

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.unique.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 diveristy values
colnames(plot_data_metadata)[1:5] <- c("Observed OTUs", "Chao","Shannon-Weiner","Inverse Simpson", "Coverage")
#Convert to long list format
alpha_long <- gather(plot_data_metadata, Alpha_diveristy_measure, value, 1:5)
#Reorder measures
alpha_long$Alpha_diveristy_measure_f <- factor(alpha_long$Alpha_diveristy_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)) + geom_boxplot(outlier.colour = NA) + theme_set(theme_gray(base_size = 8)) +facet_wrap(~ Alpha_diveristy_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(fa_b_colour[2], fa_b_colour[1]), name="Sample type") + scale_y_continuous(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
```

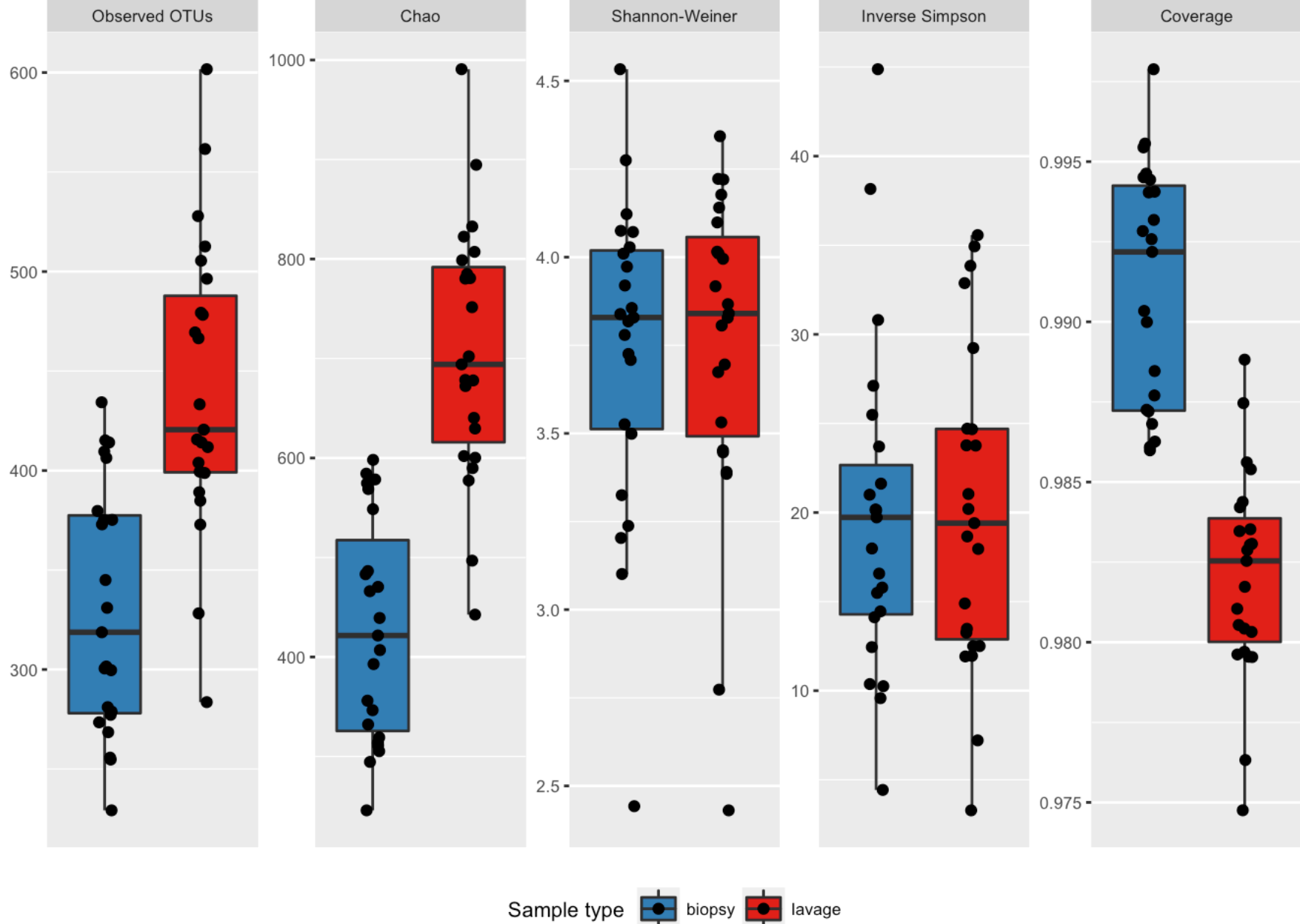


Figure 2: Relative abundance at phylum level for colonic biopsy and lavage samples.

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, "Pastell"))
#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
```



