Extending colonic mucosal microbiome analysis - Assessment of colonic lavage as a proxy for endoscopic colonic biopsies

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This document contains all figure production carried out in R for the manuscript.

All data to reproduce analysis can be found here: https://github.com/m-gemmell-uoa/Watt_etal_16sBxCL (https://github.com/m-gemmell-uoa/Watt_etal_16sBxCL)

Figure 1: Species diversity comparison between colonic biopsy and lavage sample

Wilcox test to compare the Alpha diversity values of Biopsy and Lavagae samples

```
data <- read.csv("colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter
.unique.precluster.pick.pick.an.unique_list.0.03.pick.groups.ave.txt", sep="\t", r
ow.names = 1)
Bx <- data[1:23,]
FA <- data[24:46,]
#Mannwhitney u test
data$type<-c(rep("Bx",23), rep("FA",23))
data$type <- as.factor(data$type)
wilcox.test(sobs~type, data=data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  sobs by type
## W = 68, p-value = 4.213e-06
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(chao~type, data=data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  chao by type
## W = 20, p-value = 6.593e-10
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(shannon~type, data=data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  shannon by type
## W = 249, p-value = 0.7441
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(invsimpson~type, data=data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  invsimpson by type
## W = 254, p-value = 0.8278
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(coverage~type, data=data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  coverage by type
## W = 513, p-value = 2.223e-10
## alternative hypothesis: true location shift is not equal to 0
```

Box plots for alpha diversity value comparisons

```
### Load packages or install if not present
if (!require("RColorBrewer")) {
  install.packages("RColorBrewer")
  library(RColorBrewer)}
```

```
## Loading required package: RColorBrewer
```

```
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
```

```
## Loading required package: ggplot2
```

```
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
```

```
## Loading required package: tidyr
```

```
#Files
alpha_file="colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter.uniqu
e.precluster.pick.pick.an.unique_list.0.03.pick.groups.ave.txt"
metadata_file="16sBxCL_Metadata.txt"
#Read in files
alpha_data <- read.csv(alpha_file,sep="\t", row.names = 1)
metadata <- read.csv(metadata_file,sep="\t", row.names = 1)
#Order alpha_data and metadata so they are the same order
alpha_data <- alpha_data[order(row.names(alpha_data)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match
stopifnot(identical(row.names(alpha_data), row.names(metadata)))
#Merge data frames
plot_data_metadata <- merge(x=alpha_data, y=metadata, by="row.names")
#Fix row names after merge
row.names(plot_data_metadata) <- plot_data_metadata[,1]
plot_data_metadata <- plot_data_metadata[,-1]
#Remove unwanted columns
plot_data_metadata <- plot_data_metadata[,c(-3,-4,-6,-7,-9,-10)]
#Change name of alpha diversity values
colnames(plot_data_metadata)[1:5] <- c("Observed OTUs", "Chao","Shannon-Weiner","I
nverse Simpson", "Coverage")
#Convert to long list format
alpha_long <- gather(plot_data_metadata, Alpha_diversity_measure, value, 1:5)
#Reorder measures
alpha_long$Alpha_diversity_measure_f <- factor(alpha_long$Alpha_diversity_measure,
levels=c("Observed OTUs", "Chao","Shannon-Weiner","Inverse Simpson", "Coverage"))
#Set colours
fa_b_colour <- brewer.pal(9, "Set1")
#Box Plot for alpha diversity comparing lavage against biopsy
g <- ggplot(alpha_long, aes(x=new.Sample.type, y=value, fill=new.Sample.type)) + g
eom_boxplot(outlier.colour = NA) + theme_set(theme_gray(base_size = 8)) +facet_wra
p(~ Alpha_diversity_measure_f, nrow=1, scales="free") + geom_point(position = posi
tion_jitter(width = 0.2)) + scale_x_discrete(breaks=NULL, name="") + scale_fill_ma
nual(values=c(fa_b_colour[2], fa_b_colour[1]), name="Sample type") + scale_y_conti
nuous(name="") + theme(legend.position="bottom", legend.margin=unit(0,"cm"), plot.
margin =unit(c(0,0,0,0),"mm"))
ggsave("Fig1.species_diversity_between_colonic_and_lavage_samples.pdf", g, units="
mm", height=120, width=170, dpi=300)
```

```
## [1] TRUE
```

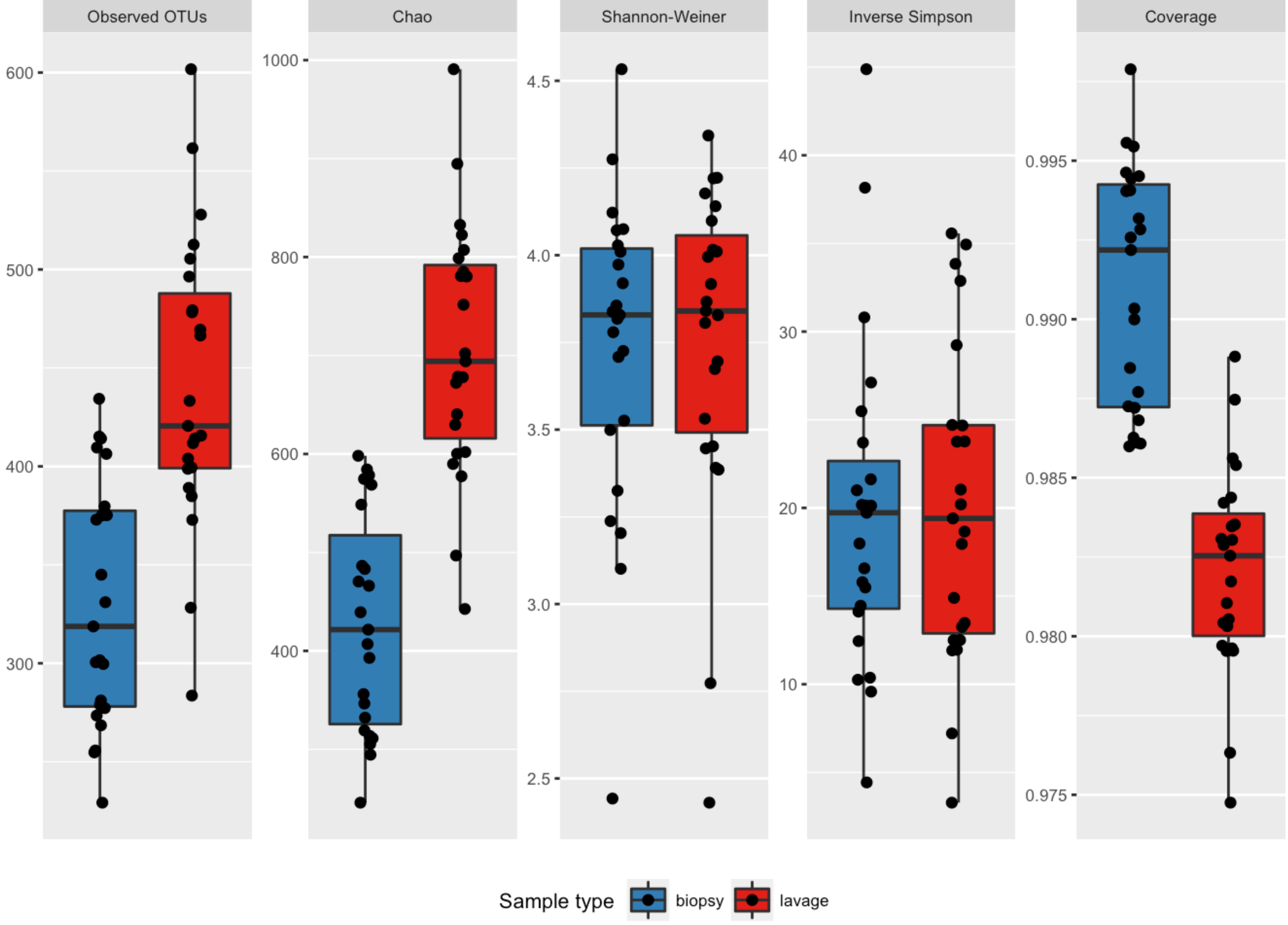
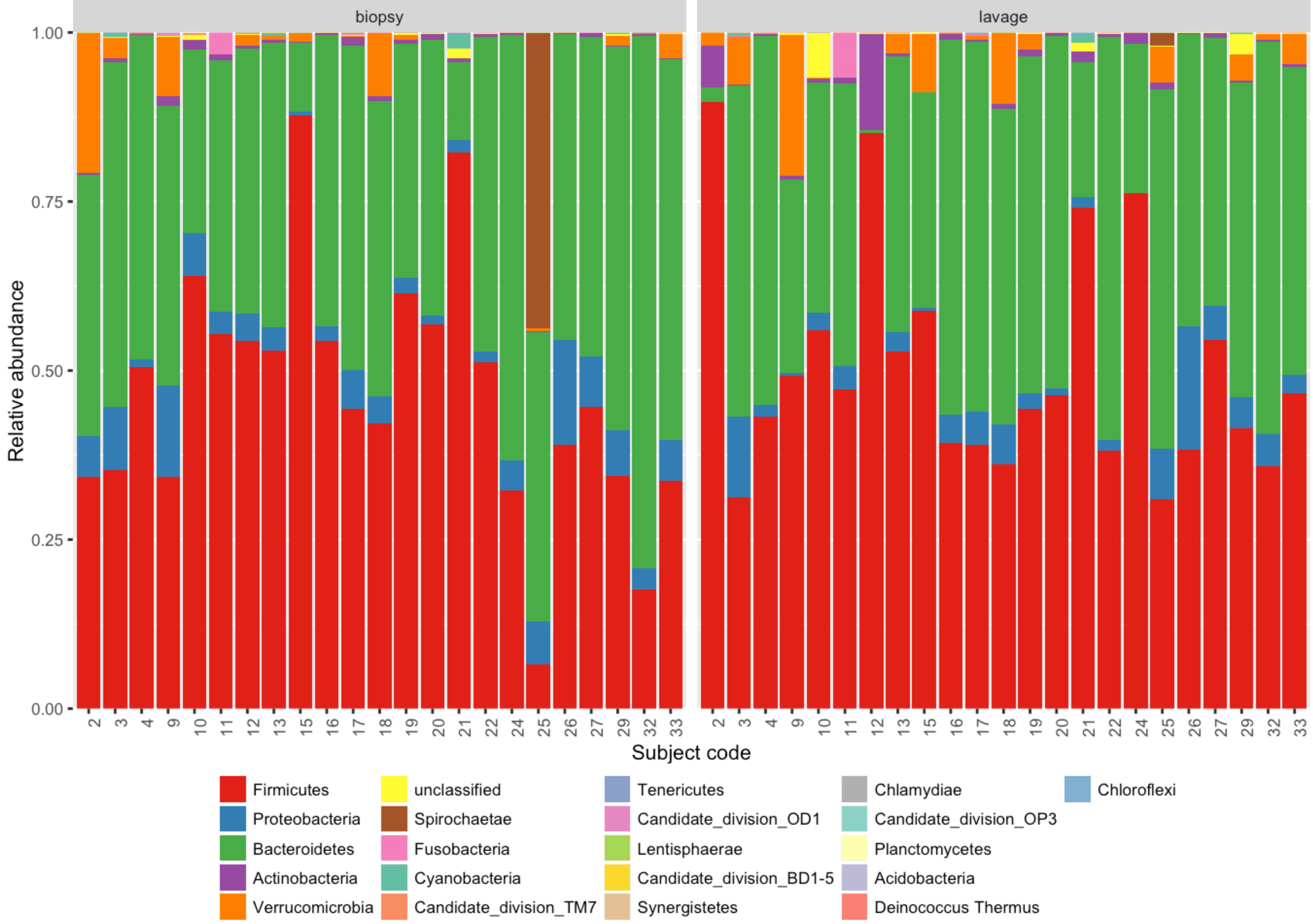


Figure 2: Relative abundance at phylum level for colonic biopsy and lavage samples.

A) Individual subject stacked bar charts

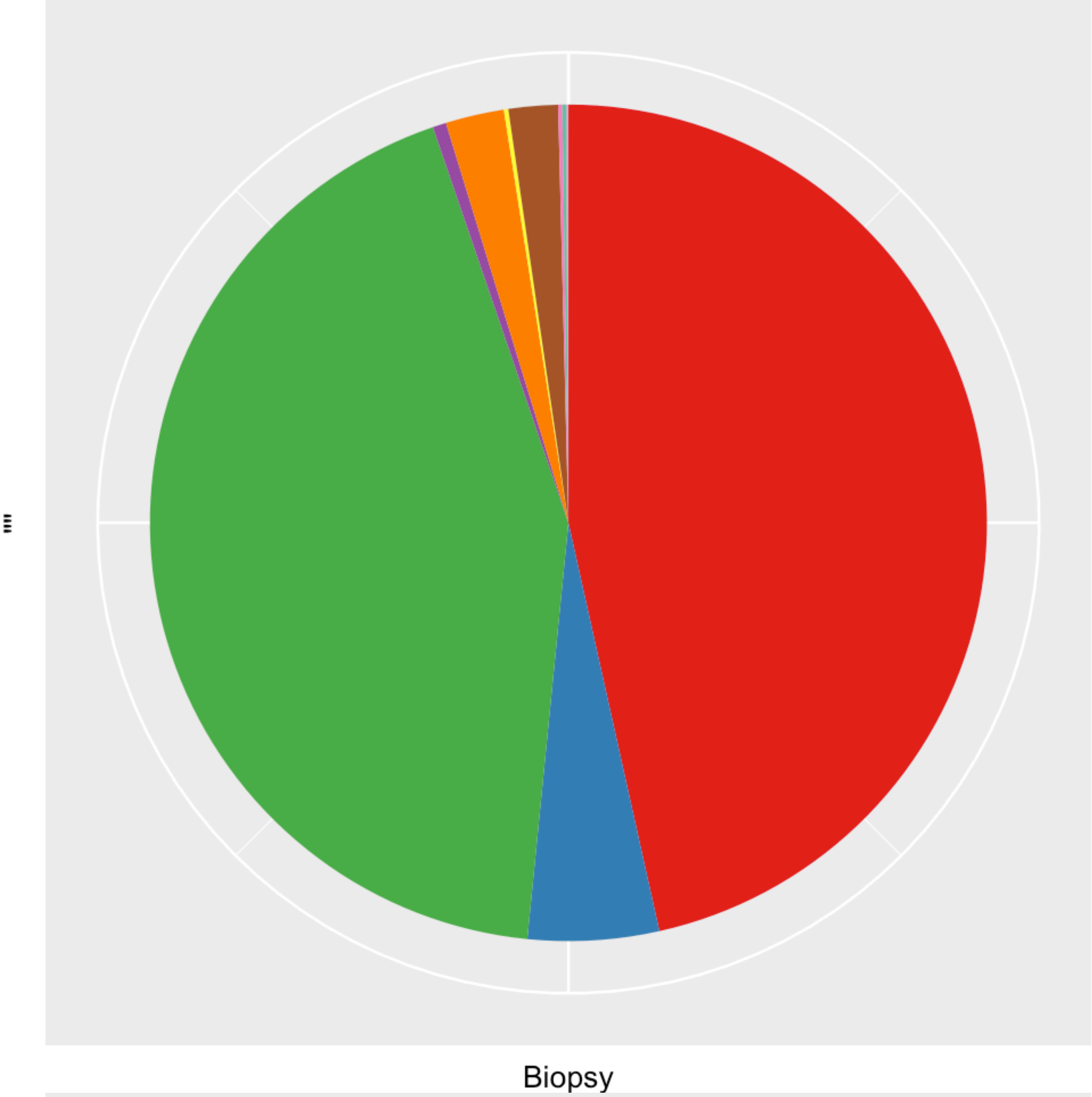
```
### Load packages or install if not present
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
#Read in files
data <- read.csv("16sBxCL_phylum_phylotype_summary.txt", sep = "\t", row.names = 1
)
metadata <- read.csv("16sBxCL_Metadata.txt", sep = "\t", row.names = 1)
#Order data and metadata so they are the same order
data <- data[order(row.names(data)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match
stopifnot(identical(row.names(metadata), row.names(data)))
#Merge data
plot_data_metadata <- merge(x=data, y=metadata, by="row.names")
row.names(plot_data_metadata) <- plot_data_metadata[,1]
plot_data_metadata <- plot_data_metadata[,-1]
#Change BD1.5 to BD1-5
colnames(plot_data_metadata)[14] <- "Candidate_division_BD1-5"
#Change Deinococcus.Thermus to Deinococcus Thermus
colnames(plot_data_metadata)[20] <- "Deinococcus Thermus"
#Change data to long list format
plot_data_metadata_long <- gather(plot_data_metadata, Phylum, relabund, Firmicutes
:Chloroflexi)
#Colours for phyla
colset <- c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set3"),
brewer.pal(9, "Pastel1"))
#Change Participant numbers into strings
plot_data_metadata_long$Individual <- as.character(plot_data_metadata_long$Individ
ual)
#Change order of phylum factor and individual factor
plot_data_metadata_long$Phylum <- factor(plot_data_metadata_long$Phylum,levels = u
nique(plot_data_metadata_long$Phylum))
plot_data_metadata_long$Individual <- factor(plot_data_metadata_long$Individual,le
vels = c(2,3,4,9:13,15:22,24:29,32,33))
#Bar chart
g_bar <- ggplot(plot_data_metadata_long, aes(x = Individual, y=relabund, fill=Phyl
um)) +geom_bar(stat = 'identity', position = 'stack', width=0.9) + facet_wrap( ~ n
ew.Sample.type, nrow=1) + scale_fill_manual(values = colset) + theme_set(theme_gra
y(base_size = 8)) + theme(axis.text.x = element_text(angle = 90, hjust = 1)) + xla
b("Subject code") + ylab("Relative abundance") + scale_y_continuous(expand = c(0,0
)) + geom_text(aes(x=1, y=1.00, label="Stretch it"), vjust=-1) + theme(legend.posi
tion="bottom", legend.key.size = unit(4, "mm"), legend.margin=unit(0,"cm"),plot.ma
rgin =unit(c(0,0,0,0),"mm")) + labs(fill='')
ggsave("Fig2A.Relative_abundance_at_phylum_level_for_colonic_biopsy_and_lavage_sam
ples.pdf", g_bar, units="cm", height=14, width=17, dpi=300)
```

```
## [1] TRUE
```



