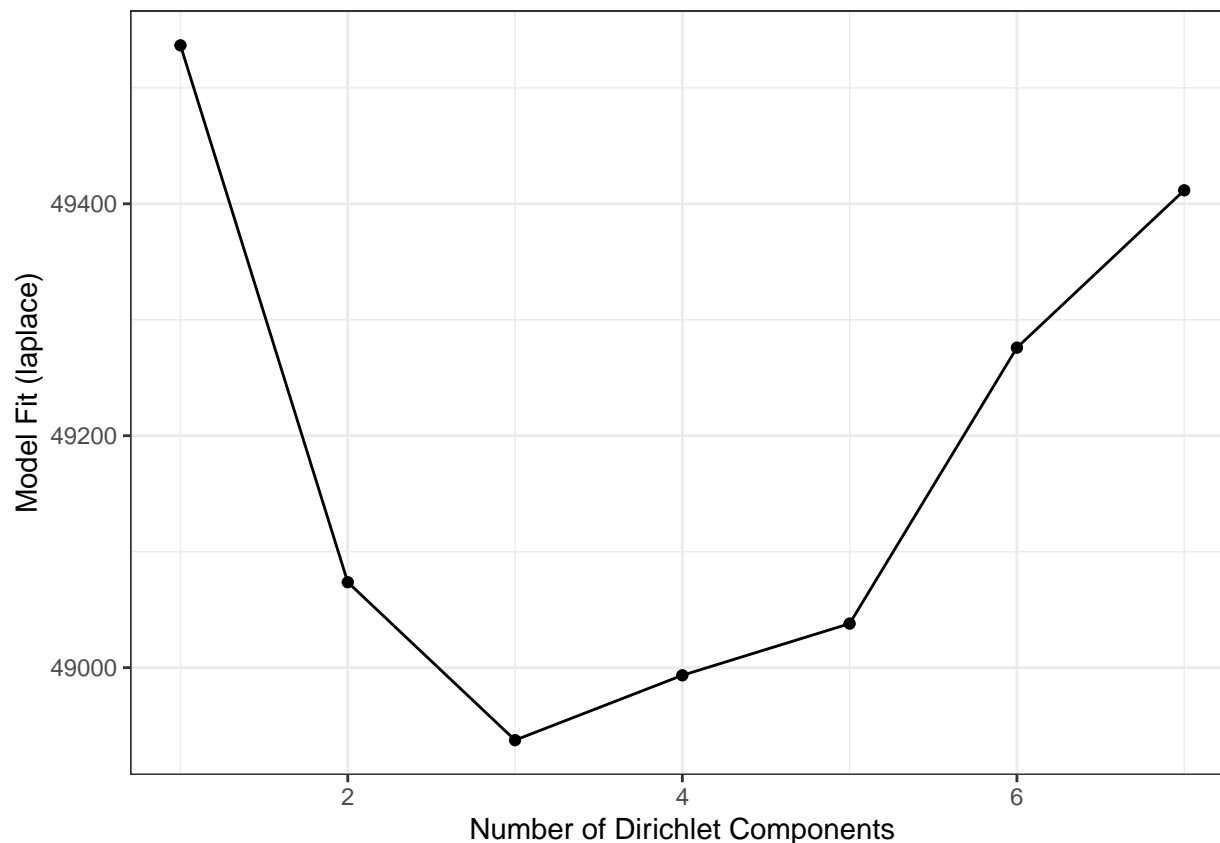
```
## class: TreeSummarizedExperiment
## dim: 119 120
## metadata(2): agglomerated_by_rank DMN
## assays(1): counts
## rownames(119): Genus:g__7_2 Genus:g__5_1 ... Genus:g__Flavonifractor
##   Genus:g__Intestinimonas
## rowData names(6): Domain Phylum ... Family Genus
## colnames(120): 2_23 2_24 ... 14_35 14_36
## colData names(27): LibraryNumber Sample_Unique ... Metagenomics Stables
## reducedDimNames(0):
## mainExpName: NULL
```

```
## altExpNames(0):  
## rowLinks: a LinkDataFrame (119 rows)  
## rowTree: 1 phylo tree(s) (119 leaves)  
## colLinks: NULL  
## colTree: NULL
```

```
getDMN(tse_dmn)
```

```
## [[1]]  
## class: DMN  
## k: 1  
## samples x taxa: 120 x 119  
## Laplace: 49536.64 BIC: 49751.56 AIC: 49585.7  
##  
## [[2]]  
## class: DMN  
## k: 2  
## samples x taxa: 120 x 119  
## Laplace: 49073.65 BIC: 49634.91 AIC: 49301.81  
##  
## [[3]]  
## class: DMN  
## k: 3  
## samples x taxa: 120 x 119  
## Laplace: 48937.44 BIC: 49800.88 AIC: 49300.53  
##  
## [[4]]  
## class: DMN  
## k: 4  
## samples x taxa: 120 x 119  
## Laplace: 48993.38 BIC: 50167.48 AIC: 49499.88  
##  
## [[5]]  
## class: DMN  
## k: 5  
## samples x taxa: 120 x 119  
## Laplace: 49037.94 BIC: 50571.21 AIC: 49736.36  
##  
## [[6]]  
## class: DMN  
## k: 6  
## samples x taxa: 120 x 119  
## Laplace: 49275.92 BIC: 51124.69 AIC: 50122.58  
##  
## [[7]]  
## class: DMN  
## k: 7  
## samples x taxa: 120 x 119  
## Laplace: 49411.63 BIC: 51635.61 AIC: 50466.26
```

```
miaViz::plotDMNFit(tse_dmn, type = "laplace")
```

```
getBestDMNFit(tse_dmn, type = "laplace") # Gives 3! as best fit for genus level data
```

```
## class: DMN
## k: 3
## samples x taxa: 120 x 119
## Laplace: 48937.44 BIC: 49800.88 AIC: 49300.53
```

```
# phylum level
```

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse_phylum <- agglomerateByRank(tse, rank = "Phylum", agglomerateTree = TRUE)
tse_dmn <- mia::runDMN(tse_phylum, name = "DMN", k = 1:7) # calculate most likely number of clusters fr
tse_dmn
```

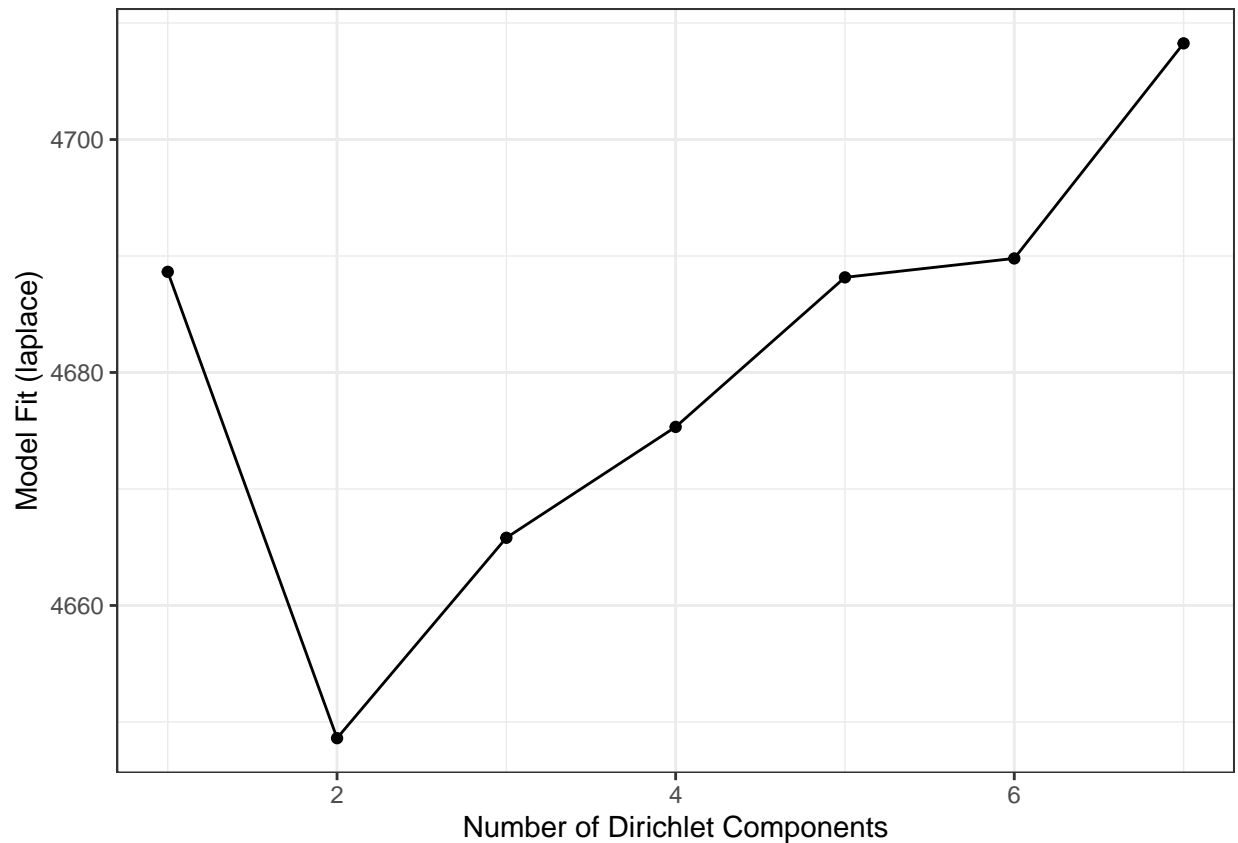
```
## class: TreeSummarizedExperiment
## dim: 7 120
## metadata(2): agglomerated_by_rank DMN
## assays(1): counts
## rownames(7): Phylum:p__Tenericutes Phylum:p__Firmicutes ...
##   Phylum:p__Verrucomicrobia Phylum:p__Cyanobacteria
## rowData names(6): Domain Phylum ... Family Genus
## colnames(120): 2_23 2_24 ... 14_35 14_36
## colData names(27): LibraryNumber Sample_Unique ... Metagenomics Stables
## reducedDimNames(0):
## mainExpName: NULL
```

```
## altExpNames(0):  
## rowLinks: a LinkDataFrame (7 rows)  
## rowTree: 1 phylo tree(s) (7 leaves)  
## colLinks: NULL  
## colTree: NULL
```

```
getDMN(tse_dmn)
```

```
## [[1]]  
## class: DMN  
## k: 1  
## samples x taxa: 120 x 7  
## Laplace: 4688.641 BIC: 4697.146 AIC: 4687.39  
##  
## [[2]]  
## class: DMN  
## k: 2  
## samples x taxa: 120 x 7  
## Laplace: 4648.607 BIC: 4667.756 AIC: 4646.85  
##  
## [[3]]  
## class: DMN  
## k: 3  
## samples x taxa: 120 x 7  
## Laplace: 4665.811 BIC: 4689.917 AIC: 4657.861  
##  
## [[4]]  
## class: DMN  
## k: 4  
## samples x taxa: 120 x 7  
## Laplace: 4675.328 BIC: 4705.498 AIC: 4662.292  
##  
## [[5]]  
## class: DMN  
## k: 5  
## samples x taxa: 120 x 7  
## Laplace: 4688.166 BIC: 4721.189 AIC: 4666.833  
##  
## [[6]]  
## class: DMN  
## k: 6  
## samples x taxa: 120 x 7  
## Laplace: 4689.791 BIC: 4746.113 AIC: 4680.607  
##  
## [[7]]  
## class: DMN  
## k: 7  
## samples x taxa: 120 x 7  
## Laplace: 4708.256 BIC: 4768.619 AIC: 4691.963
```

```
miaViz::plotDMNFit(tse_dmn, type = "laplace")
```



```
getBestDMNFit(tse_dmn, type = "laplace") # Gives 2 as best fit for phylum level data
```

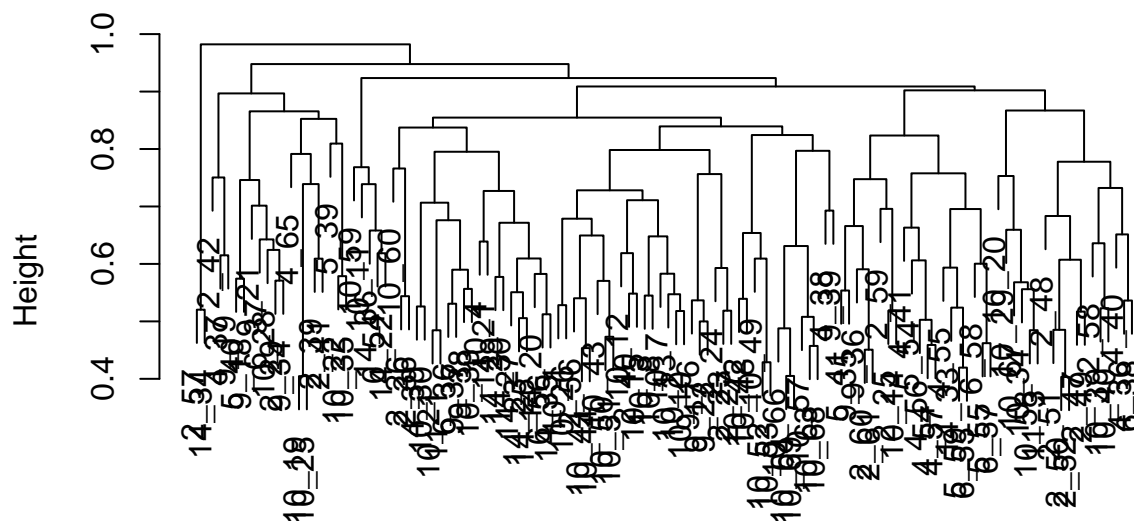
```
## class: DMN
## k: 2
## samples x taxa: 120 x 7
## Laplace: 4648.607 BIC: 4667.756 AIC: 4646.85
```

Hierarchical clustering BC on ASV level

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse <- transformCounts(tse, method = "relabundance")
tse <- runMDS(tse,
  assay.type = "relabundance",
  FUN = vegan::vegdist,
  method = "bray")

hc_bray <- hclust(vegdist(t(assay(tse, "relabundance"))), method = "bray"), method = "complete")
plot(hc_bray)
```

Cluster Dendrogram



```
vegdist(t(assay(tse, "relabundance")), method = "bray")
hclust(*, "complete")
```

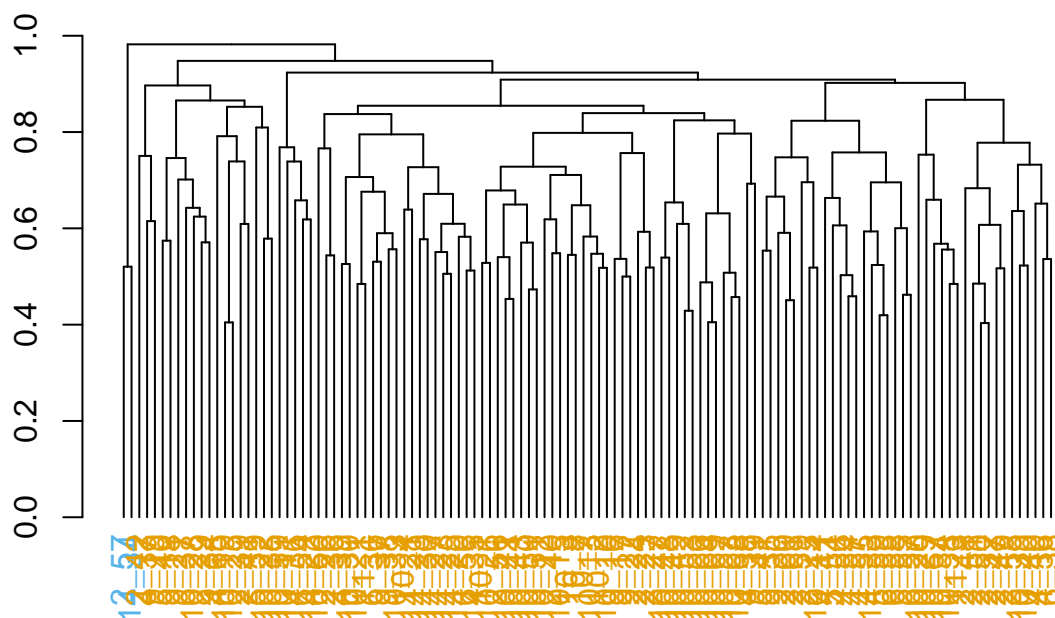
```
hcd = as.dendrogram(hc_bray)

cbPalette <- c("#999999", "#E69F00", "#56B4E9", "#009E73", "#F0E442",
              "#0072B2", "#D55E00", "#CC79A7")

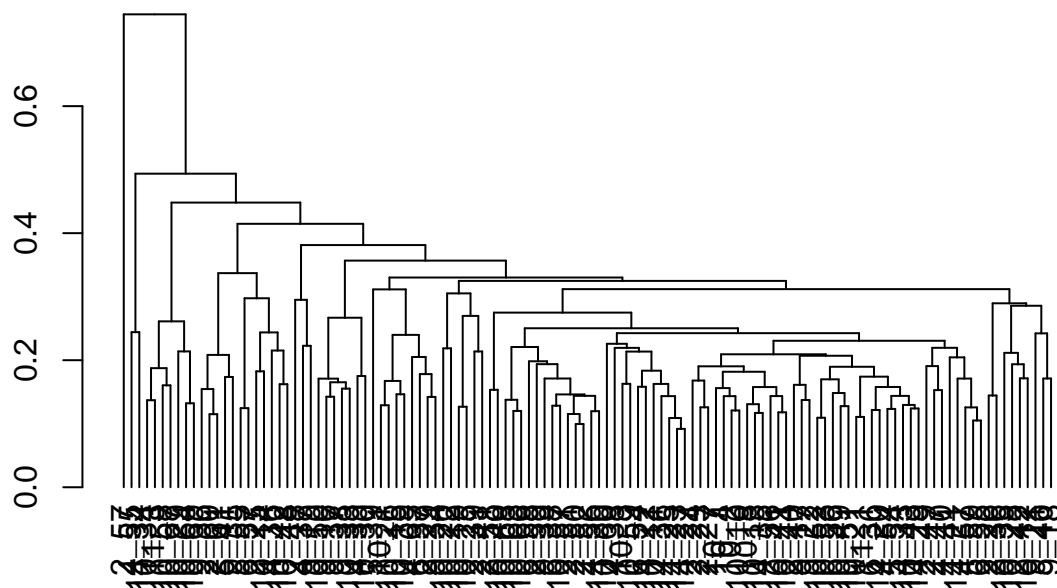
colorCode <- c(Control=cbPalette[2], CRC = cbPalette[3])

grouping = cutree(hc_bray, k = 2) # most methods gave 2 clusters, based on cuttree

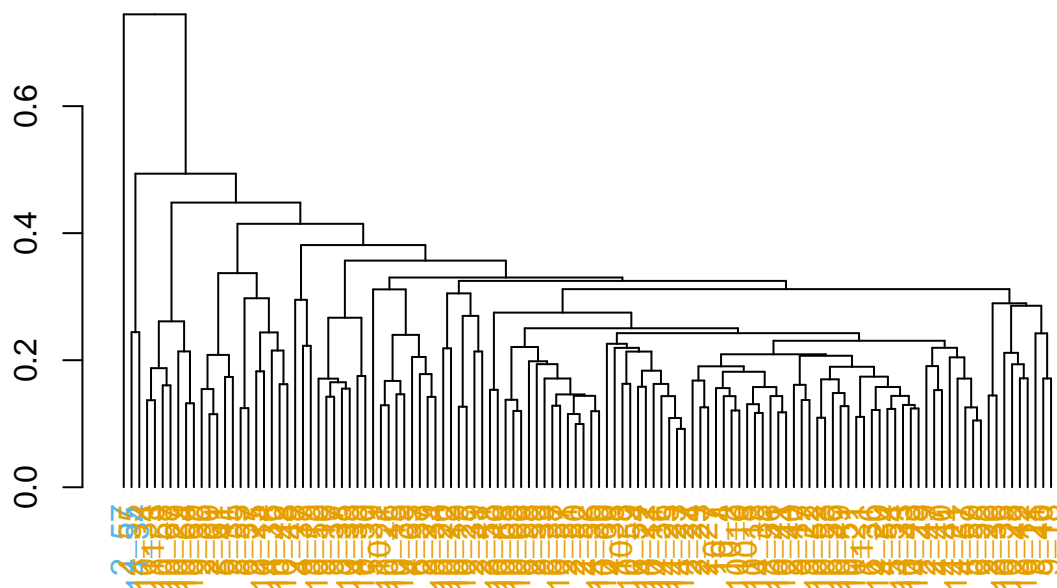
labels_colors(hcd) <- colorCode[grouping][order.dendrogram(hcd)]
plot(hcd)
```



```
hclust.out <- clusterRows(assay, HclustParam(method = "complete"), full = TRUE) # cutting based on comp
colData(tse)$clusters <- hclust.out$clusters
dendro <- as.dendrogram(hclust.out$objects$hclust)
plot(dendro)
```

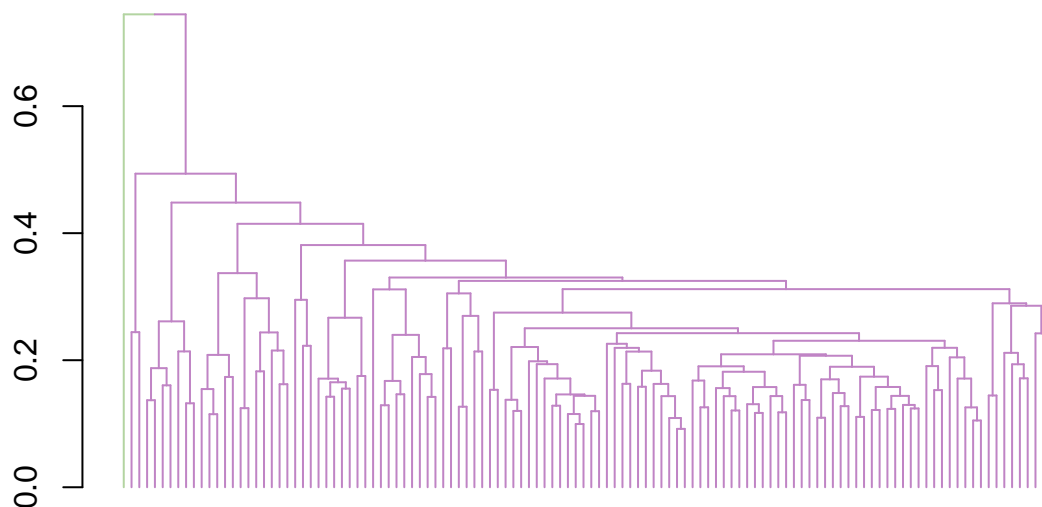


```
labels_colors(dendro) <- colorCode[grouping][order.dendrogram(dendro)]
plot(dendro)
```



```
col_val_map <- randomcoloR::distinctColorPalette("2") %>%
  as.list() %>%
  setNames(paste0("clust_", seq("2")))

dend <- color_branches(dendro, k = 2, col = unlist(col_val_map))
labels(dend) <- NULL
plot(dend) # based on all three visualisations, only a few samples are clustered distinctly, based on s
```



PAM clustering

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)

tse <- transformCounts(tse, method = "relabundance")

pam.out <- clusterCells(tse,
                        assay.type = "relabundance",
                        BLUSPARAM = PamParam(centers = 2))

pam.out
```

```
## 2_23 2_24 2_25 2_26 2_27 2_29 2_36 2_39 2_40 2_41 2_42 2_47 2_48
## 1 1 2 1 1 1 1 1 1 1 1 1 1
## 2_49 2_50 2_51 2_52 2_56 2_57 2_58 2_59 2_60 2_61 4_36 4_37 4_38
## 1 1 1 1 1 1 1 2 2 2 2 1 1
## 4_39 4_40 4_41 4_54 4_55 4_56 4_57 4_65 5_39 5_40 5_41 5_54 5_55
## 1 1 1 1 2 1 1 1 1 1 2 1 1
## 5_59 6_36 6_37 6_38 6_54 6_55 6_56 6_57 6_58 9_16 9_17 9_18 9_19
## 1 1 1 1 1 1 1 1 1 1 1 1 1
## 9_21 9_22 9_34 9_35 9_36 9_37 9_38 9_39 10_1 10_2 10_3 10_4 10_7
## 1 1 1 2 2 1 1 1 1 1 1 1 1
## 10_8 10_10 10_11 10_12 10_13 10_14 10_15 10_19 10_20 10_21 10_22 10_25 10_26
## 1 1 1 1 1 2 1 1 2 1 1 1 1
## 10_28 10_29 10_30 10_33 10_34 10_35 10_39 10_40 10_41 10_42 10_43 10_44 10_48
## 1 2 1 1 2 1 1 1 1 1 1 1 1
```



```
## 10_49 10_50 10_51 10_52 10_53 10_57 10_58 10_59 10_60 10_63 10_64 10_66 10_67
##      1      1      1      1      1      2      2      1      1      2      2      2      2
## 10_68 10_69 11_1  11_3 14_20 14_21 14_22 14_23 14_25 14_27 14_29 14_30 14_33
##      2      2      1      2      1      1      1      1      1      1      1      1      1
## 14_34 14_35 14_36
##      1      1      1
## Levels: 1 2
```

```
n_iterations <- 1000
previous_cluster_assignment <- NULL
cluster_assignments <- list()

# loop that runs PAM clusterings X times and stores the results in a list, additionally checks if any c
for (i in 1:n_iterations) {
  result <- clusterCells(tse, assay.type = "relabundance", BLUSPARAM = PamParam(centers = 2))
  cluster_assignments[[i]] <- result

  # Check if cluster assignments have changed
  if (!is.null(previous_cluster_assignment)) {
    samples_changed <- which(result != previous_cluster_assignment)
    if (length(samples_changed) > 0) {
      cat(sprintf("In iteration %d, the following samples changed clusters: %s\n", i, paste(samples_cha
    })
  }
  previous_cluster_assignment <- result
}

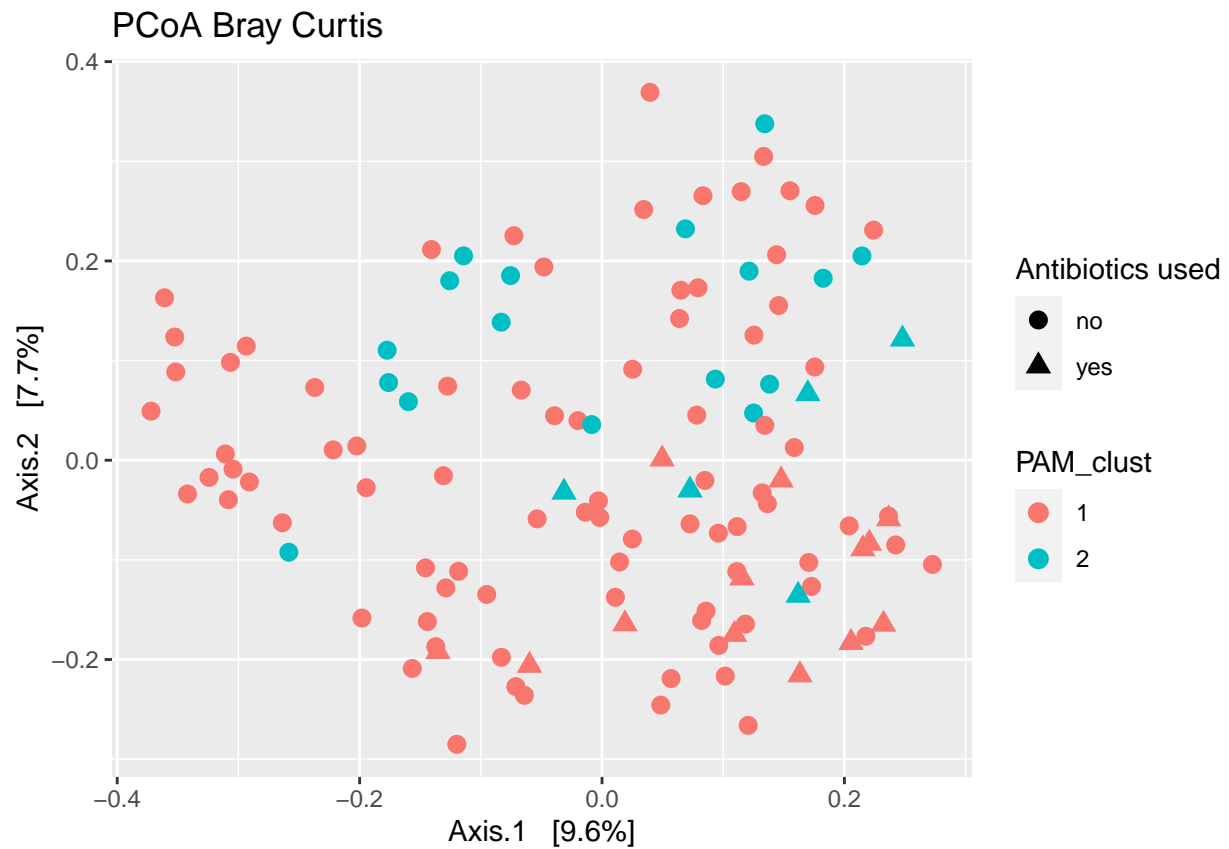
# To see if all of the clusters are the same or not
if (all(sapply(cluster_assignments, identical, cluster_assignments[[1]]))) {
  cat("All cluster assignments are the same across iterations.\n")
} else {
  cat("Cluster assignments vary across iterations.\n")
}
```

```
## All cluster assignments are the same across iterations.
```

```
# There are no differences in clusters when run 1000 times

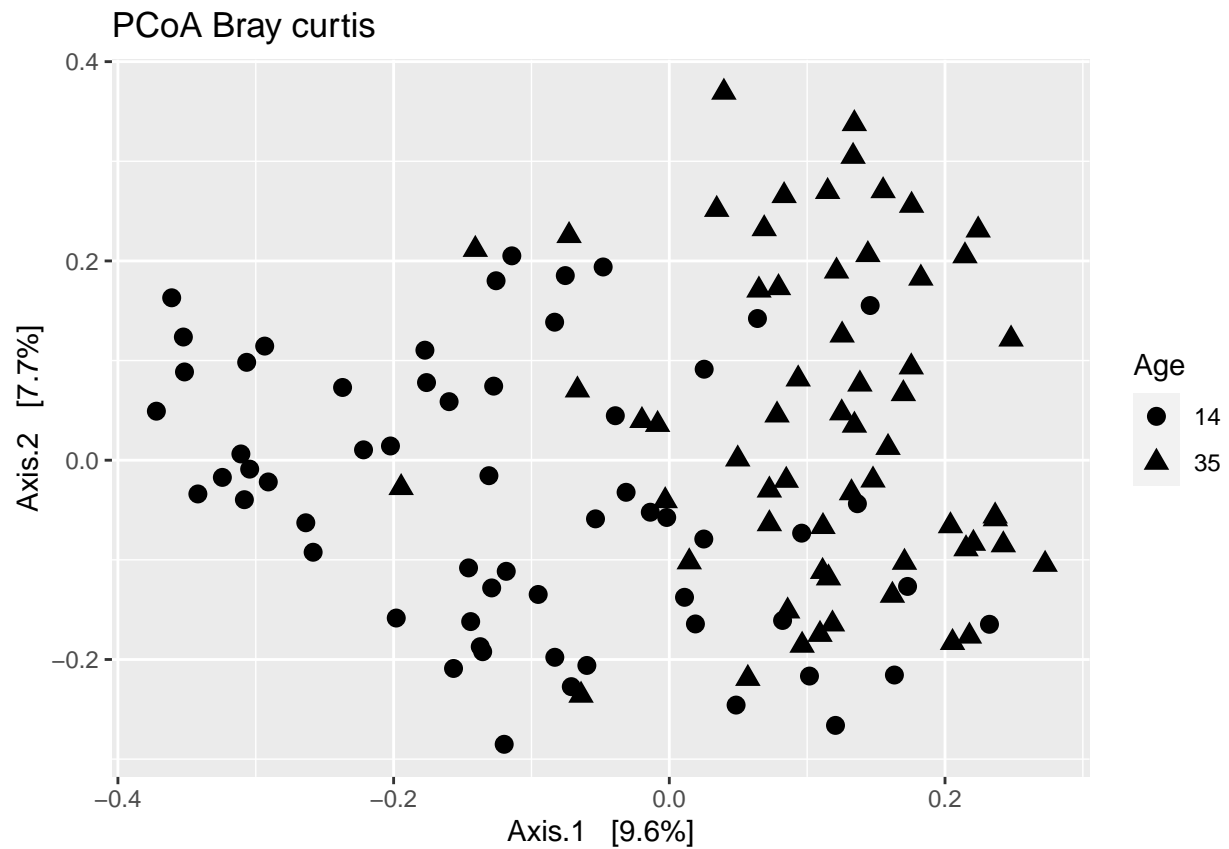
# save to metadata and make original PCoA plot
subset16S@sam_data$PAM_clust = pam.out
sample_data(subset16S)$PAM_clust = as.factor(sample_data(subset16S)$PAM_clust)
pcoa_bc = ordinate(subset16S, "PCoA", "bray")

plot_pcoa_ordination(subset16S, pcoa_bc, "PAM_clust", "PCoA Bray Curtis")
```

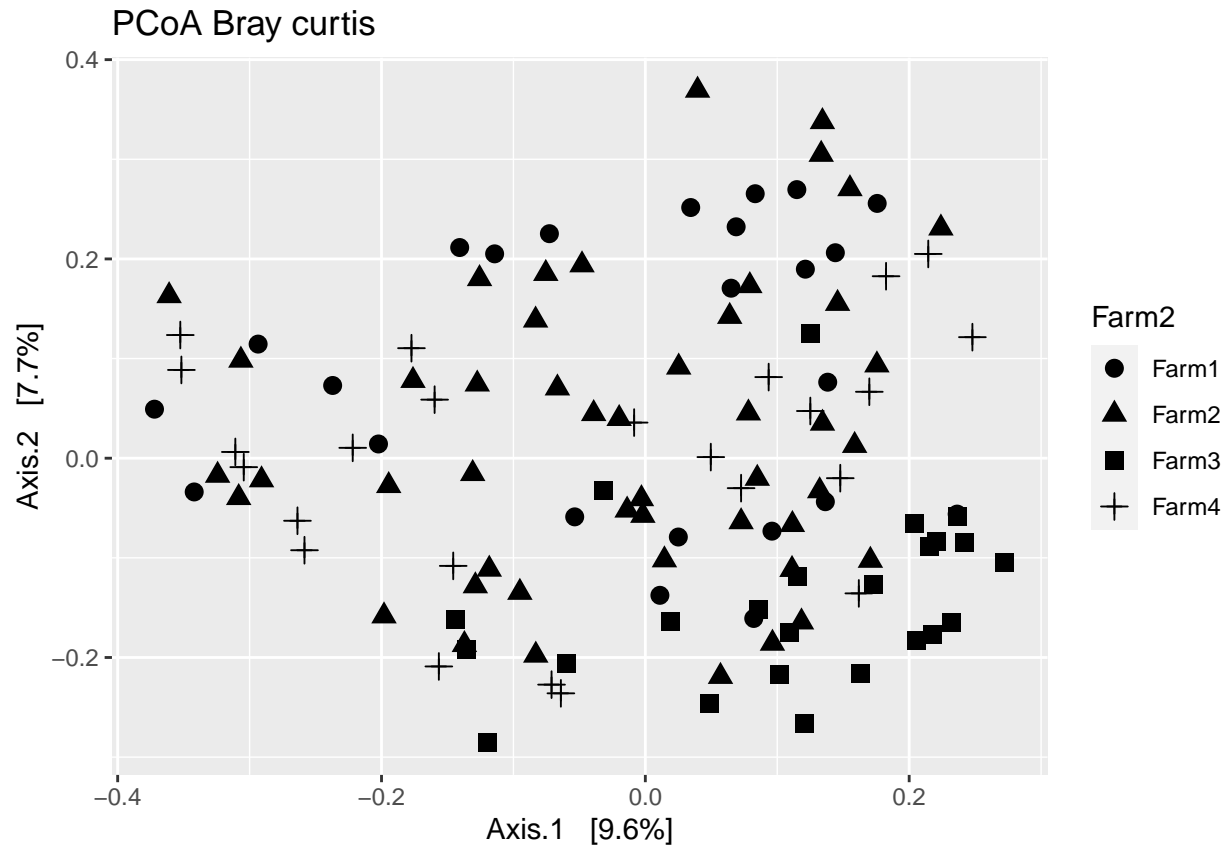


```
#plot_pcoa_ordination(subset16S, pcoa_bc, "Cluster", "PCoA Bray Curtis")

# change shape to different variables, age
plot_ordination(subset16S, pcoa_bc, color = "PAM", shape = "Age") +
  geom_point(size = 3) + labs(title = "PCoA Bray curtis", color = "AMR_class_primary")
```



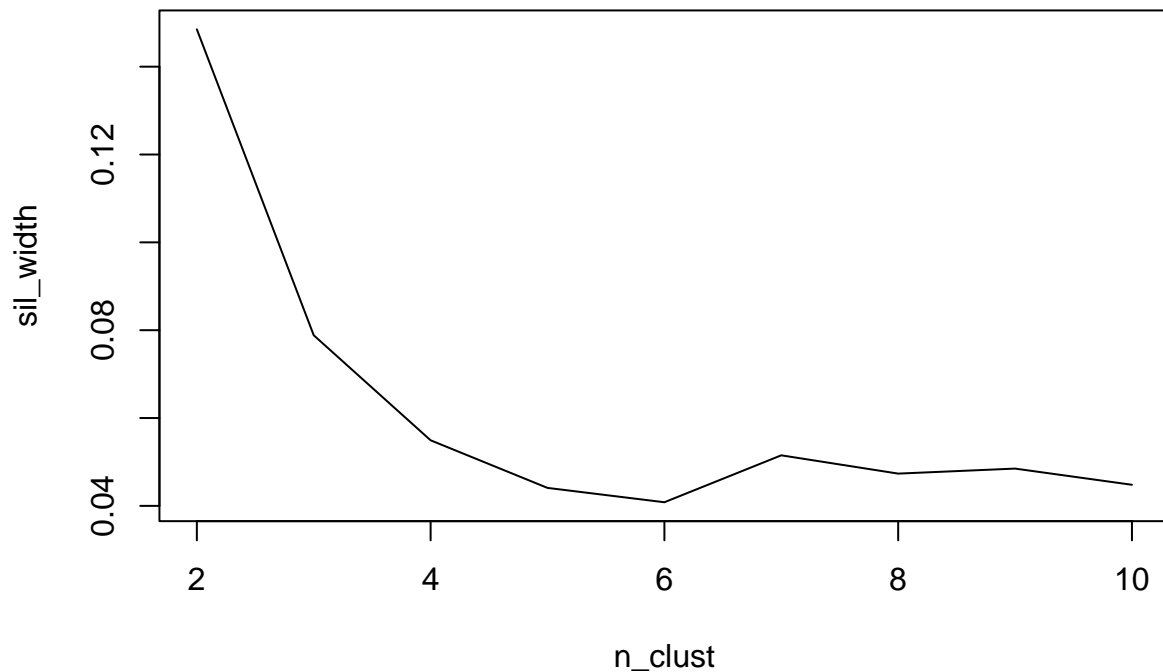
```
# change shape to different variables, farm
plot_ordination(subset16S, pcoa_bc, color = "PAM", shape = "Farm2") +
  geom_point(size = 3) + labs(title = "PCoA Bray curtis", color = "AMR_class_primary")
```



Create PAM UF PCoA - from 2 to 10 clusters

```
phy_rel <- transform_sample_counts(subset16S, function(x) log10(x+1/sum(x+1)))
UF <- UniFrac(phy_rel, weighted = TRUE)
n_clust <- 2:10
pam_list <- lapply(n_clust, function(x) pam(UF, k = x))

sil_width <- lapply(pam_list, function(x) mean(x$silinfo$widths[, "sil_width"]))
plot(n_clust, sil_width, type="l")
```



```
pcoa_data <- cmdscale(UF, eig = TRUE)
pcoa_df <- data.frame(PC1 = c(pcoa_data$points[,1]),
                     PC2 = c(pcoa_data$points[,2]),
                     Sample = rownames(pcoa_data$points))

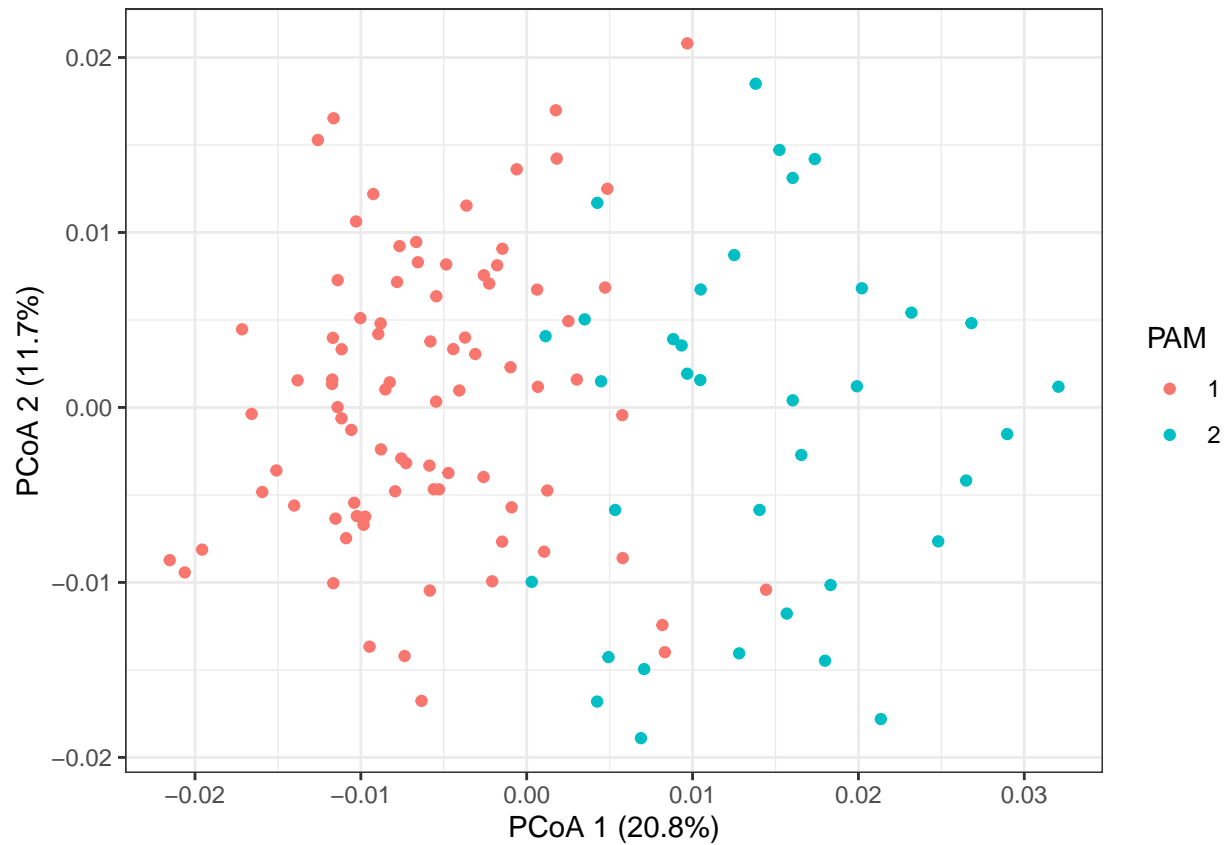
# Add sample data
Samp <- data.frame(sample_data(subset16S))
Samp$Sample <- sample_names(subset16S)

pcoa_df <- merge(pcoa_df, Samp, by = "Sample")