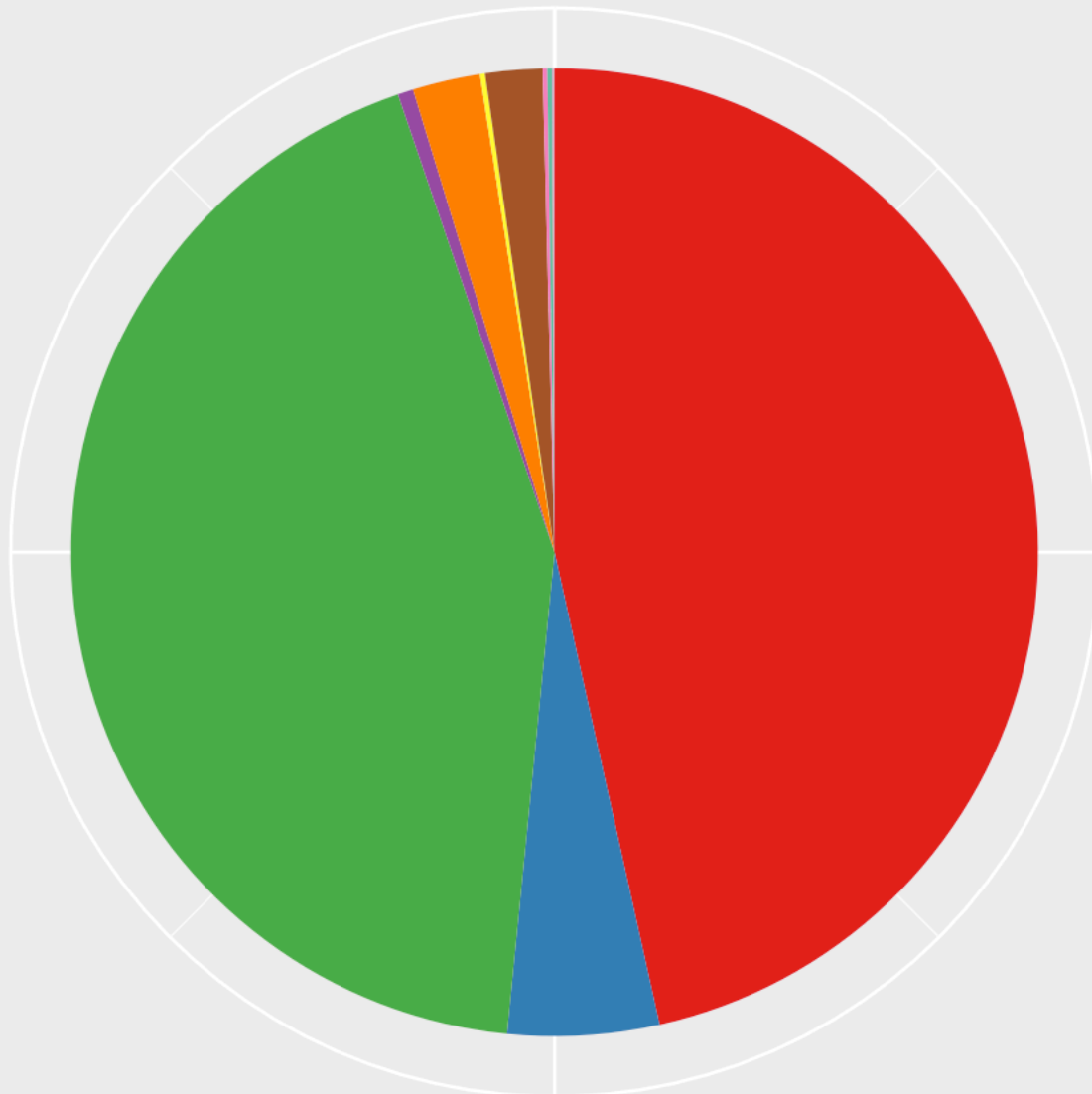
B) Collective pie chart representation.

```

#### 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", sep="\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, "Pastell"))
#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 = element_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_lavage_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 = element_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_lavage_samples.pdf",
      g_pc_2, units="cm", height=8.5, width=8.5, dpi=300)

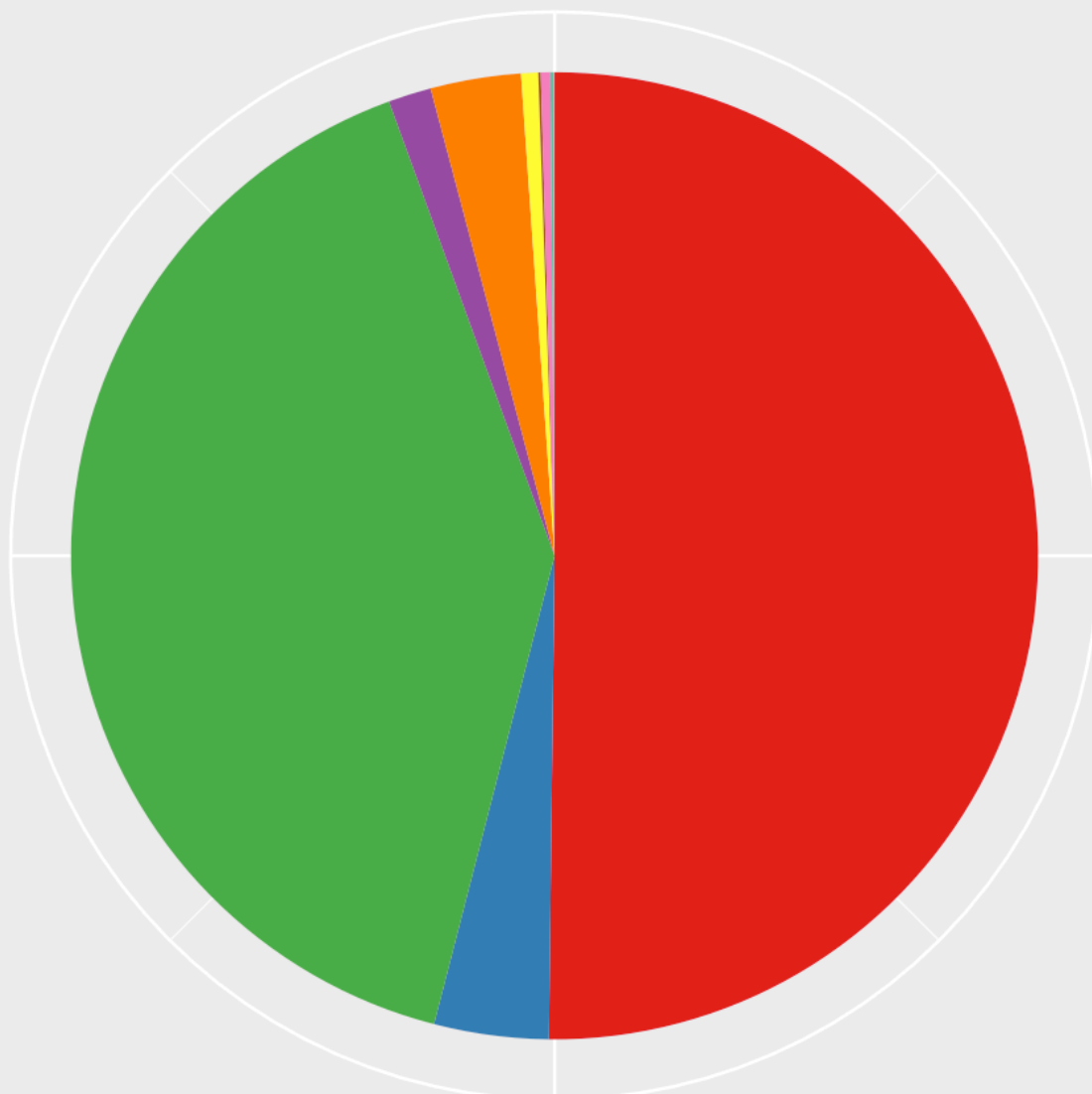
```