B) Collective pie chart representation.

```r
### Load packages or install if not present
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
#Load the data
data <- read.csv("16sBxCL_phylum_phylotype_average_by_sample_type_summary.txt", se
p="\t")
#Transform column with phyla information into a factor
data$Phylum <- factor(data$Phylum, levels = unique(data$Phylum))
#Choose a colour palette to be used in plot
colset <- c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set3"),
brewer.pal(9, "Pastel1"))
#Produce pie charts for biopsy samples and lavage samples
g_pc_1 <- ggplot(data, aes(x="", y=Biopsy, fill=Phylum)) + geom_bar(width=1, stat=
"identity") + coord_polar("y", start=0) + theme_set(theme_gray(base_size = 10)) +
scale_fill_manual(values = colset) + theme(legend.position="none", axis.ticks = el
ement_blank(), axis.text = element_blank()) + theme(plot.margin =unit(c(0,1,0,0),"
mm"))
ggsave("Fig2B.biopsy.Relative_abundance_at_phylum_level_for_colonic_biopsy_and_lav
age_samples.pdf", g_pc_1, units="cm", height=8.5, width=8.5, dpi=300)
g_pc_2 <- ggplot(data, aes(x="", y=Lavage, fill=Phylum)) + geom_bar(width=1, stat=
"identity") + coord_polar("y", start=0) + theme_set(theme_gray(base_size = 10)) +
scale_fill_manual(values = colset) + theme(legend.position="none", axis.ticks = el
ement_blank(), axis.text = element_blank()) + theme(plot.margin =unit(c(0,1,0,0),"
mm"))
ggsave("Fig2B.lavage.Relative_abundance_at_phylum_level_for_colonic_biopsy_and_lav
age_samples.pdf",
       g_pc_2, units="cm", height=8.5, width=8.5, dpi=300)
```


Biopsy

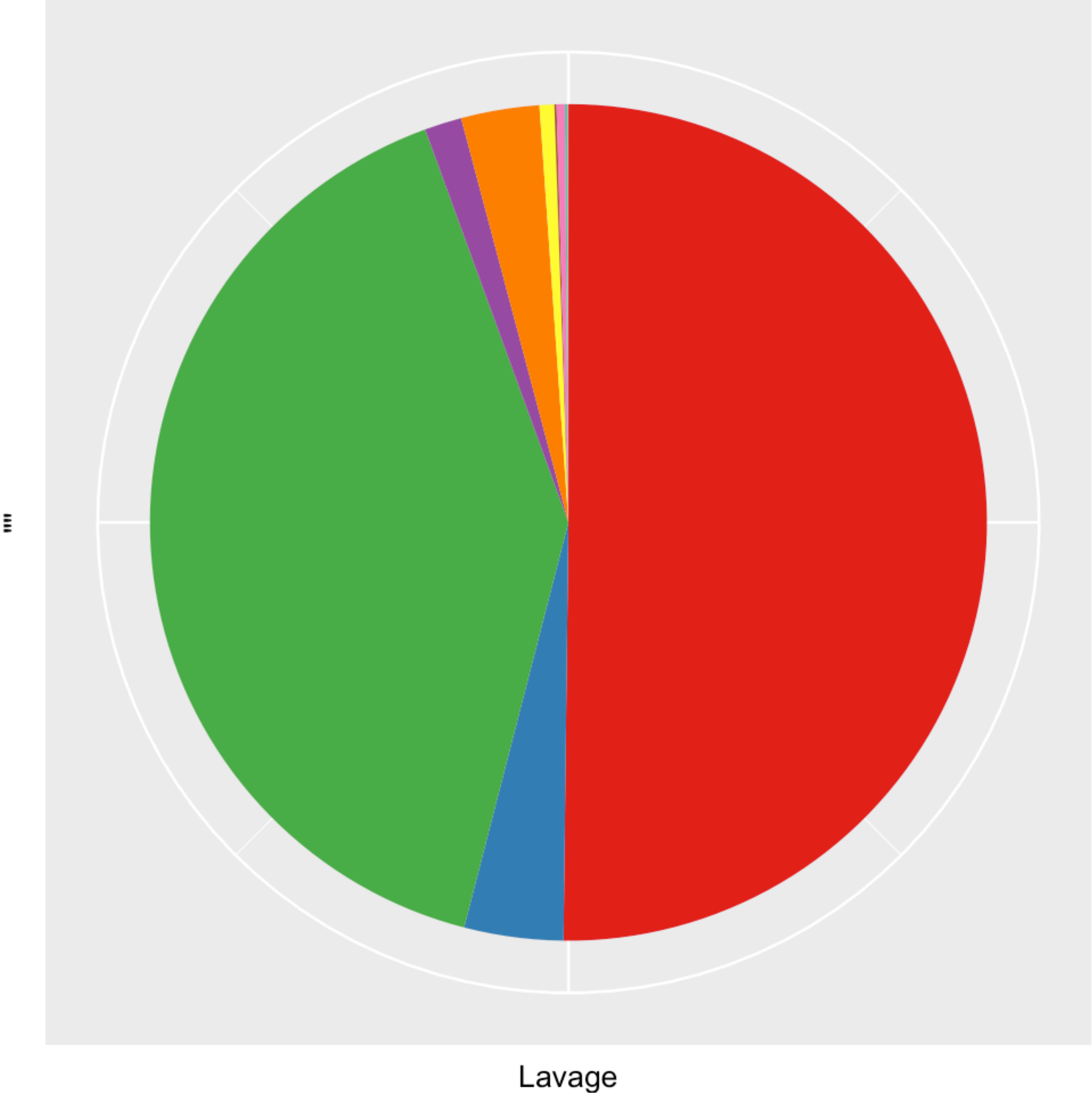

Lavage

Figure 3: The distribution of bacteria in colonic biopsy and lavage samples at phylum level

2 heatmaps produced to give the colours for sample type and particpant for each column The two heatmaps were combined using powerpoint

```
### Load the package or install if not present
if (!require("arules")) {install.packages("arules")
  library(arules)}
```

```
## Loading required package: arules
```

```
## Loading required package: Matrix
```

```
##
## Attaching package: 'Matrix'
```

```
## The following object is masked from 'package:tidyr':
##
##     expand
```

```
## The following objects are masked from 'package:base':
##
##     crossprod, tcrossprod
```

```
##
## Attaching package: 'arules'
```

```
## The following objects are masked from 'package:base':
##
##     %in%, write
```

```
if (!require("ade4")) {install.packages("ade4")
  library(ade4)}
```

```
## Loading required package: ade4
```

```
if (!require("vegan")) {install.packages("vegan")
  library(vegan)}
```

```
## Loading required package: vegan
```

```
## Loading required package: permute
## Loading required package: lattice
```

```
## This is vegan 2.3-4
```

```
##
## Attaching package: 'vegan'
```

```
## The following object is masked from 'package:ade4':
##
##     cca
```

```
if (!require("gdata")) {install.packages("gdata")
  library(gdata)}
```

```
## Loading required package: gdata
```

```
## gdata: read.xls support for 'XLS' (Excel 97-2004) files ENABLED.
```

```
##
```

```
## gdata: read.xls support for 'XLSX' (Excel 2007+) files ENABLED.
```

```
##
## Attaching package: 'gdata'
```

```
## The following object is masked from 'package:stats':
##
##     nobs
```

```
## The following object is masked from 'package:utils':
##
##     object.size
```

```
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
```

```
## Loading required package: gplots
```

```
##
## Attaching package: 'gplots'
## The following object is masked from 'package:stats':
##
##     lowess
```

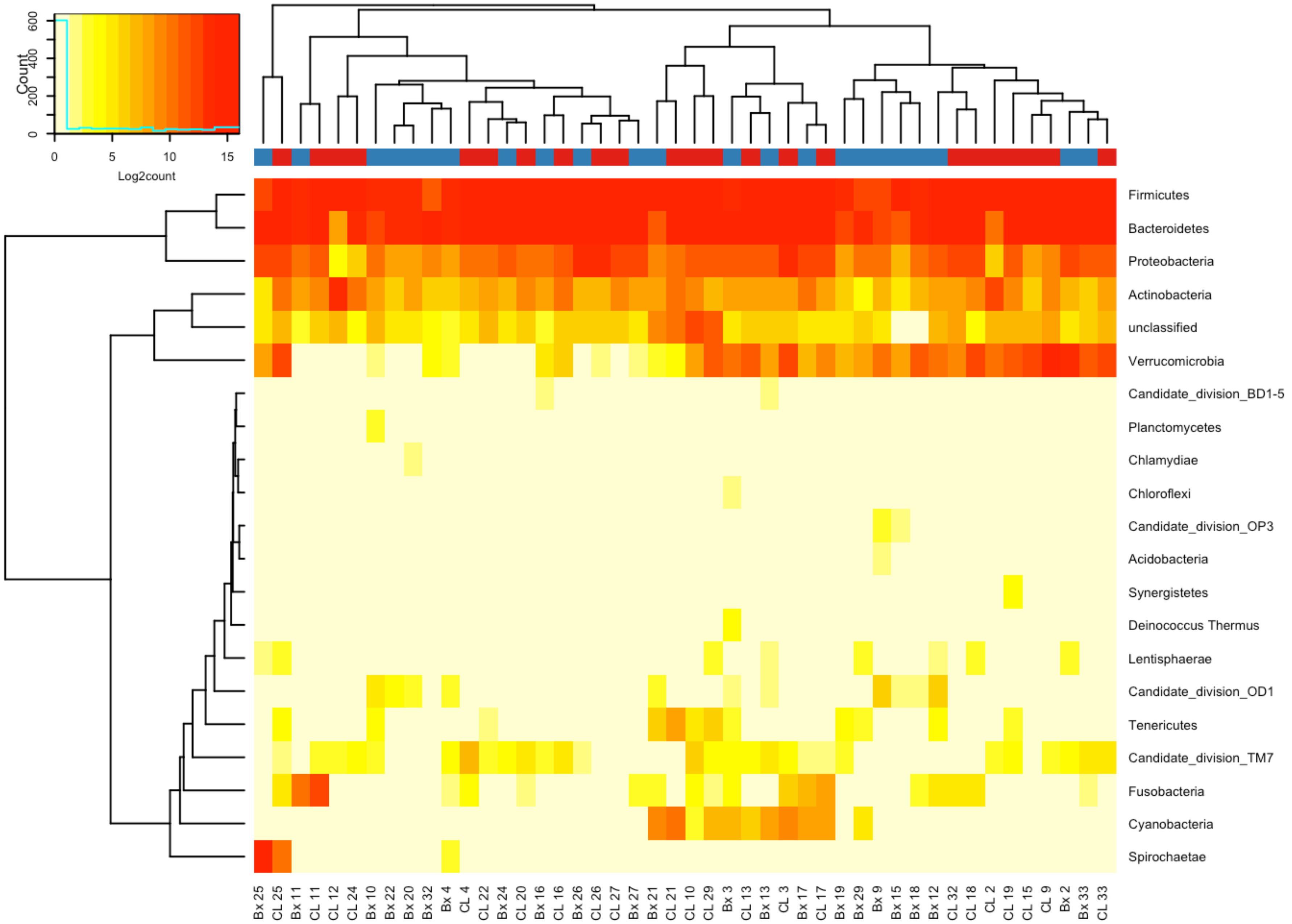
```
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
data <- read.csv("16sBxCL_phylum_phylotype_log2counts_summary.txt", sep="\t", row.
names=1)
metadata_info <- read.csv("16sBxCL_Metadata.txt", sep="\t", row.names=1)
heatmap_info <- data[,-1]
sample_info <- data$Sample_type
#Getting colours from colour brewer
brewer_colours <- brewer.pal(9, "Set1")
#Check if heatmap_info and metadata_info are in the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
##Setting colours to sample_types
sample_type_colours <- gsub("FA",brewer_colours[1], sample_info)
sample_type_colours <- gsub("Bx", brewer_colours[2], sample_type_colours)
#Changing name of BD1.5 to candidate_division_BD1-5
colnames(heatmap_info)[14] <- "Candidate_division_BD1-5"
#Changing name of Deinococcus.Thermus to Deinococcus Thermus
colnames(heatmap_info)[20] <- "Deinococcus Thermus"
#Changing
#Transposing heatmap_info
heatmap.info.t <- t(heatmap_info)
#producing heatmap
pdf("Fig3.Colour_by_sample_type.Distribution_of_bacteria_in_colonic_and_lavage_sam
ples_at_phylum_level.pdf", width = 170/25.4, height = 114/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=sample_type_colours, margins =
c(2,7), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(heat.
colors(x)), labRow = row.names(heatmap.info.t), labCol = metadata_info$new.Sample.
ID, cexRow = 0.65, cexCol = 0.65, offsetRow=0.000001, offsetCol = 0.000001, key.pa
r=list(mar=c(1,4,1,1), cex=0.4), lwid=c(1,4), lhei=c(1,5))
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
pdf("Fig3.Colour_by_participant.Distribution_of_bacteria_in_colonic_and_lavage_sam
ples_at_phylum_level.pdf", width = 170/25.4, height = 114/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=as.character(metadata_info$Colo
ur), margins = c(2,7), key.xlab="Log2count", key.title=NA, trace="none", col=funct
ion(x)rev(heat.colors(x)), labRow = row.names(heatmap.info.t), labCol = metadata_i
nfo$new.Sample.ID, cexRow = 0.65, cexCol = 0.65, offsetRow=0.000001, offsetCol = 0
.000001, key.par=list(mar=c(1,4,1,1), cex=0.4), lwid=c(1,4), lhei=c(1,5))
dev.off()
```

```
## quartz_off_screen
##                  2
```

```
## [1] TRUE
```


Count
0
200
400
600
0
5
10
15
Log2count
Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria
unclassified
Verrucomicrobia
Candidate_division_BD1-5
Planctomycetes
Chlamydiae
Chloroflexi
Candidate_division_OP3
Acidobacteria
Synergistetes
Deinococcus Thermus
Lentisphaerae
Candidate_division_OD1
Tenericutes
Candidate_division_TM7
Fusobacteria
Cyanobacteria
Spirochaetae
Bx 25
CL 25
Bx 11
CL 11
CL 12
CL 24
Bx 10
Bx 22
Bx 20
Bx 32
Bx 4
CL 4
CL 22
Bx 24
CL 20
Bx 16
CL 16
Bx 26
CL 26
CL 27
Bx 27
Bx 21
CL 21
CL 10
CL 29
Bx 3
CL 13
Bx 13
CL 3
Bx 17
CL 17
Bx 19
Bx 29
Bx 9
Bx 15
Bx 18
Bx 12
CL 32
CL 18
CL 2
CL 19
CL 15
CL 9
Bx 2
Bx 33
CL 33