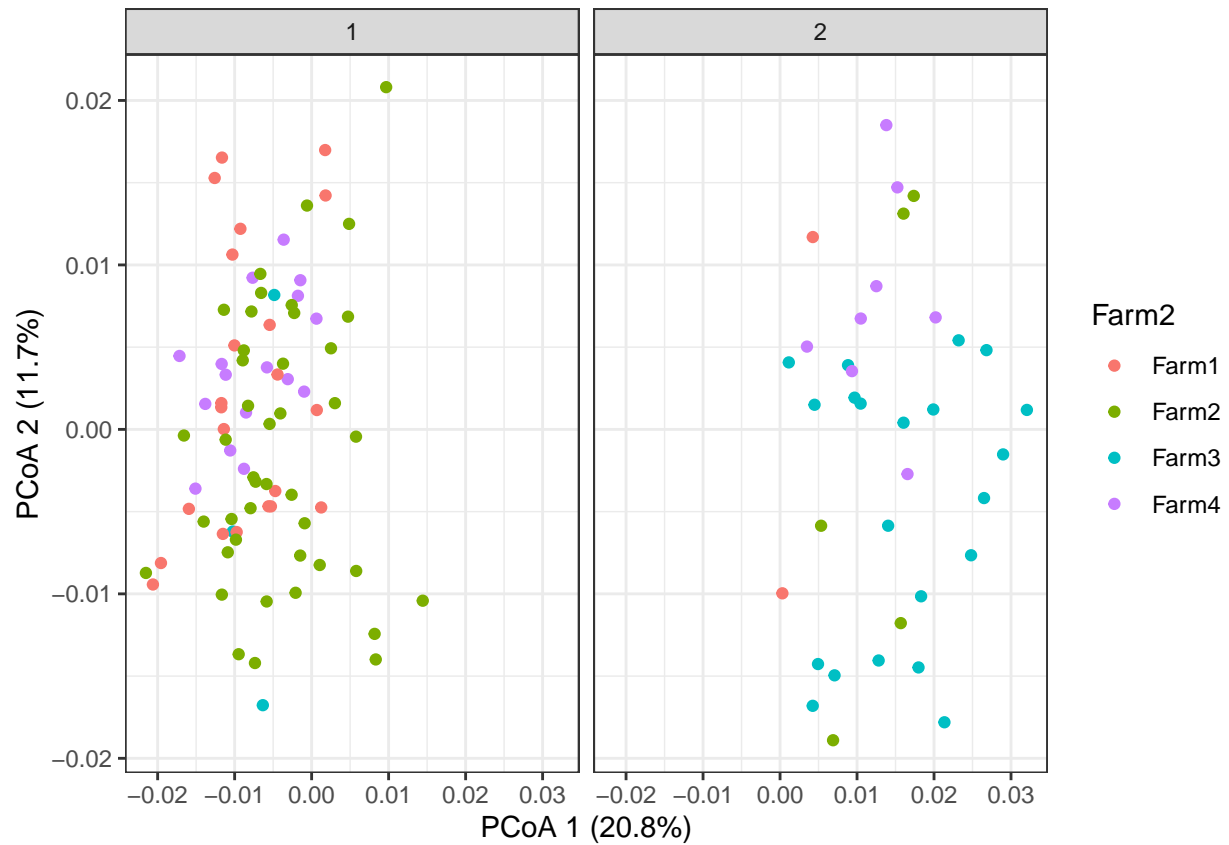
# Add cluster information
clusters <- factor(pam_list[[which.max(sil_width)]]$clustering)
pcoa_df <- merge(pcoa_df, clusters, by.x = "Sample", by.y = "row.names")
colnames(pcoa_df)[ncol(pcoa_df)] <- "PAM"

# Variance explained
ve <- pcoa_data$eig/sum(pcoa_data$eig)

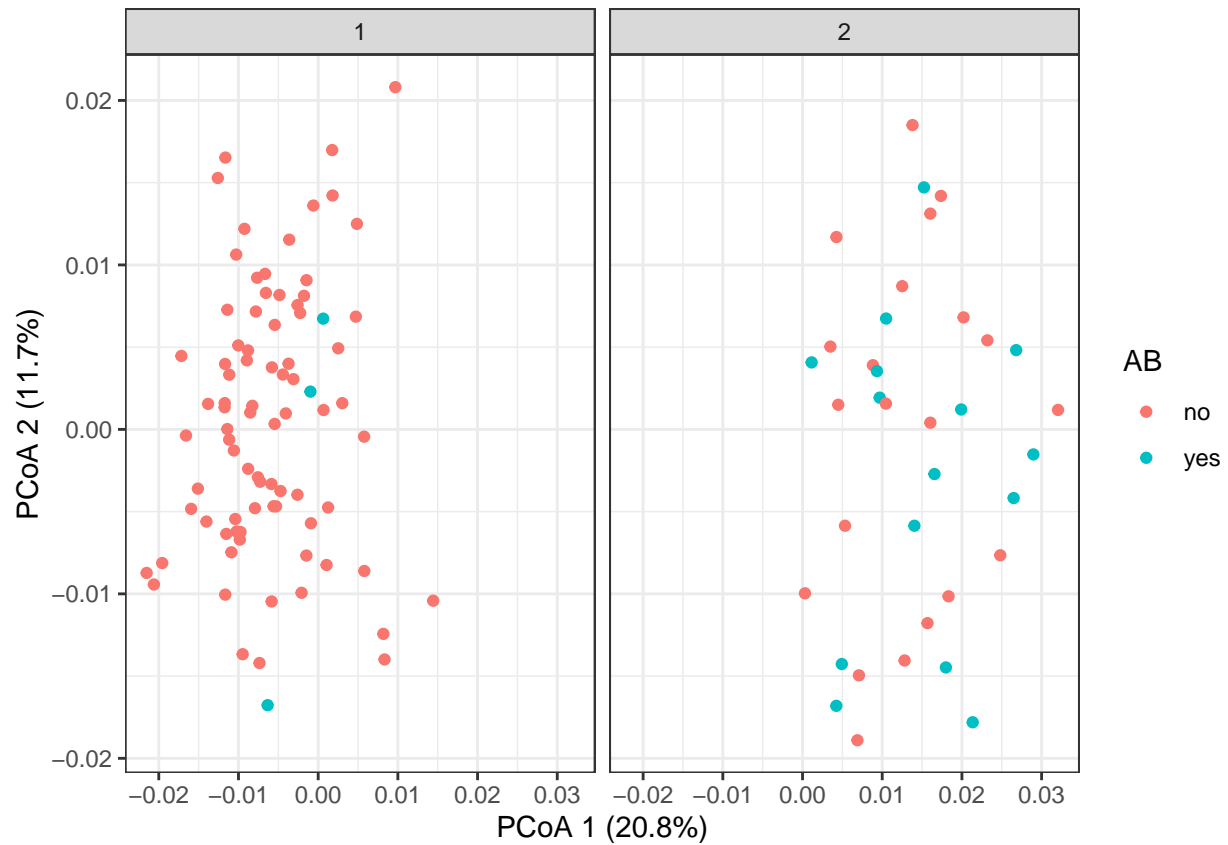
# Plot
ggplot(pcoa_df, aes(x = PC1, y = PC2, color = PAM)) +
  theme_bw() +
  geom_point() +
  xlab(paste0("PCoA 1 (", round(ve[1]*100, 1), "%)")) +
  ylab(paste0("PCoA 2 (", round(ve[2]*100, 1), "%)"))
```



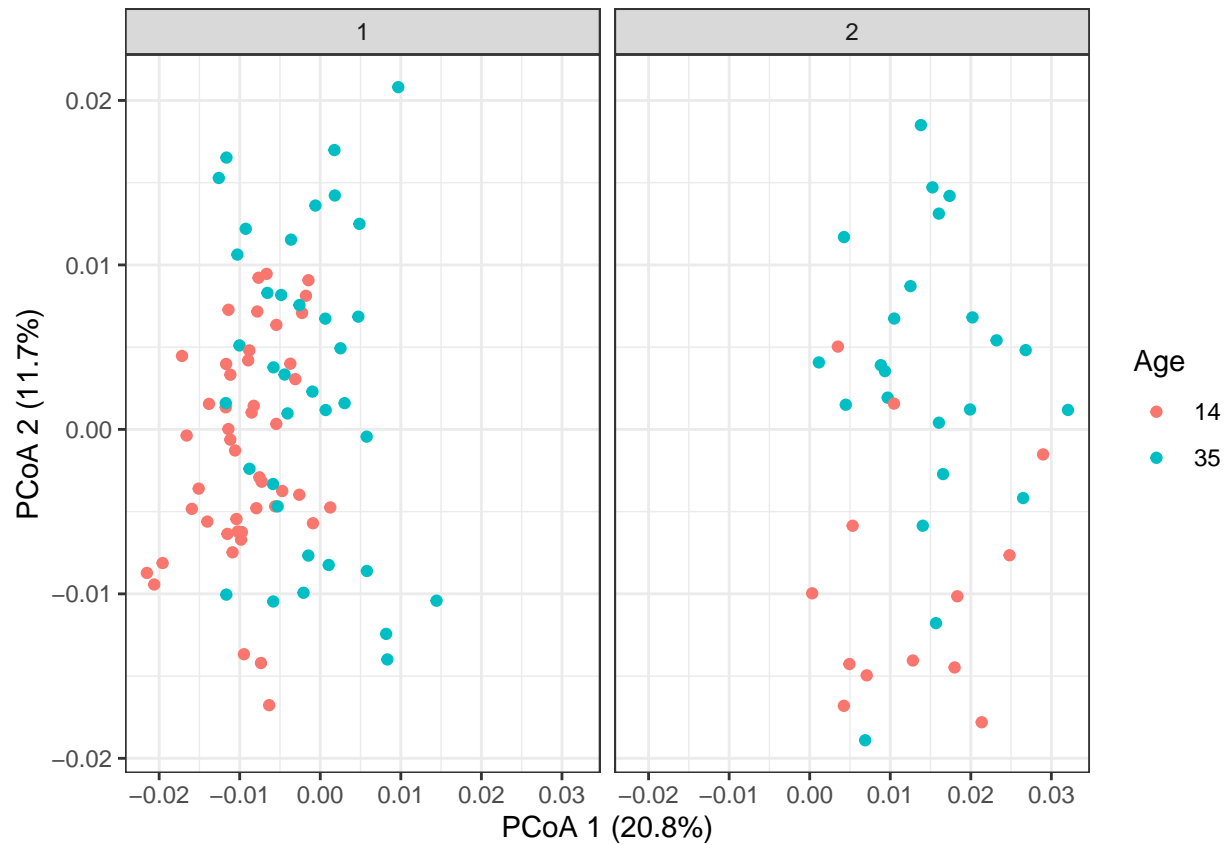
```
# facet by clusters and colour by farm
ggplot(pcoa_df, aes(x = PC1, y = PC2, color = Farm2)) +
  theme_bw() +
  geom_point() +
  xlab(paste0("PCoA 1 (",round(ve[1]*100,1),"%)" )) +
  ylab(paste0("PCoA 2 (",round(ve[2]*100,1),"%)" )) +
  facet_wrap(~PAM)
```



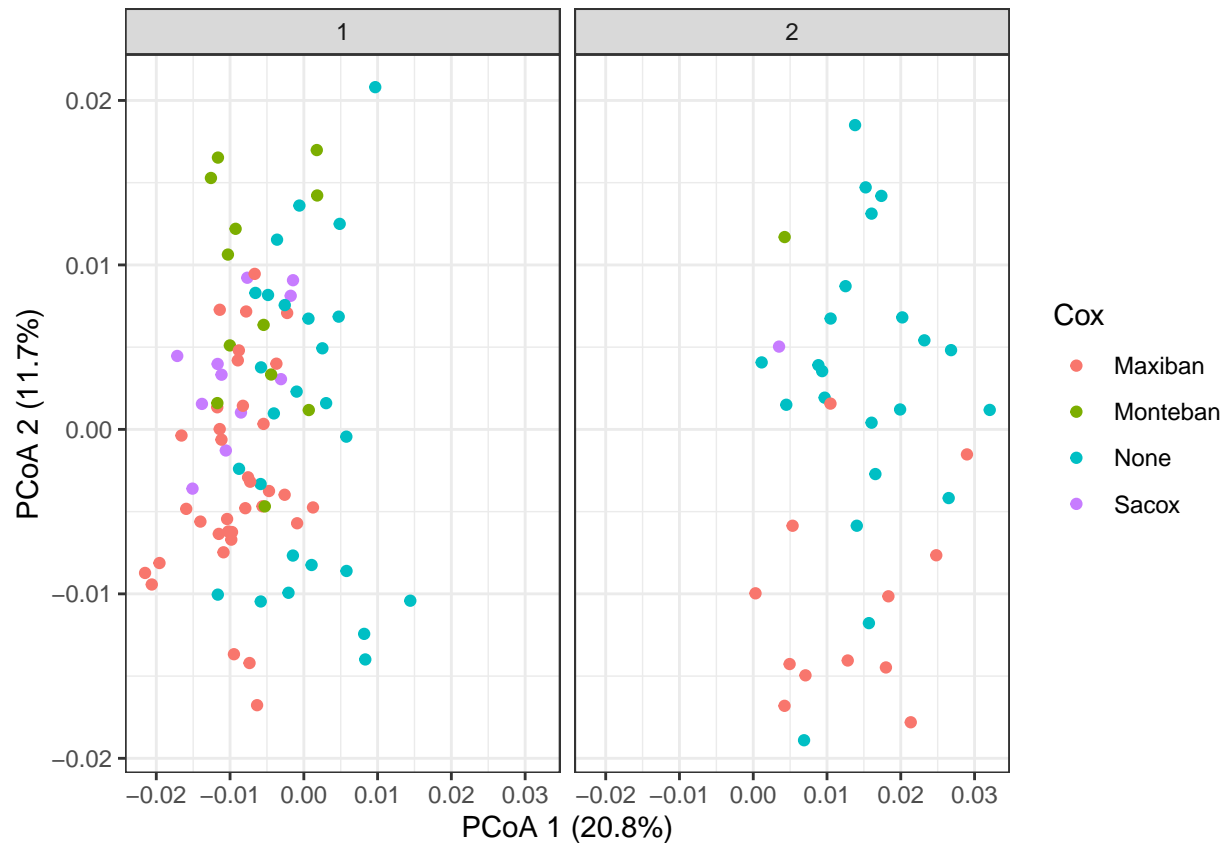
```
# facet by clusters and colour by AB
ggplot(pcoa_df, aes(x = PC1, y = PC2, color = AB)) +
  theme_bw() +
  geom_point() +
  xlab(paste0("PCoA 1 (",round(ve[1]*100,1),"%)" )) +
  ylab(paste0("PCoA 2 (",round(ve[2]*100,1),"%)" )) +
  facet_wrap(~PAM)
```



```
# facet by clusters and colour by Age
ggplot(pcoa_df, aes(x = PC1, y = PC2, color = Age)) +
  theme_bw() +
  geom_point() +
  xlab(paste0("PCoA 1 (",round(ve[1]*100,1),"%)")) +
  ylab(paste0("PCoA 2 (",round(ve[2]*100,1),"%)")) +
  facet_wrap(~PAM)
```

```
# facet by clusters and colour by Agent
ggplot(pcoa_df, aes(x = PC1, y = PC2, color = Cox)) +
  theme_bw() +
  geom_point() +
  xlab(paste0("PCoA 1 (",round(ve[1]*100,1),"%)" )) +
  ylab(paste0("PCoA 2 (",round(ve[2]*100,1),"%)" )) +
  facet_wrap(~PAM)
```



PCoA for Phylum data, BC with DMM, euclidian (can change tax level with tse_dmn)

```
dmn_group <- calculateDMNgroup(tse_dmn,
                              variable = "Age", assay.type = "counts",
                              k = 2, seed = .Machine$integer.max)

dmn_group <- calculateDMNgroup(tse_dmn,
                              variable = "Farm2", assay.type = "counts",
                              k = 2, seed = .Machine$integer.max)

dmn_group <- calculateDMNgroup(tse_dmn,
                              variable = "AB", assay.type = "counts",
                              k = 2, seed = .Machine$integer.max)

dmn_group
```

```
## class: DMNGroup
## summary:
##      k samples taxa      NLE  LogDet  Laplace      BIC      AIC
## no  2      102    7 3831.1379 59.71807 3847.2128 3865.8252 3846.1379
## yes 2       18    7  818.2031 47.31140  828.0747  839.8809  833.2031
```

```
DirichletMultinomial::mixturewt(getBestDMNFit(tse_dmn)) # measure weights
```

```
##      pi      theta
## 1 0.628418 31.806259
## 2 0.371582  6.573224
```

```
head(DirichletMultinomial::mixture(getBestDMNFit(tse_dmn))) # sample-cluster assignment probabilities
```

```
##           [,1]      [,2]
## 2_23 0.9727179084 0.02728209
## 2_24 0.0175821347 0.98241787
## 2_25 0.0010190173 0.99898098
## 2_26 0.0010546064 0.99894539
## 2_27 0.9280199742 0.07198003
## 2_29 0.0008818687 0.99911813
```

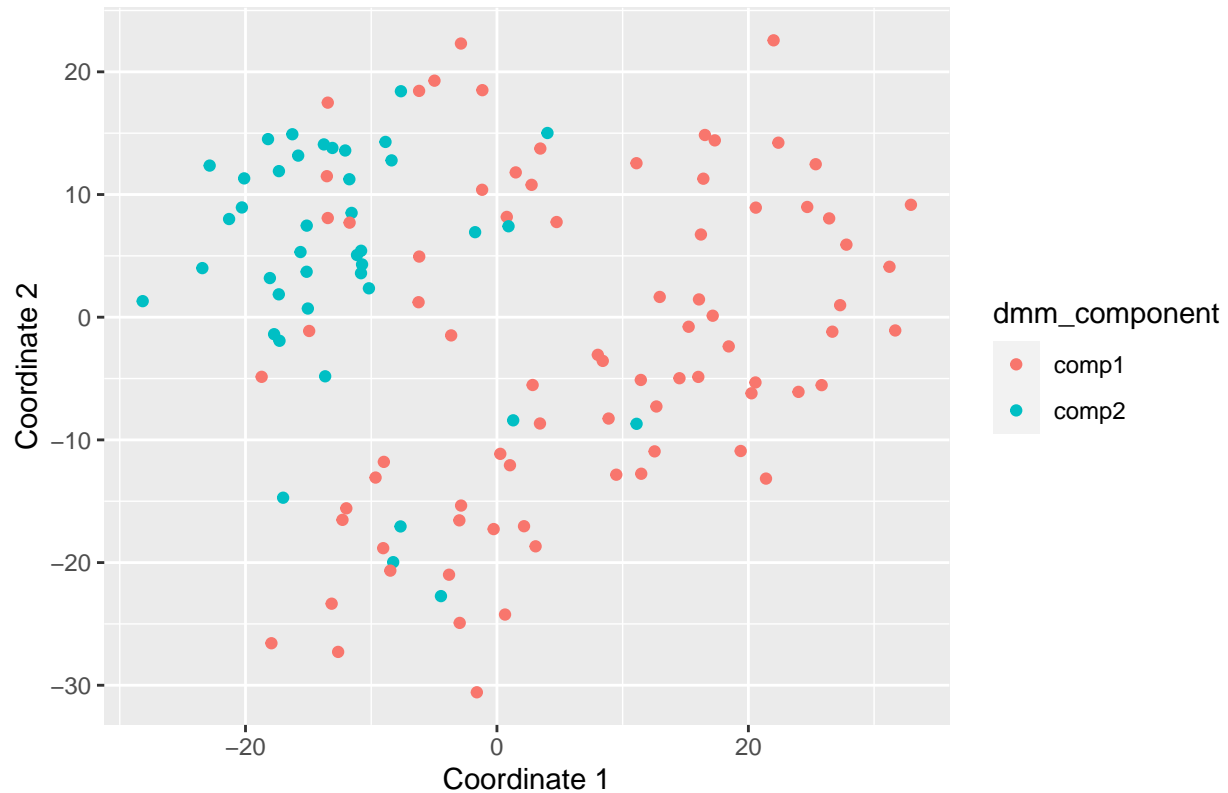
```
head(DirichletMultinomial::fitted(getBestDMNFit(tse_dmn))) # taxa contribution
```

```
##           [,1]      [,2]
## Phylum:p__Tenericutes      1.09277350 0.0842149816
## Phylum:p__Firmicutes      28.74250221 5.6267113566
## Phylum:p__Bacteroidetes    0.89459787 0.1209397315
## Phylum:p__Proteobacteria   0.28242718 0.2800613651
## Phylum:p__Actinobacteria   0.71698835 0.4555026874
## Phylum:p__Verrucomicrobia  0.01598885 0.0002660301
```

```
prob <- DirichletMultinomial::mixture(getBestDMNFit(tse_dmn))
colnames(prob) <- c("comp1", "comp2")
vec <- colnames(prob)[max.col(prob, ties.method = "first")]
assay(tse, "pseudo") <- assay(tse, "counts") + 1
tse <- transformCounts(tse, assay.type = "pseudo", method = "relabundance")
tse <- transformCounts(tse, "relabundance", method = "clr")
df <- calculateMDS(tse, assay.type = "clr", method = "euclidean")
euclidean_pcoa_df <- data.frame(
  pcoa1 = df[, 1],
  pcoa2 = df[, 2])
euclidean_dmm_pcoa_df <- cbind(euclidean_pcoa_df,
  dmm_component = vec)

ggplot(data = euclidean_dmm_pcoa_df, aes(x = pcoa1, y = pcoa2, color = dmm_component)) +
  geom_point() +
  labs(x = "Coordinate 1",
    y = "Coordinate 2",
    title = "PCoA with Aitchison distances")
```

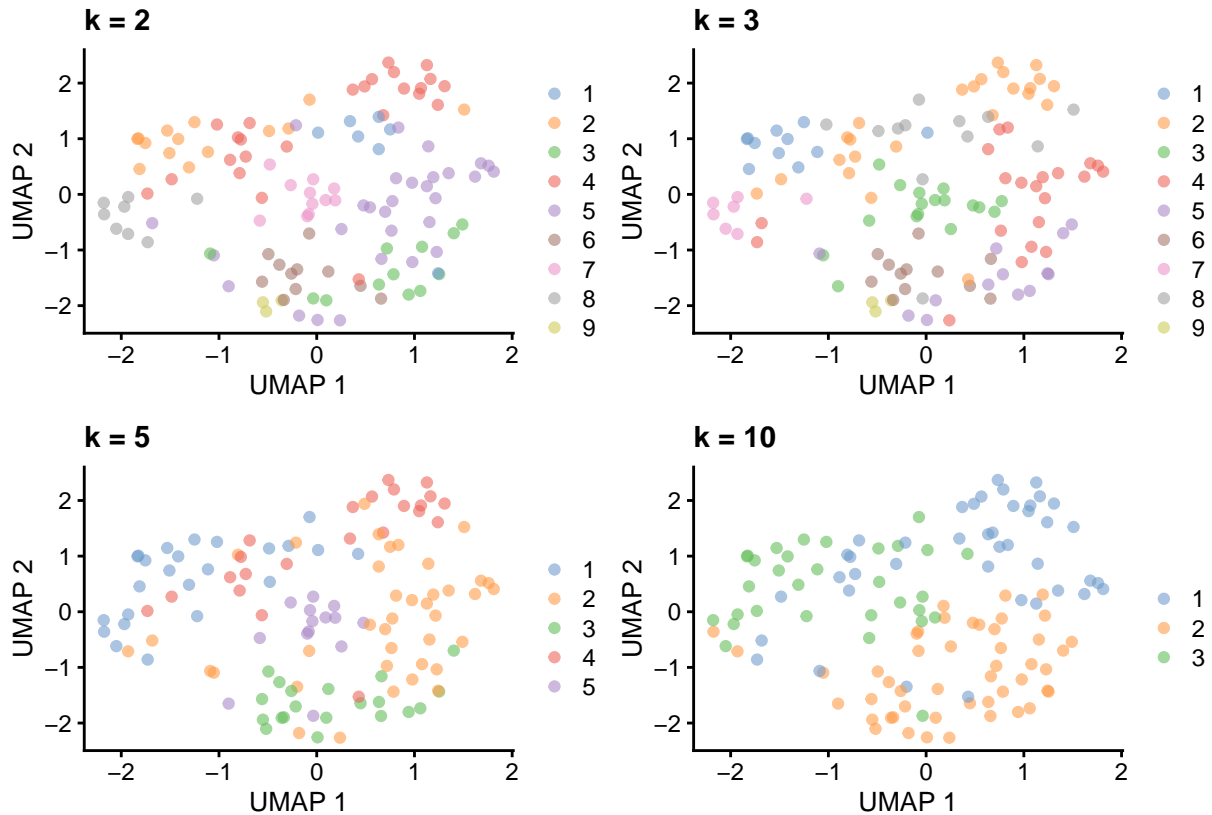
PCoA with Aitchison distances



UMAP with different ks

```
tse = makeTreeSummarizedExperimentFromPhyloseq(subset16S)
tse <- transformCounts(tse, method = "rclr")
tse <- runUMAP(tse, name = "UMAP", assay.type = "rclr")
k <- c(2, 3, 5, 10)
ClustAndPlot <- function(x) {
  # Creating the graph and running the short random walks algorithm
  graph_clusters <- clusterRows(t(assays(tse)$rclr), NNGraphParam(k = x))

  # Results of the clustering as a color for each sample
  plotUMAP(tse, colour_by = I(graph_clusters)) +
    labs(title = paste0("k = ", x))
}
plots <- lapply(k, ClustAndPlot)
(plots[[1]] + plots[[2]]) / (plots[[3]] + plots[[4]])
```

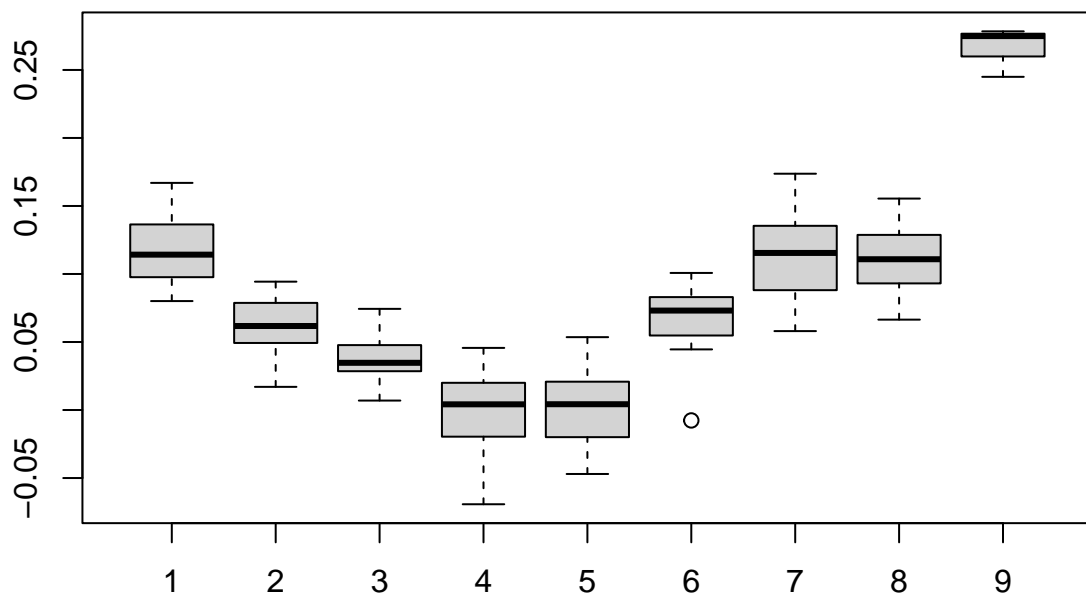


```
# boxplots
ClustDiagPlot <- function(x) {
  # Getting the clustering results
  graph_clusters <- clusterRows(t(assays(tse)$rclr), NNGraphParam(k = x))

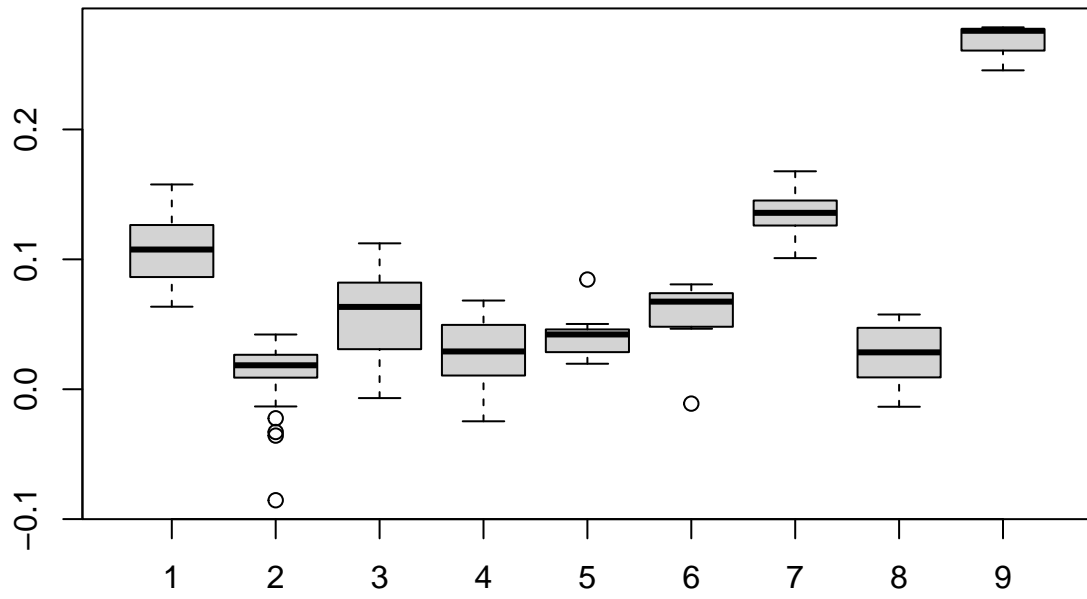
  # Computing the diagnostic info
  sil <- approxSilhouette(t(assays(tse)$rclr), graph_clusters)

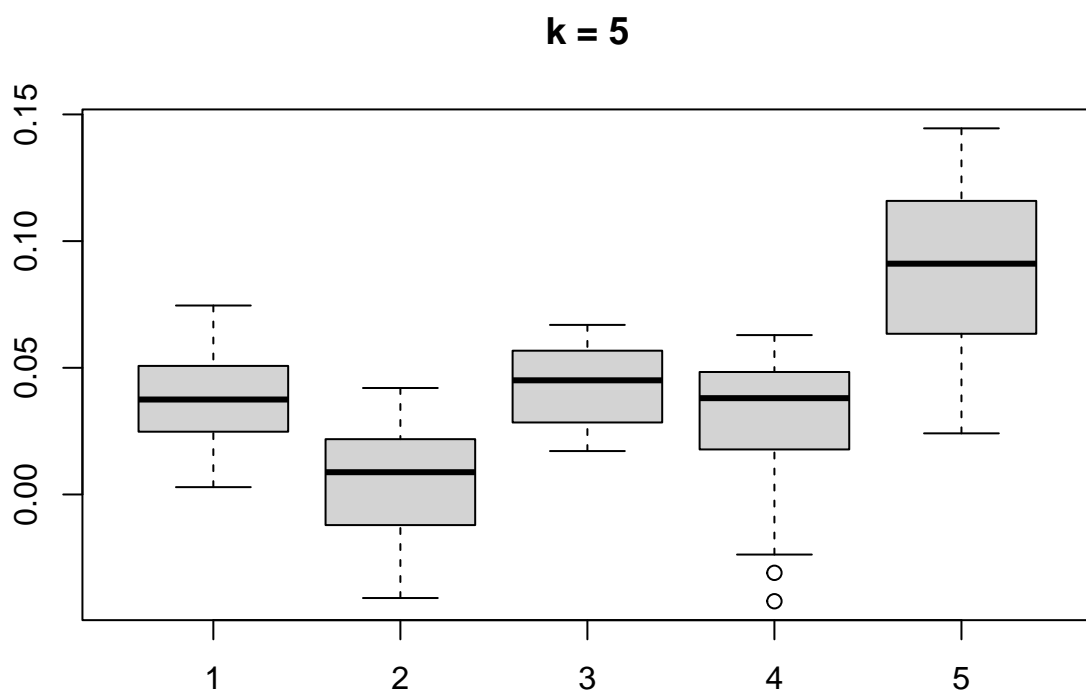
  # Plotting as a boxplot to observe cluster separation
  boxplot(split(sil$width, graph_clusters), main = paste0("k = ", x))
}
# Applying the function for different k values
res <- lapply(k, ClustDiagPlot)
```

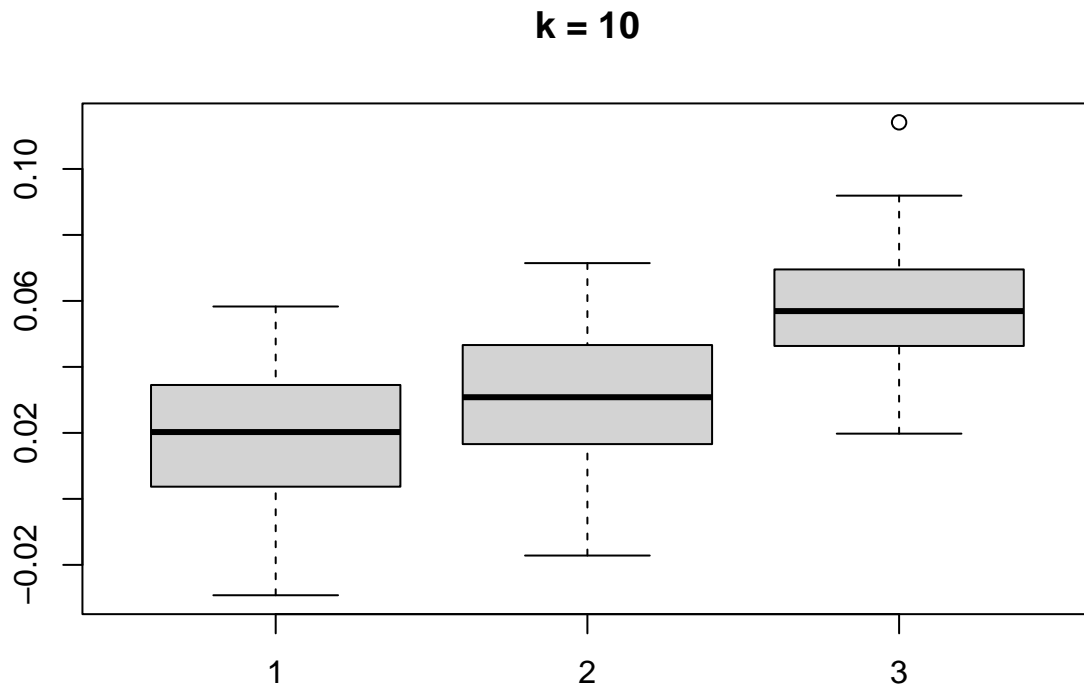
k = 2



k = 3







Metagenomic data

Loading data

```
### Loading a subset of metagenomic data into phyloseq format
subsetMG= import_biom("kraken2_output.biom") # this imports a .biom created by kraken2-biom containing

# We rewrite the sample names to a format filtering out Firm and firm and the first underscore so that
sample_names(subsetMG) = sapply(regmatches(sample_names(subsetMG), regexpr("_", sample_names(subsetMG))),
                                function(x) substr(x, 2, nchar(x)))

# Because the names in both metadata sets do not completely overlap, we need to manually edit one of them
sample_names(subsetMG)[68] = "4_65"

# reading in and combining metadata from 16S and metagenomic origins, adding missing underscores
firm_names = read_excel("./Metagenomic/FIRM_MetaNames.xlsx")
firm_names = firm_names[, -2] # Remove wrongful Raw_data_name column, to avoid confusion

meta_data = read.csv("MetaData.csv", header = TRUE, sep = ",")
meta_data_MG = dplyr::right_join(firm_names, meta_data, by="SampleID")

# using Sample_Unique as rownames so we can match the two sets in phyloseq
rownames(meta_data_MG) = meta_data_MG$Sample_Unique

# now we'll also add in microbial load
```

```

microbial_load = read.table("bacterial_load_kraken2.tab", sep = "\t", header = TRUE)
microbial_load$Sample_Unique = sapply(regmatches(microbial_load$Sample_Unique, regexpr("_",microbial_loa
microbial_load$Sample_Unique[68] = "4_65"
meta_data_MG = dplyr::right_join(meta_data_MG, microbial_load, by="Sample_Unique")

# creating tree and making phyloseq components, adding tree and sample data components to phyloseq
set.seed("878") # setting seed for reproducibility purposes
random_tree = rtree(ntaxa(subsetMG), rooted=TRUE, tip.label=taxa_names(subsetMG))
meta_data_MG = sample_data(meta_data_MG)
rownames(meta_data_MG) = meta_data_MG$Sample_Unique
subsetMG = merge_phyloseq(subsetMG, meta_data_MG, random_tree)
class(subsetMG)

## [1] "phyloseq"
## attr(,"package")
## [1] "phyloseq"

# set Rank names
colnames(tax_table(subsetMG)) = c("Domain", "Phylum", "Class", "Order", "Family", "Genus", "Species")
rank_names(subsetMG)

## [1] "Domain" "Phylum" "Class" "Order" "Family" "Genus" "Species"

### overview data
datatable(tax_table(subsetMG))

subsetMG # 7058 taxa

## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 7058 taxa and 120 samples ]
## sample_data() Sample Data: [ 120 samples by 34 sample variables ]
## tax_table() Taxonomy Table: [ 7058 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 7058 tips and 7057 internal nodes ]

# filter out non bacterial domains (no chloroplast, mitochondrial "taxa" present)
subsetMG <- subset_taxa(subsetMG, Domain!="k__Archaea")
subsetMG <- subset_taxa(subsetMG, Domain!="k__Viruses")
subsetMG <- subset_taxa(subsetMG, Domain!="k__Eukaryota")

subsetMG # 6355 taxa

## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 6355 taxa and 120 samples ]
## sample_data() Sample Data: [ 120 samples by 34 sample variables ]
## tax_table() Taxonomy Table: [ 6355 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 6355 tips and 6354 internal nodes ]

# Amount of different taxa present.
sort(table(tax_table(subsetMG)[, "Phylum"]))

```

```

##
##          p__Balneolota          p__Caldisericota
##                1                1
##          p__Calditrichota p__Candidatus Bipolaricaulota
##                1                1
##          p__Candidatus Omnitrophota          p__Chrysiogenota
##                1                1
##          p__Coprothermobacterota          p__Fibrobacterota
##                1                1
##          p__Lentisphaerota          p__Nitrospinota
##                1                1
##          p__Armatimonadota          p__Dictyoglomota
##                2                2
##          p__Kiritimatiellota          p__Thermomicrobiota
##                2                2
##          p__Elusimicrobiota          p__Rhodothermota
##                3                4
##          p__Candidatus Saccharibacteria          p__Deferribacterota
##                5                6
##          p__Gemmatimonadota          p__Aquificota
##                6                7
##          p__Nitrospirota          p__Bdellovibrionota
##                7                9
##          p__Chlamydiota          p__
##                9                10
##          p__Synergistota          p__Thermotogota
##                15                17
##          p__Chlorobiota          p__Chloroflexota
##                18                21
##          p__Acidobacteriota          p__Verrucomicrobiota
##                28                28
##          p__Fusobacteriota          p__Myxococcota
##                29                37
##          p__Deinococcota          p__Spirochaetota
##                44                59
##          p__Planctomycetota          p__Mycoplasmata
##                65                69
##          p__Campylobacterota          p__Cyanobacteriota
##                76                118
##          p__Thermodesulfobacteriota          p__Bacteroidota
##                122                414
##          p__Bacillota          p__Actinomycetota
##                1240                1488
##          p__Pseudomonadota
##                2383

```

```
sort(table(tax_table(subsetMG)[, "Order"])))
```

```

##
##          o__Acanthopleuribacterales          o__Acidothermales
##                1                1
##          o__Actinopolysporales          o__Balneolales
##                1                1
##          o__Bryobacterales          o__Caldilineales

```

##	1	1
##	o__Caldisericales	o__Calditrichales
##	1	1
##	o__Candidatus Brocadiales	o__Candidatus Izemoplasmatales
##	1	1
##	o__Candidatus Saccharimonadales	o__Candidatus Sulfidibacteriales
##	1	1
##	o__Catenulisporales	o__Chroococcidiopsidales
##	1	1
##	o__Chrysiogenales	o__Chthonomonadales
##	1	1
##	o__Coprothermobacteriales	o__Desulfarculales
##	1	1
##	o__Desulfobaccales	o__Desulfomonilales
##	1	1
##	o__Desulfurellales	o__Egibacteriales
##	1	1
##	o__Egicoccales	o__Elusimicrobiales
##	1	1
##	o__Euzebyales	o__Ferrocales
##	1	1
##	o__Fibrobacteriales	o__Fimbriimonadales
##	1	1
##	o__Gloeomargaritales	o__Haliangiales
##	1	1
##	o__Hydrogenophilales	o__Immundisolibacteriales
##	1	1
##	o__Kineosporiales	o__Kiritimatiellales
##	1	1
##	o__Koleobacteriales	o__Kordiimonadales
##	1	1
##	o__Ktedonobacteriales	o__Lentisphaerales
##	1	1
##	o__Limisphaerales	o__Limnochordales
##	1	1
##	o__Magnetococcales	o__Mesoaciditogales
##	1	1
##	o__Nannocystales	o__Nitrospinales
##	1	1
##	o__Phototrophicales	o__Salinisphaerales
##	1	1
##	o__Sneathiellales	o__Sphaerobacteriales
##	1	1
##	o__Sporichthyales	o__Syntrophales
##	1	1
##	o__Tepidisphaerales	o__Thermoleophilales
##	1	1
##	o__Thermomicrobiales	o__Thermostichales
##	1	1
##	o__Thermotomaculales	o__Tichowtungiales
##	1	1
##	o__Trueperales	o__Bacteriovoracales
##	1	2
##	o__Candidatus Pelagibacteriales	o__Dehalococcoidales

##		2		2
##	o__Desulfurobacteriales		o__Dictyoglomales	
##		2		2
##	o__Emcibacteriales		o__Endomicrobiales	
##		2		2
##	o__Kangiellales		o__Methyacidiphilales	
##		2		2
##	o__Parvularculales		o__Pleurocapsales	
##		2		2
##	o__Puniceicoccales		o__Saprospirales	
##		2		2
##	o__Syntrophobacteriales		o__Thermodesulfovibrionales	
##		2		2
##	o__Anaerolineales		o__Candidatus Babeliales	
##		3		3
##	o__Candidatus Nanopelagicales		o__Candidatus Nanosynbacteriales	
##		3		3
##	o__Glycomycetales		o__Jatrophihabitantales	
##		3		3
##	o__Jiangellales		o__Kosmotogales	
##		3		3
##	o__Mariprofundales		o__Miltoncostaeales	
##		3		3
##	o__Natranaerobiales		o__Phycisphaerales	
##		3		3
##	o__Silvanigrellales		o__Tepidiformales	
##		3		3
##	o__Vicinamibacteriales		o__Acidiferrobacteriales	
##		3		4
##	o__Bdellovibrionales		o__Bradymonadales	
##		4		4
##	o__Cardiobacteriales		o__Chlamydiales	
##		4		4
##	o__Maricaulales		o__Nakamurellales	
##		4		4
##	o__Nevskiales		o__Orbales	
##		4		4
##	o__Rhodothermales		o__Thermodesulfobacteriales	
##		4		4
##	o__Thermosediminibacteriales		o__Aquificales	
##		4		5
##	o__Brachyspirales		o__Chloroflexales	
##		5		5
##	o__Gloeobacteriales		o__Nitrospirales	
##		5		5
##	o__Parachlamydiales		o__Acidithiobacillales	
##		5		6
##	o__Deferribacteriales		o__Gemmatimonadales	
##		6		6
##	o__Holosporales		o__Hyphomonadales	
##		6		6
##	o__Isosphaerales		o__Marinilabiliales	
##		6		6
##	o__Nautiliales		o__Petrotogales	

##	6	6
##	o__Geodermatophilales	o__Halanaerobiales
##	7	7
##	o__Moorellales	o__Opitutales
##	7	7
##	o__Sedimentisphaerales	o__Thermotogales
##	7	7
##	o__Acidaminococcales	o__Frankiales
##	8	8
##	o__Gemmatales	o__Oscillatoriales
##	8	8
##	o__Solirubrobacterales	o__Acidimicrobiales
##	8	9
##	o__Polyangiales	o__Acholeplasmatales
##	9	10
##	o__Leptospirales	o__Rubrobacterales
##	10	10
##	o__Chroococcales	o__Desulfobulbales
##	11	11
##	o__Mycoplasmoidales	o__Legionellales
##	11	12
##	o__Verrucomicrobiales	o__Desulfuromonadales
##	13	14
##	o__Rickettsiales	o__Synergistales
##	14	15
##	o__Pseudanabaenales	o__Veillonellales
##	16	16
##	o__Desulfobacterales	o__Entomoplasmatales
##	17	17
##	o__Planctomycetales	o__Selenomonadales
##	17	17
##	o__Chlorobiales	o__Terriglobales
##	18	18
##	o__Thermales	o__Eggerthellales
##	18	20
##	o__Thiotrichales	o__Coriobacteriales
##	20	21
##	o__Geobacterales	o__Pirellulales
##	22	22
##	o__Chitinophagales	o__Deinococcales
##	23	25
##	o__Methylococcales	o__Nostocales
##	25	25
##	o__Tissierellales	o__Myxococcales
##	25	26
##	o__Thermoanaerobacterales	o__Aeromonadales
##	26	27
##	o__Fusobacteriales	o__Mycoplasmatales
##	29	30
##	o__Erysipelotrichales	o__Cellvibrionales
##	32	34
##	o__Pasteurellales	o__Sphingobacteriales
##	35	35
##	o__Streptosporangiales	o__Nitrosomonadales

##		36		37
##		o__Rhodocyclales		o__
##		37		38
##		o__Bifidobacteriales		o__Caulobacteriales
##		39		41
##		o__Spirochaetales		o__Synechococcales
##		44		47
##		o__Desulfovibrionales		o__Chromatiales
##		48		49
##		o__Moraxellales		o__Vibrionales
##		50		52
##		o__Actinomycetales		o__Alteromonadales
##		58		58
##		o__Pseudonocardiales		o__Campylobacteriales
##		67		69
##		o__Neisseriales		o__Micromonosporales
##		70		74
##		o__Oceanospirillales		o__Cytophagales
##		78		84
##		o__Rhodospirillales		o__Xanthomonadales
##		91		102
##		o__Propionibacteriales		o__Bacteroidales
##		108		116
##		o__Flavobacteriales		o__Rhodobacteriales
##		147		160
##		o__Sphingomonadales		o__Pseudomonadales
##		174		190
##		o__Enterobacteriales		o__Kitasatosporales
##		254		256
##		o__Lactobacillales		o__Mycobacteriales
##		296		303
##		o__Eubacteriales		o__Burkholderiales
##		308		342
##		o__Hyphomicrobiales		o__Micrococcales
##		362		436
##		o__Bacillales		
##		483		