mm



Biopsy

mm



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 participant 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$Sample_type
#Getting colours from colour brewer
brewer_colours <- brewer.pal(9, "Set1")
#Check if heatmap_info and metadata_info are in the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
##Setting colours to sample_types
sample_type_colours <- gsub("FA", brewer_colours[1], sample_info)
sample_type_colours <- gsub("Bx", brewer_colours[2], sample_type_colours)
#Changing name of BD1.5 to candidate_division_BD1-5
colnames(heatmap_info)[14] <- "Candidate_division_BD1-5"
#Chanigng name of Deinococcus.Thermus to Deinococcus Thermus
colnames(heatmap_info)[20] <- "Deinococcus Thermus"
#Changing
#Transpoing heatmap_info
heatmap.info.t <- t(heatmap_info)
#producing heatmap
pdf("Fig3.Colour_by_sample_type.Distribution_of_bacteria_in_colonic_and_lavage_samples_at_phylum_level.pdf", width = 170/25.4, height = 114/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=sample_type_colours, margins = c(2,7), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(heat.colors(x)), labRow = row.names(heatmap.info.t), labCol = metadata_info$new.Sample.ID, cexRow = 0.65, cexCol = 0.65, offsetRow=0.000001, offsetCol = 0.000001, key.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
```

```
pdf("Fig3.Colour_by_participant.Distribution_of_bacteria_in_colonic_and_lavage_samples_at_phylum_level.pdf", width = 170/25.4, height = 114/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=as.character(metadata_info$Colour), margins = c(2,7), key.xlab="Log2count", key.title=NA, trace="none", col=function(x)rev(heat.colors(x)), labRow = row.names(heatmap.info.t), labCol = metadata_info$new.Sample.ID, cexRow = 0.65, cexCol = 0.65, offsetRow=0.000001, offsetCol = 0.000001, key.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
```

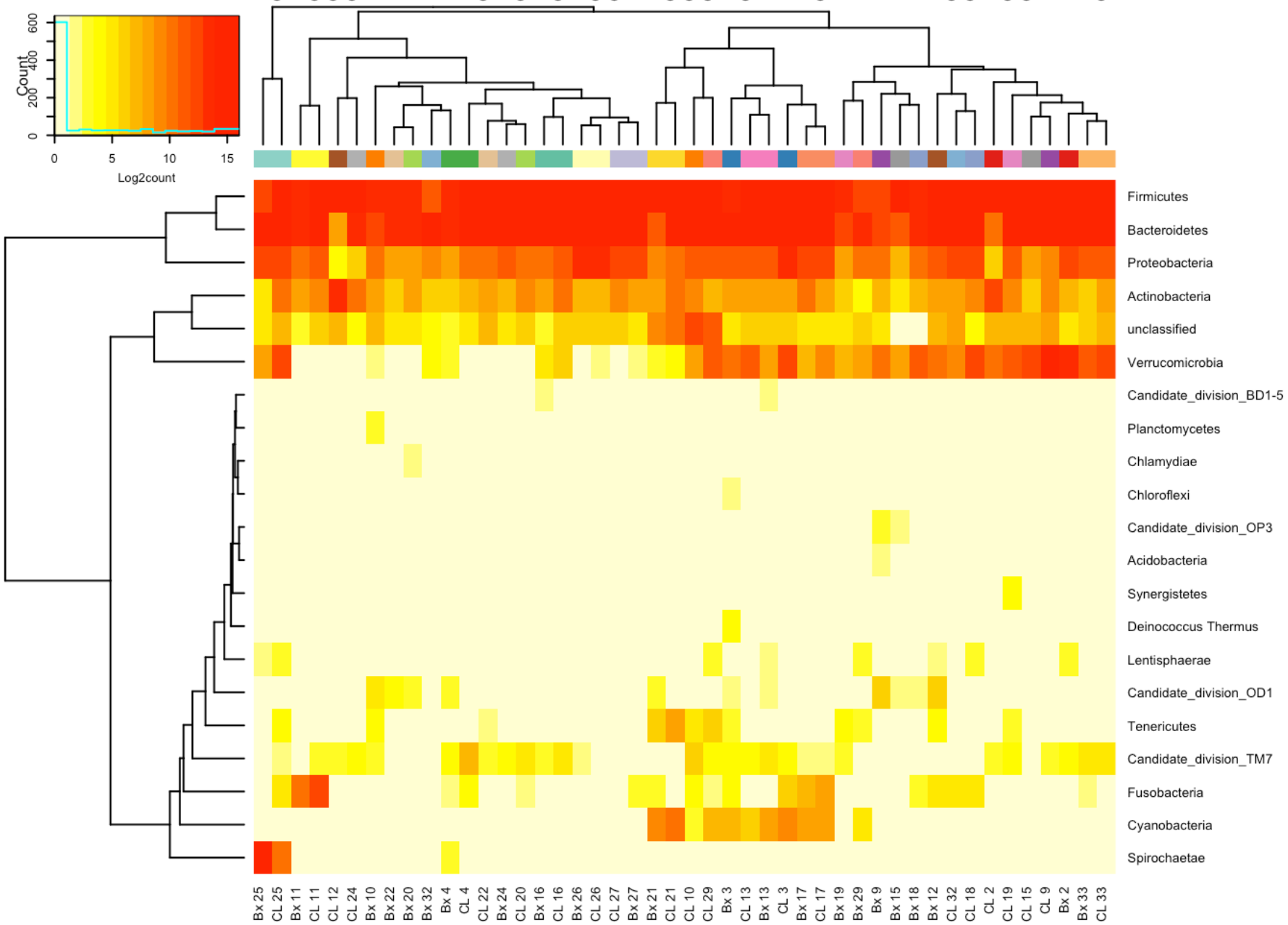
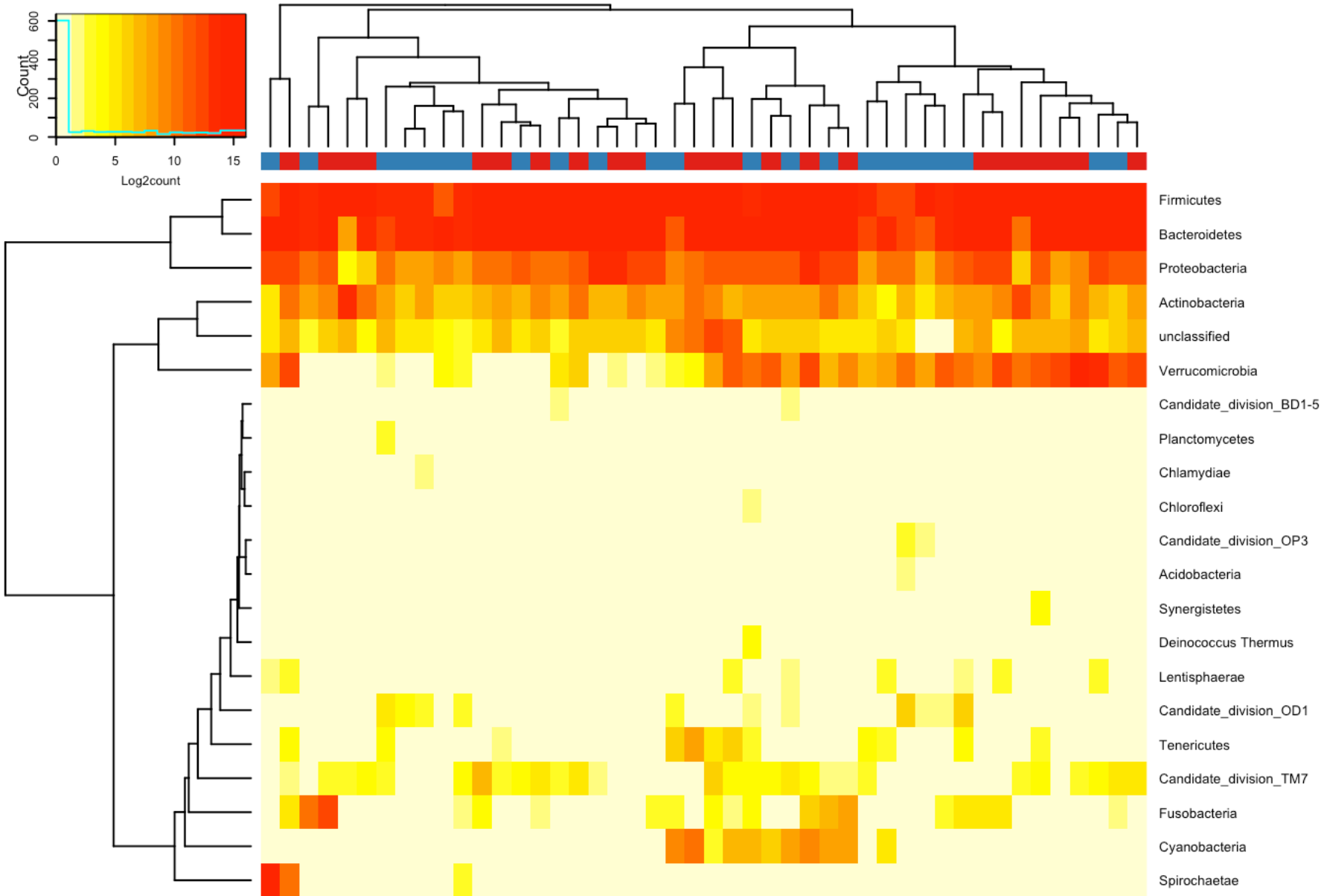


Figure 4: The distribution of bacteria in colonic biopsy and lavage samples at Family level

2 heatmaps produced to give the colours for sample type and participant 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$Sample.type
#Selecting a colour palette to be used in figure