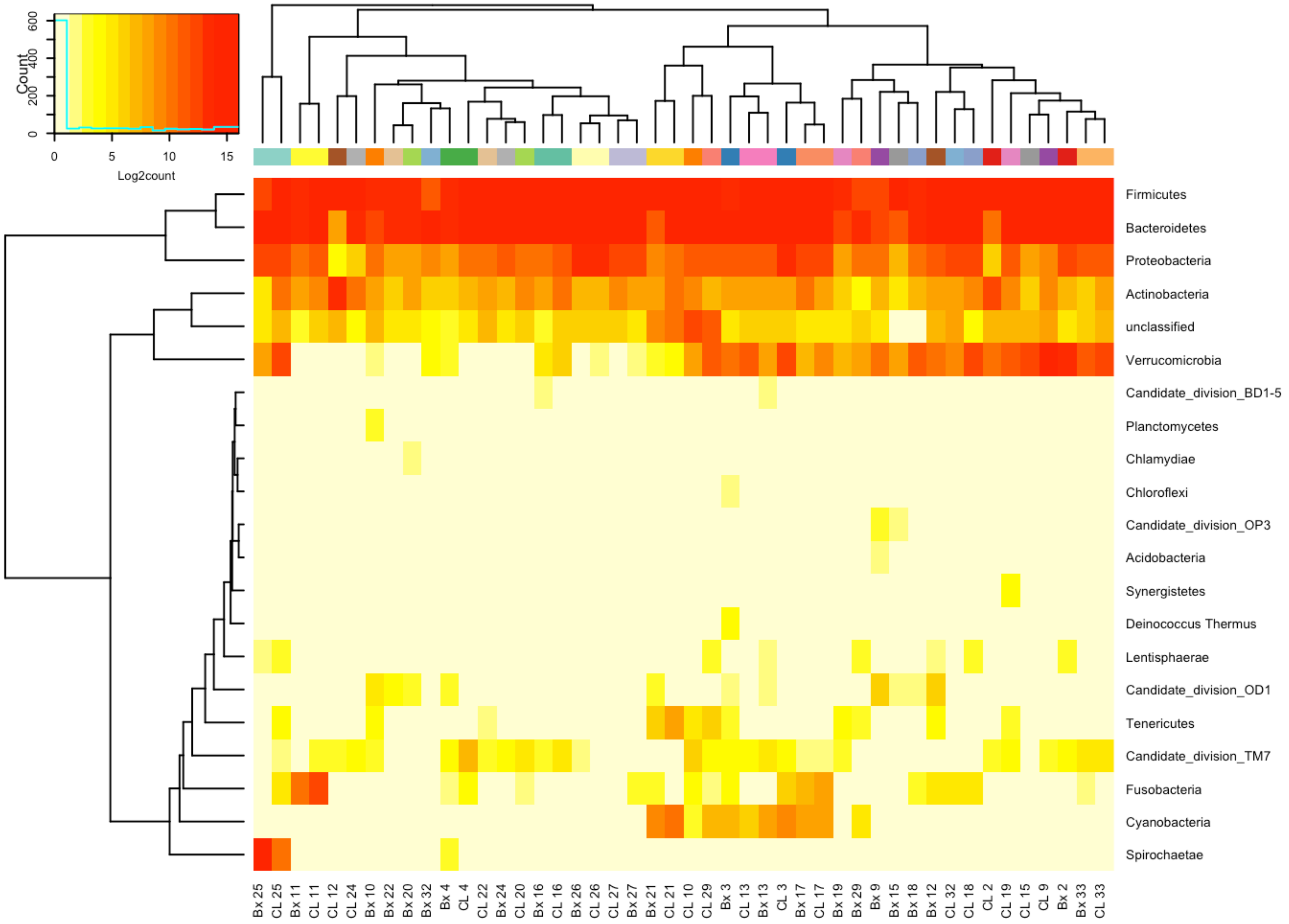

Count
0
200
400
600
0
5
10
15
Log2count
Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria
unclassified
Verrucomicrobia
Candidate_division_BD1-5
Planctomycetes
Chlamydiae
Chloroflexi
Candidate_division_OP3
Acidobacteria
Synergistetes
Deinococcus Thermus
Lentisphaerae
Candidate_division_OD1
Tenericutes
Candidate_division_TM7
Fusobacteria
Cyanobacteria
Spirochaetae
Bx 25
CL 25
Bx 11
CL 11
CL 12
CL 24
Bx 10
Bx 22
Bx 20
Bx 32
Bx 4
CL 4
CL 22
Bx 24
CL 20
Bx 16
CL 16
Bx 26
CL 26
CL 27
Bx 27
Bx 21
CL 21
CL 10
CL 29
Bx 3
CL 13
Bx 13
CL 3
Bx 17
CL 17
Bx 19
Bx 29
Bx 9
Bx 15
Bx 18
Bx 12
CL 32
CL 18
CL 2
CL 19
CL 15
CL 9
Bx 2
Bx 33
CL 33

Figure 4: The distribution of bacteria in colonic biopsy and lavage samples at Family level

2 heatmaps produced to give the colours for sample type and particpant for each column The two heatmaps were combined using powerpoint

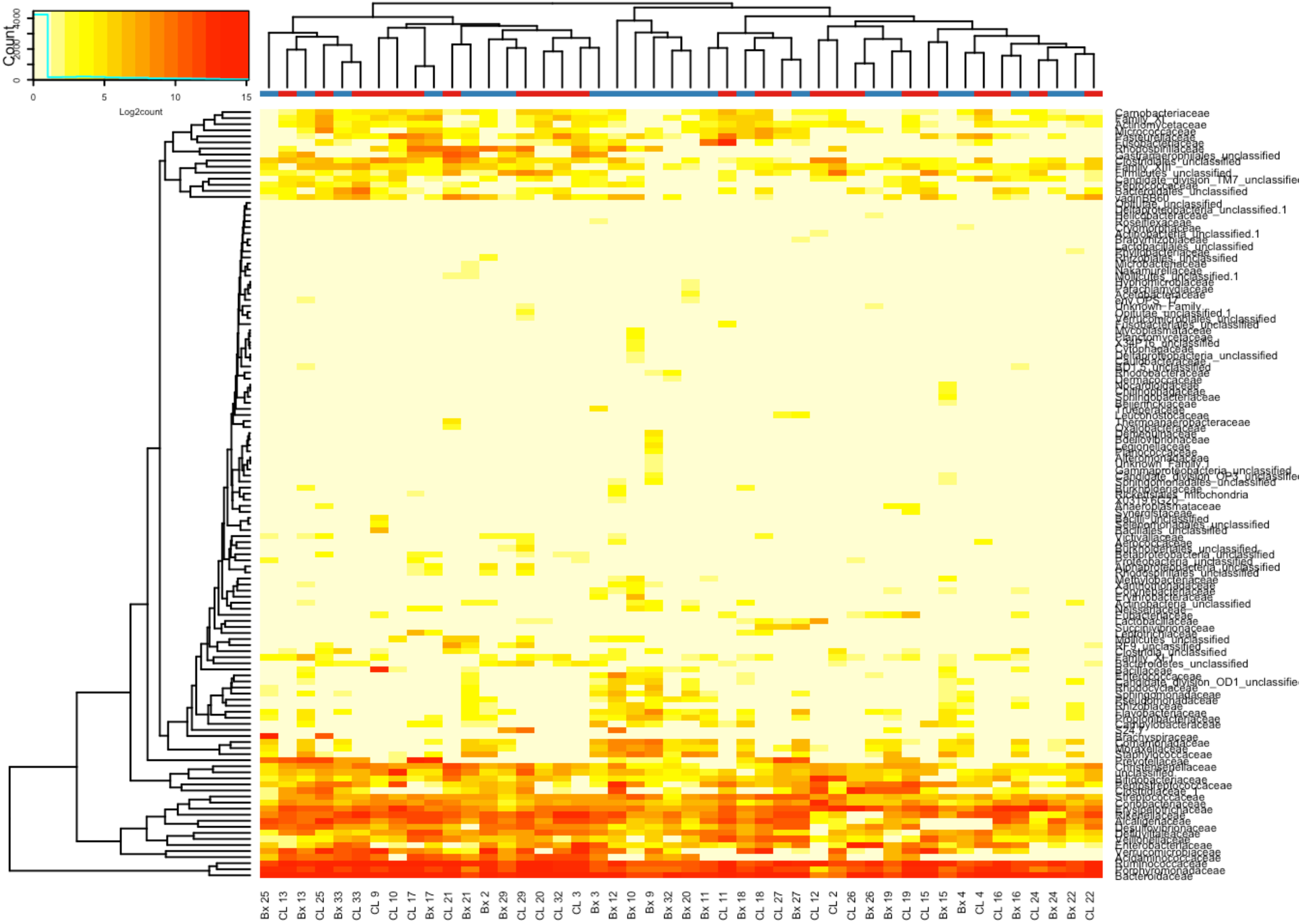
```
### Load packages or install if not present
if (!require("arules")) {install.packages("arules")
  library(arules)}
if (!require("ade4")) {install.packages("ade4")
  library(ade4)}
if (!require("vegan")) {install.packages("vegan")
  library(vegan)}
if (!require("gdata")) {install.packages("gdata")
  library(gdata)}
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
#Load in data
data <- read.csv("16sBxCL_family_phylotype_log2counts_summary.txt", sep="\t", row.
names=1)
metadata_info <- read.csv("16sBxCL_Metadata.txt", sep="\t", row.names=1)
#Remove unwanted column
heatmap_info <- data[,-1]
sample_info <- metadata_info$Sample.type
#Selecting a colour palette to be used in figure
brewer_colours <- brewer.pal(9, "Set1")
#Order heatmap_info and metadata so they are the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
#Setting colours to sample_types
sample_type_colours <- gsub("aspirate",brewer_colours[1], sample_info)
sample_type_colours <- gsub("biopsy", brewer_colours[2], sample_type_colours)
#Transposing heatmap_info
heatmap.info.t <- t(heatmap_info)
#producing heatmaps
pdf("Fig4.Colour_by_sample_type.Distribution_of_bacteria_in_colonic_and_lavage_sam
ples_at_family_level.pdf", 170/25.4,height=182.4/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=sample_type_colours,margins = c
(2,8),key.xlab="Log2count", key.title=NA,trace="none", col=function(x)rev(heat.col
ors(x)), labRow = row.names(heatmap.info.t), cexRow = 0.55, cexCol = 0.55, offsetR
ow=0.0000001, offsetCol = 0.0000001, labCol = metadata_info$new.Sample.ID, key.par
=list(mar=c(1,3,1,1), cex=0.3), lwid=c(1,4), lhei=c(1,9))
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
pdf("Fig4.Colour_by_participant.Distribution_of_bacteria_in_colonic_and_lavage_samples_at_family_level.pdf",170/25.4, height=182.4/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=as.character(metadata_info$Colour), margins = c(2,8), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(heat.colors(x)), labRow = row.names(heatmap.info.t), cexRow = 0.55, cexCol = 0.55, offsetRow=0.0000001, offsetCol = 0.0000001, labCol = metadata_info$new.Sample.ID, key.par=list(mar=c(1,3,1,1), cex=0.3), lwid=c(1,4), lhei=c(1,9))
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
## [1] TRUE
```


Count
0
2000
4000
0
5
10
15
Log2count
Carnobacteriaceae
Family_XI
Actinomycetaceae
Micrococcaceae
Pasteurellaceae
Fusobacteriaceae
Gastranaerophilales_unclassified
Clostridiales_unclassified
Firmicutes_unclassified
Candidate_division_TM7_unclassified
Bacteroidales_unclassified
Bacilli_unclassified
Deltaproteobacteria_unclassified.1
Helicobacteraceae
Roseiflexaceae
Actinobacteria_unclassified.1
Lactobacillales_unclassified
Rhizobiales_unclassified
Microbacteriaceae
Nakamurellaceae
Mollicutes_unclassified.1
Hyphomicrobiaceae
Parachlamydiaceae
Acetobacteraceae
Unknown_Family
Opitutae_unclassified.1
Verrucomicrobiales_unclassified
Mycoplasmataceae
Deltaproteobacteria_unclassified
Rhodobacteraceae
Dermacoccaceae
Sphingobacteriaceae
Leuconostocaceae
Thermoanaerobacteraceae
Desulfovibrionaceae
Legionellaceae
Alteromonadaceae
Unknown_Family.1
Gammaproteobacteria_unclassified
Sphingomonadales_unclassified
Rickettsiales_mitochondria
Anaeroplasmataceae
Bacillales_unclassified
Veillonellaceae
Betaproteobacteria_unclassified
Proteobacteria_unclassified
Methylobacteriaceae
Erythrobacteraceae
Actinobacteria_unclassified
Neisseriaceae
Lactobacillaceae
Succinivibrionaceae
Mollicutes_unclassified
Clostridia_unclassified
Bacteroidetes_unclassified
Bacillaceae
Candidate_division_OD1_unclassified
Sphingomonadaceae
Rhizobiaceae
Flavobacteriaceae
Peptostreptococcaceae
Brachyspiraceae
Prevotellaceae
Christensenellaceae
unclassified
Streptococcaceae
Erysipelotrichaceae
Alcaligenaceae
Enterobacteriaceae
Acidaminococcaceae
Porphyromonadaceae
Bacteroidaceae
Bx 25
CL 13
Bx 13
CL 25
Bx 33
CL 33
CL 9
CL 10
CL 17
Bx 17
CL 21
Bx 21
Bx 2
Bx 29
CL 29
CL 20
CL 32
CL 3
Bx 3
Bx 12
Bx 10
Bx 9
Bx 32
Bx 20
Bx 11
CL 11
Bx 18
CL 18
CL 27
Bx 27
CL 12
CL 2
CL 26
Bx 26
Bx 19
CL 19
CL 15
Bx 15
Bx 4
CL 4
CL 16
Bx 16
CL 24
Bx 24
Bx 22
CL 22