```
sort(table(tax_table(subsetMG)[, "Family"])))
```

##		f__Acanthopleuribacteraceae	
##		1	
##		f__Acetomicrobiaceae	
##		1	
##		f__Acidilutibacteraceae	
##		1	
##		f__Acidimicrobiaceae	
##		1	
##		f__Acidothermaceae	
##		1	
##		f__Actinopolymorphaceae	
##		1	
##		f__Actinopolysporaceae	

##		1
##	f__Aminithiophilaceae	
##		1
##	f__Amoebophilaceae	
##		1
##	f__Amorphaceae	
##		1
##	f__Anaerohalosphaeraceae	
##		1
##	f__Bacillales Family X. Incertae Sedis	
##		1
##	f__Breoghaniaceae	
##		1
##	f__Bryobacteraceae	
##		1
##	f__Caedimonadaceae	
##		1
##	f__Caldilineaceae	
##		1
##	f__Caldiseriaceae	
##		1
##	f__Calditerrivibrionaceae	
##		1
##	f__Calditrichaceae	
##		1
##	f__Candidatus Babeliaceae	
##		1
##	f__Candidatus Brocadiaceae	
##		1
##	f__Candidatus Chromulinivoraceae	
##		1
##	f__Candidatus Izemoplasmataceae	
##		1
##	f__Candidatus Paracaedibacteraceae	
##		1
##	f__Candidatus Saccharimonadaceae	
##		1
##	f__Capillimicrobiaceae	
##		1
##	f__Casimicrobiaceae	
##		1
##	f__Catenulisporaceae	
##		1
##	f__Celerinatantimonadaceae	
##		1
##	f__Chamaesiphonaceae	
##		1
##	f__Chloroherpetonaceae	
##		1
##	f__Christensenellaceae	
##		1
##	f__Chroococcidiopsidaceae	
##		1
##	f__Chrysiogenaceae	


```

##                                     1
##                                f__Chthonomonadaceae
##                                     1
##                f__Clostridiales Family XVI. Incertae Sedis
##                                     1
##                                f__Cohaesibacteraceae
##                                     1
##                f__Coprothermobacteraceae
##                                     1
##                                f__Coxiellaceae
##                                     1
##                                f__Cyclonatronaceae
##                                     1
##                f__Deferribacteraceae
##                                     1
##                                f__Demequinaceae
##                                     1
##                f__Dermocarpellaceae
##                                     1
##                                f__Desulfallaceae
##                                     1
##                                f__Desulfarculaceae
##                                     1
##                f__Desulfatibacillaceae
##                                     1
##                                f__Desulfobaccaceae
##                                     1
##                f__Desulfohalobiaceae
##                                     1
##                                f__Desulfolunaceae
##                                     1
##                                f__Desulfomonilaceae
##                                     1
##                                f__Desulfosudaceae
##                                     1
##                f__Desulfotomaculaceae
##                                     1
##                f__Dissulfurispiraceae
##                                     1
##                                f__Egibacteraceae
##                                     1
##                                f__Egiccoccaceae
##                                     1
##                                f__Elioraeaceae
##                                     1
##                f__Elusimicrobiaceae
##                                     1
##                                f__Euzebyaceae
##                                     1
##                                f__Ferrovaceae
##                                     1
##                                f__Fibrobacteraceae
##                                     1
##                f__Fimbriimonadaceae

```

##		1
##	f__Flexistipitaceae	
##		1
##	f__Fontisphaeraceae	
##		1
##	f__Fulvivirgaceae	
##		1
##	f__Geminicoccaceae	
##		1
##	f__Geminocystaceae	
##		1
##	f__Gloeomargaritaceae	
##		1
##	f__Gomontiellaceae	
##		1
##	f__Gottschalkiaceae	
##		1
##	f__Granulosicoccaceae	
##		1
##	f__Haliscomenobacteraceae	
##		1
##	f__Halothermotrichaceae	
##		1
##	f__Hippeaceae	
##		1
##	f__Hydrogenimonadaceae	
##		1
##	f__Hydrogenophilaceae	
##		1
##	f__Hydrogenothermaceae	
##		1
##	f__Hyellaceae	
##		1
##	f__Ichthyobacteriaceae	
##		1
##	f__Ilumatobacteraceae	
##		1
##	f__Immundisolibacteraceae	
##		1
##	f__Jonesiaceae	
##		1
##	f__Kaistiaceae	
##		1
##	f__Kiloniellaceae	
##		1
##	f__Kineosporiaceae	
##		1
##	f__Kiritimatiellaceae	
##		1
##	f__Kofleriaceae	
##		1
##	f__Koleobacteraceae	
##		1
##	f__Kordiimonadaceae	

##		1
##	f__Ktedonosporobacteraceae	
##		1
##	f__Kytococcaceae	
##		1
##	f__Labilitrichaceae	
##		1
##	f__Lawsonellaceae	
##		1
##	f__Lentisphaeraceae	
##		1
##	f__Limnochordaceae	
##		1
##	f__Magnetococcaceae	
##		1
##	f__Maliibacteriaceae	
##		1
##	f__Marivirgaceae	
##		1
##	f__Mesoaciditogaceae	
##		1
##	f__Microvenatoraceae	
##		1
##	f__Mucispirillaceae	
##		1
##	f__Nannocystaceae	
##		1
##	f__Natranaerobiaceae	
##		1
##	f__Natranaerofabaceae	
##		1
##	f__Nitrospinaceae	
##		1
##	f__Oleiphilaceae	
##		1
##	f__Paludibacteraceae	
##		1
##	f__Paraconexibacteraceae	
##		1
##	f__Parvicellaceae	
##		1
##	f__Persicobacteraceae	
##		1
##	f__Phototrophicaceae	
##		1
##	f__Pleomorphomonadaceae	
##		1
##	f__Prochlorotrichaceae	
##		1
##	f__Proteinivoraceae	
##		1
##	f__Psychromonadaceae	
##		1
##	f__Reichenbachiellaceae	

```

##                                     1
##                               f__Rivulariaceae
##                                     1
##                               f__Salinibacteraceae
##                                     1
##                               f__Salinisphaeraceae
##                                     1
##                               f__Salinivirgaceae
##                                     1
##                               f__Sandaracinaceae
##                                     1
##                               f__Saprospiraceae
##                                     1
##                               f__Schleiferiaceae
##                                     1
##                               f__Segniliparaceae
##                                     1
##                               f__Simkaniaceae
##                                     1
##                               f__Sneathiellaceae
##                                     1
##                               f__Sphaerobacteraceae
##                                     1
##                               f__Sporichthyaceae
##                                     1
##                               f__Stellaceae
##                                     1
##                               f__Steroidobacteraceae
##                                     1
##                               f__Succinivibrionaceae
##                                     1
##                               f__Syntrophaceae
##                                     1
##                               f__Tepidisphaeraceae
##                                     1
##                               f__Thalassobaculaceae
##                                     1
##                               f__Thermincolaceae
##                                     1
## f__Thermoanaerobacterales Family IV. Incertae Sedis
##                                     1
##                               f__Thermodesulfobiaceae
##                                     1
##                               f__Thermodesulfovibrionaceae
##                                     1
##                               f__Thermoguttaceae
##                                     1
##                               f__Thermohalobacteraceae
##                                     1
##                               f__Thermoleophilaceae
##                                     1
##                               f__Thermomicrobiaceae
##                                     1
##                               f__Thermosediminibacteraceae

```

##		1
##	f__Thermostichaceae	
##		1
##	f__Thermotomaculaceae	
##		1
##	f__Thermovirgaceae	
##		1
##	f__Thioalkalibacteraceae	
##		1
##	f__Thioalkalispiraceae	
##		1
##	f__Tichowtungiaceae	
##		1
##	f__Trichocoleusaceae	
##		1
##	f__Trueperaceae	
##		1
##	f__Vulgatibacteraceae	
##		1
##	f__Waddliaceae	
##		1
##	f__Woeseiaceae	
##		1
##	f__Zhaonellaceae	
##		1
##	f__Zooshikellaceae	
##		1
##	f__Zymomonadaceae	
##		1
##	f__Aminobacteriaceae	
##		2
##	f__Beutenbergiaceae	
##		2
##	f__Blattabacteriaceae	
##		2
##	f__Candidatus Midichloriaceae	
##		2
##	f__Chelatococcaceae	
##		2
##	f__Chloroflexaceae	
##		2
##	f__Chroococcaceae	
##		2
##	f__Coleofasciculaceae	
##		2
##	f__Dehalococcoidaceae	
##		2
##	f__Desulfurobacteriaceae	
##		2
##	f__Dictyoglomaceae	
##		2
##	f__Dysgonomonadaceae	
##		2
##	f__Emcibacteraceae	

##		2
##	f__Endomicrobiaceae	
##		2
##	f__Ferrimonadaceae	
##		2
##	f__Geovibrionaceae	
##		2
##	f__Hahellaceae	
##		2
##	f__Halanaerobiaceae	
##		2
##	f__Halobacteriovoraceae	
##		2
##	f__Halothiobacillaceae	
##		2
##	f__Heliobacteriaceae	
##		2
##	f__Kangiellaceae	
##		2
##	f__Lichenihabitantaceae	
##		2
##	f__Marinilabiliaceae	
##		2
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##	f__Burkholderiaceae
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##	f__Micrococcaceae
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##	f__Paenibacillaceae
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##	f__Sphingomonadaceae
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##	f__Enterobacteriaceae
##	125
##	f__


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##                                     137
##                                f__Lactobacillaceae
##                                     150
##                                f__Pseudomonadaceae
##                                     172
##                                f__Microbacteriaceae
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##                                f__Bacillaceae
##                                     200
##                                f__Streptomycetaceae
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```

```
sort(table(tax_table(subsetMG)[, "Genus"])))
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##                                g__Actinokineospora
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##	g__Candidatus Profftia	
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##	g__Candidatus Promineifilum	

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##	g__Pectinatus	

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##	g__Venatoribacter
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##	g__Verminephrobacter

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## 1
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## g__Waddlia
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## g__Wansuia
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## g__Wielerella
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## g__Williamsoniiplasma
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## g__Winkia
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## g__Woeseia
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## g__Wolbachia
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## g__Wolinella
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## g__Wujia
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## g__Xiamenia
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## g__Xianfuyuplasma
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## g__Xylella
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## g__Xylophilus
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## g__Yangia
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## g__Yimella
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## g__Yinghuangia
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## g__Yokenella
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## g__Yoonia
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## g__Youhaiella
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## g__Zhaonella
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## g__Zhongshania
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## g__Zobellella
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## g__Zobellia
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## g__Zymomonas
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## g__Acholeplasma
## 2
## g__Acidiferrobacter

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##	g__Dehalogenimonas	
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##	g__Rhodopseudomonas	
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##	g__Romboutsia	
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##	g__Roseburia	
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##	g__Ruania	
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##	g__Ruminiclostridium	
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##	g__Salipiger	
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##	g__Salmonella	
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##	g__Solidesulfovibrio	
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##	g__Spiribacter	
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##	g__Thermoanaerobacterium	
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##	g__Thiomicrothrix	

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##	g__Frankia	

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##	g__Gimesia
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##	g__Kingella
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##	g__Lachnoclostridium
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##	g__Levilactobacillus
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##	g__Limnohabitans
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##	g__Magnetospirillum
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##	g__Marinomonas
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##	g__Metabacillus
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##	g__Methylococcus
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##	g__Nonomuraea
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##	g__Oceanobacillus
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##	g__Pediococcus
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##	g__Phaeobacter
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##	g__Phocaeicola
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##	g__Raoultella
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##	g__Salinibacterium

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##	g__Shigella
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##	g__Skermanella
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##	g__Sodalis
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##	g__Sphaerochaeta
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##	g__Thermoanaerobacter
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##	g__Thioalkalivibrio
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##	g__Tsukamurella
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##	g__Turicibacter
##	5
##	g__Acidithiobacillus
##	6
##	g__Agrococcus
##	6
##	g__Anaeromyxobacter
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##	g__Ancylobacter
##	6
##	g__Arcanobacterium
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##	g__Aureimonas
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##	g__Bartonella
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##	g__Caballeronia
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##	g__Carnobacterium
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##	g__Cellulosimicrobium
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##	g__Clavibacter
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##	g__Cohnella
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##	g__Cronobacter
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##	g__Cutibacterium
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##	g__Cytobacillus
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##	g__Dactylosporangium
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##	g__Dyadobacter
##	6
##	g__Dyella

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##	g__Erysipelothrix
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##	g__Geomonas
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##	g__Janibacter
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##	g__Leifsonia
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##	g__Leptolyngbya
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##	g__Macrococcus
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##	g__Mannheimia
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##	g__Microlunatus
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##	g__Muricauda
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##	g__Myxococcus
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##	g__Parageobacillus
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##	g__Pontibacter
##	6
##	g__Prosthecochloris
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##	g__Rahnella
##	6
##	g__Rubrobacter
##	6
##	g__Ruminococcus
##	6
##	g__Sulfurimonas
##	6
##	g__Sulfurovum
##	6
##	g__Veillonella
##	6
##	g__Yersinia
##	6
##	g__Actinomadura
##	7
##	g__Aerococcus
##	7
##	g__Chitinophaga
##	7
##	g__Diaphorobacter
##	7
##	g__Dickeya
##	7
##	g__Escherichia
##	7
##	g__Geobacillus

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##	g__Halobacillus	
##		7
##	g__Halopseudomonas	
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##	g__Jeotgalibaca	
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##	g__Lacticaseibacillus	
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##	g__Leisingera	
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##	g__Methylocystis	
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##	g__Micrococcus	
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##	g__Microvirga	
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##	g__Moraxella	
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##	g__Nocardiopsis	
##		7
##	g__Peribacillus	
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##	g__Providencia	
##		7
##	g__Pseudoduganella	
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##	g__Ralstonia	
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##	g__Roseomonas	
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##	g__Roseovarius	
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##	g__Ruegeria	
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##	g__Saccharomonospora	
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##	g__Saccharopolyspora	
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##	g__Stutzerimonas	
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##	g__Acetobacter	
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##	g__Actinobacillus	
##		8
##	g__Actinoplanes	
##		8
##	g__Bosea	
##		8
##	g__Caldicellulosiruptor	
##		8
##	g__Cryobacterium	
##		8
##	g__Dietzia	

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##	g__Eubacterium	
##		8
##	g__Geobacter	
##		8
##	g__Herbaspirillum	
##		8
##	g__Kitasatospora	
##		8
##	g__Luteibacter	
##		8
##	g__Neobacillus	
##		8
##	g__Pandoraea	
##		8
##	g__Parabacteroides	
##		8
##	g__Pedobacter	
##		8
##	g__Pseudoxanthomonas	
##		8
##	g__Qipengyuania	
##		8
##	g__Selenomonas	
##		8
##	g__Shinella	
##		8
##	g__Thauera	
##		8
##	g__Capnocytophaga	
##		9
##	g__Exiguobacterium	
##		9
##	g__Kosakonia	
##		9
##	g__Legionella	
##		9
##	g__Leptospira	
##		9
##	g__Leptotrichia	
##		9
##	g__Ligilactobacillus	
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##	g__Listeria	
##		9
##	g__Luteimonas	
##		9
##	g__Mycolicibacter	
##		9
##	g__Mycoplasmopsis	
##		9
##	g__Nostoc	
##		9
##	g__Ornithinimicrobium	

##		9
##	g__Pectobacterium	
##		9
##	g__Pseudonocardia	
##		9
##	g__Psychrobacter	
##		9
##	g__Rhodoferax	
##		9
##	g__Schaalia	
##		9
##	g__Spirosoma	
##		9
##	g__Tessaracoccus	
##		9
##	g__Vagococcus	
##		9
##	g__Variovorax	
##		9
##	g__Agromyces	
##		10
##	g__Alicyclobacillus	
##		10
##	g__Brucella	
##		10
##	g__Chromobacterium	
##		10
##	g__Companilactobacillus	
##		10
##	g__Janthinobacterium	
##		10
##	g__Lactococcus	
##		10
##	g__Limosilactobacillus	
##		10
##	g__Lysinibacillus	
##		10
##	g__Methylomonas	
##		10
##	g__Mucilaginibacter	
##		10
##	g__Pseudarthrobacter	
##		10
##	g__Rothia	
##		10
##	g__Sporosarcina	
##		10
##	g__Achromobacter	
##		11
##	g__Aeromicrobium	
##		11
##	g__Agrobacterium	
##		11
##	g__Alistipes	

```

##                                11
##                                g__Blautia
##                                11
##                                g__Brevibacillus
##                                11
##                                g__Caulobacter
##                                11
##                                g__Erwinia
##                                11
##                                g__Erythrobacter
##                                11
##                                g__Faecalibacterium
##                                11
##                                g__Glutamicibacter
##                                11
##                                g__Helicobacter
##                                11
##                                g__Hydrogenophaga
##                                11
##                                g__Kocuria
##                                11
##                                g__Pseudoalteromonas
##                                11
##                                g__Rathayibacter
##                                11
##                                g__Sinorhizobium
##                                11
##                                g__Sphingobacterium
##                                11
##                                g__Thermus
##                                11
##                                g__Weissella
##                                11
##                                g__Desulfovibrio
##                                12
##                                g__Fusobacterium
##                                12
##                                g__Leuconostoc
##                                12
##                                g__Photobacterium
##                                12
##                                g__Planococcus
##                                12
##                                g__Pseudodesulfovibrio
##                                12
##                                g__Spiroplasma
##                                12
##                                g__Virgibacillus
##                                12
##                                g__Brachybacterium
##                                13
##                                g__Leucobacter
##                                13
##                                g__Polynucleobacter

```

##	13
##	g__Sulfitobacter
##	13
##	g__Azospirillum
##	14
##	g__Bordetella
##	14
##	g__Comamonas
##	14
##	g__Klebsiella
##	14
##	g__Mycoplasma
##	14
##	g__Microbulbifer
##	15
##	g__Stenotrophomonas
##	16
##	g__Xanthomonas
##	16
##	g__Curtobacterium
##	17
##	g__Devosia
##	17
##	g__Gordonia
##	17
##	g__Marinobacter
##	17
##	g__Novosphingobium
##	17
##	g__Cupriavidus
##	18
##	g__Massilia
##	18
##	g__Methylobacterium
##	18
##	g__Pantoea
##	18
##	g__Sphingobium
##	18
##	g__Treponema
##	18
##	g__Acidovorax
##	19
##	g__Aeromonas
##	19
##	g__Citrobacter
##	19
##	g__Lysobacter
##	19
##	g__Neisseria
##	19
##	g__Sphingopyxis
##	19
##	g__Enterobacter

##	20
##	g__Nocardia
##	20
##	g__Serratia
##	20
##	g__Shewanella
##	21
##	g__Brevibacterium
##	22
##	g__Brevundimonas
##	22
##	g__Cellulomonas
##	22
##	g__Amycolatopsis
##	23
##	g__Campylobacter
##	23
##	g__Actinomyces
##	24
##	g__Burkholderia
##	24
##	g__Prevotella
##	24
##	g__Deinococcus
##	25
##	g__Flavobacterium
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##	g__Paraburkholderia
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##	g__Chryseobacterium
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##	g__Enterococcus
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##	g__Rhizobium
##	28
##	g__Halomonas
##	29
##	g__Hymenobacter
##	30
##	g__Bacteroides
##	31
##	g__Rhodococcus
##	31
##	g__Paracoccus
##	32
##	g__Acinetobacter
##	33
##	g__Bifidobacterium
##	33
##	g__Synechococcus
##	33
##	g__Vibrio
##	33
##	g__Lactobacillus

```

##                                34
##                                g__Mesorhizobium
##                                35
##                                g__Arthrobacter
##                                44
##                                g__Micromonospora
##                                46
##                                g__Mycobacterium
##                                47
##                                g__Nocardioides
##                                47
##                                g__Mycolicibacterium
##                                48
##                                g__Bacillus
##                                52
##                                g__Sphingomonas
##                                52
##                                g__Bradyrhizobium
##                                53
##                                g__Staphylococcus
##                                53
##                                g__Clostridium
##                                56
##                                g__Streptococcus
##                                62
##                                g__Microbacterium
##                                70
##                                g__Paenibacillus
##                                99
##                                g__Corynebacterium
##                                101
##                                g__Pseudomonas
##                                148
##                                g__
##                                228
##                                g__Streptomyces
##                                241

```

```

# Check the amount of unique Orders in samples which have and have not been treated with antibiotics
subsetMG %>% ps_filter(AB == "no") %>% get_taxa_unique("Order") # 200 different orders for non AB treat

```

```

## [1] "o__Hyphomicrobiales"      "o__Burkholderiales"
## [3] "o__Cytophagales"         "o__Holosporales"
## [5] "o__Lactobacillales"      "o__Bacillales"
## [7] "o__Sphingobacteriales"   "o__Vibrionales"
## [9] "o__Pseudomonadales"      "o__Chromatiales"
## [11] "o__Micrococcales"        "o__Mycoplasmatales"
## [13] "o__Kitasatosporales"     "o__Rhodobacterales"
## [15] "o__Eubacteriales"        "o__Desulfuromonadales"
## [17] "o__Syntrophobacterales"  "o__Nitrosomonadales"
## [19] "o__Caulobacterales"      "o__Actinomycetales"
## [21] "o__Opitutales"           "o__Tissierellales"
## [23] "o__Nostocales"           "o__Veillonellales"
## [25] "o__Neisseriales"         "o__Myxococcales"

```

## [27]	"o__Enterobacterales"	"o__Propionibacteriales"
## [29]	"o__Methylococcales"	"o__Alteromonadales"
## [31]	"o__Natranaerobiales"	"o__Bifidobacteriales"
## [33]	"o__Sphingomonadales"	"o__Pasteurellales"
## [35]	"o__Xanthomonadales"	"o__Micromonosporales"
## [37]	"o__Cellvibrionales"	"o__Rhodospirillales"
## [39]	"o__Pseudonocardiales"	"o__Acidaminococcales"
## [41]	"o__Bacteroidales"	"o__Chitinophagales"
## [43]	"o__Oceanospirillales"	"o__Deinococcales"
## [45]	"o__Campylobacteriales"	"o__Pseudanabaenales"
## [47]	"o__Aeromonadales"	"o__Mycobacteriales"
## [49]	"o__Desulfobacteriales"	"o__Bryobacteriales"
## [51]	"o__Hyphomonadales"	"o__Streptosporangiales"
## [53]	"o__Synechococcales"	"o__Thermosediminibacteriales"
## [55]	"o__Flavobacteriales"	"o__Solirubrobacteriales"
## [57]	"o__Chlorobiales"	"o__"
## [59]	"o__Legionellales"	"o__Acidiferrobacteriales"
## [61]	"o__Terriglobales"	"o__Polyangiales"
## [63]	"o__Spirochaetales"	"o__Verrucomicrobiales"
## [65]	"o__Tepidiformales"	"o__Rubrobacteriales"
## [67]	"o__Candidatus Nanosynbacteriales"	"o__Frankiales"
## [69]	"o__Moraxellales"	"o__Eggerthellales"
## [71]	"o__Entomoplasmatales"	"o__Selenomonadales"
## [73]	"o__Desulfovibrionales"	"o__Pirellulales"
## [75]	"o__Mycoplasmoidales"	"o__Fusobacteriales"
## [77]	"o__Geobacteriales"	"o__Chlamydiales"
## [79]	"o__Rhodocyclales"	"o__Methylandiphalales"
## [81]	"o__Planctomycetales"	"o__Deferribacteriales"
## [83]	"o__Erysipelotrichales"	"o__Parachlamydiales"
## [85]	"o__Acidimicrobiales"	"o__Acidithiobacillales"
## [87]	"o__Leptospirales"	"o__Coriobacteriales"
## [89]	"o__Thiotrichales"	"o__Rickettsiales"
## [91]	"o__Thermoanaerobacteriales"	"o__Chroococcales"
## [93]	"o__Saprospirales"	"o__Cardiobacteriales"
## [95]	"o__Desulfobulbales"	"o__Nevskiales"
## [97]	"o__Thermales"	"o__Kordiimonadales"
## [99]	"o__Miltoncostaeales"	"o__Synergistales"
## [101]	"o__Ktedonobacteriales"	"o__Petrotogales"
## [103]	"o__Oscillatoriales"	"o__Puniceococcales"
## [105]	"o__Nautiliales"	"o__Thermotogales"
## [107]	"o__Sedimentisphaerales"	"o__Kineosporiales"
## [109]	"o__Acholeplasmatales"	"o__Anaerolineales"
## [111]	"o__Candidatus Babeliales"	"o__Chloroflexales"
## [113]	"o__Pleurocapsales"	"o__Nakamurellales"
## [115]	"o__Nitrospirales"	"o__Phototrophicales"
## [117]	"o__Emcibacteriales"	"o__Nitrospinales"
## [119]	"o__Gemmatimonadales"	"o__Bradymonadales"
## [121]	"o__Egibacteriales"	"o__Jatrophihabitantales"
## [123]	"o__Brachyspirales"	"o__Sphaerobacteriales"
## [125]	"o__Gemmatales"	"o__Moorellales"
## [127]	"o__Halanaerobiales"	"o__Gloeobacteriales"
## [129]	"o__Desulfarculales"	"o__Isosphaerales"
## [131]	"o__Orbales"	"o__Dictyoglomales"
## [133]	"o__Maricaulales"	"o__Aquificales"

```
## [135] "o__Kosmotogales" "o__Koleobacterales"
## [137] "o__Geodermatophilales" "o__Thermodesulfobacteriales"
## [139] "o__Marinilabiales" "o__Thermostichales"
## [141] "o__Jiangellales" "o__Rhodothermales"
## [143] "o__Bdellovibrionales" "o__Acanthopleuribacteriales"
## [145] "o__Ferrovales" "o__Glycomycetales"
## [147] "o__Thermomicrobiales" "o__Candidatus Nanopelagicales"
## [149] "o__Silvanigrellales" "o__Sneathiellales"
## [151] "o__Euzebyales" "o__Endomicrobiales"
## [153] "o__Tepidisphaerales" "o__Lentisphaerales"
## [155] "o__Thermoleophilales" "o__Kangiellales"
## [157] "o__Phycisphaerales" "o__Parvularculales"
## [159] "o__Candidatus Sulfidibacteriales" "o__Desulfobaccales"
## [161] "o__Tichowtungiales" "o__Sporichthyales"
## [163] "o__Caldisericales" "o__Dehalococcoidales"
## [165] "o__Immundisolibacteriales" "o__Desulfurobacteriales"
## [167] "o__Vicinamibacteriales" "o__Mariprofundales"
## [169] "o__Syntrophales" "o__Acidothermales"
## [171] "o__Desulfurellales" "o__Caldilineales"
## [173] "o__Trueperales" "o__Salinisphaerales"
## [175] "o__Haliangiales" "o__Bacteriovoracales"
## [177] "o__Candidatus Pelagibacteriales" "o__Chrysiogenales"
## [179] "o__Hydrogenophilales" "o__Desulfomonilales"
## [181] "o__Kiritimatiellales" "o__Elusimicrobiales"
## [183] "o__Catenulisporales" "o__Thermodesulfovibrionales"
## [185] "o__Actinopolysporales" "o__Gloeomargaritales"
## [187] "o__Nannocystales" "o__Candidatus Izemoplasmatales"
## [189] "o__Calditrichales" "o__Chthonomonadales"
## [191] "o__Limnochordales" "o__Limisphaerales"
## [193] "o__Egicoccales" "o__Fimbriimonadales"
## [195] "o__Coprothermobacteriales" "o__Fibrobacteriales"
## [197] "o__Chroococcidiopsidales" "o__Balneolales"
## [199] "o__Mesoaciditogales" "o__Magnetococcales"
```

```
subsetMG %>% ps_filter(AB == "yes") %>% get_taxa_unique("Order") # 159 different orders for AB treated
```

```
## [1] "o__Burkholderiales" "o__Cytophagales"
## [3] "o__Hyphomicrobiales" "o__Flavobacteriales"
## [5] "o__Lactobacillales" "o__Bacillales"
## [7] "o__Synechococcales" "o__Vibrionales"
## [9] "o__Pseudomonadales" "o__Chromatiales"
## [11] "o__Mycoplasmatales" "o__Kittasatosporales"
## [13] "o__Rhodobacterales" "o__Eubacteriales"
## [15] "o__Syntrophobacteriales" "o__Opitutales"
## [17] "o__Tissierellales" "o__Veillonellales"
## [19] "o__Neisseriales" "o__Myxococcales"
## [21] "o__Enterobacteriales" "o__Propionibacteriales"
## [23] "o__Bifidobacteriales" "o__Xanthomonadales"
## [25] "o__Actinomycetales" "o__Mycobacteriales"
## [27] "o__Pseudonocardiales" "o__Acidaminococcales"
## [29] "o__Bacteroidales" "o__Oceanospirillales"
## [31] "o__Rhodospirillales" "o__Aeromonadales"
## [33] "o__Desulfobacteriales" "o__Bryobacteriales"
## [35] "o__Micrococcales" "o__Pasteurellales"
```


## [37]	"o__Spirochaetales"	"o__Solirubrobacterales"
## [39]	"o__Micromonosporales"	"o__Phycisphaerales"
## [41]	"o__Methylococcales"	"o__"
## [43]	"o__Legionellales"	"o__Campylobacterales"
## [45]	"o__Terriglobales"	"o__Polyangiales"
## [47]	"o__Verrucomicrobiales"	"o__Rubrobacterales"
## [49]	"o__Alteromonadales"	"o__Nostocales"
## [51]	"o__Sphingomonadales"	"o__Candidatus Brocadiales"
## [53]	"o__Rickettsiales"	"o__Candidatus Saccharimonadales"
## [55]	"o__Acidiferrobacterales"	"o__Eggerthellales"
## [57]	"o__Entomoplasmatales"	"o__Selenomonadales"
## [59]	"o__Desulfovibrionales"	"o__Pirellulales"
## [61]	"o__Mycoplasmoidales"	"o__Geobacterales"
## [63]	"o__Rhodocyclales"	"o__Deinococcales"
## [65]	"o__Nitrosomonadales"	"o__Aquificales"
## [67]	"o__Planctomycetales"	"o__Erysipelotrichales"
## [69]	"o__Caulobacterales"	"o__Candidatus Nanopelagicales"
## [71]	"o__Acidimicrobiales"	"o__Acidithiobacillales"
## [73]	"o__Leptospirales"	"o__Coriobacterales"
## [75]	"o__Streptosporangiales"	"o__Moraxellales"
## [77]	"o__Nautiliales"	"o__Desulphuromonadales"
## [79]	"o__Cardiobacterales"	"o__Desulfobulbales"
## [81]	"o__Miltoncostaeales"	"o__Fusobacterales"
## [83]	"o__Chitinophagales"	"o__Cellvibrionales"
## [85]	"o__Sphingobacterales"	"o__Thermoanaerobacterales"
## [87]	"o__Puniceicoccales"	"o__Synergistales"
## [89]	"o__Thermales"	"o__Sedimentisphaerales"
## [91]	"o__Kineosporiales"	"o__Acholeplasmatales"
## [93]	"o__Anaerolineales"	"o__Chloroflexales"
## [95]	"o__Nakamurellales"	"o__Nitrospirales"
## [97]	"o__Parachlamydiales"	"o__Thermodesulfobacterales"
## [99]	"o__Oscillatoriales"	"o__Emcibacterales"
## [101]	"o__Nitrospinales"	"o__Bradymonadales"
## [103]	"o__Frankiales"	"o__Sphaerobacterales"
## [105]	"o__Gemmatales"	"o__Chlorobiales"
## [107]	"o__Moorellales"	"o__Halanaerobiales"
## [109]	"o__Desulfarculales"	"o__Gemmatimonadales"
## [111]	"o__Isosphaerales"	"o__Thiotrichales"
## [113]	"o__Thermosediminibacterales"	"o__Gloeobacterales"
## [115]	"o__Tepidiformales"	"o__Koleobacterales"
## [117]	"o__Geodermatophilales"	"o__Brachyspirales"
## [119]	"o__Bdellovibrionales"	"o__Acanthopleuribacterales"
## [121]	"o__Rhodothermales"	"o__Orbales"
## [123]	"o__Nevskiales"	"o__Petrotogales"
## [125]	"o__Thermotogales"	"o__Glycomycetales"
## [127]	"o__Pseudanabaenales"	"o__Sneathiellales"
## [129]	"o__Chroococcales"	"o__Euzebyales"
## [131]	"o__Parvularculales"	"o__Desulfobaccales"
## [133]	"o__Tichowtungiales"	"o__Sporichthyales"
## [135]	"o__Dehalococcoidales"	"o__Immundisolibacterales"
## [137]	"o__Marinilabiliales"	"o__Vicinamibacterales"
## [139]	"o__Candidatus Babeliales"	"o__Thermodesulfovibrionales"
## [141]	"o__Caldilineales"	"o__Haliangiales"
## [143]	"o__Candidatus Pelagibacterales"	"o__Kosmotogales"

```
## [145] "o__Thermotomaculales"      "o__Desulfomonilales"
## [147] "o__Kiritimatiellales"      "o__Gloeomargaritales"
## [149] "o__Nannocystales"          "o__Jiangellales"
## [151] "o__Candidatus Nanosynbacterales" "o__Candidatus Izemoplasmatales"
## [153] "o__Limnochordales"          "o__Limisphaerales"
## [155] "o__Egicoccales"            "o__Fimbriimonadales"
## [157] "o__Fibrobacterales"         "o__Mesoaciditogales"
## [159] "o__Maricaulales"
```

```
subsetMG %>% get_taxa_unique("Order") # 203 different order in total, so 3 orders are not found in non
```

```
## [1] "o__Hyphomicrobiales"      "o__Burkholderiales"
## [3] "o__Cytophagales"          "o__Holosporales"
## [5] "o__Flavobacteriales"      "o__Lactobacillales"
## [7] "o__Bacillales"            "o__Synechococcales"
## [9] "o__Sphingobacteriales"    "o__Vibrionales"
## [11] "o__Pseudomonadales"       "o__Chromatiales"
## [13] "o__Micrococcales"         "o__Mycoplasmatales"
## [15] "o__Kitasatosporales"      "o__Rhodobacterales"
## [17] "o__Eubacteriales"         "o__Desulfuromonadales"
## [19] "o__Syntrophobacteriales"  "o__Nitrosomonadales"
## [21] "o__Caulobacteriales"      "o__Actinomycetales"
## [23] "o__Opitutales"            "o__Tissierellales"
## [25] "o__Nostocales"            "o__Veillonellales"
## [27] "o__Neisseriales"          "o__Myxococcales"
## [29] "o__Enterobacterales"      "o__Propionibacteriales"
## [31] "o__Methylococcales"        "o__Alteromonadales"
## [33] "o__Natranaerobiales"       "o__Bifidobacteriales"
## [35] "o__Sphingomonadales"       "o__Pasteurellales"
## [37] "o__Xanthomonadales"        "o__Micromonosporales"
## [39] "o__Cellvibrionales"        "o__Rhodospirillales"
## [41] "o__Mycobacteriales"        "o__Pseudonocardiales"
## [43] "o__Acidaminococcales"      "o__Bacteroidales"
## [45] "o__Chitinophagales"        "o__Oceanospirillales"
## [47] "o__Deinococcales"          "o__Campylobacteriales"
## [49] "o__Pseudanabaenales"       "o__Aeromonadales"
## [51] "o__Desulfobacteriales"     "o__Bryobacteriales"
## [53] "o__Hyphomonadales"         "o__Streptosporangiales"
## [55] "o__Spirochaetales"         "o__Thermosediminibacteriales"
## [57] "o__Solirubrobacteriales"   "o__Chlorobiales"
## [59] "o__Phycisphaerales"        "o__"
## [61] "o__Legionellales"          "o__Acidiferrobacteriales"
## [63] "o__Terriglobales"          "o__Polyangiales"
## [65] "o__Verrucomicrobiales"     "o__Tepidiformales"
## [67] "o__Rubrobacteriales"        "o__Candidatus Nanosynbacterales"
## [69] "o__Candidatus Brocadiales" "o__Frankiales"
## [71] "o__Rickettsiales"          "o__Candidatus Saccharimonadales"
## [73] "o__Moraxellales"           "o__Eggerthellales"
## [75] "o__Entomoplasmatales"       "o__Selenomonadales"
## [77] "o__Desulfovibrionales"      "o__Pirellulales"
## [79] "o__Mycoplasmoidales"        "o__Fusobacteriales"
## [81] "o__Geobacteriales"          "o__Rhodocyclales"
## [83] "o__Aquificales"            "o__Chlamydiales"
## [85] "o__Methyacidiphilales"      "o__Planctomycetales"
```