brewer_colours <- brewer.pal(9, "Set1")
##Order heatmap_info and metadata so they are the same order
heatmap_info <- heatmap_info[order(row.names(heatmap_info)),]
metadata_info <- metadata_info[order(row.names(metadata_info)),]
#Check if row names match
stopifnot(identical(row.names(metadata_info), row.names(heatmap_info)))
#Setting colours to sample types
sample_type_colours <- gsub("aspirate",brewer_colours[1], sample_info)
sample_type_colours <- gsub("biopsy", brewer_colours[2], sample_type_colours)
#Transposing heatmap_info
heatmap.info.t <- t(heatmap_info)
#producing heatmaps
pdf("Fig4.Colour_by_sample_type.Distribution_of_bacteria_in_colonic_and_lavage_samples_at_family_level.pdf", 170/25.4,height=182.4/25.4)
heatmap.2(as.matrix(heatmap.info.t), ColSideColors=sample_type_colours,margins = c(2,8),key.xlab="Log2count", key.title=NA,trace="none", col=function(x)rev(heat.colors(x)), labRow = row.names(heatmap.info.t), cexRow = 0.55, cexCol = 0.55, offsetRow=0.0000001, offsetCol = 0.0000001, labCol = metadata_info$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
```

```
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
```


Figure 5: Spearman 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.filter.unique.precluster.pick.pick.an.unique_list.0.03.pick.shared", sep="\t", row.names = 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$new.Sample.ID
#Order by new row names
otu_info <- otu_info[order(row.names(otu_info)),]
#Transpose otu_info
trans_otu_info <- t(otu_info)
#Change Total OTU amounts into relative abundance
relabund_otu_info <- prop.table(trans_otu_info, margin=2)
#Caluclate spearman values
data_rcorr <- rcorr(as.matrix(trans_otu_info), type = "pearson")
spearman_cor <- data_rcorr$r
spearman_p <- data_rcorr$P
#Keep info interested in
subset_spearman_cor <- spearman_cor[1:23,24:46]
subset_spearman_p <- spearman_p[1:23,24:46]
#Correlation plot of FA samples against bx samples
pdf("Fig5.pearson_correlation_plot_of_biopsy_against_lavage_smamples_using_otu_counts.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_spearman_cor),type="full", mar=c(0,0,0,0), cl.pos="b",
          p.mat=subset_spearman_p, sig.level = 0.05, insig="blank")
dev.off()

```