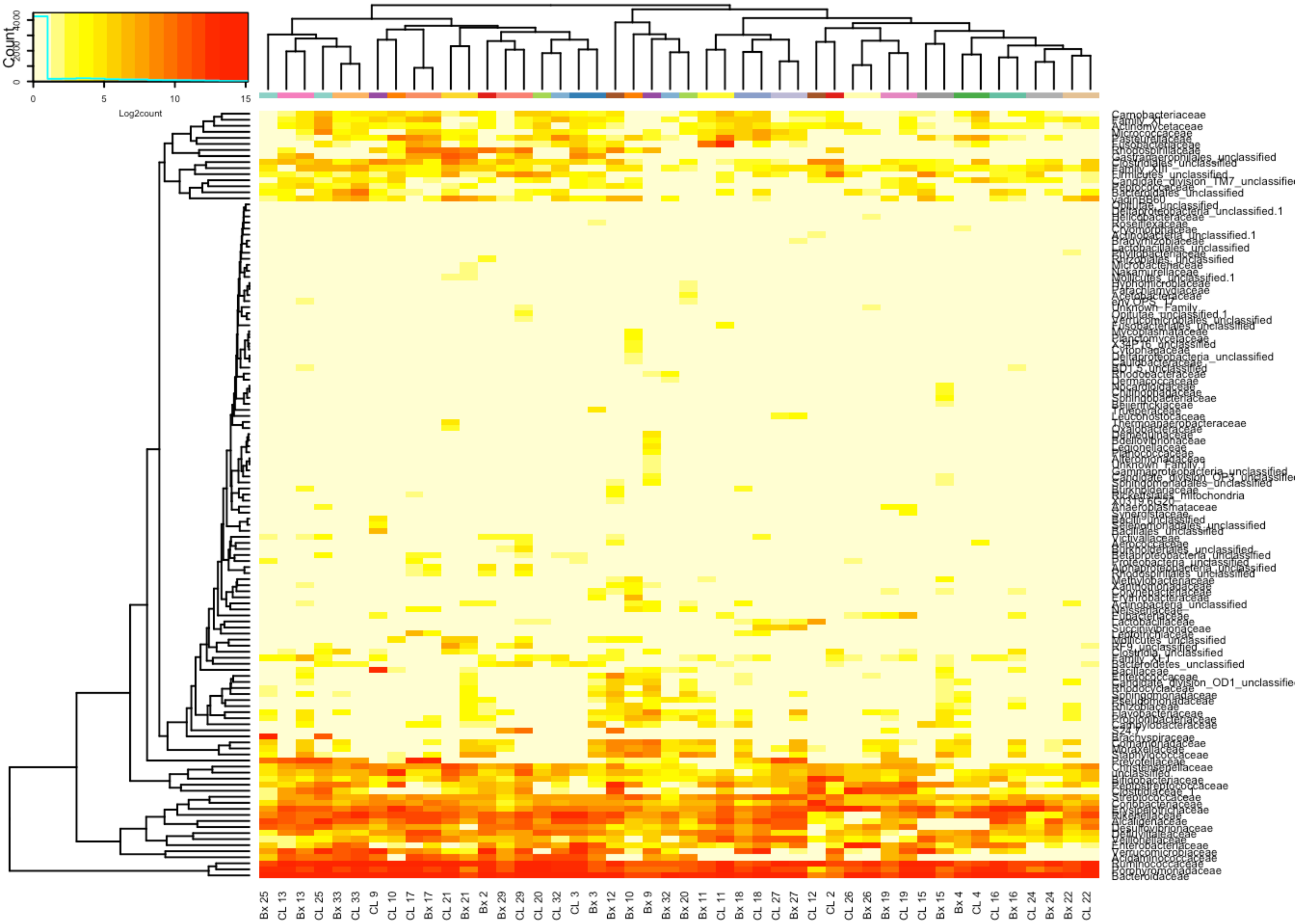

Count
0
2000
4000
0
5
10
15
Log2count
Carnobacteriaceae
Family_XI
Actinomycetaceae
Micrococcaceae
Pasteurellaceae
Fusobacteriaceae
Gastranaerophilales_unclassified
Clostridiales_unclassified
Firmicutes_unclassified
Candidate_division_TM7_unclassified
Bacteroidales_unclassified
vadinBB60
Deltaproteobacteria_unclassified.1
Helicobacteraceae
Roseiflexaceae
Actinobacteria_unclassified.1
Lactobacillales_unclassified
Rhizobiales_unclassified
Nakamurellaceae
Mollicutes_unclassified.1
Hyphomicrobiaceae
Parachlamydiaceae
Unknown_Family
Opitutae_unclassified.1
Verrucomicrobiales_unclassified
Mycoplasmataceae
Planctomycetaceae
Cytophagaceae
Deltaproteobacteria_unclassified
Rhodobacteraceae
Dermacoccaceae
Sphingobacteriaceae
Leuconostocaceae
Thermoanaerobacteraceae
Legionellaceae
Alteromonadaceae
Unknown_Family.1
Gammaproteobacteria_unclassified
Sphingomonadales_unclassified
Rickettsiales_mitochondria
Anaeroplasmataceae
Synergistaceae
Bacillales_unclassified
Victivallaceae
Betaproteobacteria_unclassified
Proteobacteria_unclassified
Erythrobacteraceae
Actinobacteria_unclassified
Neisseriaceae
Lactobacillaceae
Succinivibrionaceae
Mollicutes_unclassified
Clostridia_unclassified
Bacteroidetes_unclassified
Enterococcaceae
Candidate_division_OD1_unclassified
Rhodocyclaceae
Sphingomonadaceae
Pseudomonadaceae
Flavobacteriaceae
Peptostreptococcaceae
Brachyspiraceae
Prevotellaceae
Christensenellaceae
unclassified
Streptococcaceae
Erysipelotrichaceae
Alcaligenaceae
Desulfovibrionaceae
Enterobacteriaceae
Verrucomicrobiaceae
Ruminococcaceae
Porphyromonadaceae
Bacteroidaceae
Bx 25
CL 13
Bx 13
CL 25
Bx 33
CL 33
CL 9
CL 10
CL 17
Bx 17
CL 21
Bx 21
Bx 2
Bx 29
CL 29
CL 20
CL 32
CL 3
Bx 3
Bx 12
Bx 10
Bx 9
Bx 32
Bx 20
Bx 11
CL 11
Bx 18
CL 18
CL 27
Bx 27
CL 12
CL 2
CL 26
Bx 26
Bx 19
CL 19
CL 15
Bx 15
Bx 4
CL 4
CL 16
Bx 16
CL 24
Bx 24
Bx 22
CL 22

Figure 5: Pearson correlation plot of biopsy against lavage samples using OTU counts

```
### Load packages or install if not present
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("Hmisc")) {install.packages("Hmisc")
  library(Hmisc)}
```

```
## Loading required package: Hmisc
```

```
## Loading required package: survival
```

```
## Loading required package: Formula
```

```
##
## Attaching package: 'Hmisc'
```

```
## The following object is masked from 'package:gdata':
##
##     combine
```

```
## The following objects are masked from 'package:base':
##
##     format.pval, round.POSIXt, trunc.POSIXt, units
```

```
if (!require("corrplot")) {install.packages("corrplot")
  library(corrplot)}
```

```
## Loading required package: corrplot
```

```
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
#Load in data
otu_info <- read.csv("colonoscopy.makecontigsfile.trim.contigs.good.unique.good.fi
lter.unique.precluster.pick.pick.an.unique_list.0.03.pick.shared", sep="\t", row.n
ames = 2, header=TRUE)
metadata_info <- read.csv("16sBxCL_Metadata.txt", sep="\t", row.names=1)
#remove unwanted columns
otu_info <- otu_info[,c(-1,-2)]
otu_info <- otu_info[,-17525]
#Order otu_info and metadata_info so they are the same order
otu_info <- otu_info[order(row.names(otu_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(otu_info)))
#Change row names
row.names(otu_info) <- metadata_info$new.Sample.ID
#Order by new row names
otu_info <- otu_info[order(row.names(otu_info)),]
#Transpose otu_info
trans_otu_info <- t(otu_info)
#Change Total OTU amounts into relative abundance
relabund_otu_info <- prop.table(trans_otu_info, margin=2)
#Calculate pearson values
data_rcorr <- rcorr(as.matrix(trans_otu_info), type = "pearson")
pearson_cor <- data_rcorr$r
pearson_p <- data_rcorr$P
#Keep info interested in
subset_pearson_cor <- pearson_cor[1:23,24:46]
subset_pearson_p <- pearson_p[1:23,24:46]
#Correlation plot of FA samples against bx samples
pdf("Fig5.pearson_correlation_plot_of_biopsy_against_lavage_smaples_using_otu_coun
ts.85x85mm.pdf",height = 85/25.4, width=85/25.4)
par(mfrow=c(1,1), mar=c(0,0,0,0), cex=0.6)
corrplot((subset_pearson_cor),type="full", mar=c(0,0,0,0), cl.pos="b",
         p.mat=subset_pearson_p, sig.level = 0.05, insig="blank")
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
## [1] TRUE
```

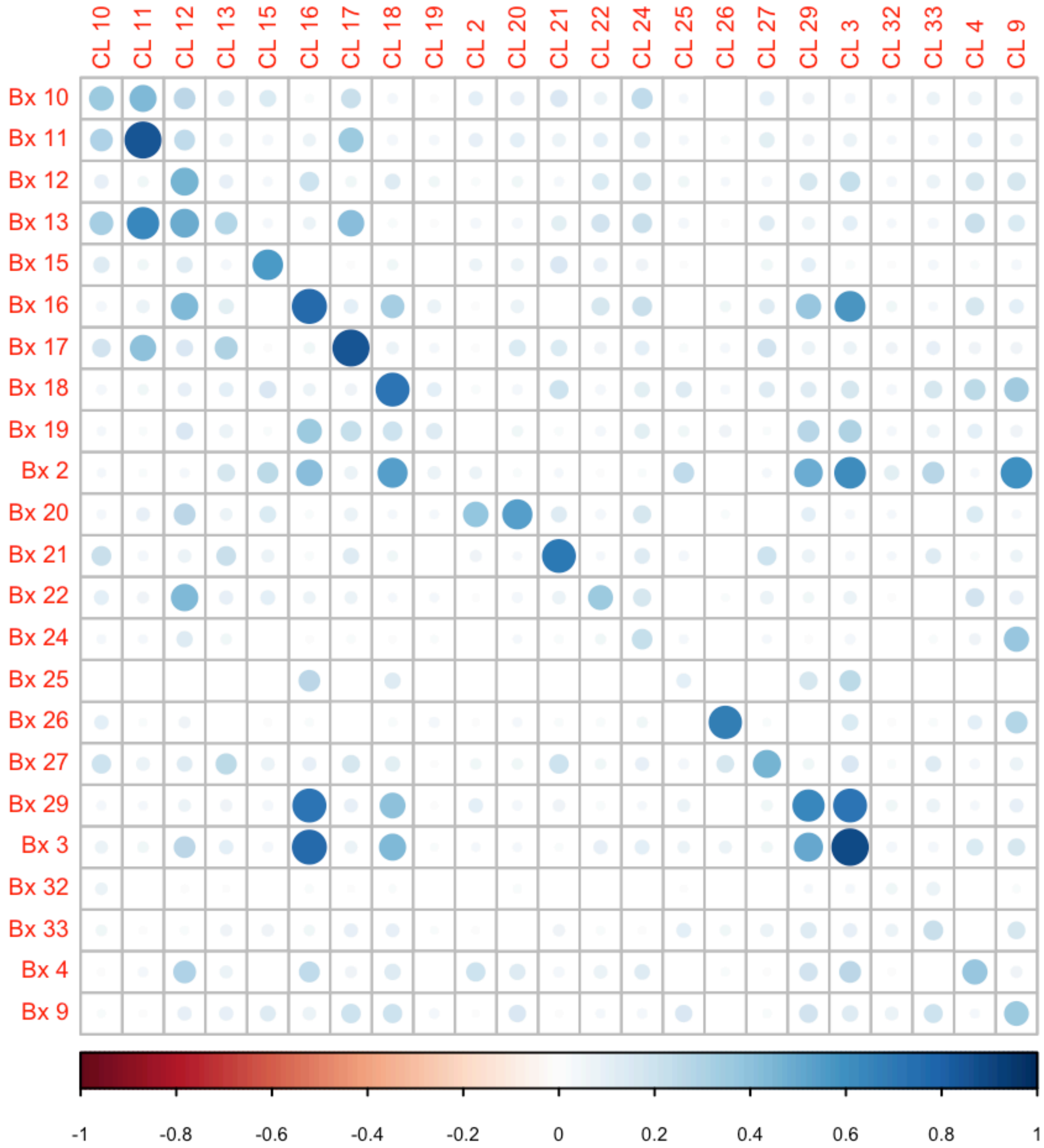
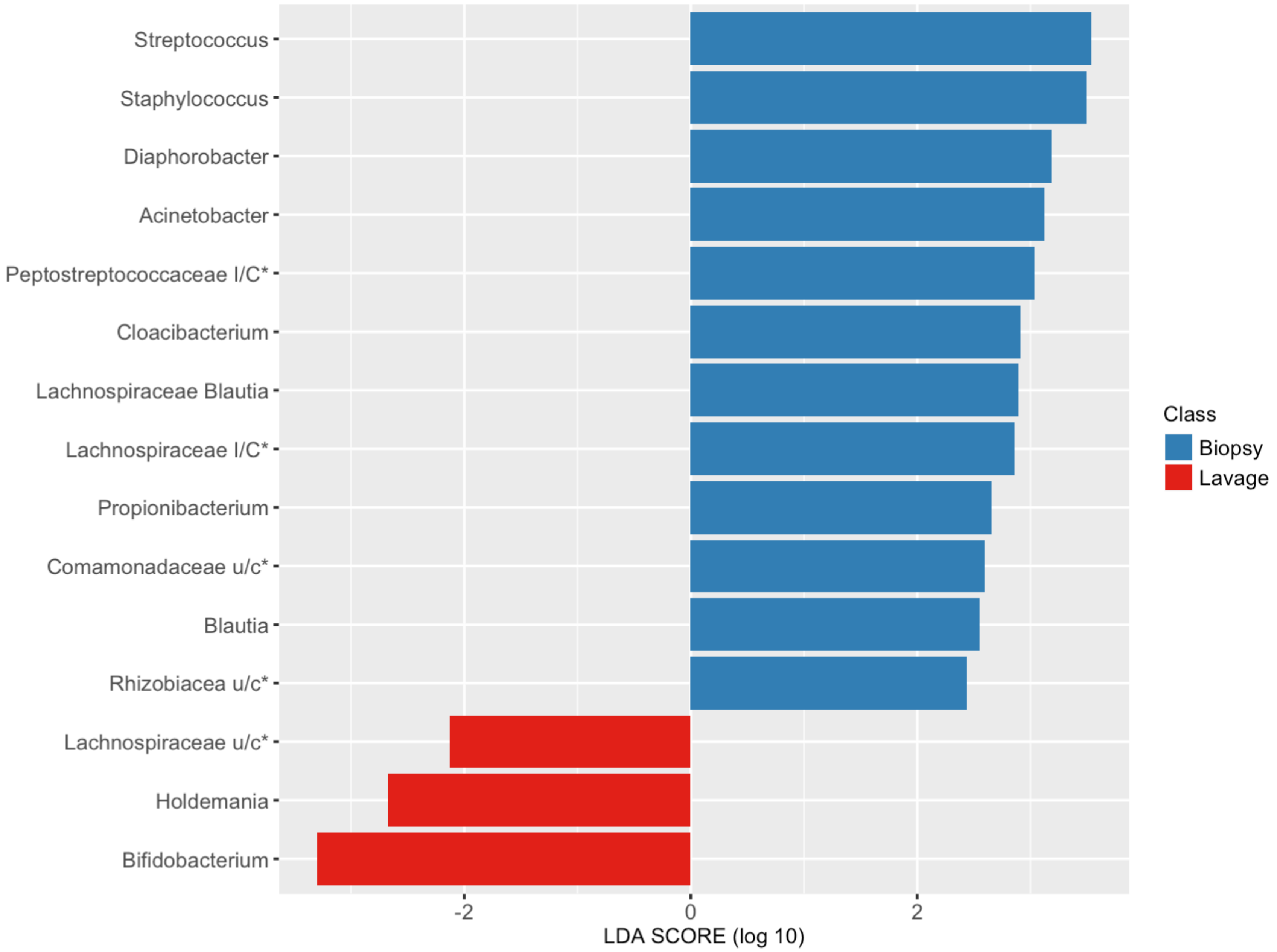


Figure 6: Differentially abundant genera between biopsy and lavage samples by LefSe

A) LEfSe LDA scores

```
### Load packages or install if not present
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
#read in data
plot_data <- read.csv("16sBxCL_LEfSe_summary_subsample.txt", sep="\t")
plot_data$Species <- factor(plot_data$Species, levels=plot_data$Species[order(plot
_data$LDA)])
#Produce Plot
g <- ggplot(plot_data, aes(x=Species, y=LDA, fill=new.Class)) + geom_bar(stat="ide
ntity", position="identity") + coord_flip() + ylab("LDA SCORE (log 10)") + xlab(""
) + theme_set(theme_gray(base_size = 8)) + scale_fill_manual(name="Class", values
= c("#377EB8","#E41A1C"), breaks=c("Biopsy", "Lavage"), labels=c("Biopsy", "Lavage
")) + theme(text= element_text(size=8), axis.text.y  = element_text(size=8), axis.
text.x  = element_text(size=8), legend.text= element_text(size = 8), legend.key.si
ze = unit(4, "mm"), plot.margin=unit(c(1,1,1,1),"mm"))
ggsave("Fig6A.Differentially_abundant_genera_between_biopsy_and_lavage_samples_by_
lefse.pdf", g, units="mm", height=70, width=170, dpi=300)
```