## [87]	"o__Deferribacterales"	"o__Erysipelotrichales"
## [89]	"o__Candidatus Nanopelagicales"	"o__Parachlamydiales"
## [91]	"o__Acidimicrobiales"	"o__Acidithiobacillales"
## [93]	"o__Leptospirales"	"o__Coriobacterales"
## [95]	"o__Thiotrichales"	"o__Thermoanaerobacterales"
## [97]	"o__Nautiliales"	"o__Chroococcales"
## [99]	"o__Saprospirales"	"o__Cardiobacterales"
## [101]	"o__Desulfobulbales"	"o__Nevskiales"
## [103]	"o__Thermales"	"o__Kordiimonadales"
## [105]	"o__Miltoncostaeales"	"o__Synergistales"
## [107]	"o__Ktedonobacterales"	"o__Petrotogales"
## [109]	"o__Oscillatoriales"	"o__Puniceicoccales"
## [111]	"o__Thermotogales"	"o__Sedimentisphaerales"
## [113]	"o__Kineosporiales"	"o__Acholeplasmatales"
## [115]	"o__Anaerolineales"	"o__Candidatus Babeliales"
## [117]	"o__Chloroflexales"	"o__Pleurocapsales"
## [119]	"o__Nakamurellales"	"o__Nitrospirales"
## [121]	"o__Phototrophicales"	"o__Thermodesulfobacterales"
## [123]	"o__Emcibacterales"	"o__Nitrospinales"
## [125]	"o__Gemmatimonadales"	"o__Bradymonadales"
## [127]	"o__Egibacterales"	"o__Jatrophihabitantales"
## [129]	"o__Brachyspirales"	"o__Sphaerobacterales"
## [131]	"o__Gemmatales"	"o__Moorellales"
## [133]	"o__Halanaerobiales"	"o__Gloeobacterales"
## [135]	"o__Desulfarculales"	"o__Isosphaerales"
## [137]	"o__Orbales"	"o__Dictyoglomales"
## [139]	"o__Maricaulales"	"o__Kosmotogales"
## [141]	"o__Koleobacterales"	"o__Geodermatophilales"
## [143]	"o__Marinilabiliales"	"o__Thermostichales"
## [145]	"o__Jiangellales"	"o__Rhodothermales"
## [147]	"o__Bdellovibrionales"	"o__Acanthopleuribacterales"
## [149]	"o__Ferrovales"	"o__Glycomycetales"
## [151]	"o__Thermomicrobiales"	"o__Silvanigrellales"
## [153]	"o__Sneathiellales"	"o__Euzebyales"
## [155]	"o__Endomicrobiales"	"o__Tepidisphaerales"
## [157]	"o__Lentisphaerales"	"o__Thermoleophilales"
## [159]	"o__Kangiellales"	"o__Parvularculales"
## [161]	"o__Candidatus Sulfidibacterales"	"o__Desulfobaccales"
## [163]	"o__Tichowtungiales"	"o__Sporichthyales"
## [165]	"o__Caldisericales"	"o__Dehalococcoidales"
## [167]	"o__Immundisolibacterales"	"o__Desulfurobacterales"
## [169]	"o__Vicinamibacterales"	"o__Mariprofundales"
## [171]	"o__Syntrophales"	"o__Acidothermales"
## [173]	"o__Desulfurellales"	"o__Thermodesulfovibrionales"
## [175]	"o__Caldilineales"	"o__Trueperales"
## [177]	"o__Salinisphaerales"	"o__Haliangiales"
## [179]	"o__Bacteriovoracales"	"o__Candidatus Pelagibacterales"
## [181]	"o__Chrysiogenales"	"o__Hydrogenophilales"
## [183]	"o__Thermotomaculales"	"o__Desulfomonilales"
## [185]	"o__Kiritimatiellales"	"o__Elusimicrobiales"
## [187]	"o__Catenulesporales"	"o__Actinopolysporales"
## [189]	"o__Gloeomargaritales"	"o__Nannocystales"
## [191]	"o__Candidatus Izemoplasmatales"	"o__Calditrichales"
## [193]	"o__Chthonomonadales"	"o__Limnochordales"

```
## [195] "o__Limisphaerales"          "o__Egicoccales"
## [197] "o__Fimbriimonadales"       "o__Coprothermobacterales"
## [199] "o__Fibrobacterales"        "o__Chroococcidiopsidales"
## [201] "o__Balneolales"           "o__Mesoaciditogales"
## [203] "o__Magnetococcales"
```

```
# Check the amount of unique Species in samples which have and have not been treated with antibiotics
subsetMG %>% ps_filter(AB == "no") %>% get_taxa_unique("Species") # 4464 different orders for non AB tr
```

```
## [1] "s__symbiodeficiens"
## [2] "s__sp. LM6"
## [3] "s__sp. 5116S-3"
## [4] "s__sp. 32K"
## [5] "s__endosymbiont of Acanthamoeba sp. UWC8"
## [6] "s__sp. CR-Ec1"
## [7] "s__rosea"
## [8] "s__malaysiensis"
## [9] "s__leguminosarum"
## [10] "s__larvae"
## [11] "s__paramultivorum"
## [12] "s__sp. TY1-4"
## [13] "s__endophytica"
## [14] "s__flavum"
## [15] "s__genomosp. 3"
## [16] "s__sp. RR6"
## [17] "s__mengziensis"
## [18] "s__"
## [19] "s__sp. ATA002"
## [20] "s__equigenitalium"
## [21] "s__sp. NEAU-sy36"
## [22] "s__sp. M2A.F.Ca.ET.046.03.2.1"
## [23] "s__gei"
## [24] "s__vestfoldensis"
## [25] "s__septicum"
## [26] "s__alkanexedens"
## [27] "s__emersonii"
## [28] "s__sp. LH3H17"
## [29] "s__sp. oral taxon 414"
## [30] "s__sp. ESL0677"
## [31] "s__sphaeroides"
## [32] "s__luteus"
## [33] "s__gaetbulicola"
## [34] "s__mirabilis"
## [35] "s__prevotii"
## [36] "s__sp. DMV24BSW_D"
## [37] "s__commune"
## [38] "s__testudinis"
## [39] "s__macrosporus"
## [40] "s__sp. SCLE84"
## [41] "s__tepida"
## [42] "s__sp. MC1595"
## [43] "s__everestensis"
## [44] "s__oryzae"
## [45] "s__caldiproteolyticus"
```

```

## [46] "s__sp. MTB7"
## [47] "s__difficile"
## [48] "s__dentium"
## [49] "s__sp. E76"
## [50] "s__gangjinensis"
## [51] "s__sp. LQ44"
## [52] "s__profundi"
## [53] "s__australis"
## [54] "s__avium"
## [55] "s__[Mannheimia] succiniciproducens"
## [56] "s__arboricola"
## [57] "s__chokoriensis"
## [58] "s__sp. PSBB023"
## [59] "s__palleroniana"
## [60] "s__abyssi"
## [61] "s__qiguomingii"
## [62] "s__tangfeifanii"
## [63] "s__cyanobacteriorum"
## [64] "s__gloriosae"
## [65] "s__intestini"
## [66] "s__glaucescens"
## [67] "s__sp. RSMS"
## [68] "s__propionicum"
## [69] "s__alhagiae"
## [70] "s__aestuarii"
## [71] "s__gelatinosa"
## [72] "s__sp. DHT3"
## [73] "s__indolicus"
## [74] "s__sp. 14171R-50"
## [75] "s__ruminicola"
## [76] "s__sp. PSKL.D1"
## [77] "s__haemaphysalidis"
## [78] "s__sp. 190D2882"
## [79] "s__actinosclerus"
## [80] "s__suis"
## [81] "s__manihotivorum"
## [82] "s__sp. VBCF_01 NA2"
## [83] "s__deleyi"
## [84] "s__sp. HN-54"
## [85] "s__sp. OT10"
## [86] "s__bestiarum"
## [87] "s__ulcerans"
## [88] "s__pogona"
## [89] "s__pseudopelargi"
## [90] "s__sp. Csp1"
## [91] "s__vannielii"
## [92] "s__lutetiensis"
## [93] "s__kobei"
## [94] "s__fermentans"
## [95] "s__sp. HMF3514"
## [96] "s__sp. CACIAM 19H1"
## [97] "s__akebiae"
## [98] "s__sp. WJP83"
## [99] "s__sp. Y-01"

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## [100] "s__thermophilus"
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## [102] "s__porcitonsillarum"
## [103] "s__farcinica"
## [104] "s__robiniae"
## [105] "s__sp. PDNC005"
## [106] "s__sp. CB0101"
## [107] "s__acidurici"
## [108] "s__radiodurans"
## [109] "s__gryphiswaldense"
## [110] "s__lacunae"
## [111] "s__acetatoxydans"
## [112] "s__taklimakanense"
## [113] "s__flava"
## [114] "s__parvum"
## [115] "s__aurantiacus"
## [116] "s__sp. KBS50"
## [117] "s__tepidum"
## [118] "s__salmonicida"
## [119] "s__sp. B53371"
## [120] "s__gilvus"
## [121] "s__sp. SL97"
## [122] "s__sanguinis"
## [123] "s__boonkerdii"
## [124] "s__endosymbiont 'TC1' of Trimyema compressum"
## [125] "s__harei"
## [126] "s__inhibens"
## [127] "s__seropedicae"
## [128] "s__ambofaciens"
## [129] "s__lytica"
## [130] "s__mobilis"
## [131] "s__cyanea"
## [132] "s__extorquens"
## [133] "s__aurantiacum"
## [134] "s__choladocola"
## [135] "s__torquis"
## [136] "s__dioxanivorans"
## [137] "s__sp. GK1"
## [138] "s__sp. FJAT-42376"
## [139] "s__sp. cx-51"
## [140] "s__sp. InS609-2"
## [141] "s__marinum"
## [142] "s__seohaensis"
## [143] "s__polymyxa"
## [144] "s__sp. N"
## [145] "s__sp. EV170708-02-1"
## [146] "s__lutea"
## [147] "s__nitratiireducens"
## [148] "s__heimbachae"
## [149] "s__halophilus"
## [150] "s__variabilis"
## [151] "s__saerimneri"
## [152] "s__griseocarneus"
## [153] "s__ultunensis"

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 ## [162] "s__sp. S09G 359"
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 ## [167] "s__sp. SK17"
 ## [168] "s__reducens"
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 ## [184] "s__koreensis"
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 ## [197] "s__balearica"
 ## [198] "s__ulvae"
 ## [199] "s__audaxviator"
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 ## [201] "s__sp. YMD87"
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 ## [205] "s__sp. 113-3-9"
 ## [206] "s__confusa"
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 ## [249] "s__sp. 5317J-9"
 ## [250] "s__sp. PAMC28688"
 ## [251] "s__aquaticus"
 ## [252] "s__rhamnosus"
 ## [253] "s__lacus"
 ## [254] "s__sp. IDR2000157661"
 ## [255] "s__terrae"
 ## [256] "s__coyleae"
 ## [257] "s__dokdonensis"
 ## [258] "s__pigmentatum"
 ## [259] "s__oceani"
 ## [260] "s__sp. A34"
 ## [261] "s__khirikhana"


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## [263] "s__sp. SMBL-WEM22"
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## [265] "s__clarkii"
## [266] "s__schleiferi"
## [267] "s__sp. 3B(2020)"
## [268] "s__finlayi"
## [269] "s__armeniacus"
## [270] "s__infernorum"
## [271] "s__pseudintermedia"
## [272] "s__chengjingii"
## [273] "s__otitidis"
## [274] "s__algincola"
## [275] "s__sp. 17Sr1-1"
## [276] "s__ponti"
## [277] "s__sp. MIT S9220"
## [278] "s__sp. C1"
## [279] "s__gambrini"
## [280] "s__sp. SH-PL14"
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## [282] "s__liaowanqingii"
## [283] "s__amarae"
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## [285] "s__sp. HS-3"
## [286] "s__sinusarabici"
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## [291] "s__intermedia"
## [292] "s__agilis"
## [293] "s__desulfuricans"
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## [299] "s__sp. S1-8"
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## [302] "s__agri"
## [303] "s__lapagei"
## [304] "s__swuensis"
## [305] "s__cuenoti"
## [306] "s__negevensis"
## [307] "s__rigui"
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## [309] "s__sp. SCSIO 61187"
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## [311] "s__sulfonilyureivorans"
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## [315] "s__tropicus"

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## [320] "s__staleyi"
## [321] "s__ruber"
## [322] "s__baltica"
## [323] "s__sp. FB-5"
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## [325] "s__amnigena"
## [326] "s__sp. PLM1"
## [327] "s__mishrai"
## [328] "s__uli"
## [329] "s__palythoae"
## [330] "s__sp. ZAC14A_NAIMI4_1"
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## [334] "s__sp. HUAS 5"
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## [336] "s__raozihei"
## [337] "s__glycinifermentans"
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## [341] "s__sp. ZFBP2030"
## [342] "s__pachyrhizi"
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## [344] "s__pecorum"
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## [346] "s__sp. TC1"
## [347] "s__sp. NIBR1757"
## [348] "s__sp. Allo2"
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## [351] "s__bonaserana"
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## [358] "s__sp. SYP-B4668"
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## [361] "s__sp. OMZ 787"
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## [363] "s__coli"
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## [365] "s__oculi"
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## [368] "s__sp. NIES-981"
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 ## [383] "s__sp. DA9"
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 ## [385] "s__dokdonellae"
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 ## [400] "s__jejuni"
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 ## [409] "s__pabuli"
 ## [410] "s__rustigianii"
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 ## [421] "s__fonticola"
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## [424] "s__sp. TSA2s"
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## [430] "s__sp. CB3481"
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## [437] "s__troglodytae"
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## [440] "s__zhangzhii"
## [441] "s__pyrrocinia"
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## [443] "s__sp. ES10-3-2-2"
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## [445] "s__filamentosa"
## [446] "s__aeruginosa"
## [447] "s__hinzii"
## [448] "s__parasuis"
## [449] "s__hydrossis"
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## [471] "s__parmentieri"
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 ## [486] "s__divergens"
 ## [487] "s__sp. SY8519"
 ## [488] "s__sp. ART55/1"
 ## [489] "s__sp. FW306-05-C"
 ## [490] "s__sp. KY-GH-1"
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 ## [492] "s__aggregatum"
 ## [493] "s__sp. PBL-H3"
 ## [494] "s__inopinata"
 ## [495] "s__lutimineralis"
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## [549] "s__oryzoeni"
## [550] "s__iowensis"
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## [574] "s__sp. MSJ-33"
## [575] "s__sp. QXT-31"
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 ## [1167] "s__kubicae"
 ## [1168] "s__rubi"
 ## [1169] "s__sp. CA-293567"
 ## [1170] "s__sp. Hama-1"
 ## [1171] "s__ossetica"
 ## [1172] "s__sp. SL4(2022)"
 ## [1173] "s__johnsonii"
 ## [1174] "s__sp. ZF2019"
 ## [1175] "s__sp. J223"
 ## [1176] "s__fragariae"
 ## [1177] "s__sp. N1-1"
 ## [1178] "s__megalosphaeroides"
 ## [1179] "s__fungicidicus"

[1180] "s__tuberculoostearicum"
 ## [1181] "s__sp. CBA3647"
 ## [1182] "s__sp. IIBBL 290-4"
 ## [1183] "s__salinus"
 ## [1184] "s__sp. AM1P"
 ## [1185] "s__otitidiscaviarum"
 ## [1186] "s__sp. DG15C"
 ## [1187] "s__genomosp. 9"
 ## [1188] "s__antarcticus"
 ## [1189] "s__glucuronolyticum"
 ## [1190] "s__calidifontis"
 ## [1191] "s__kilauensis"
 ## [1192] "s__sp. BJA-103"
 ## [1193] "s__kivui"
 ## [1194] "s__thiophilum"
 ## [1195] "s__sp. I52.16.1"
 ## [1196] "s__kefiranofaciens"
 ## [1197] "s__garvieae"
 ## [1198] "s__sp. Crenshaw"
 ## [1199] "s__jordaniae"
 ## [1200] "s__sp. CCBAU 051011"
 ## [1201] "s__pretiosum"
 ## [1202] "s__coralli"
 ## [1203] "s__sp. XCS3"
 ## [1204] "s__sp. JZ16"
 ## [1205] "s__sp. SD17-2"
 ## [1206] "s__sp. ZC-3"
 ## [1207] "s__sp. WL3"
 ## [1208] "s__fergusonii"
 ## [1209] "s__viridis"
 ## [1210] "s__[Ruminococcus] torques"
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 ## [1213] "s__violaceinigra"
 ## [1214] "s__sp. SCLZS86"
 ## [1215] "s__baarsii"
 ## [1216] "s__corrugata"
 ## [1217] "s__sp. MUD61"
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 ## [1219] "s__tunisiensis"
 ## [1220] "s__piersonii"
 ## [1221] "s__ganghwensis"
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 ## [1223] "s__paraseoulense"
 ## [1224] "s__glumae"
 ## [1225] "s__protegens"
 ## [1226] "s__pituitosa"
 ## [1227] "s__sp. PAMC 29334"
 ## [1228] "s__sp. E13-17"
 ## [1229] "s__festucae"
 ## [1230] "s__vaginalis"
 ## [1231] "s__inquinata"
 ## [1232] "s__phaseoli"
 ## [1233] "s__azorensis"

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 ## [1236] "s__caoxuetaonis"
 ## [1237] "s__thiooxydans"
 ## [1238] "s__sp. WMD882"
 ## [1239] "s__sp. CBW1107"
 ## [1240] "s__fakonensis"
 ## [1241] "s__anthropi"
 ## [1242] "s__sp. URB8-2"
 ## [1243] "s__omphalii"
 ## [1244] "s__yanjieii"
 ## [1245] "s__sp. LMS18"
 ## [1246] "s__pulveris"
 ## [1247] "s__kefiri"
 ## [1248] "s__chromogenes"
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 ## [1250] "s__sp. JQ2195"
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 ## [1252] "s__sp. AL041005-10"
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 ## [1254] "s__sp. 9128"
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 ## [1256] "s__paneuropaeus"
 ## [1257] "s__paraterrae"
 ## [1258] "s__pickettii"
 ## [1259] "s__aminophilus"
 ## [1260] "s__hydrolyticus"
 ## [1261] "s__sp. W027"
 ## [1262] "s__entomophilus"
 ## [1263] "s__kaempferiae"
 ## [1264] "s__sp. PCC 7116"
 ## [1265] "s__sp. FSL R7-0331"
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 ## [1269] "s__sp. G0186"
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 ## [1271] "s__sp. SCSIO W0465"
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 ## [1274] "s__baratii"
 ## [1275] "s__liquefaciens"
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 ## [1282] "s__turgidum"
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 ## [1284] "s__sp. OE 28.3"
 ## [1285] "s__sphenoides"
 ## [1286] "s__albidiflava"
 ## [1287] "s__pudoricolor"

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 ## [1289] "s__angustmyceticus"
 ## [1290] "s__agamarum"
 ## [1291] "s__alginolyticus"
 ## [1292] "s__sp. THN1"
 ## [1293] "s__africae"
 ## [1294] "s__umbonata"
 ## [1295] "s__radiopugnans"
 ## [1296] "s__arsenatis"
 ## [1297] "s__sp. JC009"
 ## [1298] "s__apisilvae"
 ## [1299] "s__sp. CCMP332"
 ## [1300] "s__sacchari"
 ## [1301] "s__succinogenes"
 ## [1302] "s__buecherae"
 ## [1303] "s__sp. NA04385"
 ## [1304] "s__sp. M2A.F.Ca.ET.043.05.1.1"
 ## [1305] "s__sp. GAM44"
 ## [1306] "s__varigena"
 ## [1307] "s__sp. LV10R510-11A"
 ## [1308] "s__formigenes"
 ## [1309] "s__pacaensis"
 ## [1310] "s__sauidimassiliensis"
 ## [1311] "s__sp. JMULE5"
 ## [1312] "s__resistens"
 ## [1313] "s__wenzhouensis"
 ## [1314] "s__sp. NHP19-003"
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 ## [1316] "s__daejeonense"
 ## [1317] "s__sp. HSG2"
 ## [1318] "s__sp. NCRR"
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 ## [1320] "s__sunnyaminii"
 ## [1321] "s__album"
 ## [1322] "s__veronii"
 ## [1323] "s__pulmonicola"
 ## [1324] "s__sp. LS.1a"
 ## [1325] "s__sp. 29361"
 ## [1326] "s__sp. H6"
 ## [1327] "s__sp. G2S3"
 ## [1328] "s__sp. SL250"
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 ## [1330] "s__quasipneumoniae"
 ## [1331] "s__cyaneogriseus"
 ## [1332] "s__lloydii"
 ## [1333] "s__rivuli"
 ## [1334] "s__sp. NIES-4102"
 ## [1335] "s__degradans"
 ## [1336] "s__riograndensis"
 ## [1337] "s__baixiangningiae"
 ## [1338] "s__sp. 107-1"
 ## [1339] "s__tritici"
 ## [1340] "s__sp. BT-42-2"
 ## [1341] "s__paurometabola"

[1342] "s__cloacae"
 ## [1343] "s__warabiya"
 ## [1344] "s__nigra"
 ## [1345] "s__sp. BT-123"
 ## [1346] "s__faecium"
 ## [1347] "s__phenanthrenivorans"
 ## [1348] "s__sp. WAC00303"
 ## [1349] "s__sp. XGS-02"
 ## [1350] "s__sp. ArI3"
 ## [1351] "s__carbinoliphilus"
 ## [1352] "s__mucilaginosus"
 ## [1353] "s__sp. 'Peltigera membranacea cyanobiont' N6"
 ## [1354] "s__thermosuccinogenes"
 ## [1355] "s__haemolysans"
 ## [1356] "s__elongata"
 ## [1357] "s__sp. NBH87"
 ## [1358] "s__espanaensis"
 ## [1359] "s__portucalensis"
 ## [1360] "s__alkalisoli"
 ## [1361] "s__paucimobilis"
 ## [1362] "s__sp. Pc102"
 ## [1363] "s__magna"
 ## [1364] "s__sp. FDAARGOS 1241"
 ## [1365] "s__Candidatus Protofrankia datiscae"
 ## [1366] "s__bogorensis"
 ## [1367] "s__someræ"
 ## [1368] "s__xylosoxidans"
 ## [1369] "s__indicus"
 ## [1370] "s__hominis"
 ## [1371] "s__dysgalactiae"
 ## [1372] "s__sp. LQ25"
 ## [1373] "s__sp. RTd22"
 ## [1374] "s__sp. Jing01"
 ## [1375] "s__brasiliense"
 ## [1376] "s__allomyrinae"
 ## [1377] "s__azurea"
 ## [1378] "s__baculatum"
 ## [1379] "s__phytophila"
 ## [1380] "s__yayanosii"
 ## [1381] "s__sp. DH3716P"
 ## [1382] "s__sp. BT-177"
 ## [1383] "s__aminovorans"
 ## [1384] "s__sp. F8"
 ## [1385] "s__pacifica"
 ## [1386] "s__thiotaurini"
 ## [1387] "s__goodwinii"
 ## [1388] "s__haemolytica"
 ## [1389] "s__[Ochrobactrum] quorumnocens"
 ## [1390] "s__promysalinigenes"
 ## [1391] "s__sp. CC9605"
 ## [1392] "s__metalliredigens"
 ## [1393] "s__plymuthica"
 ## [1394] "s__sp. Tan611"
 ## [1395] "s__sp. LRE541"

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## [1396] "s__urogenitale"
## [1397] "s__sp. RC67"
## [1398] "s__sp. M166"
## [1399] "s__liliifuscus"
## [1400] "s__cholerae"
## [1401] "s__flocculans"
## [1402] "s__sp. SAU14A_NAIMI4_5"
## [1403] "s__sp. ZJ106"
## [1404] "s__syringae group genomosp. 7"
## [1405] "s__telluris"
## [1406] "s__terrenum"
## [1407] "s__konosiri"
## [1408] "s__equorum"
## [1409] "s__sp. JZB09"
## [1410] "s__sp. CNQ-509"
## [1411] "s__rotundus"
## [1412] "s__lablabi"
## [1413] "s__glycaniphila"
## [1414] "s__sp. S01"
## [1415] "s__aromatica"
## [1416] "s__sp. Colony194"
## [1417] "s__caseolyticus"
## [1418] "s__sp. CZR27"
## [1419] "s__osloensis"
## [1420] "s__intracellularis"
## [1421] "s__fallax"
## [1422] "s__bifermentans"
## [1423] "s__sp. 5413J-13"
## [1424] "s__sp. SK50-23"
## [1425] "s__acetigenes"
## [1426] "s__chauvoei"
## [1427] "s__venezuelae"
## [1428] "s__sp. WAC 01438"
## [1429] "s__arginini"
## [1430] "s__oryzicola"
## [1431] "s__aureovorticillatus"
## [1432] "s__sp. QY071"
## [1433] "s__sp. SG1"
## [1434] "s__versatilis"
## [1435] "s__sp. WMD812"
## [1436] "s__sp. SA4125"
## [1437] "s__albus"
## [1438] "s__sulfuroxidans"
## [1439] "s__baengnokdamensis"
## [1440] "s__allii"
## [1441] "s__sp. OT7"
## [1442] "s__sp. FDAARGOS 1409"
## [1443] "s__urinaehominis"
## [1444] "s__sp. OxB-1"
## [1445] "s__sp. Bac332"
## [1446] "s__abscessus"
## [1447] "s__sp. JM1"
## [1448] "s__nigripulchritudo"
## [1449] "s__sp. M30-35"

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## [1450] "s__methanolica"
## [1451] "s__sp. MR_MD2014"
## [1452] "s__sp. YPW1"
## [1453] "s__phytofermentans"
## [1454] "s__sp. HUAS 15-9"
## [1455] "s__[Clostridium] colinum"
## [1456] "s__sp. 113P3"
## [1457] "s__chenweiae"
## [1458] "s__metallilatus"
## [1459] "s__hygroscopicus"
## [1460] "s__penaei"
## [1461] "s__sp. CX169"
## [1462] "s__coccoides"
## [1463] "s__guangzhouensis"
## [1464] "s__arabaticum"
## [1465] "s__sp. PAMC26645"
## [1466] "s__sp. VKM Ac-2759"
## [1467] "s__pnomenusa"
## [1468] "s__pseudoxylorus"
## [1469] "s__singaporensis"
## [1470] "s__ferrireducens"
## [1471] "s__sp. NFH-SH190041"
## [1472] "s__chenwenguii"
## [1473] "s__sp. QL22"
## [1474] "s__autotrophicum"
## [1475] "s__lutrae"
## [1476] "s__lunaelactis"
## [1477] "s__phocaeense"
## [1478] "s__alcaligenes"
## [1479] "s__aquatilis"
## [1480] "s__litorale"
## [1481] "s__sp. NSJ-69"
## [1482] "s__cervicalis"
## [1483] "s__sp. IMCC11727"
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## [1485] "s__xylanophilus"
## [1486] "s__americana"
## [1487] "s__caseinilytica"
## [1488] "s__vulnificus"
## [1489] "s__cinaedi"
## [1490] "s__rimosus"
## [1491] "s__oleovorans"
## [1492] "s__novyi"
## [1493] "s__sp. G2-5"
## [1494] "s__sp. XC 2026"
## [1495] "s__kimnyeongensis"
## [1496] "s__kermanshahensis"
## [1497] "s__sp. oral taxon 218"
## [1498] "s__brandeum"
## [1499] "s__iranensis"
## [1500] "s__similis"
## [1501] "s__japonica"
## [1502] "s__antibioticus"
## [1503] "s__mucosae"

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## [1504] "s__sp. INOP01"
## [1505] "s__piscicola"
## [1506] "s__uncultured bacterium"
## [1507] "s__sp. CY52-2"
## [1508] "s__sp. WL1"
## [1509] "s__echinospora"
## [1510] "s__opportunatum"
## [1511] "s__sp. Z423-1"
## [1512] "s__arabiense"
## [1513] "s__dextrinosolvens"
## [1514] "s__gottheilii"
## [1515] "s__lizhenjunii"
## [1516] "s__avenae"
## [1517] "s__actuosus"
## [1518] "s__sp. Bac330"
## [1519] "s__sp. D2"
## [1520] "s__cellulans"
## [1521] "s__xylaniphila"
## [1522] "s__phagedenis"
## [1523] "s__brevissima"
## [1524] "s__ignavus"
## [1525] "s__microaerophilus"
## [1526] "s__halocryophilus"
## [1527] "s__avicenniae"
## [1528] "s__sprentiae"
## [1529] "s__sp. PIV-1"
## [1530] "s__sp. FDAARGOS_375"
## [1531] "s__sp. WB-2"
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## [1533] "s__tanakiae"
## [1534] "s__sp. MEDNS5"
## [1535] "s__chagasii"
## [1536] "s__innocua"
## [1537] "s__pinensis"
## [1538] "s__sinuspersici"
## [1539] "s__cowanii"
## [1540] "s__rhodesiae"
## [1541] "s__sp. 336/3"
## [1542] "s__pectinilyticus"
## [1543] "s__tenebrarum"
## [1544] "s__richardii"
## [1545] "s__candidum"
## [1546] "s__sp. CBA3606"
## [1547] "s__temperans"
## [1548] "s__tolaasii"
## [1549] "s__violaceusniger"
## [1550] "s__sp. LG1267"
## [1551] "s__sp. KACC 23028"
## [1552] "s__atlantisensis"
## [1553] "s__fredii"
## [1554] "s__sp. WH 8020"
## [1555] "s__sp. M6A.T.Cr.TU.016.01.1.1"
## [1556] "s__sp. NRS527"
## [1557] "s__sobrinus"

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 ## [1559] "s__vicinigordonae"
 ## [1560] "s__sp. YPD9-1"
 ## [1561] "s__pumilum"
 ## [1562] "s__lithotrophicum"
 ## [1563] "s__ectocarpi"
 ## [1564] "s__sp. Y32M11"
 ## [1565] "s__toyakuensis"
 ## [1566] "s__phaeovibrioides"
 ## [1567] "s__sp. 3H"
 ## [1568] "s__sp. S1D4-14"
 ## [1569] "s__sp. CKK8"
 ## [1570] "s__lilanjuaniae"
 ## [1571] "s__sp. YG1"
 ## [1572] "s__acetoxydans"
 ## [1573] "s__sp. Hal144"
 ## [1574] "s__sp. AM 2-1-1"
 ## [1575] "s__delphini"
 ## [1576] "s__sp. CCB-MM3"
 ## [1577] "s__malmoense"
 ## [1578] "s__sp. OPL5"
 ## [1579] "s__olearia"
 ## [1580] "s__clausii"
 ## [1581] "s__brachiatum"
 ## [1582] "s__eggerthii"
 ## [1583] "s__sp. ZY201224"
 ## [1584] "s__sp. THAF30"
 ## [1585] "s__influenzae"
 ## [1586] "s__sp. S6"
 ## [1587] "s__axanthum"
 ## [1588] "s__sp. HBX-1"
 ## [1589] "s__sp. AH1"
 ## [1590] "s__towneri"
 ## [1591] "s__flexa"
 ## [1592] "s__tuirus"
 ## [1593] "s__faecalis"
 ## [1594] "s__sp. Go-475"
 ## [1595] "s__ratti"
 ## [1596] "s__sp. St316"
 ## [1597] "s__pseudogrignonensis"
 ## [1598] "s__Blochmannia endosymbiont of Polyrhachis (Hedomyrma) turneri"
 ## [1599] "s__tractuosa"
 ## [1600] "s__sp. HKU1"
 ## [1601] "s__ferriphilum"
 ## [1602] "s__damselae"
 ## [1603] "s__media"
 ## [1604] "s__Enterobacteriaceae endosymbiont of Donacia tomentosa"
 ## [1605] "s__panamensis"
 ## [1606] "s__carniphilus"
 ## [1607] "s__methoxysyntrophicus"
 ## [1608] "s__capnotolerans"
 ## [1609] "s__diazotrophicus"
 ## [1610] "s__sp. SL47"
 ## [1611] "s__phosphovorus"

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## [1612] "s__vincentii"
## [1613] "s__sp. M41"
## [1614] "s__sp. WS11"
## [1615] "s__sp. G01H"
## [1616] "s__simplex"
## [1617] "s__hydrophila"
## [1618] "s__aidingensis"
## [1619] "s__thermoresistibile"
## [1620] "s__sp. BPS33"
## [1621] "s__brasiliensis"
## [1622] "s__sp. E15-22"
## [1623] "s__jaguaris"
## [1624] "s__phocoenae"
## [1625] "s__vanderleydeniana"
## [1626] "s__sp. LM7"
## [1627] "s__zhachilii"
## [1628] "s__sp. 63ED37-2"
## [1629] "s__cytotoxicus"
## [1630] "s__saxobsidens"
## [1631] "s__terrigena"
## [1632] "s__sp. H121"
## [1633] "s__daltonii"
## [1634] "s__pediculischaeffi"
## [1635] "s__aquaticum"
## [1636] "s__humicireducens"
## [1637] "s__genisteinicus"
## [1638] "s__sp. PMCC200344"
## [1639] "s__pentosaceus"
## [1640] "s__phocisimile"
## [1641] "s__sp. CCB-ST2H9"
## [1642] "s__degensii"
## [1643] "s__sp. B32"
## [1644] "s__jordanis"
## [1645] "s__warneri"
## [1646] "s__sp. PCC 7327"
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## [1648] "s__blattae"
## [1649] "s__wieringae"
## [1650] "s__cavernae"
## [1651] "s__medellinensis"
## [1652] "s__tardum"
## [1653] "s__crateris"
## [1654] "s__senegalensis"
## [1655] "s__paucivorans"
## [1656] "s__megapolitana"
## [1657] "s__sp. WMMD987"
## [1658] "s__botulinum"
## [1659] "s__composti"
## [1660] "s__sp. 1566"
## [1661] "s__orientale"
## [1662] "s__sp. MTM4"
## [1663] "s__auricularis"
## [1664] "s__hongtaonis"
## [1665] "s__monticola"

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 ## [1668] "s__lactatiformans"
 ## [1669] "s__pukyongi"
 ## [1670] "s__stabekisii"
 ## [1671] "s__pantholopis"
 ## [1672] "s__multitudinisentens"
 ## [1673] "s__sp. No. 7"
 ## [1674] "s__urinaeequi"
 ## [1675] "s__sp. P6W"
 ## [1676] "s__nojiriensis"
 ## [1677] "s__vibrioformis"
 ## [1678] "s__tractae"
 ## [1679] "s__sp. AM 4-1-1"
 ## [1680] "s__lichenicola"
 ## [1681] "s__Verrucosispora sp. WMMD1129"
 ## [1682] "s__sp. AGMB13025"
 ## [1683] "s__hwasookii"
 ## [1684] "s__bohemicus"
 ## [1685] "s__lividus"
 ## [1686] "s__minnesotensis"
 ## [1687] "s__nucleatum"
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 ## [3274] "s__oecophyllae"
 ## [3275] "s__sp. DNDY-54"
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 ## [3277] "s__pauculus"
 ## [3278] "s__sp. L3A3"
 ## [3279] "s__crinochetorum"
 ## [3280] "s__sp. ZJ-18"
 ## [3281] "s__pusense"
 ## [3282] "s__sp. MAS-1"
 ## [3283] "s__sp. CAU 1644"
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 ## [3285] "s__sp. TF1N1"

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 ## [3288] "s__sp. AK164"
 ## [3289] "s__sp. F-323"
 ## [3290] "s__sp. Te-1"
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 ## [3292] "s__primoryensis"
 ## [3293] "s__congonensis"
 ## [3294] "s__raffinosis"
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 ## [3438] "s__soli (ex Cha et al. 2016)"
 ## [3439] "s__sp. VG12"
 ## [3440] "s__sp. JNUCC-31"
 ## [3441] "s__sp. Alg239-R112"
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 ## [3443] "s__uterequi"
 ## [3444] "s__sonnei"
 ## [3445] "s__nivis"
 ## [3446] "s__tumorigenes"
 ## [3447] "s__corydidari"

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 ## [3552] "s__sp. GSA-30"
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 ## [3554] "s__sp. 8C15b"
 ## [3555] "s__ramosus"

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## [3556] "s__herdmanii"
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## [3559] "s__frumentii"
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 ## [3619] "s__acidiformans"
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 ## [4321] "s__faecimaris"
 ## [4322] "s__macleodii"
 ## [4323] "s__thuringiensis"
 ## [4324] "s__[Ruminococcus] lactaris"
 ## [4325] "s__helvum"
 ## [4326] "s__sp. 24E2"
 ## [4327] "s__graeca"
 ## [4328] "s__taurus"
 ## [4329] "s__polytropus"
 ## [4330] "s__craniellae"
 ## [4331] "s__sp. W7"
 ## [4332] "s__sp. I71"
 ## [4333] "s__euryhalodurans"
 ## [4334] "s__corsicus"
 ## [4335] "s__elizabethae"
 ## [4336] "s__galactanivorans"
 ## [4337] "s__fengzijianii"
 ## [4338] "s__sp. CCGE532"
 ## [4339] "s__phaeum"
 ## [4340] "s__restrictus"
 ## [4341] "s__sp. VKM Ac-2760"
 ## [4342] "s__campestrisoli"
 ## [4343] "s__exhalans"
 ## [4344] "s__leprae"
 ## [4345] "s__sp. MWH-Spelu-300-X4"
 ## [4346] "s__gardneri"
 ## [4347] "s__sp. JY-7876"
 ## [4348] "s__stuartii"
 ## [4349] "s__enterica"
 ## [4350] "s__sp. CBA3102"
 ## [4351] "s__fastidiosus"
 ## [4352] "s__rhizogenes"
 ## [4353] "s__sp. L2-79-05"
 ## [4354] "s__kroppenstedtii"
 ## [4355] "s__sp. ST1015"
 ## [4356] "s__affigens"
 ## [4357] "s__sp. CS682"
 ## [4358] "s__scardovii"
 ## [4359] "s__galeata"
 ## [4360] "s__ganghwense"
 ## [4361] "s__ishigakiensis"
 ## [4362] "s__lavendulae"
 ## [4363] "s__splachnicus"
 ## [4364] "s__sp. UDSM-2020"
 ## [4365] "s__sp. S16"

[4366] "s__grossiae"
 ## [4367] "s__larrymoorei"
 ## [4368] "s__sp. 11-B-312"
 ## [4369] "s__sp. NLF-5-8"
 ## [4370] "s__proteinivorum"
 ## [4371] "s__sp. A6099"
 ## [4372] "s__serpentiformis"
 ## [4373] "s__giovannonii"
 ## [4374] "s__sp. HYN0024"
 ## [4375] "s__sp. MS455"
 ## [4376] "s__sp. BDJS001"
 ## [4377] "s__sp. NtRootA1"
 ## [4378] "s__sp. CB1024"
 ## [4379] "s__saccharophila"
 ## [4380] "s__sp. X-1"
 ## [4381] "s__sp. E4742"
 ## [4382] "s__tangerina"
 ## [4383] "s__sp. DMU1"
 ## [4384] "s__mori"
 ## [4385] "s__bethesdensis"
 ## [4386] "s__sabulinigri"
 ## [4387] "s__sp. KNUC1210"
 ## [4388] "s__sp. Mg1"
 ## [4389] "s__obscurus"
 ## [4390] "s__sp. 1608163"
 ## [4391] "s__zengyii"
 ## [4392] "s__luti"
 ## [4393] "s__sp. SCG-1"
 ## [4394] "s__sp. C5510"
 ## [4395] "s__sp. NIBR2454"
 ## [4396] "s__boletus"
 ## [4397] "s__cryptoxanthini"
 ## [4398] "s__thiophilus"
 ## [4399] "s__radicincitans"
 ## [4400] "s__sp. K1W22B-7"
 ## [4401] "s__sp. UASWS1016"
 ## [4402] "s__rhusiopathiae"
 ## [4403] "s__sp. T93"
 ## [4404] "s__sp. KIS68-7"
 ## [4405] "s__sp. WB94"
 ## [4406] "s__cryptocerci"
 ## [4407] "s__sp. CF"
 ## [4408] "s__binhaiensis"
 ## [4409] "s__sp. Marseille-Q7828"
 ## [4410] "s__sp. AAP5"
 ## [4411] "s__sp. L3-i23"
 ## [4412] "s__youngiae"
 ## [4413] "s__sp. FeN2"
 ## [4414] "s__pinxianii"
 ## [4415] "s__sp. IHBB 10380"
 ## [4416] "s__sp. Fw109-5"
 ## [4417] "s__furnissii"
 ## [4418] "s__sp. Adler-ghost"
 ## [4419] "s__canariense"

```

## [4420] "s__producens"
## [4421] "s__pontiacus"
## [4422] "s__sp. BJN0003"
## [4423] "s__multipartita"
## [4424] "s__mayonis"
## [4425] "s__roseola"
## [4426] "s__phymatum"
## [4427] "s__sedlakii"
## [4428] "s__micra"
## [4429] "s__sp. OM7"
## [4430] "s__orientis"
## [4431] "s__sp. AONIH1"
## [4432] "s__inkyongensis"
## [4433] "s__xenophagum"
## [4434] "s__sp. sptzw28"
## [4435] "s__sp. ESL0682"
## [4436] "s__carotovorum"
## [4437] "s__sp. CCBAU 51753"
## [4438] "s__lariciata"
## [4439] "s__atrarenae"
## [4440] "s__mediterranei"
## [4441] "s__sp. R24"
## [4442] "s__sp. PIA16"
## [4443] "s__ferrophilus"
## [4444] "s__chlorobenzoica"
## [4445] "s__vulturis"
## [4446] "s__drentensis"
## [4447] "s__sanyensis"
## [4448] "s__hallii"
## [4449] "s__gerontici"
## [4450] "s__sp. SCSIO52902"
## [4451] "s__obscuriglobus"
## [4452] "s__sp. TRM1-10"
## [4453] "s__sp. YIM 151385"
## [4454] "s__cetorum"
## [4455] "s__fucicola"
## [4456] "s__amoebiphila"
## [4457] "s__cellanae"
## [4458] "s__sp. LPB0260"
## [4459] "s__mysorens"
## [4460] "s__xylanus"
## [4461] "s__eburnea"
## [4462] "s__liangshanensis"
## [4463] "s__formicoaceticum"
## [4464] "s__russatus"

```

```
subsetMG %>% ps_filter(AB == "yes") %>% get_taxa_unique("Species") # 2347 different orders for AB treat
```

```

## [1] "s__sp. LM6"
## [2] "s__sp. 5116S-3"
## [3] "s__sp. 32K"
## [4] "s__sp. CJ74"
## [5] "s__sp. CR-Ec1"
## [6] "s__malaysiensis"

```

```

## [7] "s__leguminosarum"
## [8] "s__larvae"
## [9] "s__salina"
## [10] "s__sp. TY1-4"
## [11] "s__sp. RR6"
## [12] "s__mengziensis"
## [13] "s__"
## [14] "s__equigenitalium"
## [15] "s__sp. NEAU-sy36"
## [16] "s__gei"
## [17] "s__vestfoldensis"
## [18] "s__septicum"
## [19] "s__alkanexedens"
## [20] "s__sp. D15"
## [21] "s__sp. THAF1"
## [22] "s__sphaeroides"
## [23] "s__luteus"
## [24] "s__mirabilis"
## [25] "s__prevotii"
## [26] "s__testudinis"
## [27] "s__macrosporus"
## [28] "s__sp. SCLE84"
## [29] "s__tepida"
## [30] "s__salinestris"
## [31] "s__caldiproteolyticus"
## [32] "s__difficile"
## [33] "s__dentium"
## [34] "s__sp. E76"
## [35] "s__sp. LQ44"
## [36] "s__profundi"
## [37] "s__australis"
## [38] "s__avium"
## [39] "s__sp. KSB-10"
## [40] "s__arboricola"
## [41] "s__palleroniana"
## [42] "s__abyssi"
## [43] "s__qiguomingii"
## [44] "s__sihwensis"
## [45] "s__gloriosae"
## [46] "s__intestinalis"
## [47] "s__propionicum"
## [48] "s__aestuarii"
## [49] "s__indolicus"
## [50] "s__ruminicola"
## [51] "s__haemaphysalidis"
## [52] "s__sp. 190D2882"
## [53] "s__manihotivorum"
## [54] "s__deleyi"
## [55] "s__sp. OT10"
## [56] "s__bestiarum"
## [57] "s__ulcerans"
## [58] "s__sp. DTU12.3"
## [59] "s__vannielii"
## [60] "s__lutetiensis"

```

```

## [61] "s__fermentans"
## [62] "s__ruber"
## [63] "s__rotai"
## [64] "s__sp. CCBAU 05631"
## [65] "s__sp. Y-01"
## [66] "s__tsuruhatensis"
## [67] "s__porcitonsillarum"
## [68] "s__robiniae"
## [69] "s__sp. PDNC005"
## [70] "s__acidurici"
## [71] "s__gryphiswaldense"
## [72] "s__taklimakanense"
## [73] "s__parvum"
## [74] "s__aurantiacus"
## [75] "s__sp. KBS50"
## [76] "s__indica"
## [77] "s__tepidum"
## [78] "s__salmonicida"
## [79] "s__sp. B53371"
## [80] "s__corsica"
## [81] "s__gilvus"
## [82] "s__psychrotolerans"
## [83] "s__sp. SL97"
## [84] "s__sanguinis"
## [85] "s__endosymbiont 'TC1' of Trimyema compressum"
## [86] "s__harei"
## [87] "s__sp. Aquia_213"
## [88] "s__seropedicae"
## [89] "s__sp. 1_2014MBL_MicDiv"
## [90] "s__regensburgei"
## [91] "s__mobilis"
## [92] "s__extorquens"
## [93] "s__aurantiacum"
## [94] "s__dioxanivorans"
## [95] "s__sicerae"
## [96] "s__sp. cx-51"
## [97] "s__polymyxa"
## [98] "s__planticola"
## [99] "s__lutea"
## [100] "s__indicum"
## [101] "s__saerimneri"
## [102] "s__griseocarneus"
## [103] "s__sp. YIM 121038"
## [104] "s__roseus"
## [105] "s__microcysteis"
## [106] "s__sp. PL-2018"
## [107] "s__succinifaciens"
## [108] "s__sunshinyii"
## [109] "s__sp. SK17"
## [110] "s__insidiosa"
## [111] "s__thermophilum"
## [112] "s__sp. GAS474"
## [113] "s__denitrificans"
## [114] "s__sp. W1SF4"

```


[115] "s__sp. 19GGS1-52"
 ## [116] "s__ianthinogenes"
 ## [117] "s__sp. R3"
 ## [118] "s__infantis"
 ## [119] "s__sp. TCL240-02"
 ## [120] "s__changnyeongensis"
 ## [121] "s__terpenica"
 ## [122] "s__wadenswilerensis"
 ## [123] "s__thiooxidans"
 ## [124] "s__stuttgartiensis"
 ## [125] "s__bovigenitalium"
 ## [126] "s__audaxviator"
 ## [127] "s__sp. MX-AZ03"
 ## [128] "s__mitochondrii"
 ## [129] "s__confusa"
 ## [130] "s__aalborgensis"
 ## [131] "s__thiooxydans"
 ## [132] "s__carolinensis"
 ## [133] "s__sp. LMS6"
 ## [134] "s__hiranonis"
 ## [135] "s__sp. JY-X169"
 ## [136] "s__syrphidicola"
 ## [137] "s__lujinxingii"
 ## [138] "s__suranareeae"
 ## [139] "s__sp. Wa41.01b-1"
 ## [140] "s__sputigena"
 ## [141] "s__defluvii"
 ## [142] "s__nasimurium"
 ## [143] "s__sp. zrk46"
 ## [144] "s__sp. YS"
 ## [145] "s__sp. J2-11"
 ## [146] "s__musculi"
 ## [147] "s__iowae"
 ## [148] "s__fungivorans"
 ## [149] "s__vitis"
 ## [150] "s__nodosus"
 ## [151] "s__sedimenticola"
 ## [152] "s__gobiensis"
 ## [153] "s__bremense"
 ## [154] "s__stearothermophilus"
 ## [155] "s__sp. FHR47"
 ## [156] "s__sp. 5317J-9"
 ## [157] "s__sp. KH32C"
 ## [158] "s__aquaticus"
 ## [159] "s__rhamnosus"
 ## [160] "s__lacus"
 ## [161] "s__terrae"
 ## [162] "s__minervae"
 ## [163] "s__pigmentatum"
 ## [164] "s__oceanii"
 ## [165] "s__sp. 3B(2020)"
 ## [166] "s__armeniacus"
 ## [167] "s__chengjingii"
 ## [168] "s__ponti"