```

## quartz_off_screen
##                2

```

```

## [1] TRUE

```

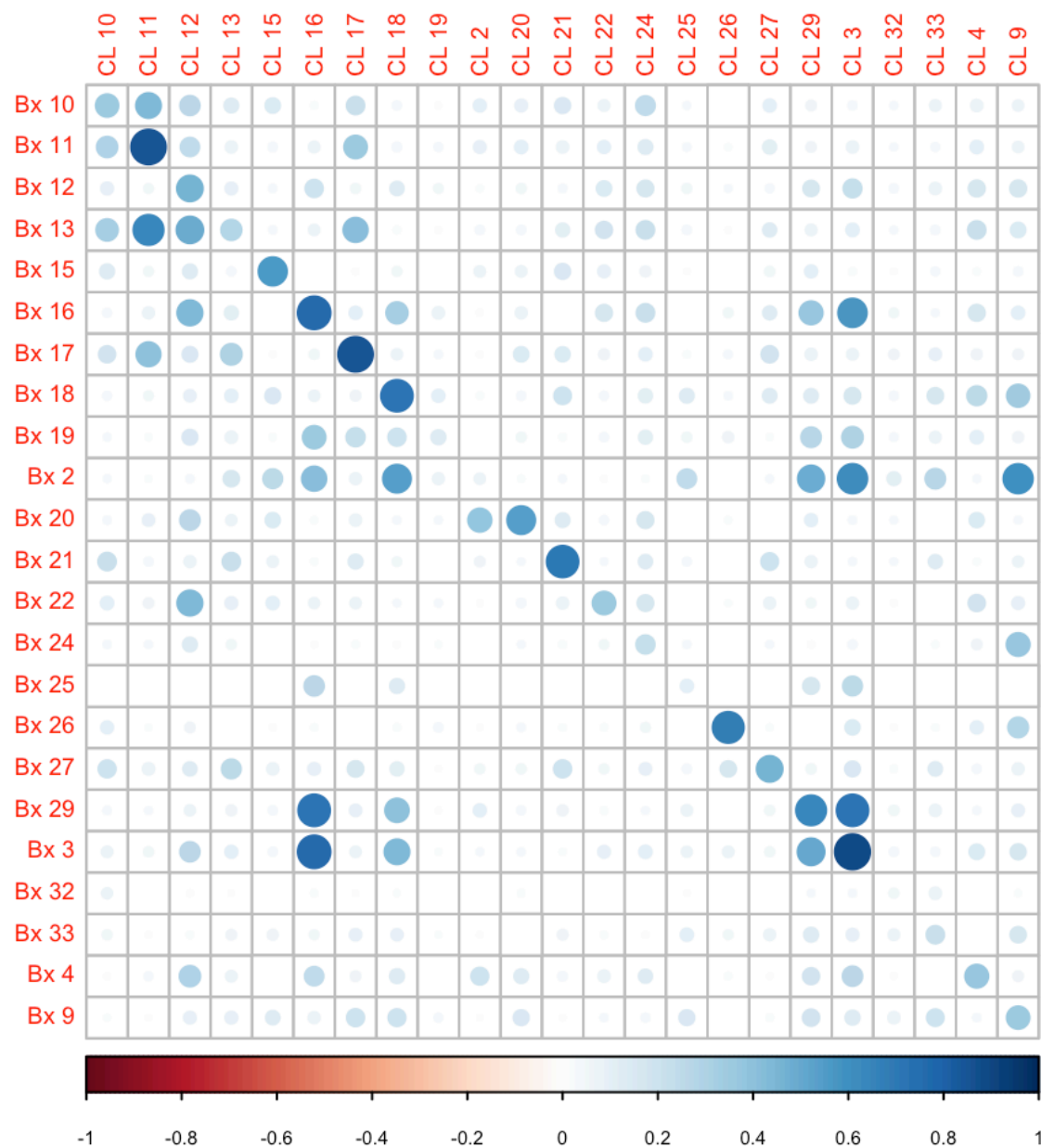


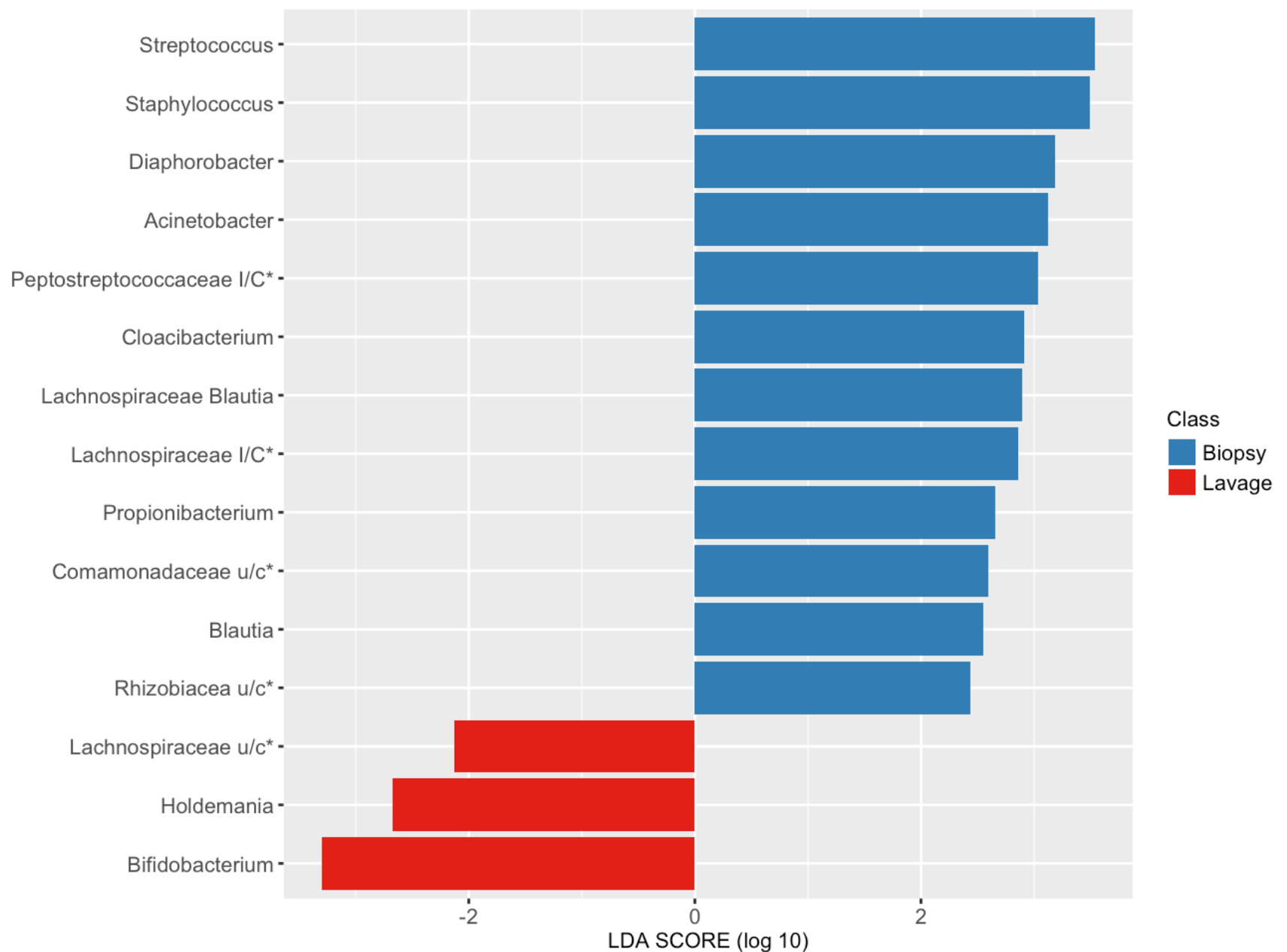
Figure 6: Differentially abundant genera between biopsy and lavage samples by LefSe

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="identity", 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.size = 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