B) Heat map of Log2count of OTUs

```
### Load packages or install if not present
if (!require("arules")) {install.packages("arules")
  library(arules)}
if (!require("ade4")) {install.packages("ade4")
  library(ade4)}
if (!require("vegan")) {install.packages("vegan")
  library(vegan)}
if (!require("gdata")) {install.packages("gdata")
  library(gdata)}
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
#Load in data and edit
data <- read.csv("16sBxCL_LEfSe_subsample_otu_log2count_info_with_taxa.txt", sep="
\t", row.names=1)
metadata_info <- read.csv("16sBxCL_Metadata.txt", sep="\t", row.names=1)
heatmap_info <- data[,-1]
sample_info <- data$Sample_type
taxa_info <- read.csv("16sBxCL_LEfSe_subsample_otu_log2count_info_with_taxa.txt",
header=FALSE, sep="\t", row.names=1)
taxa_info <- taxa_info[1,-1]
#Selecting a colour palette to be used in plot
brewer_colours <- brewer.pal(9, "Set1")
#Order heatmap_info and metadata_info so they are the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
##Setting colours to sample_types
sample_type_colours <- gsub("FA",brewer_colours[1], sample_info)
sample_type_colours <- gsub("Bx", brewer_colours[2], sample_type_colours)
#Transposing heatmap_info
heatmap.info.t <- t(heatmap_info)
#produce heatmap
pdf("Fig6B.Differentially_abundant_genera_between_biopsy_and_lavage_samples_by_lef
se.pdf",
    170/25.4, height=110/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=sample_type_colours, margins =
c(2.7,8.5), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(h
eat.colors(x)), labRow = row.names(heatmap.info.t), cexRow = 0.7, cexCol = 0.7, of
fsetRow=0.0000001, offsetCol = 0.0000001, labCol = metadata_info$new.Sample.ID, ke
y.par=list(mar=c(2,4,1,1), cex=0.4), lwid=c(1,5), lhei=c(1,5))
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
## [1] TRUE
```

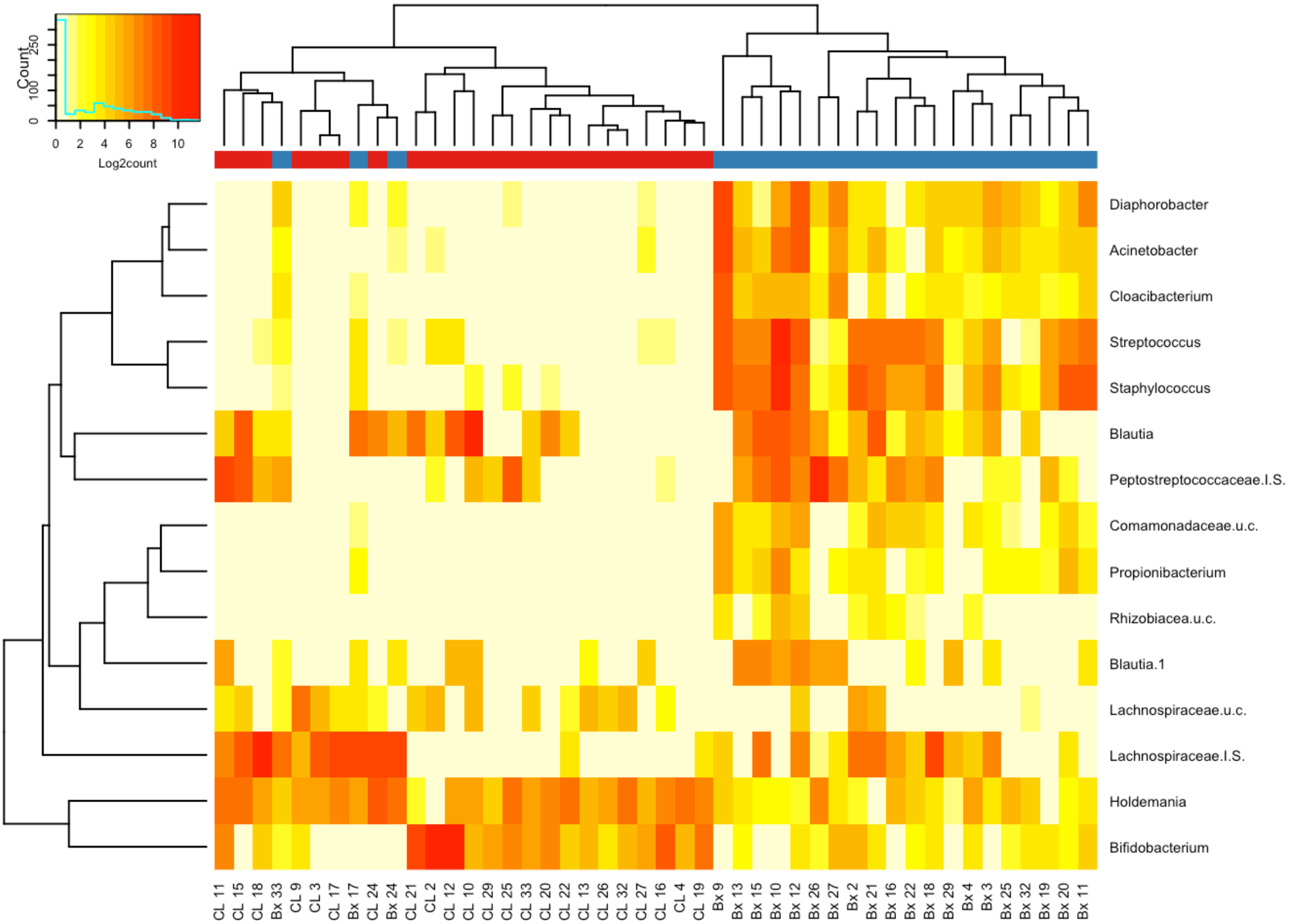


Figure 7: Heat map of Log2count of top 50 OTUs found to not be differentially abundant between biopsy and lavage samples by LefSe

```
### Load packages or install if not present
if (!require("arules")) {install.packages("arules")
  library(arules)}
if (!require("ade4")) {install.packages("ade4")
  library(ade4)}
if (!require("vegan")) {install.packages("vegan")
  library(vegan)}
if (!require("gdata")) {install.packages("gdata")
  library(gdata)}
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
#Read in data
data <- read.csv("16sBxCL_Top_50_OTU_log2count_info_minus_differentially_abundant_
otus_lefse_subsample.txt", sep="\t", row.names=1)
metadata_info <- read.csv("16sBxCL_Metadata.txt", sep="\t", row.names=1)
heatmap_info <- data[,-1]
sample_info <- data$Sample_type
taxa_info <- read.csv("16sBxCL_Top_50_OTU_taxa_info_minus_differentially_abundant_
otus_lefse_subsample.txt", sep="\t", row.names=1)
#Selecting colour palette for plot
brewer_colours <- brewer.pal(9, "Set1")
#Order heatmap_info and metadata_info so they are the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
##Set colours to sample_types
sample_type_colours <- gsub("FA",brewer_colours[1], sample_info)
sample_type_colours <- gsub("Bx", brewer_colours[2], sample_type_colours)
#Transposing heatmap_info
heatmap.info.t <- t(heatmap_info)
#Produce heatmap
pdf("Fig7.Heatmap_log2count_top50_not_differntial_between_biopsy_and_lavage.180w11
0h.pdf", 180/25.4, height=110/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=as.character(metadata_info$Colo
ur), labRow = taxa_info$Taxa.ID, labCol = metadata_info$new.Sample.ID, margins = c
(2,7.5), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(heat
.colors(x)), cexRow = 0.65, cexCol = 0.6, offsetRow=0.0000001, offsetCol = 0.00000
01, key.par=list(mar=c(1,3,1,1), cex=0.3), lwid=c(1,5), lhei=c(1,5))
dev.off()
```

```
## quartz_off_screen
##                 2
```

```
## [1] TRUE
```

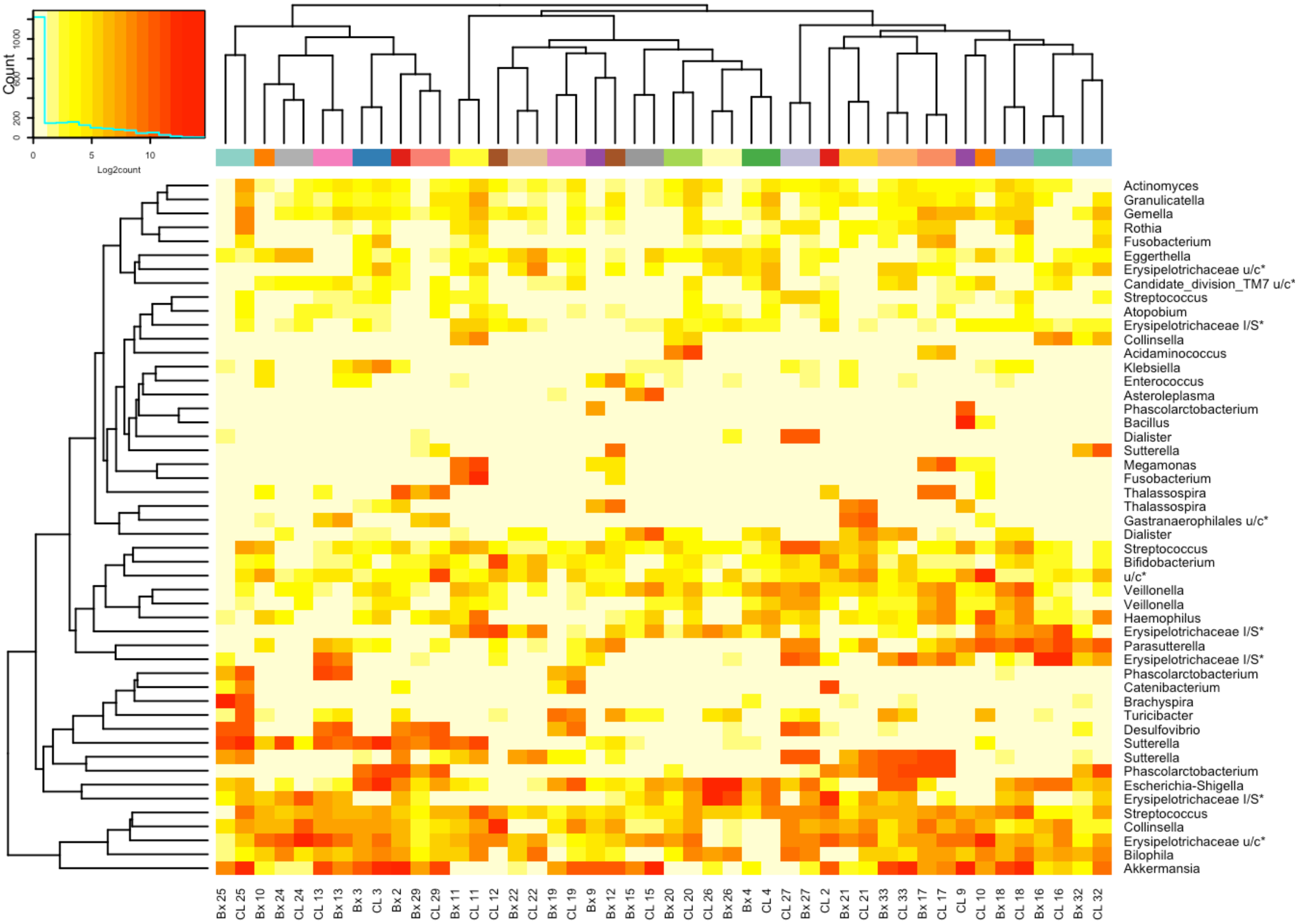
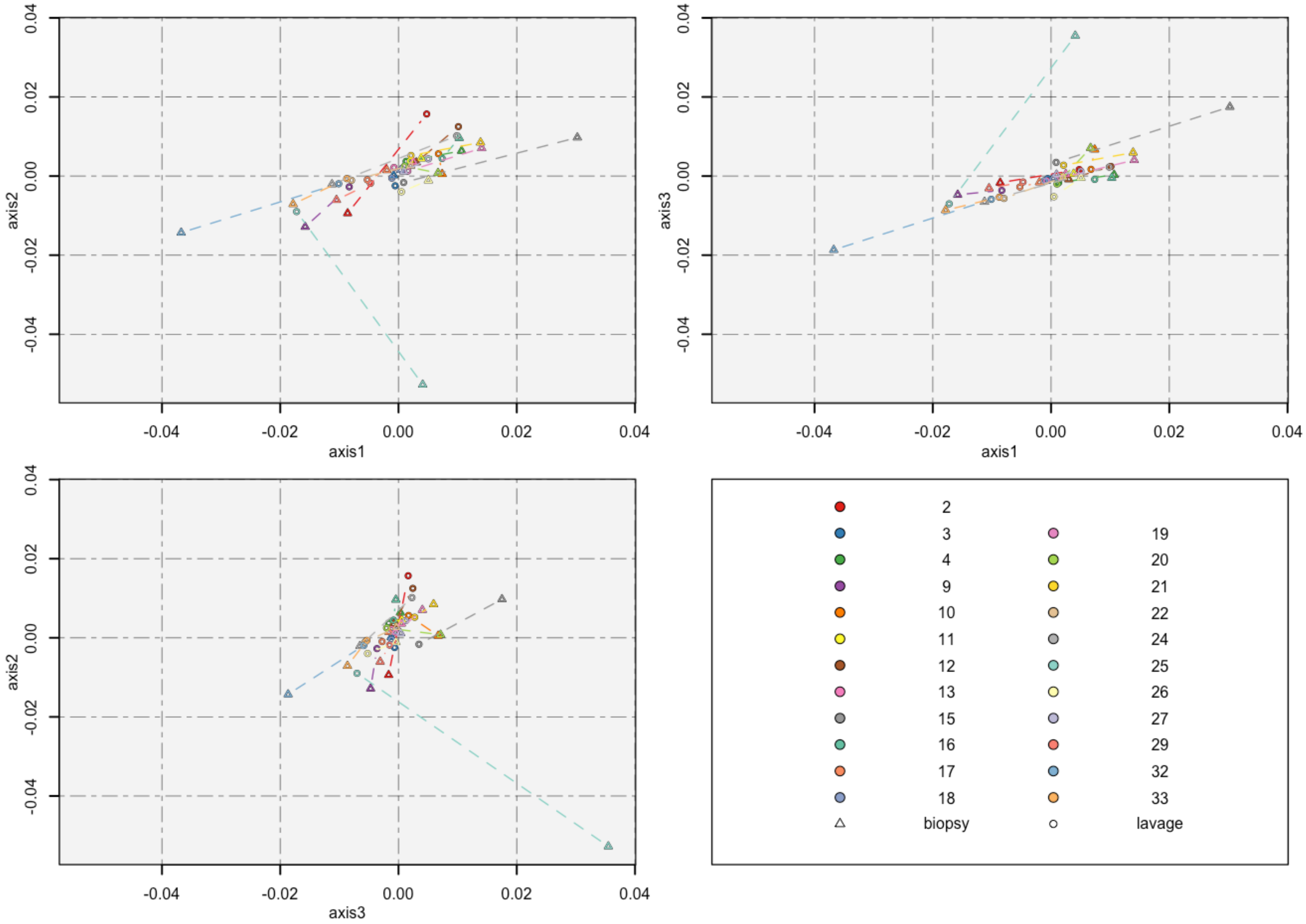