```

## [169] "s__sp. SH-PL14"
## [170] "s__liaowanqingii"
## [171] "s__amarae"
## [172] "s__gelatinosus"
## [173] "s__dokdonensis"
## [174] "s__mesenteroides"
## [175] "s__intestinalis"
## [176] "s__agilis"
## [177] "s__marina"
## [178] "s__agri"
## [179] "s__cuenoti"
## [180] "s__pyridinivorans"
## [181] "s__sp. SCSIO 61187"
## [182] "s__caldus"
## [183] "s__sulfonilyureivorans"
## [184] "s__parva"
## [185] "s__tropicus"
## [186] "s__crevioricanis"
## [187] "s__broussonetiae"
## [188] "s__sp. oral taxon 807"
## [189] "s__alboniger"
## [190] "s__baltica"
## [191] "s__sp. FB-5"
## [192] "s__uli"
## [193] "s__viscericola"
## [194] "s__sp. HUAS 5"
## [195] "s__longa"
## [196] "s__glycinifermentans"
## [197] "s__gasseri"
## [198] "s__hadrus"
## [199] "s__sp. SM18"
## [200] "s__sp. TC1"
## [201] "s__sp. Allo2"
## [202] "s__condimenti"
## [203] "s__sp. WY228"
## [204] "s__ficus"
## [205] "s__sp. SCSIO 43088"
## [206] "s__sp. SD9660Na"
## [207] "s__sp. TS-1"
## [208] "s__radiophilus"
## [209] "s__sp. OMZ 787"
## [210] "s__coli"
## [211] "s__oculi"
## [212] "s__sp. NIES-981"
## [213] "s__circulans"
## [214] "s__phyllanthi"
## [215] "s__thailandicus"
## [216] "s__sp. I507"
## [217] "s__sp. ESL0695"
## [218] "s__sp. INBF002"
## [219] "s__pedis"
## [220] "s__punjabense"
## [221] "s__ginsenosidimutans"
## [222] "s__Candidatus Sodalis pierantonius"

```

[223] "s__denticola"
 ## [224] "s__pakistanensis"
 ## [225] "s__sp. ZJ405"
 ## [226] "s__nataicola"
 ## [227] "s__amylolyticus"
 ## [228] "s__dentocariosa"
 ## [229] "s__timonensis"
 ## [230] "s__protophormiae"
 ## [231] "s__sp. ESL0732"
 ## [232] "s__crassostreae"
 ## [233] "s__rubrum"
 ## [234] "s__pabuli"
 ## [235] "s__sp. D3"
 ## [236] "s__sp. YJ01"
 ## [237] "s__sp. 12200R-103"
 ## [238] "s__melaninogenica"
 ## [239] "s__sp. 2438"
 ## [240] "s__siamensis"
 ## [241] "s__jinjuensis"
 ## [242] "s__pyogenes"
 ## [243] "s__phytohabitans"
 ## [244] "s__sp. BSN-002"
 ## [245] "s__atlanticum"
 ## [246] "s__pseudolongum"
 ## [247] "s__filamentosa"
 ## [248] "s__sp. HTF-F"
 ## [249] "s__acetylenivorans"
 ## [250] "s__sp. Marseille-Q4385"
 ## [251] "s__brevis"
 ## [252] "s__bryantii"
 ## [253] "s__sp. AP-Jannik-300A-C4"
 ## [254] "s__oligotrophicus"
 ## [255] "s__sedentarius"
 ## [256] "s__ruminantium"
 ## [257] "s__thermophilus"
 ## [258] "s__rhizoryzae"
 ## [259] "s__entomophila"
 ## [260] "s__sp. SCSIO 43204"
 ## [261] "s__sp. CA-103260"
 ## [262] "s__aliphaticivorans"
 ## [263] "s__butyriciproducens"
 ## [264] "s__ginsengisoli"
 ## [265] "s__salsilacus"
 ## [266] "s__albertii"
 ## [267] "s__variabile"
 ## [268] "s__urealyticum"
 ## [269] "s__divergens"
 ## [270] "s__sp. SY8519"
 ## [271] "s__sp. ART55/1"
 ## [272] "s__lutimineralis"
 ## [273] "s__sp. PAMC 26628"
 ## [274] "s__cortegadensis"
 ## [275] "s__paragallinarum"
 ## [276] "s__nishinomiyaensis"

```

## [277] "s__sp. AP4-R1"
## [278] "s__sp. SVR"
## [279] "s__anaerophila"
## [280] "s__sp. M54"
## [281] "s__solani"
## [282] "s__alaskensis"
## [283] "s__cryaerophilus"
## [284] "s__maiorica"
## [285] "s__salaris"
## [286] "s__cedrina"
## [287] "s__sp. oral taxon 920"
## [288] "s__panis"
## [289] "s__sp. KU26590"
## [290] "s__sp. resist"
## [291] "s__viridans"
## [292] "s__moriokaense"
## [293] "s__cicadellinicola"
## [294] "s__aestuariativum"
## [295] "s__brennaborense"
## [296] "s__sp. fd1-xmd"
## [297] "s__resinovorans"
## [298] "s__baldaniorum"
## [299] "s__caldoxylosilyticus"
## [300] "s__sp. oral taxon 126"
## [301] "s__chitae"
## [302] "s__sp. dk3624"
## [303] "s__sp. CT06"
## [304] "s__sp. AS-1"
## [305] "s__alkaliphilum"
## [306] "s__yuyongxinii"
## [307] "s__sp. 17 mud 1-3"
## [308] "s__tokaiense"
## [309] "s__bovis"
## [310] "s__frisingensis"
## [311] "s__adhaerens"
## [312] "s__pontis"
## [313] "s__insolitus"
## [314] "s__sp. IE-0392"
## [315] "s__sp. AA4"
## [316] "s__marinus"
## [317] "s__uniformis"
## [318] "s__sp. T21"
## [319] "s__sp. MSJ-33"
## [320] "s__sp. QXT-31"
## [321] "s__enoeca"
## [322] "s__brassicae"
## [323] "s__plakortidis"
## [324] "s__wilhelmae"
## [325] "s__citri"
## [326] "s__sp. SP2"
## [327] "s__sp. SFB-rat-Yit"
## [328] "s__sp. 31-12"
## [329] "s__paragasseri"
## [330] "s__pseudomultivorans"

```

```

## [331] "s__kanbiaonis"
## [332] "s__riparius"
## [333] "s__sp. JS666"
## [334] "s__subtilis"
## [335] "s__taeniospiralis"
## [336] "s__stomatis"
## [337] "s__falkenbergense"
## [338] "s__radicidentis"
## [339] "s__sp. MZ1T"
## [340] "s__niveus"
## [341] "s__halelectricus"
## [342] "s__spongiae"
## [343] "s__sp. SMC-4"
## [344] "s__sp. 313"
## [345] "s__dioscoreae"
## [346] "s__testaceum"
## [347] "s__sp. KU28468"
## [348] "s__deserti"
## [349] "s__aerofaciens"
## [350] "s__sp. T7-7"
## [351] "s__suffuscus"
## [352] "s__anyangense"
## [353] "s__sp. ATCC 8456"
## [354] "s__soli"
## [355] "s__amylovorus"
## [356] "s__rodentium"
## [357] "s__leadbetteri"
## [358] "s__jeikeium"
## [359] "s__filiformis"
## [360] "s__gallolyticus"
## [361] "s__sagamiensis"
## [362] "s__sp. HWE-109"
## [363] "s__bryophytorum"
## [364] "s__chitinilytica"
## [365] "s__kloosii"
## [366] "s__urativorans"
## [367] "s__intestinale"
## [368] "s__ptyseos"
## [369] "s__megaterium"
## [370] "s__siliguriense"
## [371] "s__binotii"
## [372] "s__vulgaris"
## [373] "s__ginsenosidivorax"
## [374] "s__sp. HUAS 3"
## [375] "s__sp. PCC 6312"
## [376] "s__novalis"
## [377] "s__sp. WMMA1423"
## [378] "s__kerstersii"
## [379] "s__albida"
## [380] "s__steedae"
## [381] "s__parmense"
## [382] "s__violascens"
## [383] "s__sp. Y33"
## [384] "s__sp. SDN3"

```

[385] "s__liquoris"
 ## [386] "s__subterraneus"
 ## [387] "s__kingae"
 ## [388] "s__coprophilus"
 ## [389] "s__tropica"
 ## [390] "s__kondratievae"
 ## [391] "s__sp. TUM22785"
 ## [392] "s__riyadhense"
 ## [393] "s__thermoglucosidasius"
 ## [394] "s__caledoniensis"
 ## [395] "s__sp. ZAC14D2_NAIMI4_7"
 ## [396] "s__diversum"
 ## [397] "s__sudanensis"
 ## [398] "s__taiwanensis"
 ## [399] "s__scoriae"
 ## [400] "s__diernhoferi"
 ## [401] "s__dendaii"
 ## [402] "s__caeni"
 ## [403] "s__sp. Arg-1"
 ## [404] "s__sp. NA02950"
 ## [405] "s__sp. zg-1228"
 ## [406] "s__saguini"
 ## [407] "s__sp. LW097"
 ## [408] "s__biformata"
 ## [409] "s__sp. S465"
 ## [410] "s__sp. KCTC 42545"
 ## [411] "s__sp. zg1085"
 ## [412] "s__sp. N1846"
 ## [413] "s__sp. PP1Y"
 ## [414] "s__sp. ZRK36"
 ## [415] "s__perfringens"
 ## [416] "s__lenta"
 ## [417] "s__Candidatus Filomicrobium marinum"
 ## [418] "s__hiltneri"
 ## [419] "s__champanellensis"
 ## [420] "s__uraniireducens"
 ## [421] "s__terrifontis"
 ## [422] "s__copri"
 ## [423] "s__cottewii"
 ## [424] "s__constellatus"
 ## [425] "s__imitans"
 ## [426] "s__muciniphila"
 ## [427] "s__sp. SGAir0287"
 ## [428] "s__concisus"
 ## [429] "s__acidaminovorans"
 ## [430] "s__lwoffii"
 ## [431] "s__sp. RF6"
 ## [432] "s__elongatus"
 ## [433] "s__fragi"
 ## [434] "s__sp. ESL0769"
 ## [435] "s__keddieii"
 ## [436] "s__antranikianii"
 ## [437] "s__radingae"
 ## [438] "s__mercurii"

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## [439] "s__huaxiensis"
## [440] "s__gauvreauii"
## [441] "s__ammoniagenes"
## [442] "s__thermautotrophica"
## [443] "s__sp. BT18"
## [444] "s__fusiformis"
## [445] "s__sp. MLAF003"
## [446] "s__xyli"
## [447] "s__quercinecans"
## [448] "s__sp. HUAS 11-8"
## [449] "s__oleivorans"
## [450] "s__acidisoli"
## [451] "s__luhongzhouii"
## [452] "s__zhejiangensis"
## [453] "s__radioresistens"
## [454] "s__stutzeri"
## [455] "s__sp. HTCC2170"
## [456] "s__mangrovi"
## [457] "s__gerenzanensis"
## [458] "s__debuckii"
## [459] "s__pigrum"
## [460] "s__koreensis"
## [461] "s__hilgardii"
## [462] "s__nedwellii"
## [463] "s__flavus"
## [464] "s__lipocalidus"
## [465] "s__lusitana"
## [466] "s__pinnipediorum"
## [467] "s__radiotolerans"
## [468] "s__sp. KUDC1026"
## [469] "s__xylanisolvens"
## [470] "s__sp. I4-3-84"
## [471] "s__nigrescens"
## [472] "s__halophila"
## [473] "s__sediminis"
## [474] "s__oryzae"
## [475] "s__sp. NAK00032"
## [476] "s__acetotolerans"
## [477] "s__krulwichiae"
## [478] "s__thermophila"
## [479] "s__piscis"
## [480] "s__aggregans"
## [481] "s__sticklandii"
## [482] "s__sp. GIMC2001"
## [483] "s__coryniformis"
## [484] "s__barguzinensis"
## [485] "s__sp. SUK 48"
## [486] "s__sp. AK26"
## [487] "s__sp. S4.7"
## [488] "s__variicola"
## [489] "s__aerolatus"
## [490] "s__accolens"
## [491] "s__Verrucosispora sp. WMMD573"
## [492] "s__iners"

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[493] "s__sp. KSW4-10"
 ## [494] "s__furukawaiii"
 ## [495] "s__sp. wkB8"
 ## [496] "s__clara"
 ## [497] "s__guaymasensis"
 ## [498] "s__benzenivorans"
 ## [499] "s__panisapium"
 ## [500] "s__portuensis"
 ## [501] "s__sp. XGS7"
 ## [502] "s__woosongensis"
 ## [503] "s__amylolytica"
 ## [504] "s__carnosus"
 ## [505] "s__sp. GSS17"
 ## [506] "s__protaetiae"
 ## [507] "s__nuruki"
 ## [508] "s__sp. 320-W"
 ## [509] "s__propionicus"
 ## [510] "s__antarctica"
 ## [511] "s__ludwigii"
 ## [512] "s__kutscheri"
 ## [513] "s__putida"
 ## [514] "s__asteroides"
 ## [515] "s__ferrooxidans"
 ## [516] "s__aquimaris"
 ## [517] "s__pseudoperiodonticum"
 ## [518] "s__vandammei"
 ## [519] "s__crispatus"
 ## [520] "s__sp. MMS16-BH015"
 ## [521] "s__persicina"
 ## [522] "s__futsaii"
 ## [523] "s__gaoshouyii"
 ## [524] "s__callunae"
 ## [525] "s__sp. 155"
 ## [526] "s__sp. Lep1P3"
 ## [527] "s__zoogloformans"
 ## [528] "s__psoromatis"
 ## [529] "s__sp. H30R-01"
 ## [530] "s__celer"
 ## [531] "s__chocolatum"
 ## [532] "s__shahii"
 ## [533] "s__thermocarboxydus"
 ## [534] "s__indistinctus"
 ## [535] "s__watsonii"
 ## [536] "s__alkaliphilus"
 ## [537] "s__inaquosorum"
 ## [538] "s__sp. PS1209"
 ## [539] "s__caribensis"
 ## [540] "s__glucanolyticus"
 ## [541] "s__nitrativorans"
 ## [542] "s__cyriacigeorgica"
 ## [543] "s__panacisegetis"
 ## [544] "s__argenteus"
 ## [545] "s__sp. QHH-9511"
 ## [546] "s__sp. HKS 07"


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## [547] "s__moscoviensis"
## [548] "s__actinocoloniiforme"
## [549] "s__coriaceae"
## [550] "s__michiganensis"
## [551] "s__sp. BRM-1"
## [552] "s__Candidatus Thiodictyon syntrophicum"
## [553] "s__papyrosolvans"
## [554] "s__anthracis"
## [555] "s__dentiae"
## [556] "s__sp. PV034"
## [557] "s__manihotivorans"
## [558] "s__proteoclasticus"
## [559] "s__sichuanensis"
## [560] "s__limicola"
## [561] "s__paradisiaca"
## [562] "s__indicoceani"
## [563] "s__okcheonensis"
## [564] "s__maricopensis"
## [565] "s__sp. SCR221107"
## [566] "s__acidovorans"
## [567] "s__dalangtanensis"
## [568] "s__sp. S13"
## [569] "s__heliothermus"
## [570] "s__sp. BHT-5-2"
## [571] "s__portus"
## [572] "s__halophilus"
## [573] "s__sp. nov. GSS16"
## [574] "s__producta"
## [575] "s__mesophilus"
## [576] "s__commune"
## [577] "s__sp. 5B5"
## [578] "s__casseliflavus"
## [579] "s__thermarum"
## [580] "s__gelatinilyticus"
## [581] "s__aurum"
## [582] "s__bolteae"
## [583] "s__frequens"
## [584] "s__algicola"
## [585] "s__irradiatisoli"
## [586] "s__sp. CdTB01"
## [587] "s__massiliensis"
## [588] "s__nakazawae"
## [589] "s__qintianiae"
## [590] "s__seeligeri"
## [591] "s__oxydans"
## [592] "s__dichloroeliminans"
## [593] "s__formosensis"
## [594] "s__sp. KNUC1026"
## [595] "s__peruense"
## [596] "s__sp. NBC_00162"
## [597] "s__homolactica"
## [598] "s__brevitalea"
## [599] "s__oris"
## [600] "s__saccharobutylicum"

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## [601] "s__agarivorans"
## [602] "s__coccineus"
## [603] "s__sp. FeAm09"
## [604] "s__sp. RD1"
## [605] "s__sp. MNS18"
## [606] "s__freundii"
## [607] "s__sp. PET-29"
## [608] "s__erythreum"
## [609] "s__hydrogenophilus"
## [610] "s__sp. NW-56"
## [611] "s__alba"
## [612] "s__aubagnense"
## [613] "s__ytuae"
## [614] "s__sp. 113-1-2"
## [615] "s__sp. AWRP"
## [616] "s__fortuitum"
## [617] "s__cynos"
## [618] "s__fuchuensis"
## [619] "s__lavamentivorans"
## [620] "s__sp. Gsoil 351"
## [621] "s__cashew"
## [622] "s__ciconiae"
## [623] "s__sp. E85"
## [624] "s__aliiiformigenes"
## [625] "s__sp. LPB0304"
## [626] "s__sp. SGAir0570"
## [627] "s__sp. TPU 3598"
## [628] "s__coleopterorum"
## [629] "s__harbinensis"
## [630] "s__crossotus"
## [631] "s__sp. PA-3-X8"
## [632] "s__sp. I3-3-89"
## [633] "s__pulmonis"
## [634] "s__sp. NJN-50"
## [635] "s__sp. SL48"
## [636] "s__delbrueckii"
## [637] "s__fastidiosum"
## [638] "s__aquilus"
## [639] "s__animalis"
## [640] "s__aureus"
## [641] "s__sp. ZAC14D2_NAIMI4_6"
## [642] "s__piscinae"
## [643] "s__sp. S8"
## [644] "s__alni"
## [645] "s__eutactus"
## [646] "s__sp. BIHB 4019"
## [647] "s__pumilus"
## [648] "s__fascians"
## [649] "s__tundricola"
## [650] "s__aeolianus"
## [651] "s__oralis"
## [652] "s__daejeonensis"
## [653] "s__sp. RM10537"
## [654] "s__sp. mosi_1"

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[655] "s__sp. Hama-1"
 ## [656] "s__ossetica"
 ## [657] "s__johnsonii"
 ## [658] "s__fragariae"
 ## [659] "s__megalosphaeroides"
 ## [660] "s__tuberculostearicum"
 ## [661] "s__sp. CBA3647"
 ## [662] "s__otitidiscaviarum"
 ## [663] "s__sp. DG15C"
 ## [664] "s__genomosp. 9"
 ## [665] "s__calidifontis"
 ## [666] "s__sp. BJA-103"
 ## [667] "s__thiophilum"
 ## [668] "s__sp. I52.16.1"
 ## [669] "s__kefiranofaciens"
 ## [670] "s__garvieae"
 ## [671] "s__coralli"
 ## [672] "s__sp. XCS3"
 ## [673] "s__sp. JZ16"
 ## [674] "s__sp. WL3"
 ## [675] "s__fergusonii"
 ## [676] "s__viridis"
 ## [677] "s__[Ruminococcus] torques"
 ## [678] "s__californiensis"
 ## [679] "s__sp. S132"
 ## [680] "s__violaceinigra"
 ## [681] "s__baarsii"
 ## [682] "s__sp. PK01"
 ## [683] "s__tunisiensis"
 ## [684] "s__ganghwensis"
 ## [685] "s__paraseoulense"
 ## [686] "s__pituitosa"
 ## [687] "s__sp. E13-17"
 ## [688] "s__festucae"
 ## [689] "s__inquinata"
 ## [690] "s__phaseoli"
 ## [691] "s__gallinarum"
 ## [692] "s__chengduensis"
 ## [693] "s__caoxuetaonis"
 ## [694] "s__sp. WMMD882"
 ## [695] "s__anthropi"
 ## [696] "s__sp. URB8-2"
 ## [697] "s__gaofuii"
 ## [698] "s__pulveris"
 ## [699] "s__kefiri"
 ## [700] "s__chromogenes"
 ## [701] "s__sp. THAF27"
 ## [702] "s__sp. JQ2195"
 ## [703] "s__sp. 9128"
 ## [704] "s__testudinoris"
 ## [705] "s__dehalogenans"
 ## [706] "s__pickettii"
 ## [707] "s__aminophilus"
 ## [708] "s__kaempferiae"

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## [709] "s__sp. FSL R7-0331"
## [710] "s__xylosus"
## [711] "s__sp. SMJS2"
## [712] "s__lovleyi"
## [713] "s__baratii"
## [714] "s__rubi"
## [715] "s__borealis"
## [716] "s__asparagiformis"
## [717] "s__mucosa"
## [718] "s__tanashiensis"
## [719] "s__salivarius"
## [720] "s__sp. OE 28.3"
## [721] "s__sphenoides"
## [722] "s__agamarum"
## [723] "s__africae"
## [724] "s__umbonata"
## [725] "s__radiopugnans"
## [726] "s__apisilvae"
## [727] "s__sp. J315"
## [728] "s__buecherae"
## [729] "s__sp. GAM44"
## [730] "s__rosea"
## [731] "s__formigenes"
## [732] "s__pacaensis"
## [733] "s__sp. AD91A"
## [734] "s__resistens"
## [735] "s__stercoris"
## [736] "s__nenjiangensis"
## [737] "s__drozdowiczii"
## [738] "s__pulmonicola"
## [739] "s__sp. G2S3"
## [740] "s__sp. SL250"
## [741] "s__quasipneumoniae"
## [742] "s__sp. 107-1"
## [743] "s__cloacae"
## [744] "s__warabiya"
## [745] "s__nigra"
## [746] "s__sp. WAC00303"
## [747] "s__sp. R56"
## [748] "s__carbinoliphilus"
## [749] "s__mucilaginosus"
## [750] "s__thermosuccinogenes"
## [751] "s__elongata"
## [752] "s__sp. NBH87"
## [753] "s__espanaensis"
## [754] "s__magna"
## [755] "s__sp. FDAARGOS 1241"
## [756] "s__Candidatus Protofrankia datiscae"
## [757] "s__albidus"
## [758] "s__paludis"
## [759] "s__somerae"
## [760] "s__xylosoxidans"
## [761] "s__indicus"
## [762] "s__hominis"

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[763] "s__dysgalactiae"
 ## [764] "s__sp. LQ25"
 ## [765] "s__sp. RTd22"
 ## [766] "s__sp. Jing01"
 ## [767] "s__rivuli"
 ## [768] "s__allomyrinae"
 ## [769] "s__azurea"
 ## [770] "s__coagulans"
 ## [771] "s__phytophila"
 ## [772] "s__sp. DH3716P"
 ## [773] "s__aminovorans"
 ## [774] "s__atlanticus"
 ## [775] "s__haemolytica"
 ## [776] "s__sp. FDAARGOS 1415"
 ## [777] "s__metalliredigens"
 ## [778] "s__bombintestini"
 ## [779] "s__sp. RC67"
 ## [780] "s__syringae group genomosp. 7"
 ## [781] "s__konosiri"
 ## [782] "s__equorum"
 ## [783] "s__mediterranea"
 ## [784] "s__sp. THAF12"
 ## [785] "s__glycaniphila"
 ## [786] "s__caseolyticus"
 ## [787] "s__zosteriae"
 ## [788] "s__osloensis"
 ## [789] "s__pasteurii"
 ## [790] "s__intracellularis"
 ## [791] "s__bifermentans"
 ## [792] "s__acetigenes"
 ## [793] "s__gingivalis"
 ## [794] "s__sp. WMMA1947"
 ## [795] "s__chauvoei"
 ## [796] "s__venezuelae"
 ## [797] "s__arginini"
 ## [798] "s__aureovorticillatus"
 ## [799] "s__sp. WMD812"
 ## [800] "s__hongkongensis"
 ## [801] "s__baengnokdamensis"
 ## [802] "s__sp. OT7"
 ## [803] "s__sp. FDAARGOS 1409"
 ## [804] "s__abscessus"
 ## [805] "s__sp. JM1"
 ## [806] "s__sp. MR_MD2014"
 ## [807] "s__sp. YPW1"
 ## [808] "s__phytofermentans"
 ## [809] "s__[Clostridium] colinum"
 ## [810] "s__chenweiae"
 ## [811] "s__metallilatus"
 ## [812] "s__sp. CX169"
 ## [813] "s__pnomenusa"
 ## [814] "s__pseudoxylorus"
 ## [815] "s__ferrireducens"
 ## [816] "s__echinicola"

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## [817] "s__abortibovis"
## [818] "s__sp. QL22"
## [819] "s__autotrophicum"
## [820] "s__phocaeense"
## [821] "s__alcaligenes"
## [822] "s__sp. NSJ-69"
## [823] "s__cervicalis"
## [824] "s__sp. ESL0785"
## [825] "s__xylanophilus"
## [826] "s__vulnificus"
## [827] "s__cinaedi"
## [828] "s__rimosus"
## [829] "s__oleovorans"
## [830] "s__sp. CB1650"
## [831] "s__mucosae"
## [832] "s__sp. INOP01"
## [833] "s__sp. WL1"
## [834] "s__echinospora"
## [835] "s__actuosus"
## [836] "s__sp. D2"
## [837] "s__cellulans"
## [838] "s__lutrae"
## [839] "s__pristinaespiralis"
## [840] "s__xylaniphila"
## [841] "s__phagedenis"
## [842] "s__brevissima"
## [843] "s__ignavus"
## [844] "s__microaerophilus"
## [845] "s__halocryophilus"
## [846] "s__sp. FDAARGOS_375"
## [847] "s__sp. WB-2"
## [848] "s__himalayensis"
## [849] "s__tanakiae"
## [850] "s__innocua"
## [851] "s__rhodesiae"
## [852] "s__pectinilyticus"
## [853] "s__richardii"
## [854] "s__candidum"
## [855] "s__temperans"
## [856] "s__violaceusniger"
## [857] "s__sp. KACC 23028"
## [858] "s__atlantisensis"
## [859] "s__sp. NRS527"
## [860] "s__sobrinus"
## [861] "s__vaginalis"
## [862] "s__vicinigordonae"
## [863] "s__sp. YPD9-1"
## [864] "s__melonis"
## [865] "s__pumilum"
## [866] "s__toyakuensis"
## [867] "s__sp. 3H"
## [868] "s__luteola"
## [869] "s__sp. CKK8"
## [870] "s__acetoxydans"

```

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## [871] "s__sp. CCB-MM3"
## [872] "s__malmoense"
## [873] "s__clausii"
## [874] "s__eggerthii"
## [875] "s__flava"
## [876] "s__sp. THAF30"
## [877] "s__axanthum"
## [878] "s__sp. HBX-1"
## [879] "s__sp. AH1"
## [880] "s__towneri"
## [881] "s__flexa"
## [882] "s__pseudogrignonensis"
## [883] "s__Blochmannia endosymbiont of Polyrhachis (Hedomyrma) turneri"
## [884] "s__poaceiphila"
## [885] "s__carniphilus"
## [886] "s__methoxysyntrophicus"
## [887] "s__capnotolerans"
## [888] "s__diazotrophicus"
## [889] "s__sp. SL47"
## [890] "s__vincentii"
## [891] "s__sp. WS11"
## [892] "s__sp. G01H"
## [893] "s__simplex"
## [894] "s__thermoresistibile"
## [895] "s__brasiliensis"
## [896] "s__zhachilii"
## [897] "s__cytotoxicus"
## [898] "s__saxobsidens"
## [899] "s__vaccinii"
## [900] "s__sp. H121"
## [901] "s__daltonii"
## [902] "s__aquaticum"
## [903] "s__humicireducens"
## [904] "s__genisteinicus"
## [905] "s__pentosaceus"
## [906] "s__degensii"
## [907] "s__warneri"
## [908] "s__blattae"
## [909] "s__wieringae"
## [910] "s__crateris"
## [911] "s__senegalensis"
## [912] "s__paucivorans"
## [913] "s__sp. 3214.6"
## [914] "s__botulinum"
## [915] "s__composti"
## [916] "s__sp. MC1825"
## [917] "s__incomptus"
## [918] "s__lactatiformans"
## [919] "s__multitudinisentens"
## [920] "s__urinaeequi"
## [921] "s__sp. P6W"
## [922] "s__lichenicola"
## [923] "s__Verrucosispora sp. WMMD1129"
## [924] "s__sp. AGMB13025"

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[925] "s__minnesotensis"
 ## [926] "s__paludicola"
 ## [927] "s__fermenticellae"
 ## [928] "s__heliotrinireducens"
 ## [929] "s__verrucosospora"
 ## [930] "s__rhamnosivorans"
 ## [931] "s__helveticus"
 ## [932] "s__callanderi"
 ## [933] "s__aerodenitrificans"
 ## [934] "s__tertiaricarbonis"
 ## [935] "s__sera"
 ## [936] "s__felsineum"
 ## [937] "s__sp. AR10"
 ## [938] "s__sp. M28"
 ## [939] "s__clevelandensis"
 ## [940] "s__sp. MB-3u-03"
 ## [941] "s__sp. HF-162"
 ## [942] "s__newyorkensis"
 ## [943] "s__anaerobius"
 ## [944] "s__asoensis"
 ## [945] "s__sp. 'AMD consortium'"
 ## [946] "s__lydicamycinicus"
 ## [947] "s__phasianinus"
 ## [948] "s__alsatica"
 ## [949] "s__schaalii"
 ## [950] "s__cadmiisoli"
 ## [951] "s__sp. 21SJ11W-1"
 ## [952] "s__endophyticus"
 ## [953] "s__setae"
 ## [954] "s__chongii"
 ## [955] "s__phragmitis"
 ## [956] "s__muris"
 ## [957] "s__hippikon"
 ## [958] "s__thermocellus"
 ## [959] "s__sp. Z2-YC6860"
 ## [960] "s__spormannii"
 ## [961] "s__sp. H1-D42"
 ## [962] "s__hoggarensis"
 ## [963] "s__sp. J780"
 ## [964] "s__sp. CBA3646"
 ## [965] "s__sp. FXJ1.172"
 ## [966] "s__novella"
 ## [967] "s__ampullae"
 ## [968] "s__sp. B7740"
 ## [969] "s__dubosii"
 ## [970] "s__porci"
 ## [971] "s__sp. KMM 9044"
 ## [972] "s__sp. N12"
 ## [973] "s__bacteriovorus"
 ## [974] "s__sp. AA-38"
 ## [975] "s__pseudolwoffii"
 ## [976] "s__sp. B01"
 ## [977] "s__otitidis"
 ## [978] "s__hordei"

[979] "s__eutropha"
 ## [980] "s__haemolyticus"
 ## [981] "s__chonburiensis"
 ## [982] "s__sp. BN140058"
 ## [983] "s__sp. AN1"
 ## [984] "s__ampelinum"
 ## [985] "s__thetaitaomicron"
 ## [986] "s__sp. KB-1"
 ## [987] "s__sordellii"
 ## [988] "s__sp. L6-1"
 ## [989] "s__sp. WH15"
 ## [990] "s__argentinense"
 ## [991] "s__ferus"
 ## [992] "s__sp. JXJ CY 41"
 ## [993] "s__alkanivorans"
 ## [994] "s__amazonense"
 ## [995] "s__sp. SAHP1"
 ## [996] "s__sp. JSBI002"
 ## [997] "s__fulvum"
 ## [998] "s__hexanoica"
 ## [999] "s__sp. Arc7-R13"
 ## [1000] "s__sp. PP30"
 ## [1001] "s__pestifer"
 ## [1002] "s__sp. HS6"
 ## [1003] "s__corallicola"
 ## [1004] "s__sp. CB01881"
 ## [1005] "s__sp. B32"
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 ## [1007] "s__parvatiensis"
 ## [1008] "s__sp. ZS110521"
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 ## [1010] "s__brockianus"
 ## [1011] "s__flavum"
 ## [1012] "s__segnis"
 ## [1013] "s__sanguinicola"
 ## [1014] "s__silvanus"
 ## [1015] "s__amylolyticum"
 ## [1016] "s__kluyveri"
 ## [1017] "s__sp. USTB-05"
 ## [1018] "s__australiense"
 ## [1019] "s__acidipiscis"
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 ## [1022] "s__faecigallinarum"
 ## [1023] "s__sp. FSL P4-0081"
 ## [1024] "s__murinus"
 ## [1025] "s__infantarius"
 ## [1026] "s__cellobiosedens"
 ## [1027] "s__gelidum"
 ## [1028] "s__herbilytica"
 ## [1029] "s__faecium"
 ## [1030] "s__pasteurianus"
 ## [1031] "s__sp. SFB-mouse-NL"
 ## [1032] "s__sp. TT6"

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 ## [1034] "s__sp. RA8"
 ## [1035] "s__sp. CIB 2401"
 ## [1036] "s__sp. YM1"
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 ## [1038] "s__ovatus"
 ## [1039] "s__hattorii"
 ## [1040] "s__centrodinii"
 ## [1041] "s__flaccumfaciens"
 ## [1042] "s__beijingensis"
 ## [1043] "s__lemovicicum"
 ## [1044] "s__onderdonkii"
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 ## [1047] "s__sp. PBC"
 ## [1048] "s__microcystinivorans"
 ## [1049] "s__sp. PGP41"
 ## [1050] "s__sp. DSM 15011"
 ## [1051] "s__sp. NBRC 113351"
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 ## [1053] "s__ureae"
 ## [1054] "s__akajimensis"
 ## [1055] "s__cystitidis"
 ## [1056] "s__pacificus"
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 ## [1058] "s__sp. AMCC400023"
 ## [1059] "s__anaerobium"
 ## [1060] "s__solisilvae"
 ## [1061] "s__terrestris"
 ## [1062] "s__psychrophila"
 ## [1063] "s__solanacearum"
 ## [1064] "s__sp. HDW16"
 ## [1065] "s__sp. (ex Biomphalaria glabrata)"
 ## [1066] "s__ihumii"
 ## [1067] "s__guodeyinii"
 ## [1068] "s__sp. L9-4"
 ## [1069] "s__sp. YRD-M1"
 ## [1070] "s__marinisabuli"
 ## [1071] "s__moniliiformis"
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 ## [1073] "s__aquatica"
 ## [1074] "s__griseochromogenes"
 ## [1075] "s__sp. BH-2-1-1"
 ## [1076] "s__urinae"
 ## [1077] "s__xanthus"
 ## [1078] "s__aespoeensis"
 ## [1079] "s__formicigenerans"
 ## [1080] "s__faecalis"
 ## [1081] "s__saurashtrense"
 ## [1082] "s__sp. ESL0704"
 ## [1083] "s__vinelandii"
 ## [1084] "s__sp. USK10"
 ## [1085] "s__dongpingensis"
 ## [1086] "s__glaciei"

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## [1087] "s__sambongensis"
## [1088] "s__sp. G11"
## [1089] "s__catena"
## [1090] "s__fodineus"
## [1091] "s__mannosilyticum"
## [1092] "s__junii"
## [1093] "s__weilii"
## [1094] "s__canadensis"
## [1095] "s__rugosus"
## [1096] "s__sp. KBS0714"
## [1097] "s__hortorum"
## [1098] "s__cerasi"
## [1099] "s__fastidiosa"
## [1100] "s__australiensis"
## [1101] "s__paradoxus"
## [1102] "s__yeei"
## [1103] "s__etheniformans"
## [1104] "s__luteolum"
## [1105] "s__aureofaciens"
## [1106] "s__gregorii"
## [1107] "s__sp. DNA4"
## [1108] "s__chlorophenolicus"
## [1109] "s__sp. LGH"
## [1110] "s__sp. 2017"
## [1111] "s__agalactiae"
## [1112] "s__canis"
## [1113] "s__nodulans"
## [1114] "s__aureum"
## [1115] "s__toyohensis"
## [1116] "s__gephyra"
## [1117] "s__aminofermentans"
## [1118] "s__genomosp. 13"
## [1119] "s__sp. Dgby_cultured_2"
## [1120] "s__arsenicus"
## [1121] "s__marisgermanici"
## [1122] "s__lari"
## [1123] "s__stantonii"
## [1124] "s__megaguti"
## [1125] "s__graminis"
## [1126] "s__cellulosilyticus"
## [1127] "s__sp. zg-Y815"
## [1128] "s__sp. I2-3-92"
## [1129] "s__sacchari"
## [1130] "s__nassauensis"
## [1131] "s__sp. ES.047"
## [1132] "s__brasiliensis"
## [1133] "s__colihominis"
## [1134] "s__maltosivorans"
## [1135] "s__sp. S09"
## [1136] "s__malefermentans"
## [1137] "s__wangleii"
## [1138] "s__sp. SL43"
## [1139] "s__sp. SGAir0207"
## [1140] "s__potens"

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## [1141] "s__tritici"
## [1142] "s__jejuensis"
## [1143] "s__sp. CBW1006"
## [1144] "s__taeniosporum"
## [1145] "s__glucosotrophus"
## [1146] "s__sp. ABG19"
## [1147] "s__sp. HM134"
## [1148] "s__rhizovicinus"
## [1149] "s__cadaveris"
## [1150] "s__navarrensis"
## [1151] "s__peucetius"
## [1152] "s__sp. zg-629"
## [1153] "s__mucilyticum"
## [1154] "s__triazinivorans"
## [1155] "s__pleomorphus"
## [1156] "s__termitida"
## [1157] "s__vulgatus"
## [1158] "s__sp. wino2"
## [1159] "s__frisingense"
## [1160] "s__paralvei"
## [1161] "s__sp. CD1"
## [1162] "s__periodonticum"
## [1163] "s__inopinata"
## [1164] "s__chartreusis"
## [1165] "s__uberis"
## [1166] "s__parauberis"
## [1167] "s__sp. R5-89-07"
## [1168] "s__antarcticus"
## [1169] "s__sp. S3-43"
## [1170] "s__acticola"
## [1171] "s__sp. WMMD937"
## [1172] "s__cynanchi"
## [1173] "s__dendritiformis"
## [1174] "s__saprophyticus"
## [1175] "s__sp. LH3U1"
## [1176] "s__choerinum"
## [1177] "s__parasitica"
## [1178] "s__sp. 7M"
## [1179] "s__sp. KGMB00164"
## [1180] "s__bronchialis"
## [1181] "s__elenkinii"
## [1182] "s__sp. N4-1P"
## [1183] "s__syzygii"
## [1184] "s__smegmatis"
## [1185] "s__eligens"
## [1186] "s__goriensis"
## [1187] "s__kitaharae"
## [1188] "s__phototrophica"
## [1189] "s__communis"
## [1190] "s__grayi"
## [1191] "s__sp. TJ11"
## [1192] "s__noguchii"
## [1193] "s__luteifluviistationis"
## [1194] "s__foliorum"

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[1195] "s__sp. UKPF54-2"
 ## [1196] "s__madurae"
 ## [1197] "s__sp. A15-44"
 ## [1198] "s__halotolerans"
 ## [1199] "s__hydrocarbonoxydans"
 ## [1200] "s__jilunlii"
 ## [1201] "s__magneticus"
 ## [1202] "s__jeddahense"
 ## [1203] "s__palustris"
 ## [1204] "s__corporis"
 ## [1205] "s__genosp. B"
 ## [1206] "s__heidelbergense"
 ## [1207] "s__madagascariense"
 ## [1208] "s__pneumoniae"
 ## [1209] "s__sp. TF02-10"
 ## [1210] "s__yangpuensis"
 ## [1211] "s__sp. BJN0001"
 ## [1212] "s__toluolica"
 ## [1213] "s__atrophaeus"
 ## [1214] "s__sp. IP-1-18"
 ## [1215] "s__sp. B183"
 ## [1216] "s__sp. CO-6"
 ## [1217] "s__sp. ATCC 39006"
 ## [1218] "s__sp. SORT26"
 ## [1219] "s__sp. MORI2"
 ## [1220] "s__sp. GU-1"
 ## [1221] "s__proteolyticus"
 ## [1222] "s__weissii"
 ## [1223] "s__vulgare"
 ## [1224] "s__verrucosa"
 ## [1225] "s__oryzihabitans"
 ## [1226] "s__sp. SSW1-51"
 ## [1227] "s__oligotrophus"
 ## [1228] "s__dongxiuzhuiae"
 ## [1229] "s__sp. MMS21-STM10"
 ## [1230] "s__sp. DG25A"
 ## [1231] "s__urinalis"
 ## [1232] "s__duncaniae"
 ## [1233] "s__pseudomesenteroides"
 ## [1234] "s__sp. I3-3-33"
 ## [1235] "s__sp. PCC 7367"
 ## [1236] "s__necrophorum"
 ## [1237] "s__cellulovorans"
 ## [1238] "s__endosymbiont of unidentified scaly snail isolate Monju"
 ## [1239] "s__backii"
 ## [1240] "s__woodii"
 ## [1241] "s__roseum"
 ## [1242] "s__sp. K90mix"
 ## [1243] "s__litoralis"
 ## [1244] "s__sp. 905_Psudmo1"
 ## [1245] "s__merdae"
 ## [1246] "s__virosa"
 ## [1247] "s__sp. 4G125"
 ## [1248] "s__gibsoniae"

[1249] "s__sp. DMF-1"
 ## [1250] "s__pacifica"
 ## [1251] "s__plantisponsor"
 ## [1252] "s__monocytogenes"
 ## [1253] "s__mucimassa"
 ## [1254] "s__hibernicus"
 ## [1255] "s__neteri"
 ## [1256] "s__phragmitetus"
 ## [1257] "s__sp. ESL0775"
 ## [1258] "s__hypogea"
 ## [1259] "s__falsenii"
 ## [1260] "s__limosum"
 ## [1261] "s__metallidurans"
 ## [1262] "s__eucalypticola"
 ## [1263] "s__gonidiaformans"
 ## [1264] "s__[Clostridium] innocuum"
 ## [1265] "s__sp. 432"
 ## [1266] "s__sp. 20-132"
 ## [1267] "s__sp. JZ18"
 ## [1268] "s__sp. SS-MA-C1-2"
 ## [1269] "s__sp. RM12651"
 ## [1270] "s__kanamyceticus"
 ## [1271] "s__sp. A10-1-5-1"
 ## [1272] "s__acidocaldarius"
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 ## [1274] "s__sp. 2447"
 ## [1275] "s__sp. AMBV1719"
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 ## [1277] "s__flavithermus"
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 ## [1279] "s__fonticola"
 ## [1280] "s__sp. SMC-8"
 ## [1281] "s__haemolyticum"
 ## [1282] "s__carnosum"
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 ## [1284] "s__sp. RerS4"
 ## [1285] "s__shinshuensis"
 ## [1286] "s__sp. YC1"
 ## [1287] "s__sp. G2-70"
 ## [1288] "s__fulva"
 ## [1289] "s__suaedae"
 ## [1290] "s__sp. XHJ-5"
 ## [1291] "s__pretoriensis"
 ## [1292] "s__mucicolens"
 ## [1293] "s__physcomitrellae"
 ## [1294] "s__capitis"
 ## [1295] "s__qitaiheensis"
 ## [1296] "s__syringae"
 ## [1297] "s__aerogenes"
 ## [1298] "s__praevalens"
 ## [1299] "s__zymae"
 ## [1300] "s__subutilus"
 ## [1301] "s__sp. NIES-970"
 ## [1302] "s__sp. I4-1-79"