```

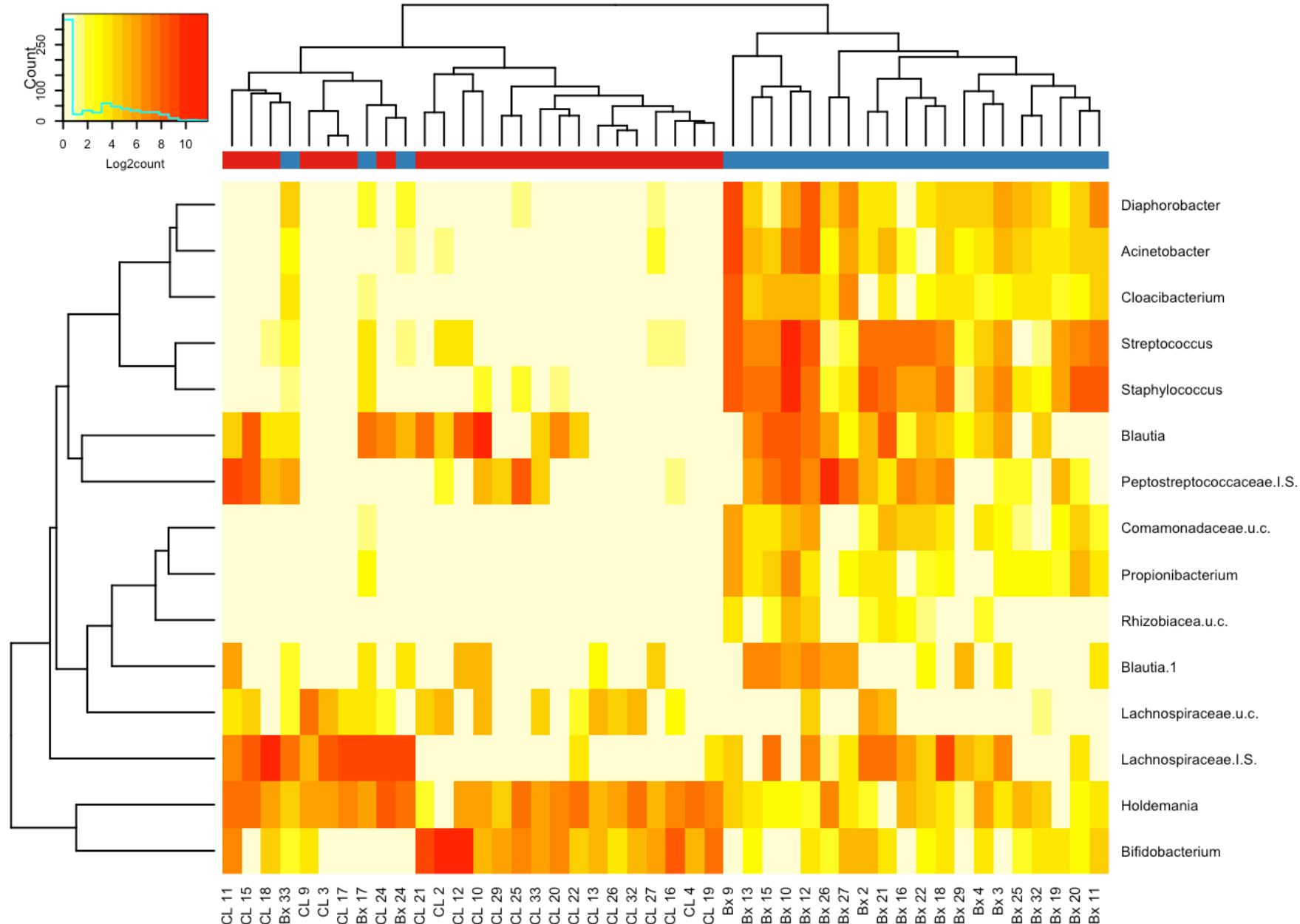


Figure 7: Heat map of Log2count of top 50 OTUs found to not be differentially abundant between biopsy and lavage samples by LefSe

```

#### 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