Figure 8: Clustering of samples' PICRUSt predicted KEGG pathways according to sample type (colonic biopsy and lavage) by NMDS, based on Yue & Clayton similarity distance.

```
#Input file names
axes_file_name <- "16sBxCL.KEGG_pathways.shared.thetayc.unique.lt.ave.nmds.axes"
metadata_file_name <- "16sBxCL_Metadata.txt"
#PDF output file name
PDF_file_name <- "Fig8.picrust_predictedKEGG_pathways_according_to_smaple_type_nmd
s_yue_clayton.85x85mm.pdf"
##Relevant metadata columns
PAIRED <- "new.Sample.type"
SAMPLE <- "Individual"
#Read in data
plot_data <- read.csv(axes_file_name,sep="\t", row.names = 1)
metadata <- read.csv(metadata_file_name,sep="\t", row.names = 1)
#Order plot_data and metadata so they are the same order
plot_data <- plot_data[order(row.names(plot_data)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match and stop if not
stopifnot(identical(row.names(plot_data), row.names(metadata)))
#Merge data frames
```

```r
plot_data_metadata <- merge(x=plot_data, y=metadata, by="row.names")
#Fix rownames after merge
row.names(plot_data_metadata) <- plot_data_metadata[,1]
plot_data_metadata <- plot_data_metadata[,-1]
#Sets column to variables
axis1 <- plot_data_metadata[,1]
axis2 <- plot_data_metadata[,2]
axis3 <- plot_data_metadata[,3]
#Set the minimum and maximum x so each plot is the same size as each other for com
parison
maxx <- max(c(max(axis1),max(axis2),max(axis3)))
minx <- min(c(min(axis1),min(axis2),min(axis3)))
#Grouping to colour by
group_samples <- as.factor(plot_data_metadata[,SAMPLE])
#Creates plot points for first and second samples
#This data will be used to connect the points together
first_level <- levels(plot_data_metadata[,PAIRED])[1]
second_level <- levels(plot_data_metadata[,PAIRED])[2]
first_rows <- plot_data_metadata[plot_data_metadata[,PAIRED]==first_level,]
second_rows <- plot_data_metadata[plot_data_metadata[,PAIRED]==second_level,]
#Order data to get colouring correct
first_rows <- first_rows[order(first_rows[,SAMPLE]),]
second_rows <- second_rows[order(second_rows[,SAMPLE]),]
first_group_samples <- as.factor(first_rows[,SAMPLE])
second_group_samples <- as.factor(second_rows[,SAMPLE])
#Selecting colour palette for plot
col.brew <- c(brewer.pal(9, "Set1"),brewer.pal(8,"Set2"),brewer.pal(12,"Set3"))
palette(col.brew)
#Assignment of axes labels
axis_1_lab <- paste("axis1")
axis_2_lab <- paste("axis2")
axis_3_lab <- paste("axis3")
#Function to produce single 2d plot
plot_production <- function(nx, ny, xlabel, ylabel){
  #Make an empty plotting area with axis labels
  plot(0,0, xlab = xlabel, ylab = ylabel, xlim=c(minx-0.001, maxx+0.001), ylim=c(m
inx-0.001, maxx+0.001), pch=19,col=NA, type='b')
  #Sets colour of background of plot
  rect(par("usr")[1], par("usr")[3], par("usr")[2], par("usr")[4], col = "#f6f6f6"
)
  #Sets gridlines that match the ticks of the axes
  grid (NULL,NULL, lty = 6, col = "black", lwd=0.3)
  #Add black borders to points
  points(first_rows[,nx], first_rows[,ny], type='p', pch=2, col="black", cex=0.8)
  points(second_rows[,nx], second_rows[,ny], type='p', pch=1, col="black", cex=0.8
)
  points(first_rows[,nx], first_rows[,ny], type='p', pch=2, col="black", cex=0.6)
  points(second_rows[,nx], second_rows[,ny], type='p', pch=1, col="black", cex=0.6
)
  #This part will plot pair by pair
  #This is so pairs only connect to each other by the dashed lines
  for (i in 1:num_samples){
    n <- i
    points(c(first_rows[n,nx],second_rows[n,nx]), c(first_rows[n,ny],second_rows[n
,ny]), type='b', pch=c(2,1), lwd=0.7, col=i, cex=0.7, lty=2)
  }
}
#Save plot to pdf file
pdf(PDF_file_name, width = 85/25.4, height = 85/25.4)
#Creates diagram to put NMDs plots into
par(mfrow=c(2,2), oma = c(0.8,0.8,0.8,0.8), mgp= c(2,1,0), mar=c(3,3,1,1), cex=0.5
, lwd=0.7)
#Variable used within function
num_samples <- nrow(first_rows)
# creating the 3 2d plots
plot_production(1,2,axis_1_lab,axis_2_lab)
plot_production(1,3,axis_1_lab,axis_3_lab)
plot_production(3,2,axis_3_lab,axis_2_lab)
#number for plotting legend
#n is the number of samples there are
n <- nlevels(group_samples)
#Determine if there is an even or odd amount of paired samples
if (n %% 2 == 0) { n1 <- n/2
n2 <- n/2 } else { n1 <- (n+1)/2
n2 <- (n-1)/2
}
x_leg <- c(rep(1, n1),rep(3, n2))
y_leg <- c((n1+1):2,(n2+1):2)
#Plot used as a legend for the overall diagram
plot(x_leg, y_leg, xlab="", ylab="", xlim=c(0,5), ylim=c(0,(n/2)+2), pch=19, col="
black",cex=1.3, xaxt='n', yaxt='n')
points(x_leg, y_leg, col=col.brew, pch=19)
text(x_leg+1,y_leg,levels(group_samples))
points(1,1, lwd=0.5, pch=2)
text(2,1, levels(plot_data_metadata[,PAIRED])[1])
points(3,1, lwd=0.5, pch=1)
text(4,1, levels(plot_data_metadata[,PAIRED])[2])
dev.off()
```

```
## quartz_off_screen
##                 2
```

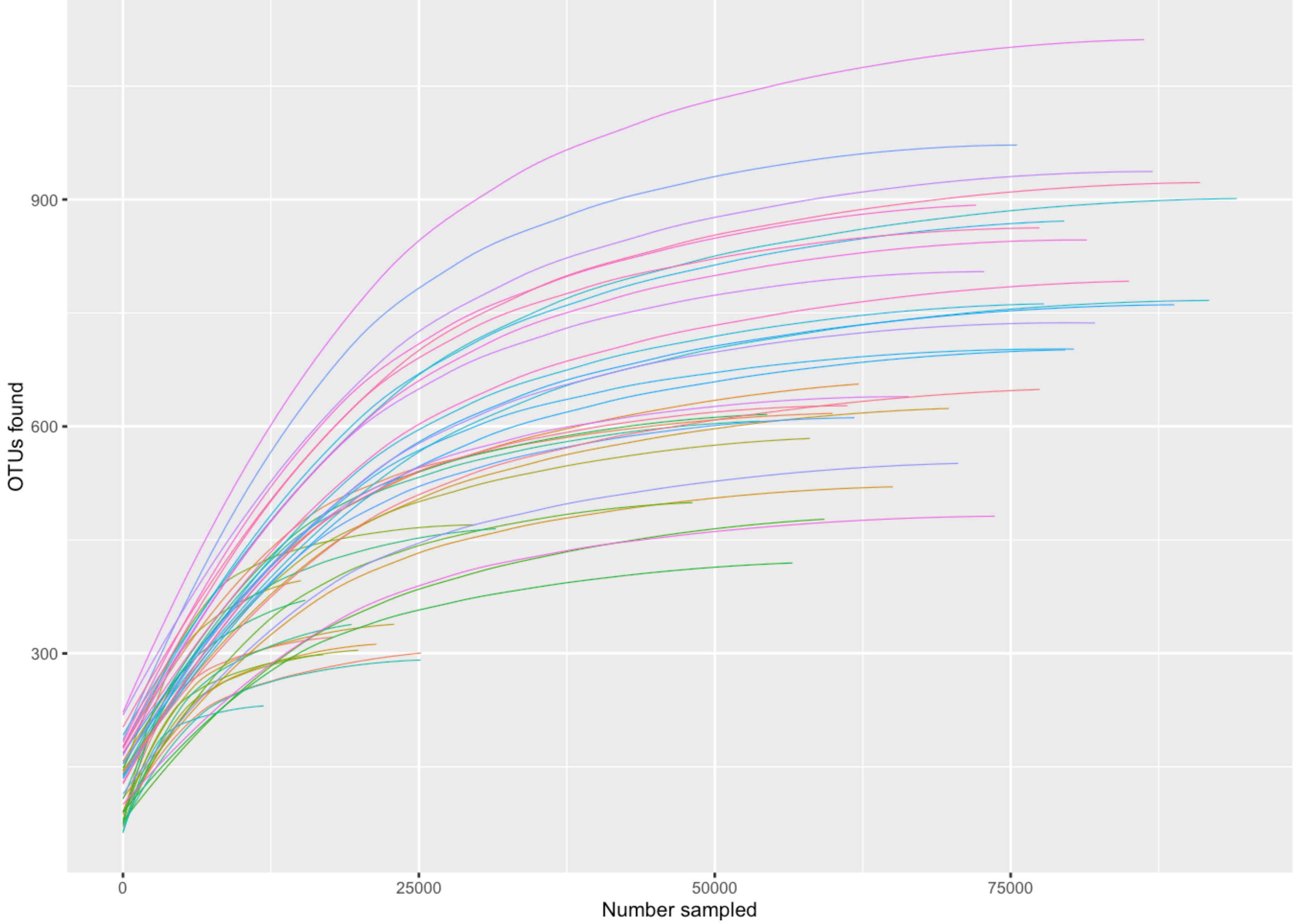


Supplementary figure 2: Rarefaction curve of all 46 samples following removal of rare OTUs

```r
### Load packages or install if not present
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
#Set name of input file
rarefaction_file="colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter
.unique.precluster.pick.pick.an.unique_list.0.03.pick.groups.rarefaction"
#Name of output PDF file
pdf_file_name="SF2.rarefaction.85x85mm.pdf"
#Read in data
rarefaction_data <- read.csv(file = rarefaction_file, sep="\t")
#Remove confidence interval information
rarefaction_data <- rarefaction_data[,c(1,seq(from=2, to= ncol(rarefaction_data),
by=3))]
#Convert to long list format
rarefaction_data_long <- gather(rarefaction_data, sample, Measure, 2:ncol(rarefact
ion_data))
#Change name of numsampled to match ggplot
colnames(rarefaction_data_long)[1] <- "numsampled"
#Select colour palette to be used in plot
colset <- c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set3"),
brewer.pal(9, "Pastel1"))
#ggplot for rarefaction curve
g <- ggplot(data = rarefaction_data_long, aes(x=numsampled, y=Measure, group=sampl
e, colour=sample)) + geom_smooth(se = FALSE, size=0.2) + theme_set(theme_gray(base
_size = 8)) + theme(text=element_text(size=8), legend.position="none", plot.margin
=unit(c(0,0,0,0),"mm")) + scale_y_continuous(name="OTUs found") + scale_x_continuo
us(name="Number sampled")
ggsave(pdf_file_name, g, units="mm", width=85, height=85)
```

```
## Warning: Removed 18554 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 18554 rows containing non-finite values (stat_smooth).
```



Supplementary figure 4: Clustering of samples according to sample type (colonic biopsy and lavage) by PCoA based on (A) Jaccard and (B) Yue & Clayton similarity distance

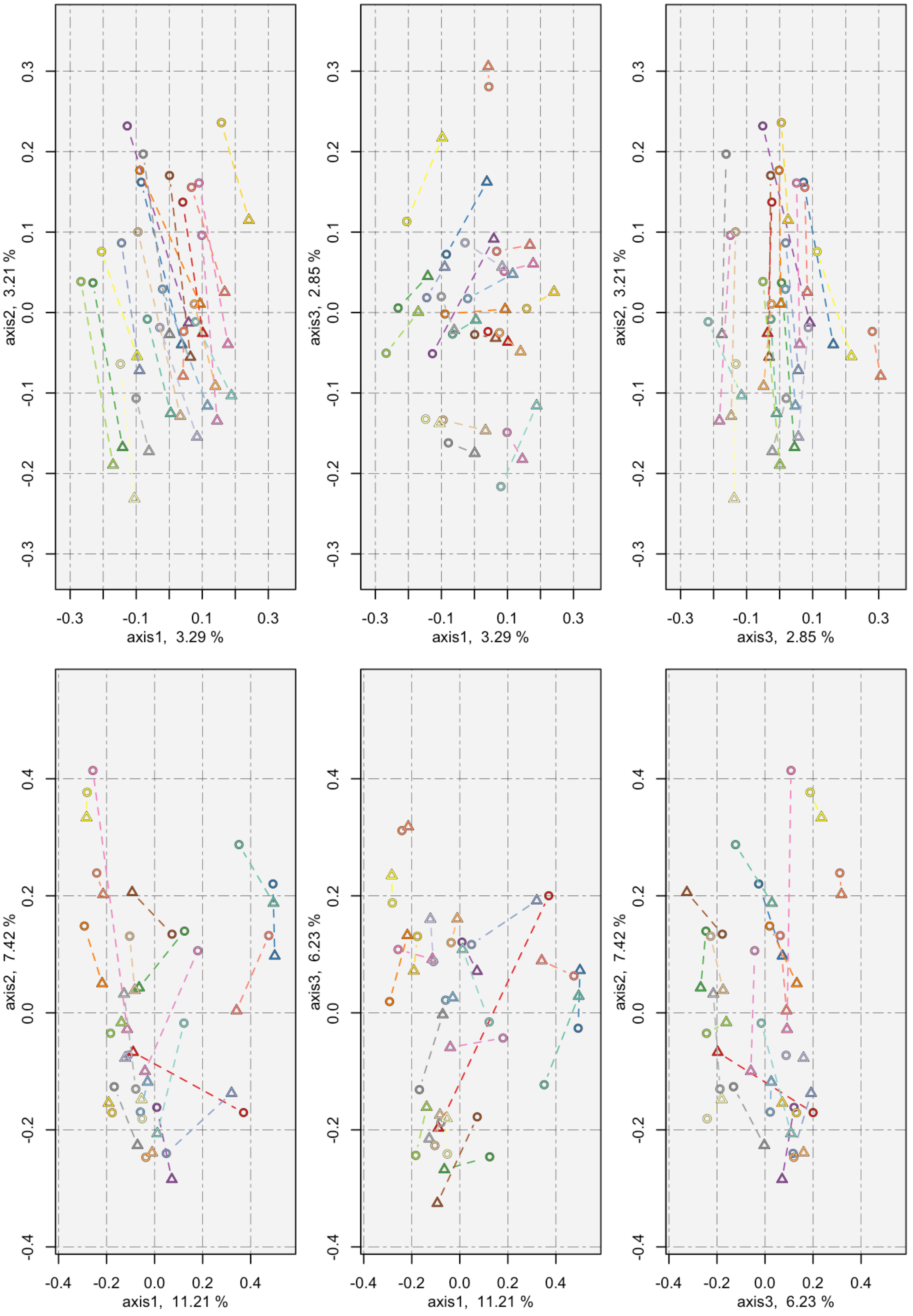
```
#Function to produce plot and PDF of plot
produce_plot <- function(axes_file, metadata_file, loadings_file, PDF_file, SAMPLE
, PAIRED){
  ### Load package or install if not present
  if (!require("RColorBrewer")) {
    install.packages("RColorBrewer")
    library(RColorBrewer)
  }
#Reads in data
plot_data <- read.csv(axes_file,sep="\t", row.names = 1)
metadata <- read.csv(metadata_file,sep="\t", row.names = 1)
loadings_info <- read.csv(loadings_file,sep="\t", row.names = 1)
#Order plot_data and metadata so they are the same order
plot_data <- plot_data[order(row.names(plot_data)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match and stop if not
stopifnot(identical(row.names(plot_data), row.names(metadata)))
#Merge data frames
```

```
plot_data_metadata <- merge(x=plot_data, y=metadata, by="row.names")
#Fix rownames after merge
row.names(plot_data_metadata) <- plot_data_metadata[,1]
plot_data_metadata <- plot_data_metadata[,-1]
#Sets columns to variables
axis1 <- plot_data_metadata[,1]
axis2 <- plot_data_metadata[,2]
axis3 <- plot_data_metadata[,3]
#Set the minimum and maximum x so each plot is the same size as each other for comparison
maxx <- max(c(max(axis1),max(axis2),max(axis3)))
minx <- min(c(min(axis1),min(axis2),min(axis3)))
#Grouping to colour by
group_samples <- as.factor(plot_data_metadata[,SAMPLE])
#Creates plot points for first and second samples
#This data will be used to connect the points together
first_level <- levels(plot_data_metadata[,PAIRED])[1]
second_level <- levels(plot_data_metadata[,PAIRED])[2]
first_rows <- plot_data_metadata[plot_data_metadata[,PAIRED]==first_level,]
second_rows <- plot_data_metadata[plot_data_metadata[,PAIRED]==second_level,]
#Order data to get colouring correct
first_rows <- first_rows[order(first_rows[,SAMPLE]),]
second_rows <- second_rows[order(second_rows[,SAMPLE]),]
first_group_samples <- as.factor(first_rows[,SAMPLE])
second_group_samples <- as.factor(second_rows[,SAMPLE])
#Select colour palette for plot
col.brew <- c(brewer.pal(9, "Set1"),brewer.pal(8,"Set2"),brewer.pal(12,"Set3"))
palette(col.brew)
#Assignment of loading values
axis_1_lab <- paste("axis1, ", round(loadings_info[1,1], digits=2), "%")
axis_2_lab <- paste("axis2, ", round(loadings_info[2,1], digits=2), "%")
axis_3_lab <- paste("axis3, ", round(loadings_info[3,1], digits=2), "%")
#Function to produce single 2d plot
plot_production <- function(nx, ny, xlabel, ylabel){
ps <- 1
  #Make an empty plotting area with axis labels
  plot(0,0, xlab = xlabel, ylab = ylabel, xlim=c(minx-0.05, maxx+0.05), ylim=c(minx-0.05, maxx+0.05), pch=19,col=NA, type='b')
  #Sets colour of background of plot
  rect(par("usr")[1], par("usr")[3], par("usr")[2], par("usr")[4], col = "#f6f6f6")
  #Sets gridlines that match the ticks of the axes
  grid (NULL,NULL, lty = 6, col = "black", lwd=0.3)
  #Add black borders to points
  points(first_rows[,nx], first_rows[,ny], type='p', pch=2, col="black", cex=ps+0.1)
  points(second_rows[,nx], second_rows[,ny], type='p', pch=1, col="black", cex=ps+0.1)
  points(first_rows[,nx], first_rows[,ny], type='p', pch=2, col="black", cex=ps-0.1)
  points(second_rows[,nx], second_rows[,ny], type='p', pch=1, col="black", cex=ps-0.1)
  #This part will plot pair by pair
  #This is so pairs only connect to each other by the dashed lines
  for (i in 1:num_samples){
    n <- i
    points(c(first_rows[n,nx],second_rows[n,nx]), c(first_rows[n,ny],second_rows[n,ny]), type='b', pch=c(2,1), lwd=ps, col=i, cex=ps, lty=2)
  }
}
#Save plot to pdf file
pdf(PDF_file, width = 170/25.4, height = 60/25.4)
#Creates diagram to put PCoA plots into
par(mfrow=c(1,3), oma = c(0.8,0.8,0,0),
    mgp= c(2,1,0), mar=c(3,3,0.5,0.5), cex=0.7)
num_samples <- nrow(first_rows)
# creating the 3 2d plots
plot_production(1,2,axis_1_lab,axis_2_lab)
plot_production(1,3,axis_1_lab,axis_3_lab)
plot_production(3,2,axis_3_lab,axis_2_lab)
dev.off()
}
#Jaccard PCoA plots
#File names of input data
axes_file_name <- "colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.an.unique_list.0.03.pick.jclass.0.03.lt.ave.pcoa.axes"
metadata_file_name <- "16sBxCL_Metadata.txt"
loadings_file_name <- "colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.an.unique_list.0.03.pick.jclass.0.03.lt.ave.pcoa.loadings"
#Name of PDF output file
pdf_file_name <- "SF4A.PCOA_jclass_thetayc_otus.pdf"
#Relevant metadata info
paired <- "Sample.type"
sample <- "Individual"
#Produce plot
produce_plot(axes_file_name, metadata_file_name, loadings_file_name, pdf_file_name, sample, paired)
```

```
## quartz_off_screen
##                 2
```

```
#yue & Clayton PCoA plots
#File names of input data
axes_file_name <- "colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filte
r.unique.precluster.pick.pick.an.unique_list.0.03.pick.thetayc.0.03.lt.ave.pcoa.ax
es"
metadata_file_name <- "16sBxCL_Metadata.txt"
loadings_file_name <- "colonoscopy.makecontigsfile.trim.contigs.good.unique.good.f
ilter.unique.precluster.pick.pick.an.unique_list.0.03.pick.thetayc.0.03.lt.ave.pco
a.loadings"
#Name of PDF output file
pdf_file_name <- "SF4B.PCOA_jclass_thetayc_otus.pdf"
#Relevant metadata info
paired <- "Sample.type"
sample <- "Individual"
#Produce plot
produce_plot(axes_file_name, metadata_file_name, loadings_file_name, pdf_file_name
, sample, paired)
```