[1303] "s__plantarum"
 ## [1304] "s__fragilis"
 ## [1305] "s__ureilytica"
 ## [1306] "s__sp. LA31"
 ## [1307] "s__equolifaciens"
 ## [1308] "s__salexigens"
 ## [1309] "s__huguangmaarenses"
 ## [1310] "s__benzoatilyticus"
 ## [1311] "s__sp. SirexAA-E"
 ## [1312] "s__butyricum"
 ## [1313] "s__furentiruminis"
 ## [1314] "s__breve"
 ## [1315] "s__sp. WMD791"
 ## [1316] "s__pseudocatenulatum"
 ## [1317] "s__dechloratans"
 ## [1318] "s__sp. XT11"
 ## [1319] "s__gummiphilus"
 ## [1320] "s__lactis"
 ## [1321] "s__[Clostridium] hylemonae"
 ## [1322] "s__magnusonii"
 ## [1323] "s__yushuensis"
 ## [1324] "s__hirae"
 ## [1325] "s__acetoxidans"
 ## [1326] "s__xenovorans"
 ## [1327] "s__sp. SCSIO 76264"
 ## [1328] "s__sp. RHB36-C18"
 ## [1329] "s__albus"
 ## [1330] "s__endosymbiont of Euscepes postfasciatus"
 ## [1331] "s__purcellii"
 ## [1332] "s__sp. NBC_00550"
 ## [1333] "s__sp. TS-293"
 ## [1334] "s__soudanensis"
 ## [1335] "s__sp. FDAARGOS 1405"
 ## [1336] "s__endosymbiont of 'Nebria riversi'"
 ## [1337] "s__sp. M4R1S46"
 ## [1338] "s__sp. AP-Nino-20-G2"
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 ## [1341] "s__sp. oral taxon 894"
 ## [1342] "s__sp. WMMB 499"
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 ## [1344] "s__sp. HDW6C"
 ## [1345] "s__acidiphilus"
 ## [1346] "s__sp. Arc7-DN-1"
 ## [1347] "s__oremlandii"
 ## [1348] "s__sp. co_0103"
 ## [1349] "s__intermedius"
 ## [1350] "s__sp. RBIITD"
 ## [1351] "s__suwonensis"
 ## [1352] "s__sp. SFB-mouse"
 ## [1353] "s__carbinolicus"
 ## [1354] "s__sp. VKM Ac-2805"
 ## [1355] "s__agaridevorans"
 ## [1356] "s__barcinonensis"

[1357] "s__sp. CP1"
 ## [1358] "s__sp. AJ005"
 ## [1359] "s__goeteborgense"
 ## [1360] "s__sp. SMBL_HHYL_HB1"
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 ## [1363] "s__spinosum"
 ## [1364] "s__pollutisolii"
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 ## [1366] "s__sabinae"
 ## [1367] "s__cardium"
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 ## [1403] "s__sp. Clip185"
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 ## [1408] "s__sp. YIM 151500-1"
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 ## [1410] "s__sp. HN38"

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 ## [1414] "s__clavuligerus"
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 ## [1422] "s__sulfoxidireducens"
 ## [1423] "s__sp. FSL H7-0357"
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 ## [1452] "s__cellulositrophicus"
 ## [1453] "s__longum"
 ## [1454] "s__showae"
 ## [1455] "s__sp. T1-3-2"
 ## [1456] "s__sp. DEMB1"
 ## [1457] "s__subvibrioides"
 ## [1458] "s__sp. zth1"
 ## [1459] "s__durans"
 ## [1460] "s__occultum"
 ## [1461] "s__sediminicola"
 ## [1462] "s__anguillarum"
 ## [1463] "s__cyclohexanicum"
 ## [1464] "s__diminuta"

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## [1465] "s__thermoamylovorans"
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## [1467] "s__sp. X23"
## [1468] "s__sp. 'Marine'"
## [1469] "s__sp. PAMC22086"
## [1470] "s__nitratioreducens"
## [1471] "s__marcescens"
## [1472] "s__sp. SYSU D00693"
## [1473] "s__rhizosphaerae"
## [1474] "s__sp. KCTC 72723"
## [1475] "s__Paradesulfovibrio bizertensis"
## [1476] "s__sp. SCSIO 80058"
## [1477] "s__winogradskyi"
## [1478] "s__woesei"
## [1479] "s__fontis"
## [1480] "s__sp. Marseille-Q7238"
## [1481] "s__ochotonae"
## [1482] "s__mytili"
## [1483] "s__phthalatica"
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## [1492] "s__sp. X19"
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## [1498] "s__schindleri"
## [1499] "s__algae"
## [1500] "s__sp. 86"
## [1501] "s__mccartyi"
## [1502] "s__sciuri"
## [1503] "s__prausnitzii"
## [1504] "s__edaphicus"
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## [1506] "s__hormaechei"
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## [1509] "s__sp. X9"
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## [1513] "s__tumefaciens"
## [1514] "s__boydii"
## [1515] "s__sp. B3.7"
## [1516] "s__detoxificans"
## [1517] "s__sp. HSL-3221"
## [1518] "s__busanensis"

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[1519] "s__caenitepidi"
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 ## [1523] "s__sp. CB2312"
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 ## [1533] "s__equigenitalis"
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 ## [1535] "s__sp. L3-i22"
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## [1663] "s__sp. SMC90"
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## [1668] "s__sp. Kuro-4"
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## [1672] "s__autotrophicus"
## [1673] "s__sp. AB-CW3"
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 ## [2045] "s__sporogenes"
 ## [2046] "s__wenshanensis"
 ## [2047] "s__hydrothermale"
 ## [2048] "s__modesticaldum"
 ## [2049] "s__shinii"
 ## [2050] "s__sp. DG01"
 ## [2051] "s__yuhuli"
 ## [2052] "s__savannae"
 ## [2053] "s__sp. BH-3-3-3"
 ## [2054] "s__sp. ME-1"
 ## [2055] "s__hyacinthi"
 ## [2056] "s__bifidum"
 ## [2057] "s__tiedjei"
 ## [2058] "s__lemurum"

[2059] "s__phaeolivaceus"
 ## [2060] "s__sp. S-1144"
 ## [2061] "s__jogaejeotgali"
 ## [2062] "s__sp. BIM B-1768"
 ## [2063] "s__sp. TH136"
 ## [2064] "s__glycovorans"
 ## [2065] "s__pentosus"
 ## [2066] "s__aegosomatissinici"
 ## [2067] "s__kirschneri"
 ## [2068] "s__hypermegale"
 ## [2069] "s__cattleyicolor"
 ## [2070] "s__isosaccharinicus"
 ## [2071] "s__isatidis"
 ## [2072] "s__sp. DMT04"
 ## [2073] "s__sp. 32-5/1"
 ## [2074] "s__sp. BNL1100"
 ## [2075] "s__acidiceleris"
 ## [2076] "s__equinus"
 ## [2077] "s__chubuense"
 ## [2078] "s__saccharolyticus"
 ## [2079] "s__vulneris"
 ## [2080] "s__trachealis"
 ## [2081] "s__polymorpha"
 ## [2082] "s__licheniformis"
 ## [2083] "s__sp. PDNC004"
 ## [2084] "s__cremoris"
 ## [2085] "s__lithophora"
 ## [2086] "s__sp. NP310"
 ## [2087] "s__varians"
 ## [2088] "s__aeruginosa"
 ## [2089] "s__milleri"
 ## [2090] "s__sp. fl3"
 ## [2091] "s__alactolyticus"
 ## [2092] "s__sp. HMT-352"
 ## [2093] "s__galegae"
 ## [2094] "s__sp. EMB200-NS6"
 ## [2095] "s__coldseepsis"
 ## [2096] "s__sp. CoE-012-22"
 ## [2097] "s__hathewayi"
 ## [2098] "s__sp. PHL 2737"
 ## [2099] "s__sp. COWG"
 ## [2100] "s__sp. LKL04"
 ## [2101] "s__naphthae"
 ## [2102] "s__xianingshaonis"
 ## [2103] "s__coprocola"
 ## [2104] "s__alkalilenta"
 ## [2105] "s__ingrahamii"
 ## [2106] "s__heyeri"
 ## [2107] "s__mutanolyticus"
 ## [2108] "s__upsaliensis"
 ## [2109] "s__chitinolyticus"
 ## [2110] "s__amalyticus"
 ## [2111] "s__amylophilus"
 ## [2112] "s__acidominimus"

[2113] "s__sandarakinum"
 ## [2114] "s__sp. MCM B-1480"
 ## [2115] "s__sp. NIES-3974"
 ## [2116] "s__silvaticum"
 ## [2117] "s__fairfieldensis"
 ## [2118] "s__piscolens"
 ## [2119] "s__sp. ESL0690"
 ## [2120] "s__sp. 6-C"
 ## [2121] "s__sp. SSW1-36"
 ## [2122] "s__sp. PCH239"
 ## [2123] "s__lydicus"
 ## [2124] "s__chlorophenolicum"
 ## [2125] "s__septicus"
 ## [2126] "s__immobilis"
 ## [2127] "s__sp. 762G35"
 ## [2128] "s__dadantii"
 ## [2129] "s__sp. Marseille-Q4147"
 ## [2130] "s__davisii"
 ## [2131] "s__sp. oral taxon 478"
 ## [2132] "s__cristatus"
 ## [2133] "s__sp. OXW06B1"
 ## [2134] "s__faviae"
 ## [2135] "s__hansenii"
 ## [2136] "s__versutus"
 ## [2137] "s__sp. 1.5R"
 ## [2138] "s__crocatu"
 ## [2139] "s__mobile"
 ## [2140] "s__ectoiniformans"
 ## [2141] "s__sp. JA-3-3Ab"
 ## [2142] "s__caccae"
 ## [2143] "s__gordoncarteri"
 ## [2144] "s__pelargi"
 ## [2145] "s__sp. PK3_47"
 ## [2146] "s__ambifaria"
 ## [2147] "s__spiroformis"
 ## [2148] "s__sp. UF01"
 ## [2149] "s__humi"
 ## [2150] "s__flexneri"
 ## [2151] "s__sp. M190262"
 ## [2152] "s__mucilaginoso"
 ## [2153] "s__sp. JBR18"
 ## [2154] "s__mitsuokai"
 ## [2155] "s__choanae"
 ## [2156] "s__sp. SynAce01"
 ## [2157] "s__coralloides"
 ## [2158] "s__sp. WCF-2"
 ## [2159] "s__persica"
 ## [2160] "s__cenocepacia"
 ## [2161] "s__sp. A4B17"
 ## [2162] "s__mantenii"
 ## [2163] "s__oxytoca"
 ## [2164] "s__fistulariae"
 ## [2165] "s__hassiacum"
 ## [2166] "s__sp. Y-9"

[2167] "s__nova"
 ## [2168] "s__caecicola"
 ## [2169] "s__arachidis"
 ## [2170] "s__sp. MG"
 ## [2171] "s__lentus"
 ## [2172] "s__tagluense"
 ## [2173] "s__sp. ORNL1"
 ## [2174] "s__phocae"
 ## [2175] "s__sp. DBS9H8"
 ## [2176] "s__orientalis"
 ## [2177] "s__sp. BDGP8"
 ## [2178] "s__castenholzii"
 ## [2179] "s__pilosa"
 ## [2180] "s__sp. P1Y"
 ## [2181] "s__sp. PAMC28650"
 ## [2182] "s__sp. Aquia_216"
 ## [2183] "s__marincola"
 ## [2184] "s__torques-reginae"
 ## [2185] "s__sp. YMA4"
 ## [2186] "s__sakei"
 ## [2187] "s__sp. ALC3"
 ## [2188] "s__azureus"
 ## [2189] "s__sp. SOS3"
 ## [2190] "s__striatum"
 ## [2191] "s__cibaria"
 ## [2192] "s__cellulosilytica"
 ## [2193] "s__peraridilitoris"
 ## [2194] "s__yamanorum"
 ## [2195] "s__stationis"
 ## [2196] "s__sp. GAS368"
 ## [2197] "s__multivorans"
 ## [2198] "s__melissae"
 ## [2199] "s__pronyensis"
 ## [2200] "s__erythrophlei"
 ## [2201] "s__unidentified bacterial endosymbiont"
 ## [2202] "s__Halalkalibacterium halodurans"
 ## [2203] "s__litorisediminis"
 ## [2204] "s__sp. WX"
 ## [2205] "s__ljungdahlii"
 ## [2206] "s__aurulentum"
 ## [2207] "s__harveyi"
 ## [2208] "s__hafniense"
 ## [2209] "s__litoris"
 ## [2210] "s__sp. JN-1"
 ## [2211] "s__bescii"
 ## [2212] "s__raffinolactis"
 ## [2213] "s__sp. BG8"
 ## [2214] "s__doosanense"
 ## [2215] "s__gigas"
 ## [2216] "s__luteum"
 ## [2217] "s__mexicana"
 ## [2218] "s__violaceum"
 ## [2219] "s__pasteurianum"
 ## [2220] "s__sp. W3-18-1"

[2221] "s__sp. KC8"
 ## [2222] "s__curvatus"
 ## [2223] "s__sp. Kera 3"
 ## [2224] "s__sp. B21-047"
 ## [2225] "s__sp. EM10"
 ## [2226] "s__glyciniphilum"
 ## [2227] "s__cereus"
 ## [2228] "s__sp. 'caverna'"
 ## [2229] "s__sophorae"
 ## [2230] "s__kefirresidentii"
 ## [2231] "s__sp. Y8"
 ## [2232] "s__sp. MA-2"
 ## [2233] "s__sp. PB12/4term"
 ## [2234] "s__sp. R8"
 ## [2235] "s__altitudinis"
 ## [2236] "s__limnaea"
 ## [2237] "s__stagnalis"
 ## [2238] "s__sp. MSR2"
 ## [2239] "s__sp. IMCC34777"
 ## [2240] "s__bilis"
 ## [2241] "s__sp. WS12"
 ## [2242] "s__ehrlichii"
 ## [2243] "s__lactucae"
 ## [2244] "s__sp. WF146"
 ## [2245] "s__sunii"
 ## [2246] "s__tetani"
 ## [2247] "s__sp. cB07"
 ## [2248] "s__cellulolyticum"
 ## [2249] "s__africana"
 ## [2250] "s__sp. KKS102"
 ## [2251] "s__sp. Z12"
 ## [2252] "s__iocasae"
 ## [2253] "s__shigaense"
 ## [2254] "s__halodurans"
 ## [2255] "s__alkalisoli"
 ## [2256] "s__arilaitensis"
 ## [2257] "s__dispersa"
 ## [2258] "s__sp. HH130629-09"
 ## [2259] "s__sp. DL-VIII"
 ## [2260] "s__sp. ALC70"
 ## [2261] "s__sp. SCPEA002"
 ## [2262] "s__succinogenes"
 ## [2263] "s__jinghuaiqii"
 ## [2264] "s__sp. M259"
 ## [2265] "s__sp. MC1862"
 ## [2266] "s__guangdongensis"
 ## [2267] "s__neonatale"
 ## [2268] "s__sp. AJA228-03"
 ## [2269] "s__odontolytica"
 ## [2270] "s__canimorsus"
 ## [2271] "s__sp. JUb54"
 ## [2272] "s__peoriae"
 ## [2273] "s__salivibrio"
 ## [2274] "s__timonense"

[2275] "s__jensenii"
 ## [2276] "s__sp. ESL0790"
 ## [2277] "s__thuringiensis"
 ## [2278] "s__[Ruminococcus] lactaris"
 ## [2279] "s__sp. 24E2"
 ## [2280] "s__graeca"
 ## [2281] "s__taurus"
 ## [2282] "s__polytropus"
 ## [2283] "s__craniellae"
 ## [2284] "s__galactanivorans"
 ## [2285] "s__phaeum"
 ## [2286] "s__restrictus"
 ## [2287] "s__sp. VKM Ac-2760"
 ## [2288] "s__campestrisoli"
 ## [2289] "s__exhalans"
 ## [2290] "s__enterica"
 ## [2291] "s__sp. NEAU-S7GS2"
 ## [2292] "s__sp. L2-79-05"
 ## [2293] "s__kroppenstedtii"
 ## [2294] "s__scardovii"
 ## [2295] "s__sp. LS1212"
 ## [2296] "s__geestiana"
 ## [2297] "s__ishigakiensis"
 ## [2298] "s__splachnicus"
 ## [2299] "s__sp. S16"
 ## [2300] "s__sp. 11-B-312"
 ## [2301] "s__aurantiaca"
 ## [2302] "s__serpentiformis"
 ## [2303] "s__sp. MS455"
 ## [2304] "s__saccharophila"
 ## [2305] "s__globerulus"
 ## [2306] "s__sp. E4742"
 ## [2307] "s__bethesdensis"
 ## [2308] "s__sp. KNUC1210"
 ## [2309] "s__obscurus"
 ## [2310] "s__luti"
 ## [2311] "s__boletus"
 ## [2312] "s__kimchii"
 ## [2313] "s__sp. K1W22B-7"
 ## [2314] "s__veronii"
 ## [2315] "s__rhusiopathiae"
 ## [2316] "s__sp. PGU16"
 ## [2317] "s__cryptocerci"
 ## [2318] "s__psychromarinicola"
 ## [2319] "s__sp. L3-i23"
 ## [2320] "s__youngiae"
 ## [2321] "s__sp. IHBB 10380"
 ## [2322] "s__sp. Fw109-5"
 ## [2323] "s__producens"
 ## [2324] "s__sp. BJN0003"
 ## [2325] "s__multipartita"
 ## [2326] "s__erdmanii"
 ## [2327] "s__micra"
 ## [2328] "s__orientis"

```
## [2329] "s__inkyongensis"
## [2330] "s__xenophagum"
## [2331] "s__sp. ESL0682"
## [2332] "s__carotovorum"
## [2333] "s__sulfidivorans"
## [2334] "s__sp. WSM4906"
## [2335] "s__atrarenae"
## [2336] "s__mediterranei"
## [2337] "s__sp. R24"
## [2338] "s__vulturis"
## [2339] "s__drentensis"
## [2340] "s__hallii"
## [2341] "s__obscuriglobus"
## [2342] "s__sp. TRM1-10"
## [2343] "s__sp. LPB0260"
## [2344] "s__eburnea"
## [2345] "s__liangshanensis"
## [2346] "s__formicoaceticum"
## [2347] "s__russatus"
```

```
subsetMG %>% get_taxa_unique("Species") # 4706 different order in total, so 242 species are not found i
```

```
## [1] "s__symbiodeficiens"
## [2] "s__sp. LM6"
## [3] "s__sp. 5116S-3"
## [4] "s__sp. 32K"
## [5] "s__endosymbiont of Acanthamoeba sp. UWC8"
## [6] "s__sp. CJ74"
## [7] "s__sp. CR-Ec1"
## [8] "s__rosea"
## [9] "s__malaysiensis"
## [10] "s__leguminosarum"
## [11] "s__larvae"
## [12] "s__salina"
## [13] "s__paramultivorum"
## [14] "s__sp. TY1-4"
## [15] "s__endophytica"
## [16] "s__flavum"
## [17] "s__genomosp. 3"
## [18] "s__sp. RR6"
## [19] "s__mengziensis"
## [20] "s__"
## [21] "s__sp. ATA002"
## [22] "s__equigenitalium"
## [23] "s__sp. NEAU-sy36"
## [24] "s__sp. M2A.F.Ca.ET.046.03.2.1"
## [25] "s__gei"
## [26] "s__vestfoldensis"
## [27] "s__septicum"
## [28] "s__alkanexedens"
## [29] "s__sp. D15"
## [30] "s__emersonii"
## [31] "s__sp. THAF1"
## [32] "s__sp. LH3H17"
```

```

## [33] "s__sp. oral taxon 414"
## [34] "s__sp. ESL0677"
## [35] "s__sphaeroides"
## [36] "s__luteus"
## [37] "s__gaetbulicola"
## [38] "s__mirabilis"
## [39] "s__prevotii"
## [40] "s__sp. DMV24BSW_D"
## [41] "s__commune"
## [42] "s__testudinis"
## [43] "s__macrosporus"
## [44] "s__sp. SCLE84"
## [45] "s__tepida"
## [46] "s__salinestris"
## [47] "s__sp. MC1595"
## [48] "s__everestensis"
## [49] "s__oryzae"
## [50] "s__caldiproteolyticus"
## [51] "s__sp. MTB7"
## [52] "s__difficile"
## [53] "s__dentium"
## [54] "s__sp. E76"
## [55] "s__gangjinensis"
## [56] "s__sp. LQ44"
## [57] "s__profundi"
## [58] "s__australis"
## [59] "s__avium"
## [60] "s__[Mannheimia] succiniciproducens"
## [61] "s__sp. KSB-10"
## [62] "s__arboricola"
## [63] "s__chokoriensis"
## [64] "s__sp. PSBB023"
## [65] "s__palleroniana"
## [66] "s__abyssi"
## [67] "s__qiguomingii"
## [68] "s__tangfeifanii"
## [69] "s__cyanobacteriorum"
## [70] "s__sihwensis"
## [71] "s__gloriosae"
## [72] "s__intestini"
## [73] "s__glaucescens"
## [74] "s__sp. RSMS"
## [75] "s__propionicum"
## [76] "s__alhagiae"
## [77] "s__aestuarii"
## [78] "s__gelatinosa"
## [79] "s__sp. DHT3"
## [80] "s__indolicus"
## [81] "s__sp. 14171R-50"
## [82] "s__ruminicola"
## [83] "s__sp. PSKL.D1"
## [84] "s__haemaphysalidis"
## [85] "s__sp. 190D2882"
## [86] "s__actinosclerus"

```

```

## [87] "s__suis"
## [88] "s__manihotivorum"
## [89] "s__sp. VBCF_01 NA2"
## [90] "s__deleyi"
## [91] "s__sp. HN-54"
## [92] "s__sp. OT10"
## [93] "s__bestiarum"
## [94] "s__ulcerans"
## [95] "s__pogona"
## [96] "s__sp. DTU12.3"
## [97] "s__pseudopelargi"
## [98] "s__sp. Csp1"
## [99] "s__vannielii"
## [100] "s__lutetiensis"
## [101] "s__kobei"
## [102] "s__fermentans"
## [103] "s__sp. HMF3514"
## [104] "s__sp. CACIAM 19H1"
## [105] "s__ruber"
## [106] "s__rotai"
## [107] "s__akebiae"
## [108] "s__sp. CCBAU 05631"
## [109] "s__sp. WJP83"
## [110] "s__sp. Y-01"
## [111] "s__thermophilus"
## [112] "s__tsuruhatensis"
## [113] "s__porcitonsillarum"
## [114] "s__farcinica"
## [115] "s__robiniae"
## [116] "s__sp. PDNC005"
## [117] "s__sp. CB0101"
## [118] "s__acidurici"
## [119] "s__radiodurans"
## [120] "s__gryphiswaldense"
## [121] "s__lacunae"
## [122] "s__acetatoxydans"
## [123] "s__taklimakanense"
## [124] "s__flava"
## [125] "s__parvum"
## [126] "s__aurantiacus"
## [127] "s__sp. KBS50"
## [128] "s__indica"
## [129] "s__tepidum"
## [130] "s__salmonicida"
## [131] "s__sp. B53371"
## [132] "s__corsica"
## [133] "s__gilvus"
## [134] "s__psychrotolerans"
## [135] "s__sp. SL97"
## [136] "s__sanguinis"
## [137] "s__boonkerdii"
## [138] "s__endosymbiont 'TC1' of Trimyema compressum"
## [139] "s__harei"
## [140] "s__sp. Aquia_213"

```

[141] "s__inhibens"
 ## [142] "s__seropedicae"
 ## [143] "s__sp. 1_2014MBL_MicDiv"
 ## [144] "s__regensburgei"
 ## [145] "s__ambofaciens"
 ## [146] "s__lytica"
 ## [147] "s__mobilis"
 ## [148] "s__cyanea"
 ## [149] "s__extorquens"
 ## [150] "s__aurantiacum"
 ## [151] "s__choladocola"
 ## [152] "s__torquis"
 ## [153] "s__dioxanivorans"
 ## [154] "s__sp. GK1"
 ## [155] "s__sp. FJAT-42376"
 ## [156] "s__sicerae"
 ## [157] "s__sp. cx-51"
 ## [158] "s__sp. InS609-2"
 ## [159] "s__marinum"
 ## [160] "s__seohaensis"
 ## [161] "s__polymyxa"
 ## [162] "s__sp. N"
 ## [163] "s__planticola"
 ## [164] "s__sp. EV170708-02-1"
 ## [165] "s__lutea"
 ## [166] "s__nitratioreducens"
 ## [167] "s__heimbachae"
 ## [168] "s__halophilus"
 ## [169] "s__indicum"
 ## [170] "s__variabilis"
 ## [171] "s__saerimneri"
 ## [172] "s__griseocarneus"
 ## [173] "s__ultunensis"
 ## [174] "s__sp. YIM 121038"
 ## [175] "s__roseus"
 ## [176] "s__guangdongense"
 ## [177] "s__sp. SN-593"
 ## [178] "s__waltersii"
 ## [179] "s__microcysteis"
 ## [180] "s__plicata"
 ## [181] "s__sp. PL-2018"
 ## [182] "s__sp. S09G 359"
 ## [183] "s__succinifaciens"
 ## [184] "s__sunshinyii"
 ## [185] "s__naejangsanensis"
 ## [186] "s__weaveri"
 ## [187] "s__sp. SK17"
 ## [188] "s__reducens"
 ## [189] "s__insidiosa"
 ## [190] "s__sp. YPW16"
 ## [191] "s__curvus"
 ## [192] "s__thermophilum"
 ## [193] "s__Candidatus Arsenophonus lipoptenae"
 ## [194] "s__sp. GAS474"

[195] "s__denitrificans"
 ## [196] "s__sp. H1-7"
 ## [197] "s__geothermalis"
 ## [198] "s__sp. W1SF4"
 ## [199] "s__sp. D3"
 ## [200] "s__aestuariivivens"
 ## [201] "s__caledonica"
 ## [202] "s__sp. 19GGS1-52"
 ## [203] "s__bonchosmolovskayae"
 ## [204] "s__koreensis"
 ## [205] "s__ianthinogenes"
 ## [206] "s__sp. Xi13"
 ## [207] "s__lacustris"
 ## [208] "s__agarilyticus"
 ## [209] "s__sp. R3"
 ## [210] "s__sp. IBH004"
 ## [211] "s__infantis"
 ## [212] "s__sp. TCL240-02"
 ## [213] "s__changnyeongensis"
 ## [214] "s__featherlites"
 ## [215] "s__terpenica"
 ## [216] "s__wadenswilerensis"
 ## [217] "s__thiooxidans"
 ## [218] "s__balearica"
 ## [219] "s__stuttgartiensis"
 ## [220] "s__ulvae"
 ## [221] "s__bovigenitalium"
 ## [222] "s__audaxviator"
 ## [223] "s__sp. AJA081-3"
 ## [224] "s__sp. YMD87"
 ## [225] "s__longhuiensis"
 ## [226] "s__inefficax"
 ## [227] "s__sp. MX-AZ03"
 ## [228] "s__mitochondrii"
 ## [229] "s__sp. 113-3-9"
 ## [230] "s__confusa"
 ## [231] "s__aalborgensis"
 ## [232] "s__sp. M92"
 ## [233] "s__thiooxydans"
 ## [234] "s__carolinensis"
 ## [235] "s__gilva"
 ## [236] "s__sp. TGL-Y2"
 ## [237] "s__sp. E35C"
 ## [238] "s__riegelii"
 ## [239] "s__sp. LMS6"
 ## [240] "s__hiranonis"
 ## [241] "s__sp. Z13"
 ## [242] "s__sp. JY-X169"
 ## [243] "s__syrphidicola"
 ## [244] "s__lujinxingii"
 ## [245] "s__adiacens"
 ## [246] "s__suranareeae"
 ## [247] "s__sp. Wa41.01b-1"
 ## [248] "s__frigoritolerans"

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## [249] "s__sputigena"
## [250] "s__defluvii"
## [251] "s__nasimurium"
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## [254] "s__sp. zrk46"
## [255] "s__sp. StoSoilB20"
## [256] "s__sp. YS"
## [257] "s__sp. J2-11"
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## [259] "s__musculi"
## [260] "s__iowae"
## [261] "s__fungivorans"
## [262] "s__polymorphum"
## [263] "s__soli"
## [264] "s__vitis"
## [265] "s__hyicus"
## [266] "s__nodosus"
## [267] "s__freneyi"
## [268] "s__sedimenticola"
## [269] "s__delphinicola"
## [270] "s__haeundaensis"
## [271] "s__gobiensis"
## [272] "s__zundukense"
## [273] "s__bremense"
## [274] "s__stearothermophilus"
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## [276] "s__sp. 5317J-9"
## [277] "s__sp. PAMC28688"
## [278] "s__sp. KH32C"
## [279] "s__aquaticus"
## [280] "s__rhamnosus"
## [281] "s__lacus"
## [282] "s__sp. IDR2000157661"
## [283] "s__terrae"
## [284] "s__coyleae"
## [285] "s__minervae"
## [286] "s__dokdonensis"
## [287] "s__pigmentatum"
## [288] "s__oceani"
## [289] "s__sp. A34"
## [290] "s__khirikhana"
## [291] "s__sp. YST-16"
## [292] "s__sp. SMBL-WEM22"
## [293] "s__polymorphus"
## [294] "s__clarkii"
## [295] "s__schleiferi"
## [296] "s__sp. 3B(2020)"
## [297] "s__finlayi"
## [298] "s__armeniacus"
## [299] "s__infernorum"
## [300] "s__pseudintermedia"
## [301] "s__chengjingii"
## [302] "s__otitidis"

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[303] "s__alginicola"
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 ## [316] "s__sp. FJAT-22090"
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 ## [321] "s__agilis"
 ## [322] "s__desulfuricans"
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 ## [327] "s__izadpanahii"
 ## [328] "s__sp. S1-8"
 ## [329] "s__pinatubonensis"
 ## [330] "s__marina"
 ## [331] "s__agri"
 ## [332] "s__lapagei"
 ## [333] "s__swuensis"
 ## [334] "s__cuenoti"
 ## [335] "s__negevensis"
 ## [336] "s__rigui"
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 ## [348] "s__sp. oral taxon 807"
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 ## [350] "s__alboniger"
 ## [351] "s__baltica"
 ## [352] "s__sp. FB-5"
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 ## [354] "s__amnigena"
 ## [355] "s__sp. PLM1"
 ## [356] "s__mishrai"


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## [359] "s__sp. ZAC14A_NAIMI4_1"
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## [365] "s__raozihei"
## [366] "s__longa"
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## [368] "s__gasseri"
## [369] "s__sp. M317"
## [370] "s__doucetiae"
## [371] "s__sp. ZFBP2030"
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## [373] "s__hadrus"
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## [375] "s__sp. SM18"
## [376] "s__sp. TC1"
## [377] "s__sp. NIBR1757"
## [378] "s__sp. Allo2"
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## [381] "s__bonaserana"
## [382] "s__sp. WY228"
## [383] "s__ficus"
## [384] "s__sp. ASNIH4"
## [385] "s__sp. WAC 06738"
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## [388] "s__sp. SD9660Na"
## [389] "s__sp. SYP-B4668"
## [390] "s__sp. TS-1"
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## [392] "s__sp. OMZ 787"
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## [397] "s__natechei"
## [398] "s__sp. SDW2"
## [399] "s__sp. NIES-981"
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## [401] "s__paranthracis"
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## [403] "s__phyllanthi"
## [404] "s__sp. 20G"
## [405] "s__sp. CAP-1"
## [406] "s__salmonis"
## [407] "s__sp. M344"
## [408] "s__thailandicus"
## [409] "s__sp. I507"
## [410] "s__dauci"

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## [413] "s__sp. ESL0695"
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## [416] "s__rhizoryzae"
## [417] "s__sp. DA9"
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## [420] "s__pedis"
## [421] "s__cucullus"
## [422] "s__sp."
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## [424] "s__punjabense"
## [425] "s__ginsenosidimutans"
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## [428] "s__naphthalenivorans"
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## [430] "s__pakistanensis"
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## [434] "s__dentocariosa"
## [435] "s__jejuni"
## [436] "s__timonensis"
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## [442] "s__crassostreae"
## [443] "s__rubrum"
## [444] "s__pabuli"
## [445] "s__rustigianii"
## [446] "s__fischeri"
## [447] "s__sp. YJ01"
## [448] "s__coelicolor"
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## [450] "s__africanus"
## [451] "s__sp. 12200R-103"
## [452] "s__melaninogenica"
## [453] "s__sp. MG-5-Ahmo-C2"
## [454] "s__sp. N902-109"
## [455] "s__sp. 2438"
## [456] "s__siamensis"
## [457] "s__fonticola"
## [458] "s__sp. YTS05"
## [459] "s__jinjuensis"
## [460] "s__sp. TSA2s"
## [461] "s__pyogenes"
## [462] "s__flavibacter"
## [463] "s__phytohabitans"
## [464] "s__sp. ORS 285"

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## [465] "s__zeae"
## [466] "s__sp. CB3481"
## [467] "s__faecale"
## [468] "s__Candidatus Desulfovibrio trichonymphae"
## [469] "s__immobilis"
## [470] "s__carboxidivorans"
## [471] "s__sp. BSN-002"
## [472] "s__sp. WMD975"
## [473] "s__kansasii"
## [474] "s__atlanticum"
## [475] "s__troglodytae"
## [476] "s__profunda"
## [477] "s__butanolivorans"
## [478] "s__zhangzhongyongii"
## [479] "s__pyrrocinia"
## [480] "s__sp. Marseille-Q3772"
## [481] "s__sp. ES10-3-2-2"
## [482] "s__pseudolongum"
## [483] "s__filamentosa"
## [484] "s__aeruginosa"
## [485] "s__hinzii"
## [486] "s__parasuis"
## [487] "s__hydrossis"
## [488] "s__sp. HTF-F"
## [489] "s__sp. KUDC0406"
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## [492] "s__eiseniae"
## [493] "s__sp. Marseille-Q4385"
## [494] "s__brevis"
## [495] "s__bryantii"
## [496] "s__erythropolis"
## [497] "s__testosteroni"
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## [500] "s__sedentarius"
## [501] "s__branderi"
## [502] "s__ruminantium"
## [503] "s__sp. S5"
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## [505] "s__Candidatus Pantoea carbekii"
## [506] "s__autotrophica"
## [507] "s__atypica"
## [508] "s__sp. erpn"
## [509] "s__entomophila"
## [510] "s__leopoldii"
## [511] "s__sp. SCSIO 43204"
## [512] "s__parmentieri"
## [513] "s__sp. CA-103260"
## [514] "s__normanense"
## [515] "s__aliphaticivorans"
## [516] "s__sp. 891-h"
## [517] "s__butyriciproducens"
## [518] "s__ginsengisoli"

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## [519] "s__salsilacus"
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## [522] "s__variabile"
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## [524] "s__urealyticum"
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## [526] "s__divergens"
## [527] "s__sp. SY8519"
## [528] "s__sp. ART55/1"
## [529] "s__sp. FW306-05-C"
## [530] "s__sp. KY-GH-1"
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## [532] "s__aggregatum"
## [533] "s__sp. PBL-H3"
## [534] "s__inopinata"
## [535] "s__lutimineralis"
## [536] "s__sp. PROS-U-1"
## [537] "s__antioxidans"
## [538] "s__sp. FDAARGOS_506"
## [539] "s__sp. PAMC 26628"
## [540] "s__sp. TMPB413"
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## [546] "s__nishinomiyaensis"
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## [548] "s__sp. SVR"
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## [550] "s__sp. M54"
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## [563] "s__sp. KU26590"
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## [565] "s__sp. HDW4A"
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## [567] "s__armeniaca"
## [568] "s__mucosa"
## [569] "s__sp. resist"
## [570] "s__viridans"
## [571] "s__sp. StoSoilA2"
## [572] "s__moriokaense"

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## [577] "s__sp. GL2"
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## [581] "s__sp. oral taxon 126"
## [582] "s__chitae"
## [583] "s__sp. dk3624"
## [584] "s__halotolerans"
## [585] "s__sp. LMS-CY"
## [586] "s__litoralis"
## [587] "s__sp. CT06"
## [588] "s__sp. NEB1569"
## [589] "s__brunensis"
## [590] "s__jostii"
## [591] "s__sp. B21-053"
## [592] "s__sp. AS-1"
## [593] "s__alkaliphilum"
## [594] "s__yuyongxinii"
## [595] "s__sp. SCSIO W1101"
## [596] "s__sp. 17 mud 1-3"
## [597] "s__tokaiense"
## [598] "s__oryzoeni"
## [599] "s__iowensis"
## [600] "s__bovis"
## [601] "s__frisingensis"
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## [606] "s__stewartii"
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## [609] "s__sp. PSB04"
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## [611] "s__sp. BMK-MC-1"
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## [614] "s__sp. IE-0392"
## [615] "s__sp. WJP1"
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## [617] "s__alcaliphila"
## [618] "s__sp. E2T0"
## [619] "s__sp. AA4"
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## [621] "s__marinus"
## [622] "s__funiformis"
## [623] "s__sp. T21"
## [624] "s__sp. MSJ-33"
## [625] "s__sp. QXT-31"
## [626] "s__enoeca"

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 ## [633] "s__sp. VNUA24"
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 ## [635] "s__pneumosintes"
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 ## [637] "s__sp. SP2"
 ## [638] "s__sp. SFB-rat-Yit"
 ## [639] "s__sp. HL-NP1"
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 ## [642] "s__sp. WS"
 ## [643] "s__paragasseri"
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 ## [647] "s__riparius"
 ## [648] "s__sp. JS666"
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 ## [655] "s__platys"
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 ## [673] "s__aerofaciens"
 ## [674] "s__neapolitanus"
 ## [675] "s__sp. MUSA4"
 ## [676] "s__sp. T7-7"
 ## [677] "s__sp. SWIR-1"
 ## [678] "s__suffuscus"
 ## [679] "s__anyangense"
 ## [680] "s__sp. ATCC 8456"

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## [681] "s__coagulans"
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## [683] "s__sp. 15-184"
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## [686] "s__rodentium"
## [687] "s__leadbetteri"
## [688] "s__jeikeium"
## [689] "s__sp. WM"
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## [691] "s__filiformis"
## [692] "s__gallolyticus"
## [693] "s__bryophila"
## [694] "s__sagamiensis"
## [695] "s__glossinidius"
## [696] "s__cryptum"
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## [704] "s__pygmaeum"
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## [708] "s__megaterium"
## [709] "s__sp. AT1b"
## [710] "s__siliguriense"
## [711] "s__binotii"
## [712] "s__sp. CF8"
## [713] "s__vulgaris"
## [714] "s__ginsenosidivorax"
## [715] "s__sp. HUAS 3"
## [716] "s__sp. PCC 6312"
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## [724] "s__sediminis"
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## [728] "s__steedae"
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## [732] "s__sp. Y33"
## [733] "s__sp. SSS035"
## [734] "s__dehalogenans"

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 ## [740] "s__sp. W8901"
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 ## [747] "s__kondratievae"
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 ## [749] "s__sp. L1A9"
 ## [750] "s__pseudoryzae"
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 ## [752] "s__sp. TUM22785"
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 ## [764] "s__taiwanensis"
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 ## [767] "s__diernhoferi"
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 ## [772] "s__sp. 3211"
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 ## [775] "s__yunxiaonensis"
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 ## [782] "s__sp. zg-1228"
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 ## [788] "s__sp. LW097"


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## [798] "s__sp. FWC26"
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## [800] "s__sp. PP1Y"
## [801] "s__sp. ZRK36"
## [802] "s__sp. MTM3W5.2"
## [803] "s__perfringens"
## [804] "s__cannabina"
## [805] "s__lenta"
## [806] "s__diekertiae"
## [807] "s__Candidatus Filomicrobium marinum"
## [808] "s__protaetiae"
## [809] "s__hiltneri"
## [810] "s__propinquum"
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## [812] "s__uraniireducens"
## [813] "s__terrifontis"
## [814] "s__copri"
## [815] "s__cottewii"
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## [817] "s__brasiliense"
## [818] "s__sp. SB155-2"
## [819] "s__imitans"
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## [821] "s__muciniphila"
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## [823] "s__sp. SGAir0287"
## [824] "s__concisus"
## [825] "s__sp. TH-20"
## [826] "s__amalonaticus"
## [827] "s__sp. FB24"
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## [831] "s__sp. MV4-Y"
## [832] "s__sp. RF6"
## [833] "s__sp. KI723T1"
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## [835] "s__marchantiae"
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## [837] "s__fragi"
## [838] "s__altamirensis"
## [839] "s__sp. ESL0769"
## [840] "s__sp. M7A.F.Ce.TU.012.03.2.1"
## [841] "s__sp. KS 6"
## [842] "s__kddieii"

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[843] "s__antranikianii"
 ## [844] "s__radingae"
 ## [845] "s__mercurii"
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 ## [847] "s__gauvreauii"
 ## [848] "s__ammoniagenes"
 ## [849] "s__thermautotrophica"
 ## [850] "s__sp. EGB"
 ## [851] "s__sp. BT18"
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 ## [853] "s__sp. MLAF003"
 ## [854] "s__xyli"
 ## [855] "s__quercinecans"
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 ## [858] "s__sp. HUAS 11-8"
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 ## [862] "s__acidisoli"
 ## [863] "s__brassicacearum"
 ## [864] "s__panacisoli"
 ## [865] "s__luhongzhouii"
 ## [866] "s__zhejiangensis"
 ## [867] "s__radioresistens"
 ## [868] "s__flavus"
 ## [869] "s__stutzeri"
 ## [870] "s__sp. HTCC2170"
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 ## [873] "s__luteovorticillatus"
 ## [874] "s__gerenzanensis"
 ## [875] "s__debuckii"
 ## [876] "s__sp. MYC101"
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 ## [878] "s__sp. L1SW"
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 ## [888] "s__sp. RS9902"
 ## [889] "s__sp. FDAARGOS_553"
 ## [890] "s__pinnipediorum"
 ## [891] "s__radiotolerans"
 ## [892] "s__sp. KUDC1026"
 ## [893] "s__xylanisolvens"
 ## [894] "s__sp. I4-3-84"
 ## [895] "s__nigrescens"
 ## [896] "s__sp. HMP6"

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## [898] "s__sp. DA14"
## [899] "s__cathodiphilus"
## [900] "s__sp. NAK00032"
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## [902] "s__acetotolerans"
## [903] "s__krulwichiae"
## [904] "s__thermophila"
## [905] "s__destructor"
## [906] "s__piscis"
## [907] "s__sp. PAMC 26508"
## [908] "s__seminalis"
## [909] "s__gingivalis"
## [910] "s__sp. BC42"
## [911] "s__sticklandii"
## [912] "s__sp. GIMC2001"
## [913] "s__coryniformis"
## [914] "s__sp. Colony322"
## [915] "s__vignae"
## [916] "s__barguzinensis"
## [917] "s__sp. ANAM02"
## [918] "s__cyaneochromogenes"
## [919] "s__selenitireducens"
## [920] "s__sp. SUK 48"
## [921] "s__sp. ADAK13"
## [922] "s__sp. AK26"
## [923] "s__sp. S4.7"
## [924] "s__sp. Leaf245"
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## [926] "s__aerolatus"
## [927] "s__spongiicola"
## [928] "s__palaemonis"
## [929] "s__aerolatum"
## [930] "s__sp. FDAARGOS_737"
## [931] "s__plutonius"
## [932] "s__maritimus"
## [933] "s__accolens"
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## [935] "s__iners"
## [936] "s__sp. KSW4-10"
## [937] "s__thermotolerans"
## [938] "s__furukawaii"
## [939] "s__sp. PS18"
## [940] "s__sp. wkB8"
## [941] "s__clara"
## [942] "s__purpuratum"
## [943] "s__guaymasensis"
## [944] "s__sp. M3A.F.Ca.ET.080.04.2.1"
## [945] "s__pohangensis"
## [946] "s__benzenivorans"
## [947] "s__sp. RAC05"
## [948] "s__sp. FJ2-5-3"
## [949] "s__silvestris"
## [950] "s__sp. NS1(2017)"