```

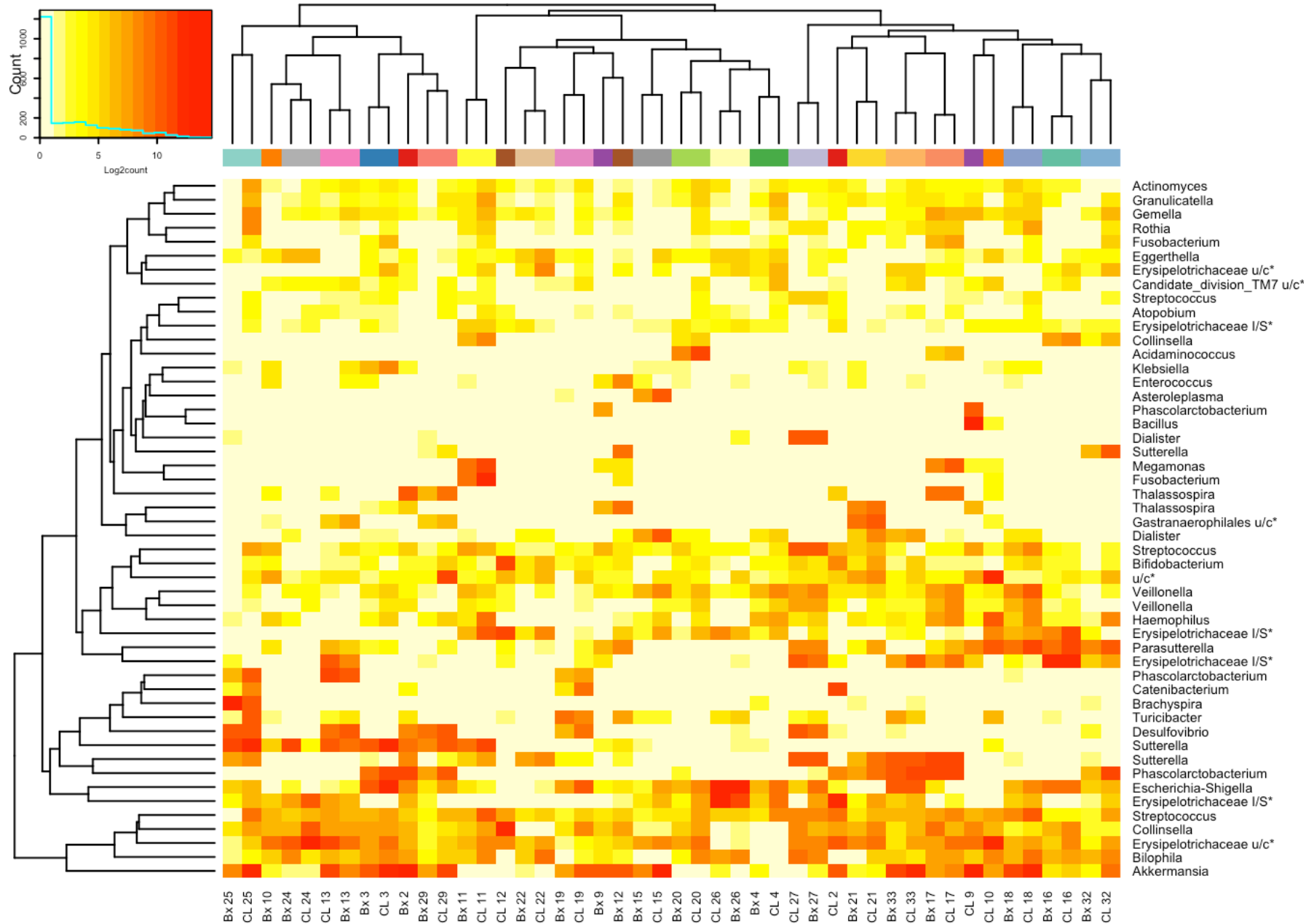



Figure 8: Clustering of samples' PICRUSt predicted KEGG pathways according to sample type (colonic biopsy and lavage) by NMDS, based on Yue & Clayton similarity distance.

```
#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
```