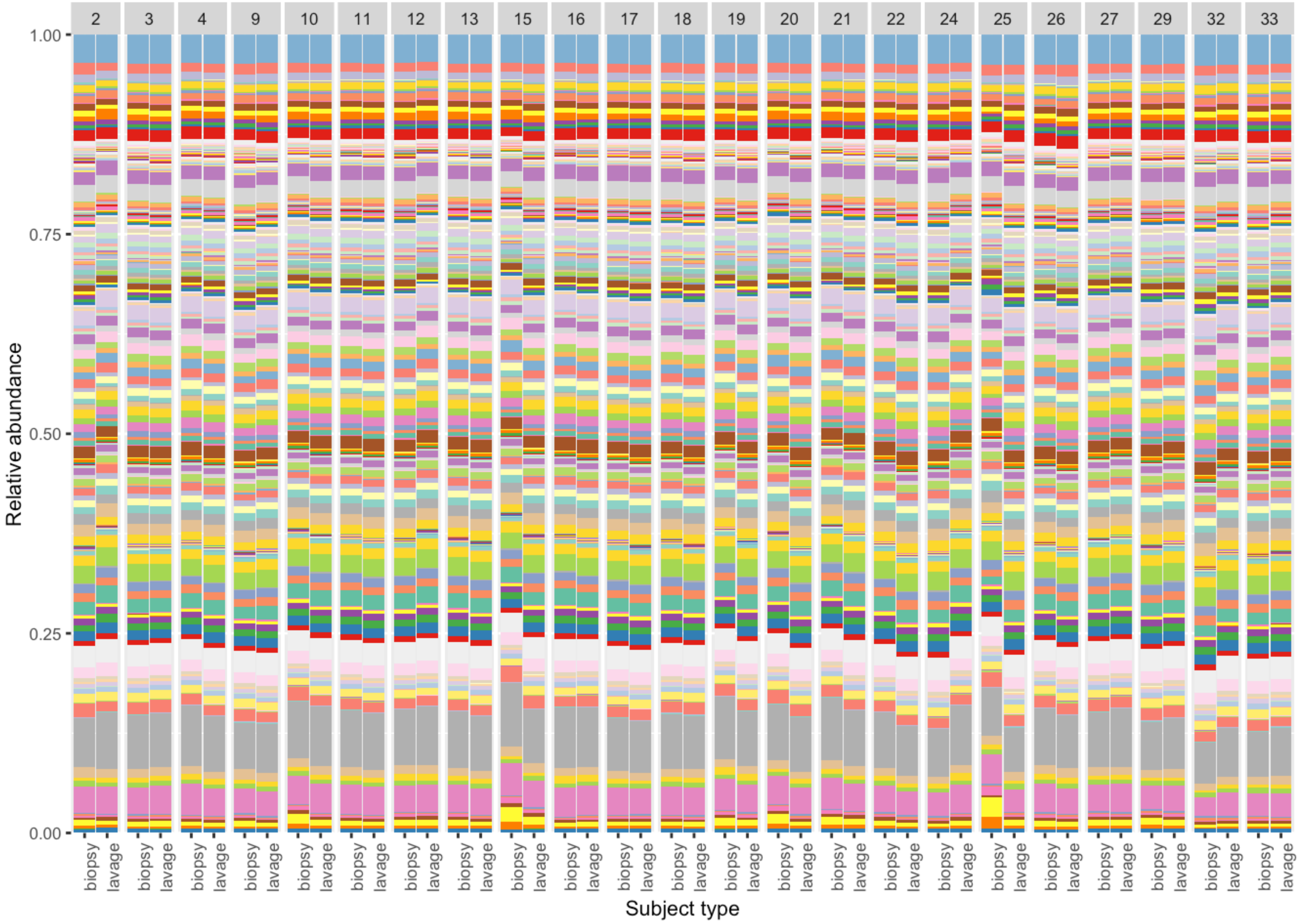
```
## quartz_off_screen
##                 2
```


axis1, 3.29 %
axis2, 3.21 %
axis3, 2.85 %
axis3, 2.85 %
axis2, 3.21 %
-0.3
-0.1
0.1
0.3
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
axis1, 11.21 %
axis2, 7.42 %
axis3, 6.23 %
axis3, 6.23 %
axis2, 7.42 %
-0.4
-0.2
0.0
0.2
0.4

Supplementary figure 5: Predicted KEGG pathway comparison between colonic biopsy and lavage sample

A) KEGG pathway abundance

```
### Load packages or install if not present
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
#File names
taxa_table_file <- "16sBxCL_picrust_predicted_metagenome.KEGG_pathways_L3.relabund
.txt"
metadata_file <- "16sBxCL_Metadata.txt"
PDF_file <- "SF5A.Picrust_kegg_data.pdf"
#Read in files
taxa_data <- read.csv(taxa_table_file, sep = "\t", row.names = 1)
metadata <- read.csv(metadata_file, sep = "\t", row.names = 1)
#Remove empty rows
taxa.no0 <- taxa_data[ rowSums(taxa_data)!=0,]
#Transpose taxa data
taxa.no0.t <- t(taxa.no0)
#Check if heatmap_info and metadata_info are in the same order
taxa.no0.t <- taxa.no0.t[order(row.names(taxa.no0.t)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match
stopifnot(identical(row.names(taxa.no0.t), row.names(metadata)))
#Merge data
plot_data_metadata <- merge(x=taxa.no0.t, y=metadata, by="row.names")
#Change data to long list format
plot_data_metadata_long <- gather(plot_data_metadata, Taxa, Measure, (2:281))
#Select colour palette for plot
colset <- rep (c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set
3"), brewer.pal(9, "Pastel1")), 10)
#Plot the data
g_bar <- ggplot(plot_data_metadata_long, aes(x =new.Sample.type, y=Measure, fill=T
axa)) + geom_bar(stat = 'identity', position = 'fill', width=0.95) + facet_wrap( ~
Individual, nrow=1) + theme_set(theme_grey(base_size = 8)) + theme(legend.position
="none", panel.margin = unit(0.1, "lines"), axis.text.x = element_text(angle = 90,
hjust = 1)) + scale_fill_manual(values = colset) + xlab("Subject type") + ylab("Re
lative abundance") + scale_y_continuous(expand = c(0,0)) + geom_text(aes(x=1, y=1.
00, label="Stretch it"), vjust=-1)
ggsave(PDF_file, g_bar, units="mm", height=105, width=170, dpi=300)
```



B) Alpha diversity scores

```
### Load packages or install if not present
if (!require("RColorBrewer")) {
  install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
#Name of input files
alpha_file="16sBxCL.KEGG_pathways.shared.groups.ave-std.summary"
metadata_file="16sBxCL_Metadata.txt"
#name of output PDF file
pdf_file_box_plot="SF5B.Picrust_kegg_data.pdf"
#Read in data and manipulate
alpha_data <- read.csv(alpha_file,sep="\t")
alpha_data <- alpha_data[alpha_data$method == "ave",]
rownames(alpha_data) <- alpha_data$group
#Keep relevant columns
alpha_data <- alpha_data[,c("sobs", "chao", "shannon", "invsimpson", "coverage")]
#Rename columns
colnames(alpha_data) <- c("KEGG Pathways", "Chao","Shannon-Weiner", "Inverse Simps
on", "Coverage")
#To determine order of alpha diversity for plot
alpha_order <- c("KEGG Pathways", "Chao","Shannon-Weiner", "Inverse Simpson", "Cov
erage")
#Function to manipulate data
produce_plot <- function(ALPHA_DATA, METADATA_FILE, ORDER_ALPHA){
### Load package or install if not present
if (!require("tidyr")) {install.packages("tidyr")
    library(tidyr)}
#Read in Metadata file
metadata <- read.csv(METADATA_FILE,sep="\t", row.names = 1)
#Order plot_data and metadata so they are the same order
alpha_data_t <- ALPHA_DATA[order(row.names(ALPHA_DATA)),]
metadata <- metadata[order(row.names(metadata)),]
#Check if row names match and stop if not
stopifnot(identical(row.names(alpha_data_t), row.names(metadata)))
#Merge data frames
plot_data_metadata <- merge(x=alpha_data_t, y=metadata, by="row.names")
#Fix rownames after merge
row.names(plot_data_metadata) <- plot_data_metadata[,1]
colnames(plot_data_metadata)[1] <- "Sample_ID"
#Convert to long
alpha_long <- gather(plot_data_metadata, Alpha_diversity_measure, value, 2:(ncol(A
LPHA_DATA)+1))
#Reorder measures
alpha_long$Alpha_diversity_measure_f <- factor(alpha_long$Alpha_diversity_measure,
levels=ORDER_ALPHA)
return(alpha_long)
}
#Function to manipulate data to make it ready for ggplot
alpha_long <- produce_plot(alpha_data, metadata_file, alpha_order)

#Mann whitney u test
alpha_long_t <- alpha_long[,-11]
mann_whitney_data <- spread(alpha_long_t, Alpha_diversity_measure_f, value)
colnames(mann_whitney_data)[13:16] <- c("KEGG", "Chao", "ShannonWeiner", "InvSimps
on")
wilcox.test(KEGG ~ new.Sample.type, data=mann_whitney_data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  KEGG by new.Sample.type
## W = 405, p-value = 0.001632
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(Chao ~ new.Sample.type, data=mann_whitney_data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  Chao by new.Sample.type
## W = 441, p-value = 4.988e-05
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(ShannonWeiner ~ new.Sample.type, data=mann_whitney_data)
```

```
##
##  Wilcoxon rank sum test
##
## data:  ShannonWeiner by new.Sample.type
## W = 256, p-value = 0.8618
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(InvSimpson ~ new.Sample.type, data=mann_whitney_data)
```

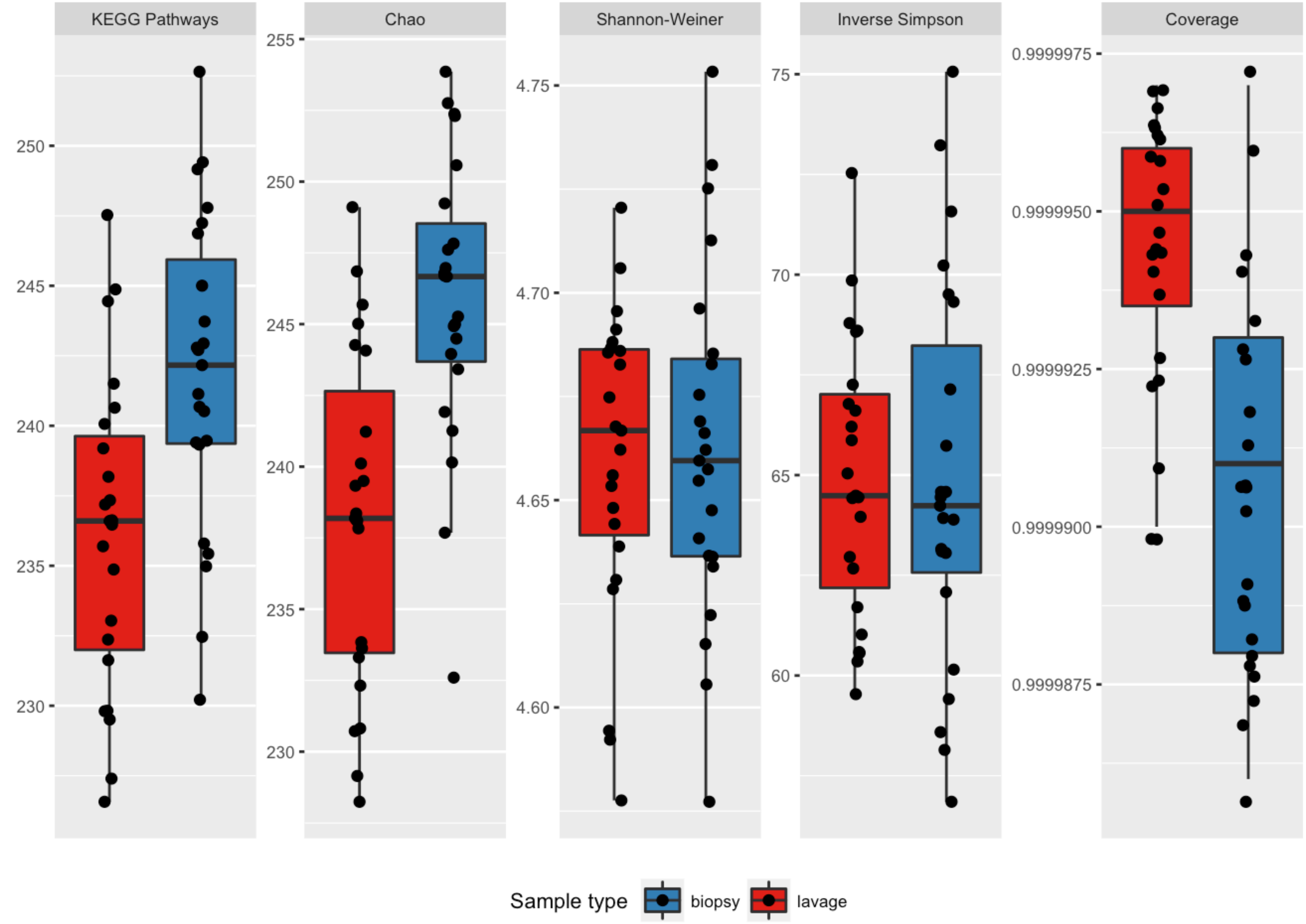
```
##
##  Wilcoxon rank sum test
##
## data:  InvSimpson by new.Sample.type
## W = 253, p-value = 0.8109
## alternative hypothesis: true location shift is not equal to 0
```

```
wilcox.test(Coverage ~ new.Sample.type, data=mann_whitney_data)
```

```
## Warning in wilcox.test.default(x = c(0.999991, 0.99999, 0.999991,
## 0.999987, : cannot compute exact p-value with ties
```

```
##
##  Wilcoxon rank sum test with continuity correction
##
## data:  Coverage by new.Sample.type
## W = 87, p-value = 9.222e-05
## alternative hypothesis: true location shift is not equal to 0
```

```
#Select colour palette for plot
col.brew <- c(brewer.pal(9, "Set1"),brewer.pal(8,"Set2"),brewer.pal(12,"Set3"))
#Box Plot for alpha diversity comparing aspirate and biopsy
g_box <- ggplot(alpha_long, aes(x=Sample.type, y=value, fill=new.Sample.type)) + geom_boxplot(outlier.colour = NA) + theme_set(theme_gray(base_size = 8)) + facet_wrap(~ Alpha_diversity_measure_f, nrow=1, scales="free") + geom_point(position = position_jitter(width = 0.2)) + scale_x_discrete(breaks=NULL, name="") + scale_fill_manual(values=c(col.brew[2], col.brew[1]), name="Sample type") + scale_y_continuous(name="") + theme_grey(base_size = 8) + theme(legend.position="bottom", legend.margin=unit(0,"cm"))
ggsave(pdf_file_box_plot, g_box, units="mm", height=75, width=170, dpi=300)
```