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 ## [953] "s__sp. MP-37"
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 ## [955] "s__sp. XGS7"
 ## [956] "s__sp. 4G"
 ## [957] "s__sp. SK012"
 ## [958] "s__woosongensis"
 ## [959] "s__amylolytica"
 ## [960] "s__carnosus"
 ## [961] "s__sp. GSS17"
 ## [962] "s__nuruki"
 ## [963] "s__ruckeri"
 ## [964] "s__sp. 320-W"
 ## [965] "s__propionicus"
 ## [966] "s__sp. LS44"
 ## [967] "s__chitinolytica"
 ## [968] "s__antarctica"
 ## [969] "s__sp. S190"
 ## [970] "s__ludwigii"
 ## [971] "s__kutscheri"
 ## [972] "s__putida"
 ## [973] "s__sp. NCPPB 2350"
 ## [974] "s__asteroides"
 ## [975] "s__incerta"
 ## [976] "s__woluwensis"
 ## [977] "s__haemolyticus"
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 ## [979] "s__aquimaris"
 ## [980] "s__manosquense"
 ## [981] "s__pseudoperiodonticum"
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 ## [983] "s__canicola"
 ## [984] "s__vandammei"
 ## [985] "s__dumoffii"
 ## [986] "s__endopervernicosa"
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 ## [991] "s__persicina"
 ## [992] "s__mediterraneus"
 ## [993] "s__algae"
 ## [994] "s__carbinolica"
 ## [995] "s__sp. BS20"
 ## [996] "s__futsaii"
 ## [997] "s__gaoshouyii"
 ## [998] "s__callunae"
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 ## [1000] "s__sp. 155"
 ## [1001] "s__sp. Lep1P3"
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 ## [1004] "s__argentoratensis"

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 ## [1007] "s__rubeus"
 ## [1008] "s__sp. H30R-01"
 ## [1009] "s__sp. AU20"
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 ## [1013] "s__thermocarboxydus"
 ## [1014] "s__japonicus"
 ## [1015] "s__defectiva"
 ## [1016] "s__indistinctus"
 ## [1017] "s__watsonii"
 ## [1018] "s__sp. NA07423"
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 ## [1020] "s__inaquosorum"
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 ## [1034] "s__sp. V7"
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 ## [1118] "s__frequens"
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 ## [1340] "s__sp. JQ2195"
 ## [1341] "s__palmae"
 ## [1342] "s__sp. AL041005-10"
 ## [1343] "s__albata"
 ## [1344] "s__sp. 9128"
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 ## [1346] "s__paneuropaeus"
 ## [1347] "s__paraterrae"
 ## [1348] "s__pickettii"
 ## [1349] "s__aminophilus"
 ## [1350] "s__hydrolyticus"
 ## [1351] "s__sp. W027"
 ## [1352] "s__entomophilus"
 ## [1353] "s__kaempferiae"
 ## [1354] "s__sp. PCC 7116"
 ## [1355] "s__sp. FSL R7-0331"
 ## [1356] "s__xylosus"
 ## [1357] "s__sp. SMJS2"
 ## [1358] "s__nantongensis"
 ## [1359] "s__sp. G0186"
 ## [1360] "s__pseudoflava"
 ## [1361] "s__sp. SCSIO W0465"
 ## [1362] "s__lovleyi"
 ## [1363] "s__mediterranea"
 ## [1364] "s__baratii"
 ## [1365] "s__liquefaciens"
 ## [1366] "s__donggukensis"
 ## [1367] "s__borealis"
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 ## [1370] "s__tanashiensis"
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 ## [1372] "s__turgidum"
 ## [1373] "s__xanthii"
 ## [1374] "s__sp. OE 28.3"
 ## [1375] "s__sphenoides"
 ## [1376] "s__albidiflava"
 ## [1377] "s__pudoricolor"
 ## [1378] "s__sp. SCSIO W1103"
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 ## [1380] "s__agamarum"
 ## [1381] "s__alginolyticus"
 ## [1382] "s__sp. THN1"

[1383] "s__africae"
 ## [1384] "s__umbonata"
 ## [1385] "s__radiopugnans"
 ## [1386] "s__arsenatis"
 ## [1387] "s__sp. JC009"
 ## [1388] "s__apisilvae"
 ## [1389] "s__sp. CCMP332"
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 ## [1391] "s__sp. J315"
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 ## [1394] "s__sp. NA04385"
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 ## [1398] "s__sp. LV10R510-11A"
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 ## [1402] "s__sp. AD91A"
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 ## [1409] "s__daejeonense"
 ## [1410] "s__sp. HSG2"
 ## [1411] "s__sp. NCRR"
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 ## [1414] "s__album"
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 ## [1416] "s__pulmonicola"
 ## [1417] "s__sp. LS.1a"
 ## [1418] "s__sp. 29361"
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 ## [1420] "s__sp. G2S3"
 ## [1421] "s__sp. SL250"
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 ## [1429] "s__riograndensis"
 ## [1430] "s__baixiangningiae"
 ## [1431] "s__sp. 107-1"
 ## [1432] "s__tritici"
 ## [1433] "s__sp. BT-42-2"
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 ## [1435] "s__cloacae"
 ## [1436] "s__warabiya"

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## [1437] "s__nigra"
## [1438] "s__sp. BT-123"
## [1439] "s__faecium"
## [1440] "s__phenanthrenivorans"
## [1441] "s__sp. WAC00303"
## [1442] "s__sp. XGS-02"
## [1443] "s__sp. ArI3"
## [1444] "s__sp. R56"
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## [1446] "s__mucilaginosus"
## [1447] "s__sp. 'Peltigera membranacea cyanobiont' N6"
## [1448] "s__thermosuccinogenes"
## [1449] "s__haemolysans"
## [1450] "s__elongata"
## [1451] "s__sp. NBH87"
## [1452] "s__espanaensis"
## [1453] "s__portucalensis"
## [1454] "s__alkalisoli"
## [1455] "s__paucimobilis"
## [1456] "s__sp. Pc102"
## [1457] "s__magna"
## [1458] "s__sp. FDAARGOS 1241"
## [1459] "s__Candidatus Protofrankia datisciae"
## [1460] "s__albidus"
## [1461] "s__bogorensis"
## [1462] "s__someræ"
## [1463] "s__xylosoxidans"
## [1464] "s__indicus"
## [1465] "s__hominis"
## [1466] "s__dysgalactiae"
## [1467] "s__sp. LQ25"
## [1468] "s__sp. RTd22"
## [1469] "s__sp. Jing01"
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## [1471] "s__allomyrinae"
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## [1473] "s__baculatum"
## [1474] "s__phytophila"
## [1475] "s__yayanosii"
## [1476] "s__sp. DH3716P"
## [1477] "s__sp. BT-177"
## [1478] "s__aminovorans"
## [1479] "s__sp. F8"
## [1480] "s__pacifica"
## [1481] "s__thiotaurini"
## [1482] "s__goodwinii"
## [1483] "s__haemolytica"
## [1484] "s__[Ochrobactrum] quorumnocens"
## [1485] "s__promysalinigenes"
## [1486] "s__sp. CC9605"
## [1487] "s__sp. FDAARGOS 1415"
## [1488] "s__metalliredigens"
## [1489] "s__plymuthica"
## [1490] "s__sp. Tan611"

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## [1492] "s__sp. LRE541"
## [1493] "s__urogenitale"
## [1494] "s__sp. RC67"
## [1495] "s__sp. M166"
## [1496] "s__liliifuscus"
## [1497] "s__cholerae"
## [1498] "s__flocculans"
## [1499] "s__sp. SAU14A_NAIMI4_5"
## [1500] "s__sp. ZJ106"
## [1501] "s__syringae group genomosp. 7"
## [1502] "s__telluris"
## [1503] "s__terrenum"
## [1504] "s__konosiri"
## [1505] "s__equorum"
## [1506] "s__sp. JZB09"
## [1507] "s__sp. THAF12"
## [1508] "s__sp. CNQ-509"
## [1509] "s__rotundus"
## [1510] "s__lablabi"
## [1511] "s__glycaniphila"
## [1512] "s__sp. S01"
## [1513] "s__aromatica"
## [1514] "s__sp. Colony194"
## [1515] "s__caseolyticus"
## [1516] "s__zosteriae"
## [1517] "s__sp. CZR27"
## [1518] "s__osloensis"
## [1519] "s__pasteurii"
## [1520] "s__intracellularis"
## [1521] "s__fallax"
## [1522] "s__bifermentans"
## [1523] "s__sp. 5413J-13"
## [1524] "s__sp. SK50-23"
## [1525] "s__acetigenes"
## [1526] "s__sp. WMMA1947"
## [1527] "s__chauvoei"
## [1528] "s__venezuelae"
## [1529] "s__sp. WAC 01438"
## [1530] "s__arginini"
## [1531] "s__oryzicola"
## [1532] "s__aureovorticillatus"
## [1533] "s__sp. QY071"
## [1534] "s__sp. SG1"
## [1535] "s__versatilis"
## [1536] "s__sp. WMMD812"
## [1537] "s__sp. SA4125"
## [1538] "s__albus"
## [1539] "s__sulfuroxidans"
## [1540] "s__baengnokdamensis"
## [1541] "s__allii"
## [1542] "s__sp. OT7"
## [1543] "s__sp. FDAARGOS 1409"
## [1544] "s__urinaehominis"

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## [1545] "s__sp. 0xB-1"
## [1546] "s__sp. Bac332"
## [1547] "s__abscessus"
## [1548] "s__sp. JM1"
## [1549] "s__nigripulchritudo"
## [1550] "s__sp. M30-35"
## [1551] "s__methanolica"
## [1552] "s__sp. MR_MD2014"
## [1553] "s__sp. YPW1"
## [1554] "s__phytofermentans"
## [1555] "s__sp. HUAS 15-9"
## [1556] "s__[Clostridium] colinum"
## [1557] "s__sp. 113P3"
## [1558] "s__chenweiae"
## [1559] "s__metallilatus"
## [1560] "s__hygroscopicus"
## [1561] "s__penaei"
## [1562] "s__sp. CX169"
## [1563] "s__coccoides"
## [1564] "s__guangzhouensis"
## [1565] "s__arabaticum"
## [1566] "s__sp. PAMC26645"
## [1567] "s__sp. VKM Ac-2759"
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## [1570] "s__singaporensis"
## [1571] "s__ferrireducens"
## [1572] "s__sp. NFH-SH190041"
## [1573] "s__chenwenguii"
## [1574] "s__echinicola"
## [1575] "s__abortibovis"
## [1576] "s__sp. QL22"
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## [1578] "s__lutrae"
## [1579] "s__lunaelactis"
## [1580] "s__phocaeense"
## [1581] "s__alcaligenes"
## [1582] "s__aquatilis"
## [1583] "s__litorale"
## [1584] "s__sp. NSJ-69"
## [1585] "s__cervicalis"
## [1586] "s__sp. IMCC11727"
## [1587] "s__aquaemixtae"
## [1588] "s__sp. ESL0785"
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## [1590] "s__americana"
## [1591] "s__caseinilytica"
## [1592] "s__vulnificus"
## [1593] "s__cinaedi"
## [1594] "s__rimosus"
## [1595] "s__oleovorans"
## [1596] "s__novyi"
## [1597] "s__sp. G2-5"
## [1598] "s__sp. XC 2026"

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[1599] "s__kimnyeongensis"
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 ## [1601] "s__sp. oral taxon 218"
 ## [1602] "s__brandeum"
 ## [1603] "s__iranensis"
 ## [1604] "s__similis"
 ## [1605] "s__japonica"
 ## [1606] "s__sp. CB1650"
 ## [1607] "s__antibioticus"
 ## [1608] "s__mucosae"
 ## [1609] "s__sp. INOP01"
 ## [1610] "s__piscicola"
 ## [1611] "s__uncultured bacterium"
 ## [1612] "s__sp. CY52-2"
 ## [1613] "s__sp. WL1"
 ## [1614] "s__echinospora"
 ## [1615] "s__opportunatum"
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 ## [1617] "s__arabiense"
 ## [1618] "s__dextrinosolvens"
 ## [1619] "s__gottheilii"
 ## [1620] "s__lizhenjunii"
 ## [1621] "s__avenae"
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 ## [1624] "s__sp. D2"
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 ## [1630] "s__ignavus"
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 ## [1634] "s__sprentiae"
 ## [1635] "s__sp. PIV-1"
 ## [1636] "s__sp. FDAARGOS_375"
 ## [1637] "s__sp. WB-2"
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 ## [1639] "s__himalayensis"
 ## [1640] "s__tanakiae"
 ## [1641] "s__sp. MEDNS5"
 ## [1642] "s__chagasii"
 ## [1643] "s__innocua"
 ## [1644] "s__pinensis"
 ## [1645] "s__sinuspersici"
 ## [1646] "s__cowanii"
 ## [1647] "s__rhodesiae"
 ## [1648] "s__sp. 336/3"
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 ## [1650] "s__tenebrarum"
 ## [1651] "s__richardii"
 ## [1652] "s__candidum"

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 ## [1655] "s__tolaasii"
 ## [1656] "s__violaceusniger"
 ## [1657] "s__sp. LG1267"
 ## [1658] "s__sp. KACC 23028"
 ## [1659] "s__atlantisensis"
 ## [1660] "s__fredii"
 ## [1661] "s__sp. WH 8020"
 ## [1662] "s__sp. M6A.T.Cr.TU.016.01.1.1"
 ## [1663] "s__sp. NRS527"
 ## [1664] "s__sobrinus"
 ## [1665] "s__allegheense"
 ## [1666] "s__vicinigordonae"
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 ## [1669] "s__lithotrophicum"
 ## [1670] "s__ectocarpus"
 ## [1671] "s__sp. Y32M11"
 ## [1672] "s__toyakuensis"
 ## [1673] "s__phaeovibrioides"
 ## [1674] "s__sp. 3H"
 ## [1675] "s__sp. S1D4-14"
 ## [1676] "s__sp. CKK8"
 ## [1677] "s__lilanzuaniae"
 ## [1678] "s__sp. YG1"
 ## [1679] "s__acetoxydans"
 ## [1680] "s__sp. Hal144"
 ## [1681] "s__sp. AM 2-1-1"
 ## [1682] "s__delphini"
 ## [1683] "s__sp. CCB-MM3"
 ## [1684] "s__malmoense"
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 ## [1687] "s__clausii"
 ## [1688] "s__brachiatum"
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 ## [1691] "s__sp. THAF30"
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 ## [1695] "s__sp. HBX-1"
 ## [1696] "s__sp. AH1"
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 ## [1699] "s__tuirus"
 ## [1700] "s__faecalis"
 ## [1701] "s__sp. Go-475"
 ## [1702] "s__ratti"
 ## [1703] "s__sp. St316"
 ## [1704] "s__pseudogrignonensis"
 ## [1705] "s__Blochmannia endosymbiont of Polyrhachis (Hedomyrma) turneri"
 ## [1706] "s__tractuosa"

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## [1709] "s__ferriphilum"
## [1710] "s__damselae"
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## [1712] "s__Enterobacteriaceae endosymbiont of Donacia tomentosa"
## [1713] "s__panamensis"
## [1714] "s__carniphilus"
## [1715] "s__methoxysyntrophicus"
## [1716] "s__capnotolerans"
## [1717] "s__diazotrophicus"
## [1718] "s__sp. SL47"
## [1719] "s__phosphovorus"
## [1720] "s__vincentii"
## [1721] "s__sp. M41"
## [1722] "s__sp. WS11"
## [1723] "s__sp. G01H"
## [1724] "s__simplex"
## [1725] "s__hydrophila"
## [1726] "s__aidingensis"
## [1727] "s__thermoresistibile"
## [1728] "s__sp. BPS33"
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## [1730] "s__sp. E15-22"
## [1731] "s__jaguaris"
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## [1733] "s__vanderleydeniana"
## [1734] "s__sp. LM7"
## [1735] "s__zhachilii"
## [1736] "s__sp. 63ED37-2"
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## [1738] "s__saxobsidens"
## [1739] "s__terrigena"
## [1740] "s__vaccinii"
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## [1743] "s__pediculischaeffi"
## [1744] "s__aquaticum"
## [1745] "s__humicireducens"
## [1746] "s__genisteinicus"
## [1747] "s__sp. PMCC200344"
## [1748] "s__pentosaceus"
## [1749] "s__phocisimile"
## [1750] "s__sp. CCB-ST2H9"
## [1751] "s__degensii"
## [1752] "s__sp. B32"
## [1753] "s__jordanis"
## [1754] "s__warneri"
## [1755] "s__sp. PCC 7327"
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## [1757] "s__blattae"
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## [1759] "s__cavernae"
## [1760] "s__medellinensis"

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[1761] "s__tardum"
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 ## [1765] "s__megapolitana"
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 ## [1767] "s__sp. 3214.6"
 ## [1768] "s__botulinum"
 ## [1769] "s__composti"
 ## [1770] "s__sp. 1566"
 ## [1771] "s__orientale"
 ## [1772] "s__sp. MTM4"
 ## [1773] "s__auricularis"
 ## [1774] "s__hongtaonis"
 ## [1775] "s__monticola"
 ## [1776] "s__sp. MC1825"
 ## [1777] "s__incomptus"
 ## [1778] "s__lactatiformans"
 ## [1779] "s__pukyongi"
 ## [1780] "s__stabekisii"
 ## [1781] "s__pantholopis"
 ## [1782] "s__multitudinisentens"
 ## [1783] "s__sp. No. 7"
 ## [1784] "s__urinaeequi"
 ## [1785] "s__sp. P6W"
 ## [1786] "s__nojiriensis"
 ## [1787] "s__vibrioformis"
 ## [1788] "s__tructae"
 ## [1789] "s__sp. AM 4-1-1"
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 ## [1791] "s__Verrucosispora sp. WMMD1129"
 ## [1792] "s__sp. AGMB13025"
 ## [1793] "s__hwasookii"
 ## [1794] "s__bohemicus"
 ## [1795] "s__lividus"
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 ## [1801] "s__sp. JS3050"
 ## [1802] "s__[Acidovorax] ebreus"
 ## [1803] "s__furrinae"
 ## [1804] "s__sp. QJXJ"
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 ## [1807] "s__carboxydovora"
 ## [1808] "s__barranii"
 ## [1809] "s__helveticus"
 ## [1810] "s__callanderi"
 ## [1811] "s__capsulatus"
 ## [1812] "s__aerodenitrificans"
 ## [1813] "s__griseus"
 ## [1814] "s__tertiaricarbonis"

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## [1815] "s__sera"
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## [1819] "s__sp. MI1205"
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## [1821] "s__sp. AR10"
## [1822] "s__sp. GDN1"
## [1823] "s__sp. M28"
## [1824] "s__clevelandensis"
## [1825] "s__sp. MB-3u-03"
## [1826] "s__sp. 116-D4"
## [1827] "s__sp. BIM B-2242"
## [1828] "s__sp. HF-162"
## [1829] "s__tubbatahanensis"
## [1830] "s__sp. RPA4-2"
## [1831] "s__newyorkensis"
## [1832] "s__anaerobius"
## [1833] "s__sp. 8"
## [1834] "s__halichoeri"
## [1835] "s__sp. ZAC14D1_NAIMI4_6"
## [1836] "s__asoensis"
## [1837] "s__sp. 'AMD consortium'"
## [1838] "s__sp. NIV53"
## [1839] "s__lydicamycinicus"
## [1840] "s__fuliginis"
## [1841] "s__phasianinus"
## [1842] "s__sp. XAAS-72"
## [1843] "s__flavescens"
## [1844] "s__alvei"
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## [1848] "s__schaalii"
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 ## [2421] "s__backii"
 ## [2422] "s__woodii"
 ## [2423] "s__septentrionale"
 ## [2424] "s__sp. M1242"
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 ## [2513] "s__sp. G2-70"
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 ## [2564] "s__sp. HC6"
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 ## [2567] "s__sp. TEGF004"
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 ## [2573] "s__blasticum"
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 ## [2575] "s__sp. SirexAA-E"
 ## [2576] "s__butyricum"
 ## [2577] "s__sp. AP8"
 ## [2578] "s__crocea"
 ## [2579] "s__shinjukuense"
 ## [2580] "s__sp. HN-2-9-2"
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 ## [2585] "s__sp. RKMC-009"
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 ## [2603] "s__sp. GF20"
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 ## [2610] "s__resinovorum"
 ## [2611] "s__fontium"
 ## [2612] "s__purcellii"
 ## [2613] "s__sp. NBC_00550"
 ## [2614] "s__sp. SW4"
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 ## [2623] "s__petrolearium"
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 ## [2654] "s__rubneri"
 ## [2655] "s__carbinolicus"
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 ## [2675] "s__sp. SMBL_HHYL_HB1"
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 ## [2777] "s__clavuligerus"
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 ## [2780] "s__ihbetae"
 ## [2781] "s__safensis"
 ## [2782] "s__baliensis"
 ## [2783] "s__sp. SMR4y"
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 ## [2888] "s__obesiensis"
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 ## [2892] "s__auxotrophicus"
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 ## [2894] "s__sp. PTS2502"

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 ## [2905] "s__sp. B30-1"
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 ## [2911] "s__phaeoluteigriseus"
 ## [2912] "s__sp. SCSIO 80058"
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## [3557] "s__glauca"
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 ## [4490] "s__sabuli"
 ## [4491] "s__ehrlichii"
 ## [4492] "s__sp. P2"
 ## [4493] "s__sp. KUDC0405"
 ## [4494] "s__canalis"
 ## [4495] "s__lactucae"
 ## [4496] "s__tsukubensis"
 ## [4497] "s__sp. WF146"
 ## [4498] "s__sunii"
 ## [4499] "s__tetani"
 ## [4500] "s__sp. WKF16"
 ## [4501] "s__sp. cB07"
 ## [4502] "s__sp. MFBS3-15"
 ## [4503] "s__cellulolyticum"
 ## [4504] "s__sp. RAC03"
 ## [4505] "s__sp. NP-4(2019)"
 ## [4506] "s__alkalaceticum"
 ## [4507] "s__activa"
 ## [4508] "s__oriscaviae"
 ## [4509] "s__sp. YN"
 ## [4510] "s__sp. Pch-S"
 ## [4511] "s__sp. KKS102"
 ## [4512] "s__sp. Z12"
 ## [4513] "s__sp. TF02-7"
 ## [4514] "s__theicola"

[4515] "s__sp. YB324"
 ## [4516] "s__gallisepticum"
 ## [4517] "s__xanthomarina"
 ## [4518] "s__psittacipulmonis"
 ## [4519] "s__necator"
 ## [4520] "s__shigaense"
 ## [4521] "s__halodurans"
 ## [4522] "s__sp. XY-2"
 ## [4523] "s__arilaitensis"
 ## [4524] "s__dispersa"
 ## [4525] "s__sp. HH130629-09"
 ## [4526] "s__arcticus"
 ## [4527] "s__rhodochrous"
 ## [4528] "s__sp. ABRD_28"
 ## [4529] "s__sp. THAF82"
 ## [4530] "s__bemidjiense"
 ## [4531] "s__alhagi"
 ## [4532] "s__sp. 4R-513"
 ## [4533] "s__sp. DL-VIII"
 ## [4534] "s__sp. ALC70"
 ## [4535] "s__sp. SCPEA002"
 ## [4536] "s__jinghuaiqii"
 ## [4537] "s__sp. M259"
 ## [4538] "s__alkylphenolica"
 ## [4539] "s__sp. MC1862"
 ## [4540] "s__guangdongensis"
 ## [4541] "s__neonatale"
 ## [4542] "s__sp. AJA228-03"
 ## [4543] "s__roseirectus"
 ## [4544] "s__shigelloides"
 ## [4545] "s__odontolytica"
 ## [4546] "s__canimorsus"
 ## [4547] "s__sp. NMCA1"
 ## [4548] "s__sp. JUb54"
 ## [4549] "s__peoriae"
 ## [4550] "s__salivibrio"
 ## [4551] "s__timonense"
 ## [4552] "s__sp. Ricciae_BoGa-3"
 ## [4553] "s__sp. ESL0790"
 ## [4554] "s__sp. 2125159857"
 ## [4555] "s__faecimaris"
 ## [4556] "s__macleodii"
 ## [4557] "s__thuringiensis"
 ## [4558] "s__[Ruminococcus] lactaris"
 ## [4559] "s__helvum"
 ## [4560] "s__sp. 24E2"
 ## [4561] "s__graeca"
 ## [4562] "s__taurus"
 ## [4563] "s__polytropus"
 ## [4564] "s__craniellae"
 ## [4565] "s__sp. W7"
 ## [4566] "s__sp. I71"
 ## [4567] "s__euryhalodurans"
 ## [4568] "s__corsicus"

[4569] "s__elizabethae"
 ## [4570] "s__galactanivorans"
 ## [4571] "s__fengzijianii"
 ## [4572] "s__sp. CCGE532"
 ## [4573] "s__phaeum"
 ## [4574] "s__restrictus"
 ## [4575] "s__sp. VKM Ac-2760"
 ## [4576] "s__campestrisoli"
 ## [4577] "s__exhalans"
 ## [4578] "s__leprae"
 ## [4579] "s__sp. MWH-Spelu-300-X4"
 ## [4580] "s__gardneri"
 ## [4581] "s__sp. JY-7876"
 ## [4582] "s__stuartii"
 ## [4583] "s__enterica"
 ## [4584] "s__sp. NEAU-S7GS2"
 ## [4585] "s__sp. CBA3102"
 ## [4586] "s__fastidiosus"
 ## [4587] "s__rhizogenes"
 ## [4588] "s__sp. L2-79-05"
 ## [4589] "s__kroppenstedtii"
 ## [4590] "s__sp. ST1015"
 ## [4591] "s__affigens"
 ## [4592] "s__sp. CS682"
 ## [4593] "s__scardovii"
 ## [4594] "s__galeata"
 ## [4595] "s__ganghwense"
 ## [4596] "s__sp. LS1212"
 ## [4597] "s__geestiana"
 ## [4598] "s__ishigakiensis"
 ## [4599] "s__lavendulae"
 ## [4600] "s__splachnicus"
 ## [4601] "s__sp. UDSM-2020"
 ## [4602] "s__sp. S16"
 ## [4603] "s__grossiae"
 ## [4604] "s__larrymoorei"
 ## [4605] "s__sp. 11-B-312"
 ## [4606] "s__sp. NLF-5-8"
 ## [4607] "s__proteinivorum"
 ## [4608] "s__sp. A6099"
 ## [4609] "s__serpentiformis"
 ## [4610] "s__giovannonii"
 ## [4611] "s__sp. HYN0024"
 ## [4612] "s__sp. MS455"
 ## [4613] "s__sp. BDJS001"
 ## [4614] "s__sp. NtRootA1"
 ## [4615] "s__sp. CB1024"
 ## [4616] "s__saccharophila"
 ## [4617] "s__globerulus"
 ## [4618] "s__sp. X-1"
 ## [4619] "s__sp. E4742"
 ## [4620] "s__tangerina"
 ## [4621] "s__sp. DMU1"
 ## [4622] "s__mori"

[4623] "s__bethesdensis"
 ## [4624] "s__sabulinigri"
 ## [4625] "s__sp. KNUC1210"
 ## [4626] "s__sp. Mg1"
 ## [4627] "s__obscurus"
 ## [4628] "s__sp. 1608163"
 ## [4629] "s__zengyii"
 ## [4630] "s__luti"
 ## [4631] "s__sp. SCG-1"
 ## [4632] "s__sp. C5510"
 ## [4633] "s__sp. NIBR2454"
 ## [4634] "s__boletus"
 ## [4635] "s__cryptoxanthini"
 ## [4636] "s__thiophilus"
 ## [4637] "s__radicincitans"
 ## [4638] "s__sp. K1W22B-7"
 ## [4639] "s__sp. UASWS1016"
 ## [4640] "s__rhusiopathiae"
 ## [4641] "s__sp. PGU16"
 ## [4642] "s__sp. T93"
 ## [4643] "s__sp. KIS68-7"
 ## [4644] "s__sp. WB94"
 ## [4645] "s__cryptocerci"
 ## [4646] "s__psychromarinicola"
 ## [4647] "s__sp. CF"
 ## [4648] "s__binhaiensis"
 ## [4649] "s__sp. Marseille-Q7828"
 ## [4650] "s__sp. AAP5"
 ## [4651] "s__sp. L3-i23"
 ## [4652] "s__youngiae"
 ## [4653] "s__sp. FeN2"
 ## [4654] "s__pinxianii"
 ## [4655] "s__sp. IHBB 10380"
 ## [4656] "s__sp. Fw109-5"
 ## [4657] "s__furnissii"
 ## [4658] "s__sp. Adler-ghost"
 ## [4659] "s__canariense"
 ## [4660] "s__producens"
 ## [4661] "s__pontiacus"
 ## [4662] "s__sp. BJN0003"
 ## [4663] "s__multipartita"
 ## [4664] "s__erdmanii"
 ## [4665] "s__mayonis"
 ## [4666] "s__roseola"
 ## [4667] "s__phymatum"
 ## [4668] "s__sedlakii"
 ## [4669] "s__micra"
 ## [4670] "s__sp. OM7"
 ## [4671] "s__orientis"
 ## [4672] "s__sp. AONIH1"
 ## [4673] "s__inkyongensis"
 ## [4674] "s__xenophagum"
 ## [4675] "s__sp. sptzw28"
 ## [4676] "s__sp. ESL0682"


```
## [4677] "s__carotovorum"
## [4678] "s__sp. WSM4906"
## [4679] "s__sp. CCBAU 51753"
## [4680] "s__lariciata"
## [4681] "s__atrarenae"
## [4682] "s__mediterranei"
## [4683] "s__sp. R24"
## [4684] "s__sp. PIA16"
## [4685] "s__ferrophilus"
## [4686] "s__chlorobenzoica"
## [4687] "s__vulturis"
## [4688] "s__drentensis"
## [4689] "s__sanyensis"
## [4690] "s__hallii"
## [4691] "s__gerontici"
## [4692] "s__sp. SCSIO52902"
## [4693] "s__obscuriglobus"
## [4694] "s__sp. TRM1-10"
## [4695] "s__sp. YIM 151385"
## [4696] "s__cetorum"
## [4697] "s__fucicola"
## [4698] "s__amoebiphila"
## [4699] "s__cellanae"
## [4700] "s__sp. LPB0260"
## [4701] "s__mysorens"
## [4702] "s__xylanus"
## [4703] "s__eburnea"
## [4704] "s__liangshanensis"
## [4705] "s__formicoaceticum"
## [4706] "s__russatus"
```

```
# Check the amount of unique taxa in samples which have and have not been treated with antibiotics
subsetMG %>% ps_filter(AB == "no") # 6014 different taxa for non AB treated
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 6014 taxa and 102 samples ]
## sample_data() Sample Data: [ 102 samples by 34 sample variables ]
## tax_table() Taxonomy Table: [ 6014 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 6014 tips and 6013 internal nodes ]
```

```
subsetMG %>% ps_filter(AB == "yes") # 3148 different taxa for AB treated
```

```
## phyloseq-class experiment-level object
## otu_table() OTU Table: [ 3148 taxa and 18 samples ]
## sample_data() Sample Data: [ 18 samples by 34 sample variables ]
## tax_table() Taxonomy Table: [ 3148 taxa by 7 taxonomic ranks ]
## phy_tree() Phylogenetic Tree: [ 3148 tips and 3147 internal nodes ]
```

```
# 6355 different taxa in total, so 341 taxa are not found in non AB
```

```
# Stable "Farm2R1S1" has the three lowest sampling depths of the dataset, the other nine samples are f
subsetMG %>% ps_filter(FarmRoundStable == c("Farm2R1S1")) %>% sample_sums() %>% sort()
```

```
# factorizing variables as not to create problems with visualization later down the line
sample_data(subsetMG)$Cluster = as.factor(sample_data(subsetMG)$Cluster)
sample_data(subsetMG)$FlockSize = as.factor(sample_data(subsetMG)$FlockSize)
sample_data(subsetMG)$AgeParentStock = as.factor(sample_data(subsetMG)$AgeParentStock)
sample_data(subsetMG)$Age = as.factor(sample_data(subsetMG)$Age)
sample_data(subsetMG)$LibraryNumber = as.factor(sample_data(subsetMG)$LibraryNumber)

# add stable column with shorter names
sample_data(subsetMG)$FarmRoundStable = as.factor(sample_data(subsetMG)$FarmRoundStable)
subsetMG@sam_data$Stables = revalue(sample_data(subsetMG)$FarmRoundStable, c("Farm1R1S1"=
                                                                              "Farm2R2S1"=
                                                                              "Farm4R1S1"=
                                                                              ))

# Shortening agent names
subsetMG@sam_data$Cox[subsetMG@sam_data$Cox == "narasinandnicarbazin(maxiban)"] = "Maxiba"
subsetMG@sam_data$Cox[subsetMG@sam_data$Cox == "narasin(monteban)"] = "Monteban"
subsetMG@sam_data$Cox[subsetMG@sam_data$Cox == "salinomycin(Sacox120microGranulate)"] = "
```

loading data

```

# now we'll also add in microbial load
microbial_load = read.table("bacterial_load_kraken2.tab", sep = "\t", header = TRUE)
microbial_load$Sample_Unique = sapply(regmatches(microbial_load$Sample_Unique, regexpr("_",microbial_loa
microbial_load$Sample_Unique[68] = "4_65"
meta_data_R = dplyr::right_join(meta_data_R, microbial_load, by="Sample_Unique")

# creating tree and making phyloseq components, adding tree and sample data components to phyloseq
set.seed("877") # setting seed for reproducibility purposes
random_tree = rtree(ntaxa(Rps), rooted=TRUE, tip.label=taxa_names(Rps))
meta_data_R = sample_data(meta_data_R)
rownames(meta_data_R) = meta_data_R$Sample_Unique
Rps = merge_phyloseq(Rps, meta_data_R, random_tree)

# repeat for mp
set.seed("878") # setting seed for reproducibility purposes
random_tree2 = rtree(ntaxa(Rps_mp), rooted=TRUE, tip.label=taxa_names(Rps_mp))
Rps_mp = merge_phyloseq(Rps_mp, meta_data_R, random_tree2)

# repeat for tpm
set.seed("879") # setting seed for reproducibility purposes
random_tree3 = rtree(ntaxa(Rps_tpm), rooted=TRUE, tip.label=taxa_names(Rps_tpm))
Rps_tpm = merge_phyloseq(Rps_tpm, meta_data_R, random_tree3)

# overview data
datatable(tax_table(Rps))

```

```
rank_names(Rps) # Shows classes and ARGs
```

```
## [1] "AMR_class_primary" "AMR_class_secondary" "ARGCluster90"
## [4] "ID_ClustRefSequence"
```

```
sort(get_taxa_unique(Rps, "AMR_class_primary")) # Shows primary AMR classes
```

```
## [1] "Aminoglycoside" "Beta-lactam" "Fosfomycin" "Glycopeptide"
## [5] "Lincosamide" "Macrolide" "Not determined" "Oxazolidinone"
## [9] "Phenicol" "Quinolone" "Streptogramin" "Sulphonamide"
## [13] "Tetracycline" "Trimethoprim"
```

```
sort(sample_sums(Rps)) # Amount of unique "taxa" per sample, the min is 1365.913 and max 44483.138, whi
```

```
##      4_55      4_57      4_36      4_39      4_37      14_34      5_55      5_41
## 1365.915 1746.186 2162.499 2332.954 2466.228 2718.590 3066.586 3172.468
##      10_26      10_22      10_20      14_22      10_67      2_57      14_21      4_56
## 3673.885 3685.018 3729.530 3765.937 3823.575 3839.225 3978.278 4001.996
##      9_17      6_37      11_1      10_59      10_14      10_29      10_12      10_15
## 4093.433 4180.804 4345.176 4424.335 4498.027 4651.786 4689.540 4755.647
##      10_19      10_4      6_55      10_7      14_30      10_69      2_48      14_36
## 4869.370 4877.468 4903.775 4918.347 4936.114 5041.872 5072.382 5106.032
##      4_54      10_8      6_57      10_40      5_40      14_35      9_22      14_20
## 5129.624 5167.848 5190.200 5214.607 5270.470 5336.436 5395.266 5410.307
##      5_54      10_68      14_33      10_34      10_57      2_41      9_16      5_59
```

```
## 5581.891 5584.014 5595.311 5619.167 5671.777 5753.916 5758.922 5795.072
## 10_11 4_65 4_40 10_58 9_21 14_25 10_10 14_29
## 5816.317 5826.670 5838.197 5885.278 5977.274 6024.628 6044.767 6065.843
## 10_44 10_63 2_49 6_36 2_58 14_23 11_3 2_40
## 6105.070 6124.063 6206.665 6234.746 6254.150 6267.295 6268.648 6326.603
## 10_30 10_35 10_13 6_58 6_54 6_56 5_39 10_21
## 6375.700 6392.360 6431.761 6452.046 6506.618 6516.612 6522.346 6581.651
## 6_38 10_25 2_26 2_36 10_51 10_64 4_38 2_56
## 6643.052 6768.292 6862.483 6869.818 6924.205 6937.682 6956.992 6984.465
## 10_52 2_25 10_39 2_29 2_51 2_59 9_19 14_27
## 7060.573 7067.078 7091.217 7169.829 7383.892 7463.646 7528.052 7542.397
## 2_24 4_41 2_52 9_37 10_42 9_38 10_48 10_53
## 7543.974 7558.754 7639.814 7741.943 7759.352 7851.401 7890.425 7894.041
## 10_66 2_50 10_41 9_36 10_33 2_27 10_60 2_39
## 8025.357 8083.126 8104.861 8142.604 8192.787 8365.139 8412.984 8511.399
## 2_23 10_28 9_34 10_43 9_39 2_42 9_35 2_47
## 8613.113 8811.783 8881.043 8927.401 9038.993 9095.792 9217.621 9321.329
## 2_61 10_49 9_18 2_60 10_50 10_2 10_3 10_1
## 9503.493 9672.041 9749.846 9753.045 10461.847 23616.052 27684.887 44483.138
```

```
summary(sample_sums(Rps)) # summary of the sampling depths
```

```
## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 1366 5065 6244 6827 7579 44483
```

```
summary(sample_sums(Rps_mp)) # there are big differences between kraken2 and metaphlan counts data, with
```

```
## Min. 1st Qu. Median Mean 3rd Qu. Max.
## 125.5 6816.4 11282.9 16409.8 26186.9 93167.6
```

```
sample_variables(Rps) # metadata variables
```

```
## [1] "SampleIdentifier" "ResCap" "Conc...ng..pl." "SampleID"
## [5] "LibraryNumber" "Sample_Unique" "LibraryName" "Farm"
## [9] "Farm2" "Stable" "FarmRoundStable" "Days"
## [13] "Age" "Sname" "WeightAnimal" "Gender"
## [17] "AgeParentStock" "Hatchery" "Researcher" "AB"
## [21] "Abday" "FlockSize" "FeedF" "FeedType"
## [25] "FeedProducent" "Cox" "OPG" "Cluster"
## [29] "LitterType" "Metagenomics" "ReadPerc" "ReadTot"
```

```
taxa_names(Rps) # ARGs (662)
```

```
## [1] "blaTEM-210_1_KJ484630" "blaSHV-183_1_HG934764"
## [3] "aac(6)-Iih_1_AJ584701" "blaSED1_1_AF321608"
## [5] "aadA5_1_AF137361" "blaCTX-M-36_1_AB177384"
## [7] "tet(M)_3_U08812" "tet(B)_2_AF326777"
## [9] "dfrA14_1_KF921535" "aac(6)-Iw_1_AF031331"
## [11] "lsa(E)_1_JX560992" "dfrA5_1_X12868"
## [13] "tet(44)_1_NZ_ABDU01000081" "dfrA1_12_AY141977"
## [15] "aph(2)-Ib_3_KF652098" "lnu(A)_1_M14039"
```

## [17]	"cepA-44_1_U05885"	"aph(3)-Ia_10_EU855787"
## [19]	"mecC_4_HG515014"	"vat(E)_3_AF153312"
## [21]	"blaTEM-182_1_HQ317449"	"blaTEM-127_1_AY368236"
## [23]	"blaZ_1_CP000704"	"cepA_1_U05887"
## [25]	"dfrA5_4_AY139589"	"blaOXA-453_1_KR061507"
## [27]	"tet(O)_1_M18896"	"erm(B)_18_X66468"
## [29]	"ant(9)-Ia_2_M69221"	"aac(3)-IV_1_DQ241380"
## [31]	"blaOXA-474_1_KR182167"	"cat_5_U35036"
## [33]	"blaSHV-50_1_AY288915"	"blaOXA-473_1_KR182166"
## [35]	"blaTEM-213_1_LJEE02000034"	"VanC2XY_2_EU151755"
## [37]	"tet(X4)_1_MK134376"	"tet(G)_1_AF133139"
## [39]	"sul2_5_AY524415"	"blaZ_6_AP003139"
## [41]	"erm(T)_1_M64090"	"blaOXA-322_1_KF203096"
## [43]	"blaOXA-461_1_KR061509"	"tet(Z)_1_AF121000"
## [45]	"tetA(P)_3_AB001076"	"aph(3)-Ia_3_EF015636"
## [47]	"sul2_8_AJ877041"	"blaZ_78_KU607301"
## [49]	"erm(D)_1_M29832"	"cfr(B)_1_KM359439"
## [51]	"blaTEM-197_1_HQ877606"	"blaTEM-17_1_Y14574"
## [53]	"erm(Q)_1_L22689"	"blaOXA-230_1_JQ422054"
## [55]	"blaZ_59_NZ_JVBT01000063"	"tet(M)_4_X75073"
## [57]	"blaOXA-193_1_CP013032"	"blaTEM-225_1_KY432403"
## [59]	"tet(W)_2_AY049983"	"lnu(F)_3_AJ561197"
## [61]	"tet(O/W/32/O/W/O)_2_DQ679926"	"aac(3)-IVa_1_X01385"
## [63]	"dfrA1_6_FJ215857"	"blaCTX-M-28_6_AJ549244"
## [65]	"blaTEM-168_1_FJ919776"	"aph(3)-Ia_7_X62115"
## [67]	"aph(3)-Ib_2_AF024602"	"dfrA8_1_U10186"
## [69]	"mecA_9_AB505630"	"mecC_2_FR823292"
## [71]	"blaTEM-11_1_AY874537"	"dfrA12_4_EU650399"
## [73]	"sul2_3_HQ840942"	"cfiA4_1_AB087229"
## [75]	"dfrA17_6_AF180469"	"mecA2_1_AM048811"
## [77]	"qnrS8_1_KF730652"	"blaPED0-2_1_KP109678"
## [79]	"msr(A)_1_X52085"	"aadA13_1_AY713504"
## [81]	"blaTEM-1C_1_FJ560503"	"lnu(C)_1_AY928180"
## [83]	"sul1_35_AB281182"	"mph(C)_2_AF167161"
## [85]	"blaCTX-M-30_1_AY292654"	"tet(X3)_1_MK134375"
## [87]	"sul1_17_AM746675"	"sul2_11_AY232670"
## [89]	"mef(C)_1_AB571865"	"blaZ_16_JBTH01000015"
## [91]	"VanC3XY_1_AY033764"	"dfrA12_1_FJ763641"
## [93]	"tet(32)_2_EF626943"	"tet(O/W)_3_AM889120"
## [95]	"erm(T)_2_AY894138"	"erm(B)_2_K00551"
## [97]	"blaTEM-35_1_KP860986"	"blaTEM-88_1_AY027590"
## [99]	"qnrB10_2_HM439644"	"VanG2XY_1_FJ872410"
## [101]	"tet(M)_13_AM990992"	"blaTEM-166_1_FJ197316"
## [103]	"aph(3)-Ib_4_AF313472"	"blaTEM-196_1_JQ034306"
## [105]	"cat(pC221)_1_X02529"	"dfrA1_9_AJ238350"
## [107]	"dfrA16_1_AF077008"	"aph(3)-Ia_9_EU722351"
## [109]	"sul2_1_AF542061"	"blaTEM-83_1_AF427129"
## [111]	"cfiA3_1_AB087228"	"cmx_1_U85507"
## [113]	"dfrA12_8_AM040708"	"mph(G)_1_AB571865"
## [115]	"sul1_3_EU855787"	"blaTEM-176_1_GU550123"
## [117]	"tetA(P)_2_L20800"	"tet(K)_1_U38656"
## [119]	"blaTEM-104_1_AF516719"	"cfr(C)_1_KX686749"
## [121]	"erm(B)_1_JN899585"	"VanHDX_5_AY489045"
## [123]	"tet(40)_1_FJ158002"	"blaTEM-70_1_AF188199"