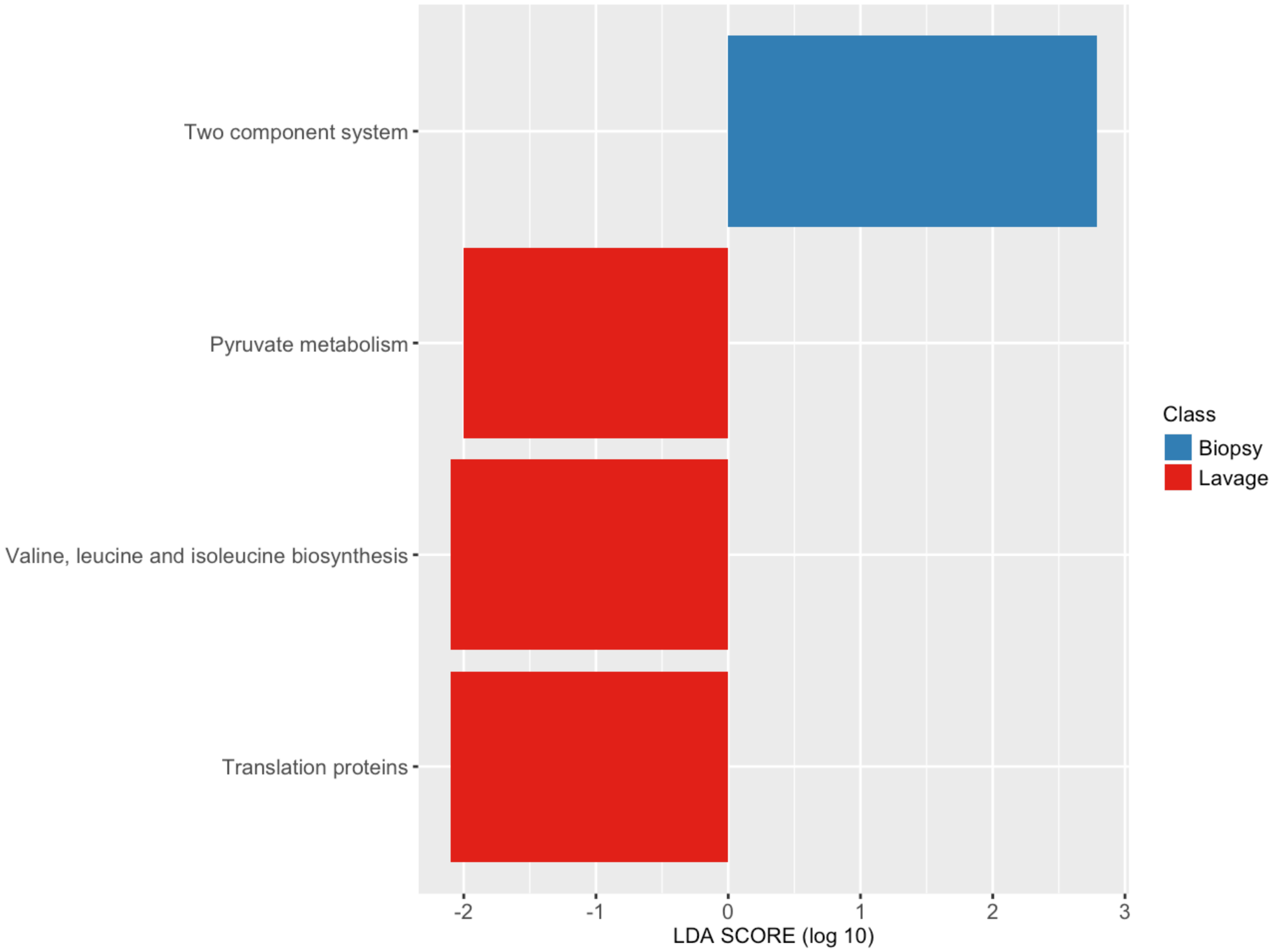
C) LEfSe LDA scores

```
### Load packages or install if not present
if (!require("gplots")) {install.packages("gplots")
  library(gplots)}
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
#read in data and manipulate
plot_data <- read.csv("16sBxCL_LEfSe_kegg_pathway_discovered_biomarkers.txt", sep=
"\t", header=FALSE)
```

```
## Warning in read.table(file = file, header = header, sep = sep,
## quote = quote, : incomplete final line found by readTableHeader on
## '16sBxCL_LEfSe_kegg_pathway_discovered_biomarkers.txt'
```

```
colnames(plot_data) <- c("KEGG", "LogMaxMean", "Class", "LDA", "pvalue", "new.Clas
s")
plot_data$KEGG <- factor(plot_data$KEGG, levels=plot_data$KEGG[order(plot_data$LDA
)])
#Produce plot
g <- ggplot(plot_data, aes(x=KEGG, y=LDA, fill=new.Class)) + geom_bar(stat="identi
ty", position="identity") + theme_set(theme_gray(base_size = 8)) + coord_flip() +
ylab("LDA SCORE (log 10)") + xlab("") + scale_fill_manual(name="Class", values = c
("#377EB8","#E41A1C"), breaks=c("Biopsy", "Lavage"), labels=c("Biopsy", "Lavage"))
+ theme(text= element_text(size=8), axis.text.y  = element_text(size=8), axis.text
.x  = element_text(size=8), legend.text= element_text(size = 8), legend.key.size =
unit(4, "mm"), plot.margin=unit(c(1,1,1,1),"mm"))
ggsave("SF5C.Picrust_kegg_data.pdf", g, units="mm", height=50, width=170, dpi=300)
```

```
## Warning in read.table(file = file, header = header, sep = sep,
## quote = quote, : incomplete final line found by readTableHeader on
## '16sBxCL_LEfSe_kegg_pathway_discovered_biomarkers.txt'
```



Supplementary figure 6: Average relative abundance of genera within biopsy and lavage samples matching contaminant genera from

Salter et al 2014

```
### Load packages or install if not present
if (!require("RColorBrewer")) {install.packages("RColorBrewer")
  library(RColorBrewer)}
if (!require("ggplot2")) {install.packages("ggplot2")
  library(ggplot2)}
if (!require("tidyr")) {install.packages("tidyr")
  library(tidyr)}
#Input file names
taxa_table_file <- "16sBxCL_contamination_genera_bar_chart.txt"
#Set name of PDF output file
PDF_file <- "SF6.Average_relabund_of_genera_withitn_biopsy_and_lavage_smaples_matc
hing_contaminant_genera_from_Salter et al 2014.170x140mm.pdf"
#Read in files
taxa_data <- read.csv(taxa_table_file, sep = "\t")
```

```
## Warning in read.table(file = file, header = header, sep = sep,
## quote = quote, : incomplete final line found by readTableHeader on
## '16sBxCL_contamination_genera_bar_chart.txt'
```

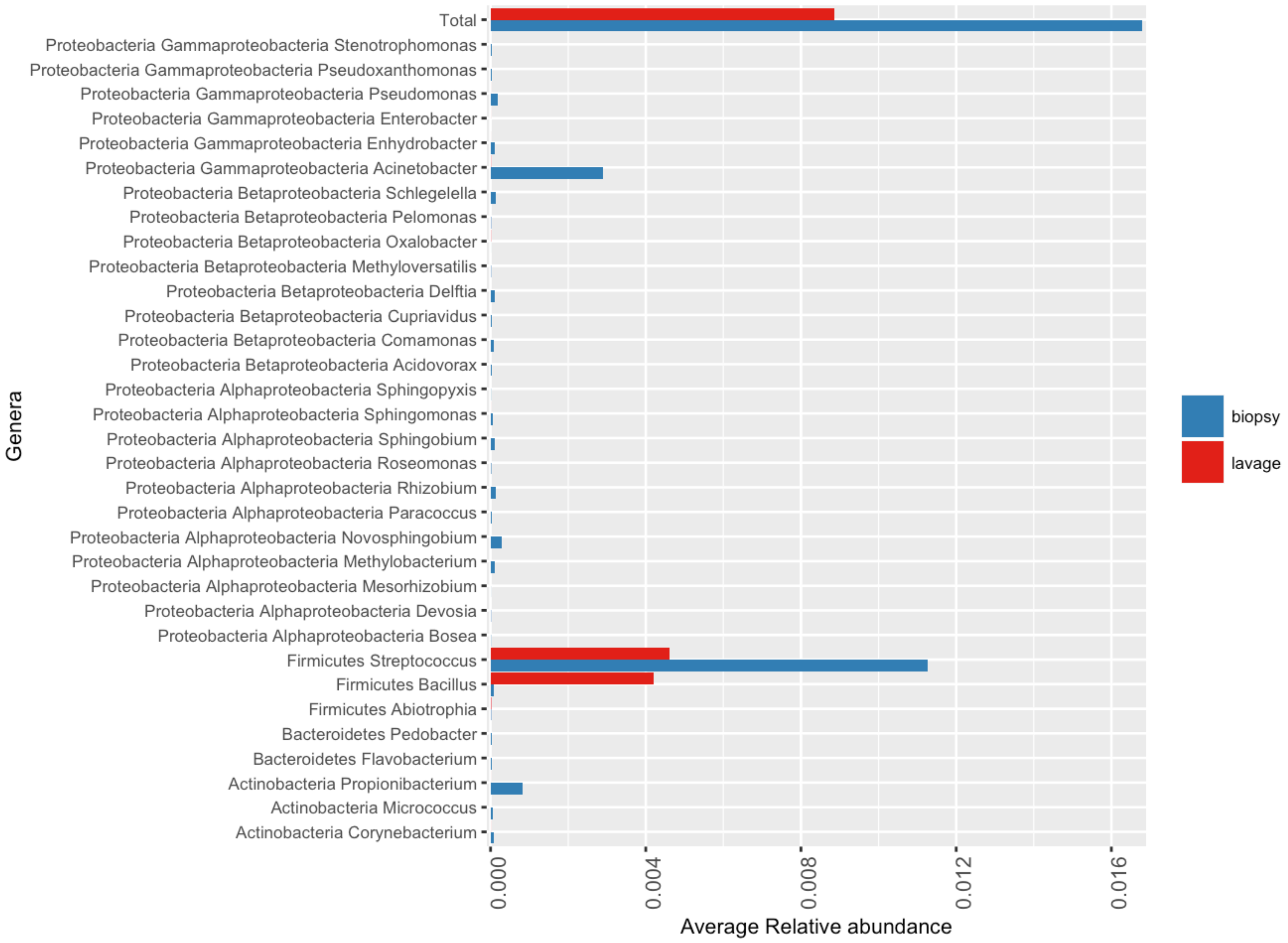
```
#Get ID info
ID.info <- taxa_data[3,]
#Only relabund info
relabund.info <- taxa_data[1:2,]
#Change data to long list format
relabund.info_long <- gather(relabund.info, Genera, relabund, (2:(ncol(relabund.in
fo))))
```

```
## Warning: attributes are not identical across measure variables; they will
## be dropped
```

```
#Change . in genera names to space
relabund.info_long$Genera <- gsub('([[:punct:]])|\\s+', ' ', relabund.info_long$Ge
nera)
#Select colour palette for plot
colset <- c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set3"),
brewer.pal(9, "Pastel1"))
#Plot the data
g_bar <- ggplot(relabund.info_long, aes(x=Genera, y=(as.numeric(relabund)), fill=S
ample.type)) + geom_bar(stat = 'identity', position = 'dodge', width=0.95) + scale
_fill_manual(values = c(colset[2], colset[1])) + theme_set(theme_gray(base_size =
8)) + theme(axis.text.x = element_text(angle = 90, hjust = 1, size=8)) + xlab("Gen
era") + ylab("Average Relative abundance") + scale_y_continuous(expand = c(0,0.000
1)) + labs(fill='') + coord_flip()
ggsave(PDF_file, g_bar, units="mm", height=140, width=170, dpi=300)
```

```
## Warning in read.table(file = file, header = header, sep = sep,
## quote = quote, : incomplete final line found by readTableHeader on
## '16sBxCL_contamination_genera_bar_chart.txt'
```

```
## Warning: attributes are not identical across measure variables; they will
## be dropped
```


Total
Proteobacteria Gammaproteobacteria Stenotrophomonas
Proteobacteria Gammaproteobacteria Pseudoxanthomonas
Proteobacteria Gammaproteobacteria Pseudomonas
Proteobacteria Gammaproteobacteria Enterobacter
Proteobacteria Gammaproteobacteria Enhydrobacter
Proteobacteria Gammaproteobacteria Acinetobacter
Proteobacteria Betaproteobacteria Schlegelella
Proteobacteria Betaproteobacteria Pelomonas
Proteobacteria Betaproteobacteria Oxalobacter
Proteobacteria Betaproteobacteria Methyloversatilis
Proteobacteria Betaproteobacteria Delftia
Proteobacteria Betaproteobacteria Cupriavidus
Proteobacteria Betaproteobacteria Comamonas
Proteobacteria Betaproteobacteria Acidovorax
Proteobacteria Alphaproteobacteria Sphingopyxis
Proteobacteria Alphaproteobacteria Sphingomonas
Proteobacteria Alphaproteobacteria Sphingobium
Proteobacteria Alphaproteobacteria Roseomonas
Proteobacteria Alphaproteobacteria Rhizobium
Proteobacteria Alphaproteobacteria Paracoccus
Proteobacteria Alphaproteobacteria Novosphingobium
Proteobacteria Alphaproteobacteria Methylobacterium
Proteobacteria Alphaproteobacteria Mesorhizobium
Proteobacteria Alphaproteobacteria Devosia
Proteobacteria Alphaproteobacteria Bosea
Firmicutes Streptococcus
Firmicutes Bacillus
Firmicutes Abiotrophia
Bacteroidetes Pedobacter
Bacteroidetes Flavobacterium
Actinobacteria Propionibacterium
Actinobacteria Micrococcus
Actinobacteria Corynebacterium
Genera
Average Relative abundance
biopsy
lavage