## [125]	"tet(W/32/O)_4_AM710605"	"dfrA17_7_AB196349"
## [127]	"blaSHV-26_1_AF227204"	"cfiA2_1_AB087226"
## [129]	"mph(B)_1_D85892"	"blaCTX-M-12_1_DQ821704"
## [131]	"tet(O/W)-2_1_AY485122"	"msr(C)_2_AF313494"
## [133]	"aph(3)-Ia_6_L05392"	"aph(3)-Ia_5_AP004237"
## [135]	"aac(6)-aph(2)_1_M13771"	"tet(W/32/O)_3_AM710603"
## [137]	"cfxA5_1_AY769934"	"blaCGB-1_1_EF672680"
## [139]	"msr(A)_2_AB013298"	"VanC3XY_2_EU151759"
## [141]	"sul1_2_U12338"	"vgb(B)_1_AF015628"
## [143]	"blaZ_57_NZ_JURP01000126"	"aadA2b_1_D43625"
## [145]	"blaTEM-63_1_AF332513"	"blaZ_30_JGUQ01000012"
## [147]	"erm(34)_1_AY234334"	"blaOXA-493_1_CP007774"
## [149]	"blaTEM-57_1_FJ405211"	"vat(E)_10_AY043212"
## [151]	"dfrA1_8_X00926"	"aadA13_2_NC010643"
## [153]	"blaOXA-58_1_AY665723"	"blaTEM-153_1_KC149518"
## [155]	"tet(X)_3_AB097942"	"poxTA_1_MF095097"
## [157]	"aadA2_2_JQ364967"	"dfrA17_1_FJ460238"
## [159]	"blaTEM-128_1_AY368237"	"cml_1_M22614"
## [161]	"tet(L)_1_HM235948"	"VanC1XY_2_DQ022190"
## [163]	"lnu(B)_1_AJ238249"	"catP_1_U15027"
## [165]	"erm(A)_1_X03216"	"blaTEM-227_1_KY418040"
## [167]	"erm(C)_15_U82607"	"blaTEM-40_1_FR717535"
## [169]	"erm(B)_6_AF242872"	"blaSHV-2a_1_X98102"
## [171]	"aadA1_5_JX185132"	"blaOXA-349_1_KF297578"
## [173]	"tet(O/W)_1_AM889118"	"sul1_8_JN581942"
## [175]	"aph(2)-Ig_1_CP004067"	"blaSHV-64_1_DQ174304"
## [177]	"lsa(B)_1_AJ579365"	"tet(O/W/O)-2_1_AY196920"
## [179]	"dfrA15_4_AJ867237"	"blaOXA-308_1_APPN01000080"
## [181]	"blaZ_8_HE993884"	"cepA-29_1_U05884"
## [183]	"mecC_3_KR732654"	"catA1_1_V00622"
## [185]	"msr(D)_2_AF274302"	"blaTEM-92_1_AF143804"
## [187]	"tetA(P)_1_AB054980"	"erm(D)_3_M77505"
## [189]	"aadA17_1_FJ460181"	"vat(E)_5_AJ488494"
## [191]	"blaTEM-72_1_AF157553"	"sul2_19_AJ319822"
## [193]	"tet(A)_4_AJ517790"	"dfrA29_1_AM237806"
## [195]	"lnu(P)_1_FJ589781"	"oqx_B_1_EU370913"
## [197]	"aph(3)-IIIa_2_AJ490186"	"blaSHV-5_1_X55640"
## [199]	"dfrA12_3_KJ568502"	"sul2_9_FJ197818"
## [201]	"aadA21_1_AY171244"	"tet(M)_7_FN433596"
## [203]	"blaOXA-490_1_KU721147"	"sul2_13_AJ289135"
## [205]	"aac(3)-Ia_1_X15852"	"tet(M)_2_X90939"
## [207]	"tet(M)_10_EU182585"	"blaOXA-489_1_CP013733"
## [209]	"erm(B)_20_AF109075"	"blaSHV-61_1_AJ866284"
## [211]	"vanXmurFvanKWI_2_AP008230"	"dfrA12_2_KJ546436"
## [213]	"dfrA17_11_JN645876"	"aadA7_1_AF224733"
## [215]	"cat(pC233)_1_AY355285"	"blaTEM-47_1_Y10279"
## [217]	"blaTEM-138_1_AY853593"	"tet(O)_3_Y07780"
## [219]	"VanHDX_1_AF130997"	"dfrA1_15_FM207631"
## [221]	"VanHDX_3_AF175293"	"erm(F)_4_M62487"
## [223]	"blaTEM-6_1_X57972"	"erm(36)_1_AF462611"
## [225]	"aadA2_1_NC_010870"	"sul2_10_AM183225"
## [227]	"blaTEM-54_1_AF104442"	"mdf(A)_1_Y08743"
## [229]	"erm(F)_3_M17808"	"tet(M)_6_M21136"
## [231]	"blaZ_5_AJ302698"	"tet(W/32/O)_2_AM710602"

## [233]	"blaSHV-160_1_JX121127"	"sul1_5_EU780013"
## [235]	"blaOXA-323_1_KF203097"	"blaTEM-36_1_KY305958"
## [237]	"tet(O/W)_5_AM889122"	"tet(Q)_4_Z21523"
## [239]	"blaTEM-107_1_AY101764"	"aph(3)-Ia_2_EU287476"
## [241]	"erm(F)_1_M14730"	"qnrB19_1_EU432277"
## [243]	"dfrA17_2_JN645879"	"blaTEM-85_1_AJ277414"
## [245]	"mecA_8_NC_007168"	"tet(A)_2_X00006"
## [247]	"cfxA4_1_AY769933"	"ant(6)-Ib_1_FN594949"
## [249]	"lsa(A)_1_AY225127"	"aph(3)-Ia_4_AF498082"
## [251]	"blaIND-3_1_AF219131"	"tet(S/M)_1_HM367711"
## [253]	"tet(W)_1_DQ060146"	"fexB_1_JN201336"
## [255]	"erm(C)_1_V01278"	"dfrA1_5_EU089668"
## [257]	"dfrA15_2_AF221900"	"aac(3)-VIa_2_NC_009838"
## [259]	"aph(2)-If_1_KF652097"	"aac(6)-Iid_1_AJ584700"
## [261]	"blaOXA-437_1_KP410856"	"dfrA1_10_AF203818"
## [263]	"tet(X)_2_M37699"	"blaZ_21_JGQH01000014"
## [265]	"qnrB47_1_JQ349155"	"mecC_1_FR821779"
## [267]	"aph(2)-Id_1_AF016483"	"blaOXA-348_1_KF297577"
## [269]	"blaCTX-M-23_1_AF488377"	"dfrA14_4_AF393510"
## [271]	"blaOXA-331_1_KF203105"	"blaTEM-2_1_X54606"
## [273]	"sul1_20_JF262165"	"cfiA1_1_AB087225"
## [275]	"dfrA17_4_JQ837988"	"tet(O/32/O)_1_JQ740052"
## [277]	"dfrA1_4_AB188271"	"blaZ_39_JHTU01000073"
## [279]	"blaTEM-8_1_X65252"	"VanHAX_PA_1_DQ018711"
## [281]	"tet(O/W/O)-3_1_EF065524"	"dfrD_1_Z50141"
## [283]	"VanH_bc_1_Y15705"	"dfrK_1_FN377602"
## [285]	"mecA_3_Y13095"	"blaIND-14_1_HM367709"
## [287]	"tetB(P)_1_NC_010937"	"sul1_15_EF667294"
## [289]	"blaTEM-55_1_DQ286729"	"VanHDX_2_EU999036"
## [291]	"blaOXA-228_1_JQ422053"	"blaOXA-452_1_KR061505"
## [293]	"cfiA9_1_AB087234"	"qnrS7_1_KF730651"
## [295]	"blaTEM-12_1_M88143"	"tet(A)_1_AJ313332"
## [297]	"tet(L)_3_M11036"	"aadA8_1_AF326210"
## [299]	"erm(X)_4_NC_005206"	"tet(M)_12_FR671418"
## [301]	"blaTEM-99_1_AF397066"	"VanC4XY_1_EU151752"
## [303]	"aadA24_1_DQ677333"	"sul2_14_AJ514834"
## [305]	"blaTEM-124_1_AY327540"	"dfrA16_3_AY878718"
## [307]	"blaCTX-M-54_1_DQ303459"	"tet(B)_3_AJ277653"
## [309]	"blaTEM-123_1_AY327539"	"blaTEM-106_1_AY101578"
## [311]	"tet(L)_4_D00006"	"floR_2_AF118107"
## [313]	"blaTEM-157_1_DQ909059"	"tet(W)_5_AJ427422"
## [315]	"erm(B)_26_AF080450"	"sul2_7_HM486907"
## [317]	"sul1_14_AJ517791"	"mef(B)_1_FJ196385"
## [319]	"tet(J)_1_ACLE01000065"	"qnrS1_1_AB187515"
## [321]	"erm(B)_9_AF299292"	"dfrA17_9_FJ807902"
## [323]	"ant(6)-Ia_3_KF864551"	"cfiA10_1_AB087227"
## [325]	"tet(S)_2_L09756"	"dfrA14_5_DQ388123"
## [327]	"aph(3)-Ia_1_V00359"	"tet(S/M)_2_AY534326"
## [329]	"VanC1XY_1_AF162694"	"erm(B)_7_AF368302"
## [331]	"blaTEM-34_1_KC292503"	"ant(6)-Ia_5_AB247327"
## [333]	"cfr(B)_3_KR610408"	"tet(Q)_3_U73497"
## [335]	"blaSHV-18_1_AF132290"	"blaOXA-24_1_AJ239129"
## [337]	"mph(A)_1_D16251"	"blaTEM-3_1_X64523"
## [339]	"qnrS3_1_EU077611"	"erm(X)_3_U21300"

## [341]	"aph(2)-Ia_2_AP009486"	"rmtE_1_GU201947"
## [343]	"blaOXA-470_1_KR182163"	"blaTEM-122_1_AY307100"
## [345]	"tet(W/32/0)_1_AM710601"	"blaTEM-159_1_EF136376"
## [347]	"dfrA12_6_AY551331"	"blaTEM-135_1_GQ896333"
## [349]	"blaZ_11_AJ400722"	"erm(X)_2_X51472"
## [351]	"blaSHV-28_1_AF299299"	"tet(O/32/0)_5_FP929050"
## [353]	"ARR-3_4_FM207631"	"sul3_2_AJ459418"
## [355]	"tet(M)_9_X56353"	"tet(O/W/O)-1_1_AY196921"
## [357]	"erm(T)_4_AJ488494"	"dfrA6_1_Z86002"
## [359]	"blaSHV-13_1_AF164577"	"sul1_10_DQ143913"
## [361]	"blaOXA-333_1_KF203107"	"blaCTX-M-61_1_EF219142"
## [363]	"aph(3)-Ib_5_AF321551"	"aph(6)-Id_1_M28829"
## [365]	"dfrG_1_AB205645"	"mecA_2_NC_002951"
## [367]	"aph(2)-Ib_2_AF207840"	"lnu(F)_2_DQ836009"
## [369]	"sul2_12_AF497970"	"tet(Y)_1_EF495198"
## [371]	"tet(44)_2_FN594949"	"dfrA14_3_Z50804"
## [373]	"aadA3_1_AF047479"	"aadA8b_2_AM040708"
## [375]	"ant(6)-Ia_1_AF330699"	"erm(C)_13_M13761"
## [377]	"aac(2)-IIa_1_AB669090"	"blaOXA-512_1_KU726870"
## [379]	"erm(B)_23_X72021"	"vat(E)_9_AY043210"
## [381]	"str_1_X92946"	"blaTEM-113_1_AY589494"
## [383]	"blaOXA-464_1_KU721146"	"blaTEM-16_1_X65254"
## [385]	"qnrB36_1_JN173058"	"erm(B)_12_U18931"
## [387]	"blaTEM-93_1_AJ318093"	"aph(2)-Ia_3_AJ536195"
## [389]	"cfr(C)_2_CANB01000378"	"blaTEM-33_1_GU371926"
## [391]	"tet(O/W/32/0)_2_FM164392"	"aac(3)-VIa_1_M88012"
## [393]	"blaOXA-9_1_KQ089875"	"sul1_7_FJ715937"
## [395]	"dfrA16_4_EU158182"	"blaSHV-16_1_AF072684"
## [397]	"tet(L)_7_X60828"	"blaTEM-186_1_JN227084"
## [399]	"blaTEM-29_1_DQ269440"	"aph(2)-Ib_1_AF337947"
## [401]	"aph(6)-Id_5_18676889"	"mef(A)_2_U83667"
## [403]	"blaTEM-94_1_AJ318094"	"aac(6)-Im_1_AF337947"
## [405]	"blaOXA-460_1_KR061508"	"blaCTX-M-116_1_JF966749"
## [407]	"blaCTX-M-158_1_KM211691"	"blaOXA-25_1_AF201826"
## [409]	"erm(D)_2_L08389"	"dfrA12_7_AB196348"
## [411]	"blaTEM-22_1_Y17583"	"cfr(B)_2_KM359438"
## [413]	"blaCTX-M-58_1_EF210159"	"blaOXA-451_1_KR061504"
## [415]	"mecA_6_BX571856"	"dfrA15_1_AF156486"
## [417]	"dfrA16_2_AF174129"	"tet(M)_8_X04388"
## [419]	"erm(35)_1_AF319779"	"vat(E)_7_AY043211"
## [421]	"blaTEM-76_1_AF190694"	"blaTEM-95_1_AJ308558"
## [423]	"cat_2_M35190"	"blaOXA-471_1_EU086833"
## [425]	"fosD_1_KC989517"	"tet(A)_6_AF534183"
## [427]	"vat(E)_8_AY043209"	"cfiA6_1_AB087231"
## [429]	"aac(6)-Ian_1_AP014611"	"aph(4)-Ia_1_V01499"
## [431]	"blaSHV-15_1_AJ011428"	"lsa(A)_3_AY737526"
## [433]	"aadA8b_1_AY139603"	"tet(A)_3_AY196695"
## [435]	"dfrA1_17_FJ489928"	"blaSHV-55_1_DQ054528"
## [437]	"blaTEM-1B_1_AY458016"	"blaTEM-52_1_Y13612"
## [439]	"ant(3)-Ia_1_X02340"	"VanGXY_1_AY271782"
## [441]	"blaOXA-491_1_KU721148"	"cat(pC194)_1_NC_002013"
## [443]	"blaSHV-29_1_AF301532"	"blaTEM-1D_1_AF188200"
## [445]	"blaOXA-229_1_JQ422052"	"tet(M)_11_JN846696"
## [447]	"cfxA2_1_AF504914"	"dfrA5_2_FJ001870"

## [449]	"blaSHV-129_1_GU827715"	"blaTEM-20_1_EU527189"
## [451]	"tet(O/W)-1_1_AY485126"	"blaCTX-M-42_1_DQ061159"
## [453]	"fmr0_1_Q08325"	"aadA1_3_JQ414041"
## [455]	"aph(6)-Id_3_AB109805"	"aadA24_1_AM711129"
## [457]	"blaSHV-24_1_AB023477"	"aph(2)-Ie_1_AY743255"
## [459]	"blaOXA-448_1_KR061497"	"tet(B)_1_AP000342"
## [461]	"blaTEM-181_1_KM977568"	"erm(C)_9_Y09001"
## [463]	"cmlA1_1_M64556"	"tet(M)_5_U58985"
## [465]	"lnu(B)_2_JQ861959"	"tet(S)_1_DQ377340"
## [467]	"lnu(G)_1_KX470419"	"VanHBX_2_U35369"
## [469]	"str_2_FN435330"	"tet(33)_1_AY255627"
## [471]	"mecA1_3_Y13094"	"blaCTX-M-138_1_KF526119"
## [473]	"tet(O/32/O)_4_AIOQ01000025"	"blaTEM-112_1_AY589493"
## [475]	"blaCTX-M-166_1_KU978909"	"erm(C)_3_M17990"
## [477]	"blaTEM-164_1_EU274580"	"tet(Q)_2_X58717"
## [479]	"aadA22_1_AM261837"	"aadA12_1_AY665771"
## [481]	"aph(6)-Id_2_AF024602"	"erm(43)_1_HE650138"
## [483]	"blaSHV-31_1_AY277255"	"cfiA14_1_FM200789"
## [485]	"dfrA12_10_FM877486"	"tet(K)_2_J01764"
## [487]	"sul2_6_FN995456"	"aac(6)-Iak_1_AB894482"
## [489]	"tet(O/32/O)_3_NZ_AUJS01000017"	"tet(Q)_1_L33696"
## [491]	"aadA1_4_JQ480156"	"vat(E)_11_AY043213"
## [493]	"erm(C)_14_M12730"	"tet(O/32/O)_2_AJ295238"
## [495]	"aac(6)-Iaj_1_AB709942"	"cmlA1_2_AB212941"
## [497]	"blaTEM-86_1_AJ277415"	"tet(L)_2_M29725"
## [499]	"blaOXA-418_1_KJ997966"	"blaZ_10_FR823292"
## [501]	"tet(40)_2_AM419751"	"sul1_9_AY963803"
## [503]	"cepA_1_L13472"	"tet(O)_2_M20925"
## [505]	"aadD_2_M19465"	"aph(3)-Ib_3_AF321550"
## [507]	"tet(A)_5_AJ419171"	"blaCTX-M-29_1_AY267213"
## [509]	"blaOXA-499_1_KT964029"	"aph(3)-IIIA_3_AB247327"
## [511]	"mph(C)_1_AB013298"	"blaOXA-493_1_KU739135"
## [513]	"aac(3)-Ib_1_L06157"	"mph(A)_2_U36578"
## [515]	"blaZ_7_AP004832"	"dfrA12_5_FR875302"
## [517]	"VanC2XY_3_EU151757"	"blaTEM-162_1_EF468463"
## [519]	"erm(B)_11_M19270"	"tet(33)_2_DQ390458"
## [521]	"tet(O/W/32/O)_5_JQ740053"	"tet(X)_1_GU014535"
## [523]	"erm(B)_22_X52632"	"sul2_2_AY034138"
## [525]	"mecA_4_AB546266"	"aac(6)-Ii_1_L12710"
## [527]	"blaSHV-14_1_AF226622"	"tet(32)_1_EU722333"
## [529]	"erm(G)_1_M15332"	"dfrA1_16_FJ001872"
## [531]	"blaSHV-25_1_AF208796"	"qnrB82_1_KX372672"
## [533]	"blaZ_4_X04121"	"sul1_11_DQ914960"
## [535]	"tet(O/W/32/O)_1_EF065523"	"blaTEM-30_1_AJ437107"
## [537]	"dfrA1_1_FJ591049"	"tet(L)_8_AY081910"
## [539]	"VanHBX_1_AF192329"	"blaZ_3_CP000732"
## [541]	"aadA4_1_Z50802"	"blaCTX-M-1_1_DQ915955"
## [543]	"blaSHV-8_1_U92041"	"blaTEM-15_1_AM849805"
## [545]	"blaSHV-60_1_AB302939"	"blaCTX-M-32_2_AJ557142"
## [547]	"catB_1_M93113"	"blaOXA-61_1_AY587956"
## [549]	"erm(B)_15_U48430"	"blaOXA-397_1_KM087865"
## [551]	"dfrA17_10_AM937244"	"aadA6_1_AF140629"
## [553]	"blaTEM-1A_1_HM749966"	"vat(D)_1_L12033"
## [555]	"blaTEM-24_1_GQ293500"	"blaSHV-45_1_AF547625"

## [557]	"aph(3)-Ib_1_M28829"	"cfxA_1_U38243"
## [559]	"blaOXA-96_1_DQ519090"	"blaOXA-450_1_KR061502"
## [561]	"blaSHV-40_1_AF535128"	"tet(O/W/32/O/W/O)_1_DQ525023"
## [563]	"msr(C)_1_AY004350"	"lnu(F)_1_EU118119"
## [565]	"mecA_5_AB546267"	"blaCTX-M-175_1_KT997887"
## [567]	"dfrA15_3_DQ647028"	"dfrA1_13_DQ018382"
## [569]	"erm(C)_16_AF019140"	"blaSHV-7_1_U20270"
## [571]	"erm(X)_1_M36726"	"blaIND-5_1_AY504627"
## [573]	"erm(G)_2_L42817"	"msr(D)_3_AF227520"
## [575]	"erm(B)_21_U35228"	"ant(6)-Ia_2_KF421157"
## [577]	"blaTEM-52C_2_EF141186"	"dfrA1_11_AJ419168"
## [579]	"dfrA12_9_DQ995286"	"aph(3)-IIa_1_AF330699"
## [581]	"blaTEM-2_2_AJ251946"	"aadA1_2_FJ591054"
## [583]	"blaZ_38_NZ_JEMM01000040"	"tet(O/W)_2_AM889119"
## [585]	"mecC2_1_KF955540"	"blaOXA-420_1_AB983359"
## [587]	"floR_1_AF071555"	"blaZ_2_CP000731"
## [589]	"mef(A)_1_AJ971089"	"blaSHV-46_1_AY210887"
## [591]	"blaOXA-26_1_AF201827"	"sul2_21_AB366440"
## [593]	"blaTEM-116_1_AY425988"	"aph(3)-IIa_1_X57709"
## [595]	"ant(9)-Ia_1_X02588"	"blaTEM-82_1_AF427128"
## [597]	"aph(2)-If_2_AY701528"	"aph(3)-IIa_2_V00618"
## [599]	"VanLXY_1_EU250284"	"dfrA1_14_AB199789"
## [601]	"cepA-49_1_U05886"	"aadD_1_AF181950"
## [603]	"aph(3)-VIIa_1_M29953"	"blaSHV-66_1_DQ174306"
## [605]	"blaSHV-128_1_GU932590"	"tet(W)_4_FN396364"
## [607]	"blaTEM-21_1_Y17582"	"blaOXA-472_1_KR182165"
## [609]	"blaTEM-111_1_AF468003"	"cat_3_S48276"
## [611]	"blaTEM-96_1_AY092401"	"blaSHV-134_1_HM559945"
## [613]	"npmA_1_AB261016"	"erm(B)_10_U86375"
## [615]	"blaSHV-35_1_AY070258"	"blaTEM-43_1_U95363"
## [617]	"blaACT-9_1_HQ693810"	"qnrB5_1_DQ303919"
## [619]	"tet(M)_1_X92947"	"blaTEM-87_1_AF250872"
## [621]	"blaOXA-518_1_KU739134"	"cfiA8_1_AB087233"
## [623]	"dfrA17_3_JQ414038"	"erm(33)_1_AJ579365"
## [625]	"aadA23_1_AJ809407"	"aph(3)-III_1_M26832"
## [627]	"sul1_38_BX248359"	"blaZ_68_NZ_CUHK01000055"
## [629]	"dfrA14_2_Z50805"	"cepA_6_FR688022"
## [631]	"blaTEM-52B_1_AF027199"	"dfrA1_3_GU726913"
## [633]	"blaTEM-84_1_AF427130"	"blaTEM-28_1_U37195"
## [635]	"tet(W)_3_AJ427421"	"catS_1_X74948"
## [637]	"blaOXA-257_1_KC567681"	"mef(A)_4_HG423652"
## [639]	"blaZ_34_JJA001000008"	"aadA7_2_AB114632"
## [641]	"tet(S)_3_X92946"	"dfrA17_8_AM932673"
## [643]	"tet(O/W)_4_AM889121"	"aadA15_1_DQ393783"
## [645]	"blaTEM-198_1_AB700703"	"aph(6)-Ic_1_X01702"
## [647]	"aph(6)-Id_4_CP000971"	"erm(C)_2_M19652"
## [649]	"mef(A)_3_AF227520"	"aph(3)-Ia_8_Y00452"
## [651]	"dfrA1_2_HM055363"	"cfxA3_1_AF472622"
## [653]	"erm(C)_10_Y09002"	"qnrS9_1_KF732714"
## [655]	"mecA_1_NC_002745"	"dfrA17_5_GU358475"
## [657]	"blaTEM-10_1_AF093512"	"VanHAX_PT_1_DQ018710"
## [659]	"qnrB81_1_KX372671"	"aadA1b_1_M95287"
## [661]	"dfrA1_7_AJ400733"	"qnrS4_1_FJ418153"

```
# Stable "Farm2R1S1" has the five lowest sampling depths of the dataset, some other samples are also v
Rps %>% ps_filter(FarmRoundStable == c("Farm2R1S1")) %>% sample_sums() %>% sort()
```

```
##      4_55      4_57      4_36      4_39      4_37      5_55      4_56      4_54
## 1365.915 1746.186 2162.499 2332.954 2466.228 3066.586 4001.996 5129.624
##      5_54      4_40      4_38      4_41
## 5581.891 5838.197 6956.992 7558.754
```

```
# For metaphlan data, Stable "Farm2R1S1 has 11/12 lowest sampling depths of the dataset, and there are
Rps_mp %>% ps_filter(FarmRoundStable == c("Farm2R1S1")) %>% sample_sums() %>% sort()
```

```
##      4_57      4_55      4_36      4_39      4_56      4_37      4_54      5_55
## 125.4769 190.1299 198.9479 211.1739 305.2175 345.8083 394.6092 495.9399
##      5_54      4_40      4_38      4_41
## 521.6233 572.6645 635.2437 822.9736
```

```
# factorizing variables as not to create problems with visualisation later down the line
sample_data(Rps)$Cluster = as.factor(sample_data(Rps)$Cluster)
sample_data(Rps)$FlockSize = as.factor(sample_data(Rps)$FlockSize)
sample_data(Rps)$AgeParentStock = as.factor(sample_data(Rps)$AgeParentStock)
sample_data(Rps)$Age = as.factor(sample_data(Rps)$Age)
sample_data(Rps)$LibraryNumber = as.factor(sample_data(Rps)$LibraryNumber)
```

```
# repeat for MP
```

```
sample_data(Rps_mp)$Cluster = as.factor(sample_data(Rps_mp)$Cluster)
sample_data(Rps_mp)$FlockSize = as.factor(sample_data(Rps_mp)$FlockSize)
sample_data(Rps_mp)$AgeParentStock = as.factor(sample_data(Rps_mp)$AgeParentStock)
sample_data(Rps_mp)$Age = as.factor(sample_data(Rps_mp)$Age)
sample_data(Rps_mp)$LibraryNumber = as.factor(sample_data(Rps_mp)$LibraryNumber)
```

```
# repeat for TPM
```

```
sample_data(Rps_tpm)$Cluster = as.factor(sample_data(Rps_tpm)$Cluster)
sample_data(Rps_tpm)$FlockSize = as.factor(sample_data(Rps_tpm)$FlockSize)
sample_data(Rps_tpm)$AgeParentStock = as.factor(sample_data(Rps_tpm)$AgeParentStock)
sample_data(Rps_tpm)$Age = as.factor(sample_data(Rps_tpm)$Age)
sample_data(Rps_tpm)$LibraryNumber = as.factor(sample_data(Rps_tpm)$LibraryNumber)
```

```
# add stable column with shorter names
```

```
sample_data(Rps)$FarmRoundStable = as.factor(sample_data(Rps)$FarmRoundStable)
Rps@sam_data$Stables = revalue(sample_data(Rps)$FarmRoundStable, c("Farm1R1S1"="Stable1", "Farm1R1S2"="F",
                                                                    "Farm2R2S1"="Stable5", "F",
                                                                    "Farm4R1S1"="Stable9", "F"))
```

```
# Shortening agent names
```

```
Rps@sam_data$Cox[Rps@sam_data$Cox == "narsinandnicarbazin(maxiban)"] = "Maxiban"
Rps@sam_data$Cox[Rps@sam_data$Cox == "narsin(monteban)"] = "Monteban"
Rps@sam_data$Cox[Rps@sam_data$Cox == "salinomycin(Sacox120microGranulate)"] = "Sacox"
```

```
# repeat
```

```
sample_data(Rps_mp)$FarmRoundStable = as.factor(sample_data(Rps_mp)$FarmRoundStable)
Rps_mp@sam_data$Stables = revalue(sample_data(Rps_mp)$FarmRoundStable, c("Farm1R1S1"="Stable1", "Farm1R",
                                                                    "Farm2R2S1"="Stable5", "F",
                                                                    "Farm4R1S1"="Stable9", "F"))
```

```

Rps_mp@sam_data$Cox[Rps_mp@sam_data$Cox == "narasinandnicarbazin(maxiban)"] = "Maxiban"
Rps_mp@sam_data$Cox[Rps_mp@sam_data$Cox == "narasin(monteban)"] = "Monteban"
Rps_mp@sam_data$Cox[Rps_mp@sam_data$Cox == "salinomycin(Sacox120microGranulate)"] = "Sacox"

sample_data(Rps_tpm)$FarmRoundStable = as.factor(sample_data(Rps_tpm)$FarmRoundStable)
Rps_tpm@sam_data$Stables = revalue(sample_data(Rps_tpm)$FarmRoundStable, c("Farm1R1S1"="Stable1", "Farm1R2S1"="Stable2", "Farm2R1S1"="Stable3", "Farm2R2S1"="Stable5", "Farm3R1S1"="Stable6", "Farm3R2S1"="Stable7", "Farm4R1S1"="Stable9", "Farm4R2S1"="Stable10"))

Rps_tpm@sam_data$Cox[Rps_tpm@sam_data$Cox == "narasinandnicarbazin(maxiban)"] = "Maxiban"
Rps_tpm@sam_data$Cox[Rps_tpm@sam_data$Cox == "narasin(monteban)"] = "Monteban"
Rps_tpm@sam_data$Cox[Rps_tpm@sam_data$Cox == "salinomycin(Sacox120microGranulate)"] = "Sacox"

# In order to create taxa prevalence plots, and many more functions, we need to change our "taxa" level.
# We'll make a copy to fulfill these purposes
# Phylum = AMR_class_primary, Class = AMR_class_secondary, Order = ARGCluster90, Family = ID_Clust_Refs
Rps_copy = Rps
colnames(Rps_copy@tax_table) = c("Phylum", "Class", "Order", "Family")
Rps_mp_copy = Rps_mp
colnames(Rps_mp_copy@tax_table) = c("Phylum", "Class", "Order", "Family")

```

Procrustes plots

```

#Procrustes analyses
copy16S = subset16S
# get the samples in the same order
sample_names(copy16S) = sample_names(subsetMG)

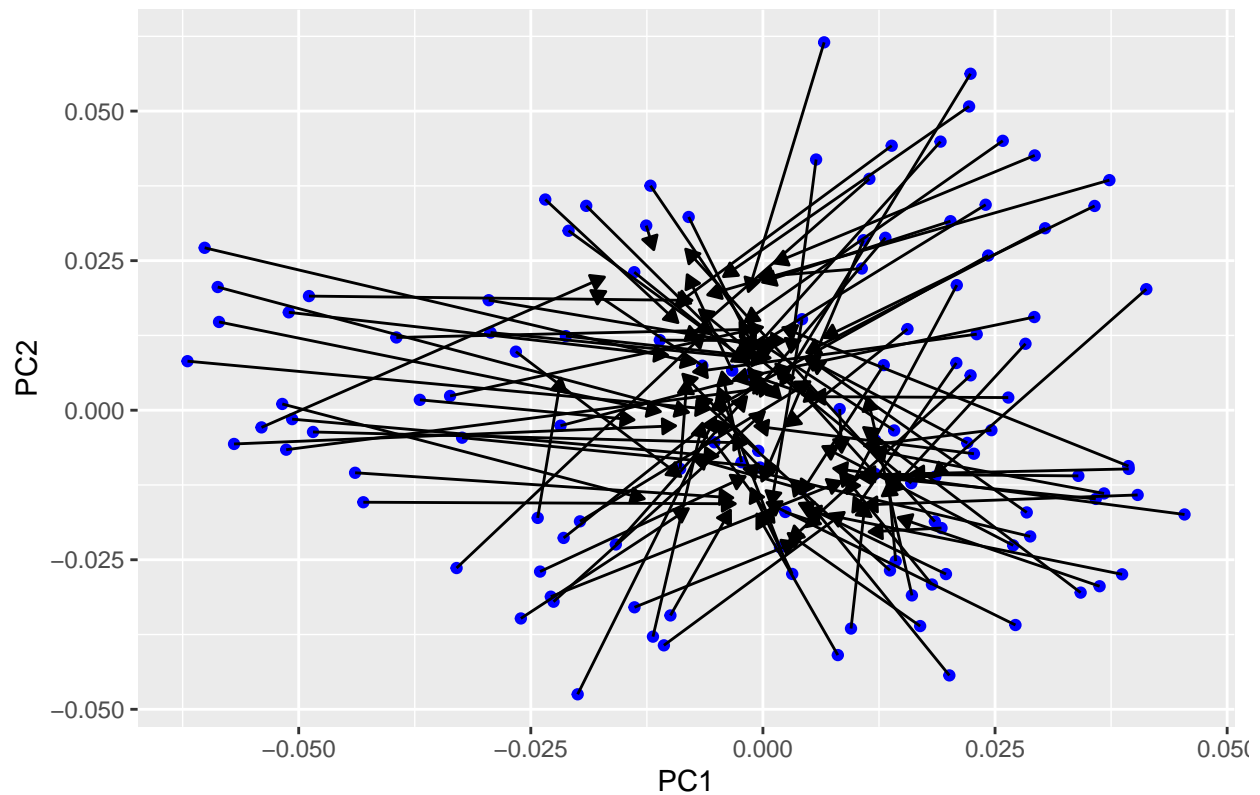
PCoA_BC_16s = ordinate(copy16S, "PCoA")
PCoA_BC_MG = ordinate(subsetMG, "PCoA")
procrustes = protest(PCoA_BC_16s$vectors, PCoA_BC_MG$vectors)

plot_data <- data.frame(
  MT_PC1 = procrustes$X[, 1],
  MT_PC2 = procrustes$X[, 2],
  MG_PC1 = procrustes$Yrot[, 1],
  MG_PC2 = procrustes$Yrot[, 2])

# with arrows pointing from MG to 16S
ggplot(plot_data) +
  geom_point(aes(x=MT_PC1, y=MT_PC2), color = "blue") +
  geom_segment(aes(x=MT_PC1, y=MT_PC2, xend=MG_PC1, yend=MG_PC2), arrow=arrow(type = "closed", length=unit(1.5)),
  labs(title = "Procrustes Plot metagenomic vs. metataxonomic", x = "PC1", y = "PC2") +
  scale_color_manual(values = c("16S" = "black", "MG" = "blue"))

```

Procrustes Plot metagenomic vs. metataxonomic



```
# plot with both points
#ggplot(plot_data) +
#  geom_point(aes(x=MT_PC1, y=MT_PC2), color = "green") +
#  geom_point(aes(x=MG_PC1, y=MG_PC2), color = "blue") +
#  geom_segment(aes(x = MT_PC1, xend = MG_PC1, y = MT_PC2, yend = MG_PC2), linetype = "solid") +
#  labs(title = "Procrustes Plot Metataxonomic vs metagenomic")

# Resistome vs MG
copyRps = Rps
# get the samples in the same order
sample_names(copyRps) = sample_names(subsetMG)

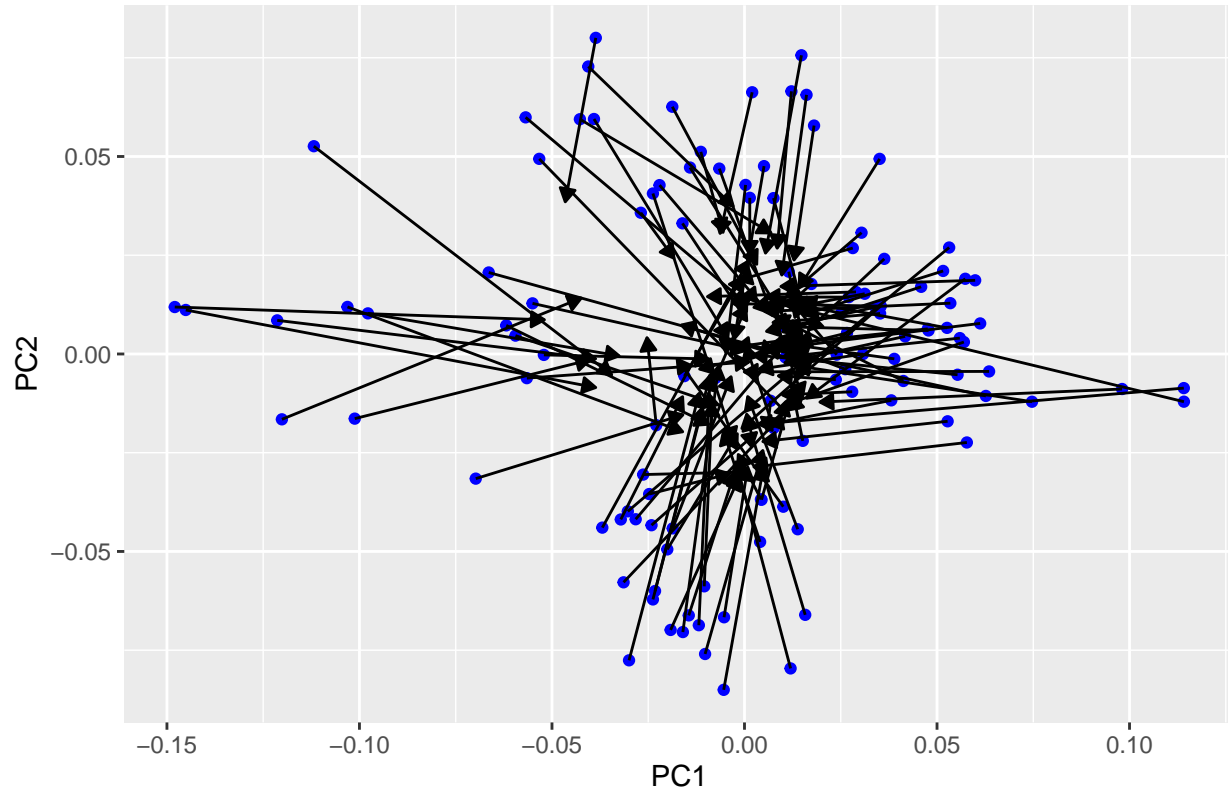
PCoA_BC_Rps = ordinate(copyRps, "PCoA")
PCoA_BC_MG = ordinate(subsetMG, "PCoA")
procrustes = protest(PCoA_BC_Rps$vectors, PCoA_BC_MG$vectors)

plot_data <- data.frame(
  R_PC1 = procrustes$X[, 1],
  R_PC2 = procrustes$X[, 2],
  MG_PC1 = procrustes$Yrot[, 1],
  MG_PC2 = procrustes$Yrot[, 2])

# resistome (k2) points to MG
ggplot(plot_data) +
  geom_point(aes(x=R_PC1, y=R_PC2), color = "blue") +
  geom_segment(aes(x=R_PC1,y=R_PC2,xend=MG_PC1,yend=MG_PC2),arrow=arrow(type = "closed", length=unit(0.1)))
```

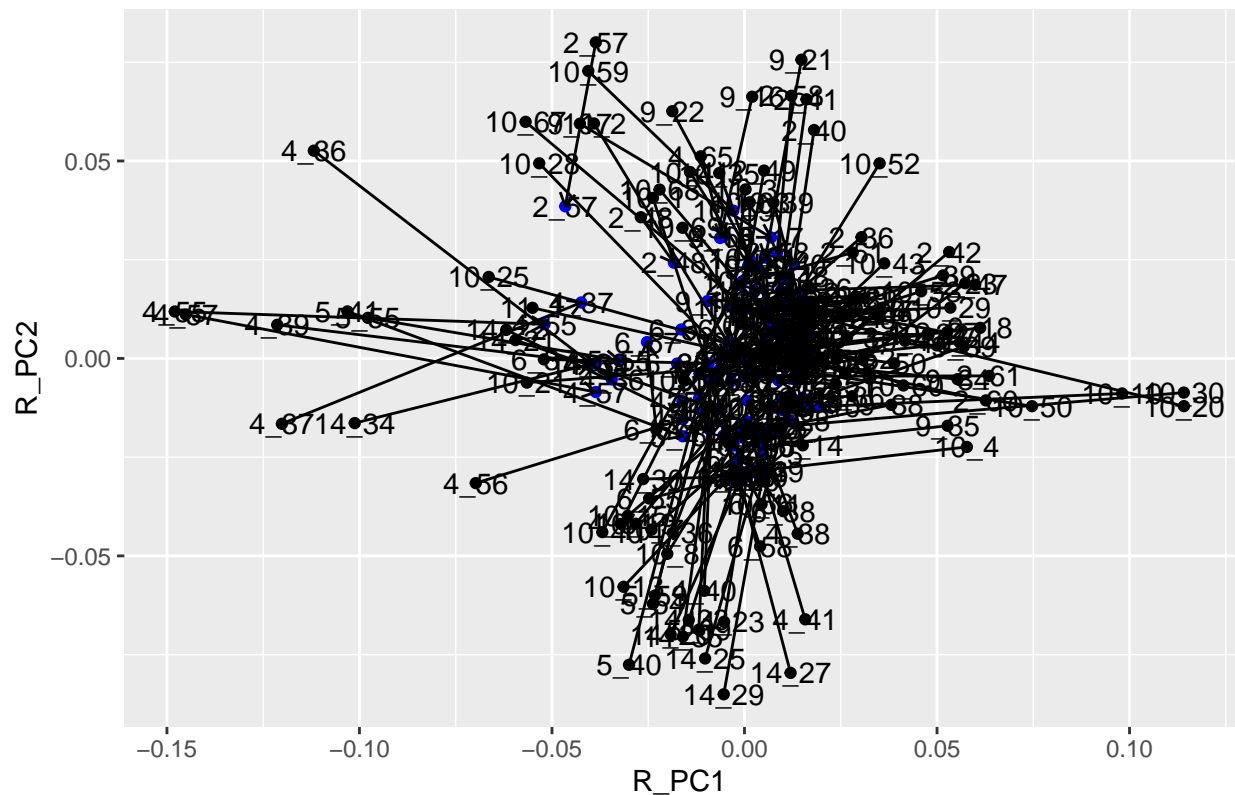
```
scale_color_manual(values = c("16S" = "black", "MG" = "blue")) +
guides(color = guide_legend(title = "Data Type")) +
labs(title = "Procrustes Plot resistomic vs. metagenomic", x = "PC1", y = "PC2")
```

Procrustes Plot resistomic vs. metagenomic



```
# adds labels to see if samples line up
ggplot(plot_data) +
  geom_point(aes(x=R_PC1, y=R_PC2)) +
  geom_point(aes(x=MG_PC1, y=MG_PC2), color = "blue")+
  geom_segment(aes(x=R_PC1,y=R_PC2,xend=MG_PC1,yend=MG_PC2),arrow=arrow(length=unit(0.2,"cm")))+
  geom_text(aes(x = MG_PC1, y = MG_PC2, label = rownames(plot_data))) +
  geom_text(aes(x = R_PC1, y = R_PC2, label = rownames(plot_data))) +
  labs(title = "Procrustes Plot")
```

Procrustes Plot



```
# MP vs k2
PCoA_BC_MP = ordinate(Rps_mp, "PCoA")
PCoA_BC_k2 = ordinate(Rps, "PCoA")
procrustes = protest(PCoA_BC_MP$vectors, PCoA_BC_k2$vectors)

plot_data <- data.frame(
  MP_PC1 = procrustes$X[, 1],
  MP_PC2 = procrustes$X[, 2],
  k2_PC1 = procrustes$Yrot[, 1],
  k2_PC2 = procrustes$Yrot[, 2])

ggplot(plot_data) +
  geom_point(aes(x=MP_PC1, y=MP_PC2), color = "blue") +
  geom_segment(aes(x=MP_PC1, y=MP_PC2, xend=k2_PC1, yend=k2_PC2), arrow=arrow(type = "closed", length=unit(
  labs(title = "Procrustes Plot MetaPhlAn vs Kraken 2", x = "PC1", y = "PC2") +
  scale_color_manual(values = c("16S" = "black", "MG" = "blue"))) +
  guides(color = guide_legend(title = "Data Type"))
```

Procrustes Plot MetaPhlAn vs Kraken 2