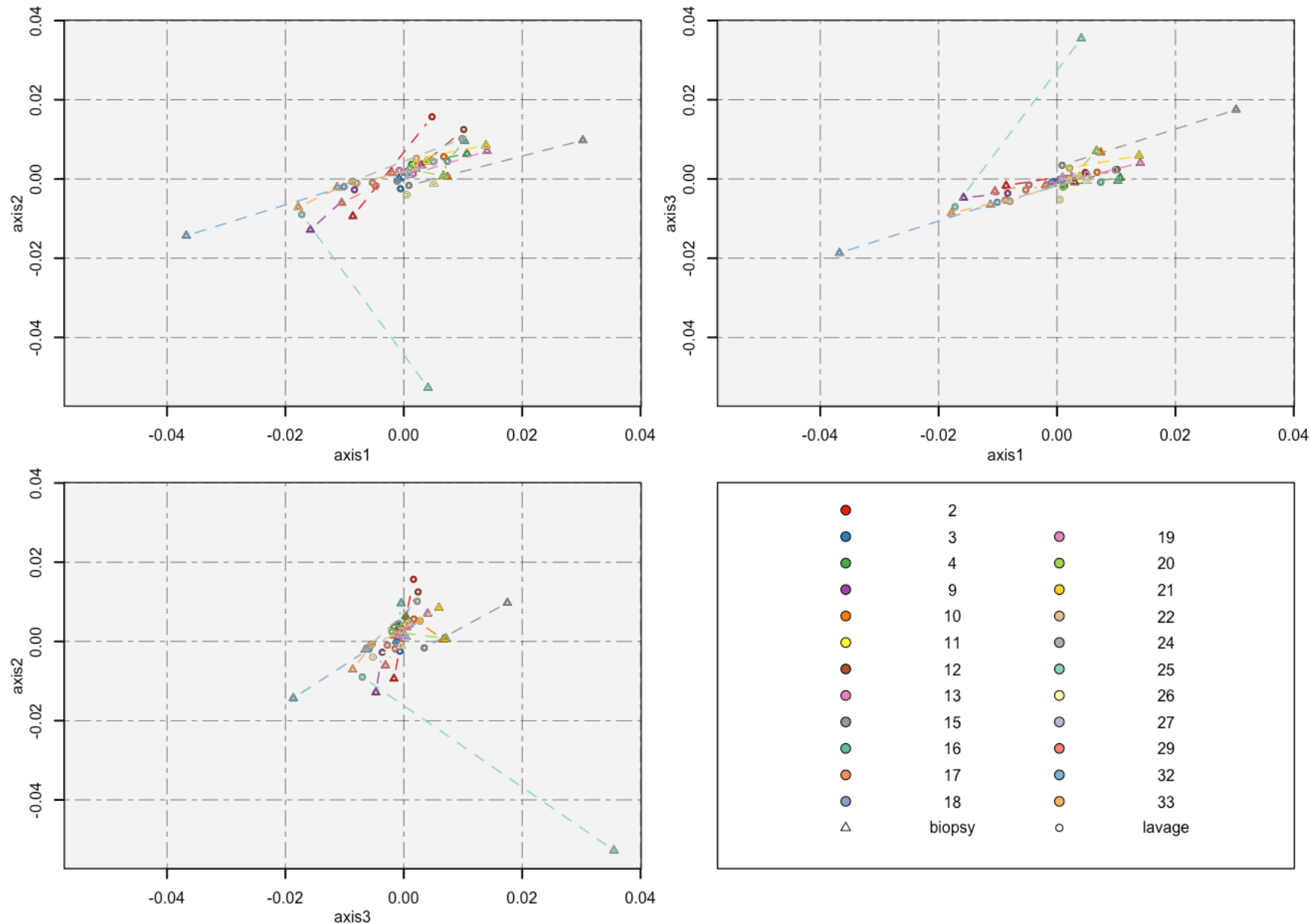
```

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 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])
#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(minx-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

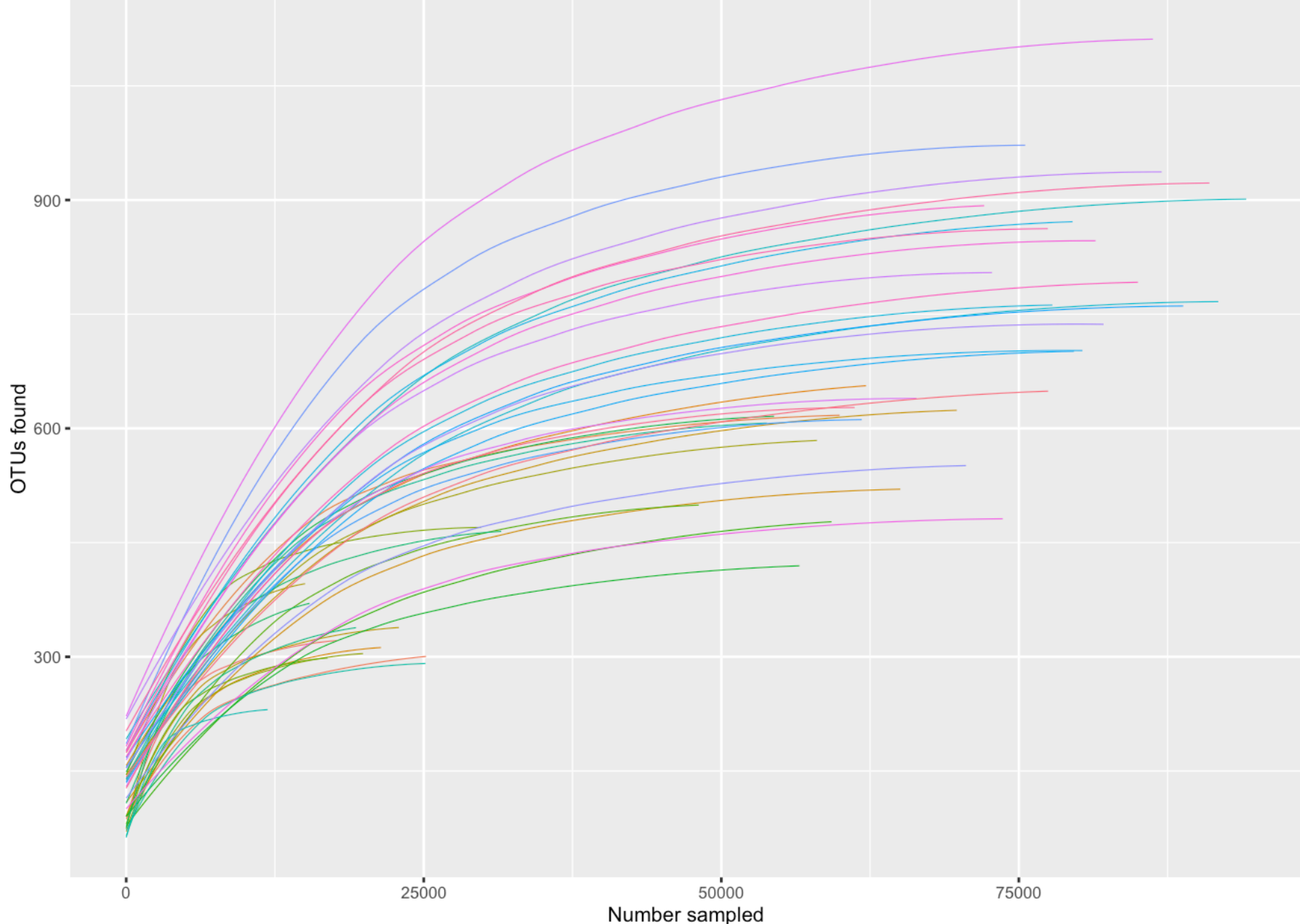
```

#### 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, "Pastell"))
#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(min
x-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.filte
r.unique.precluster.pick.pick.an.unique_list.0.03.pick.jclass.0.03.lt.ave.pcoa.axe
s"
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.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)

```

```

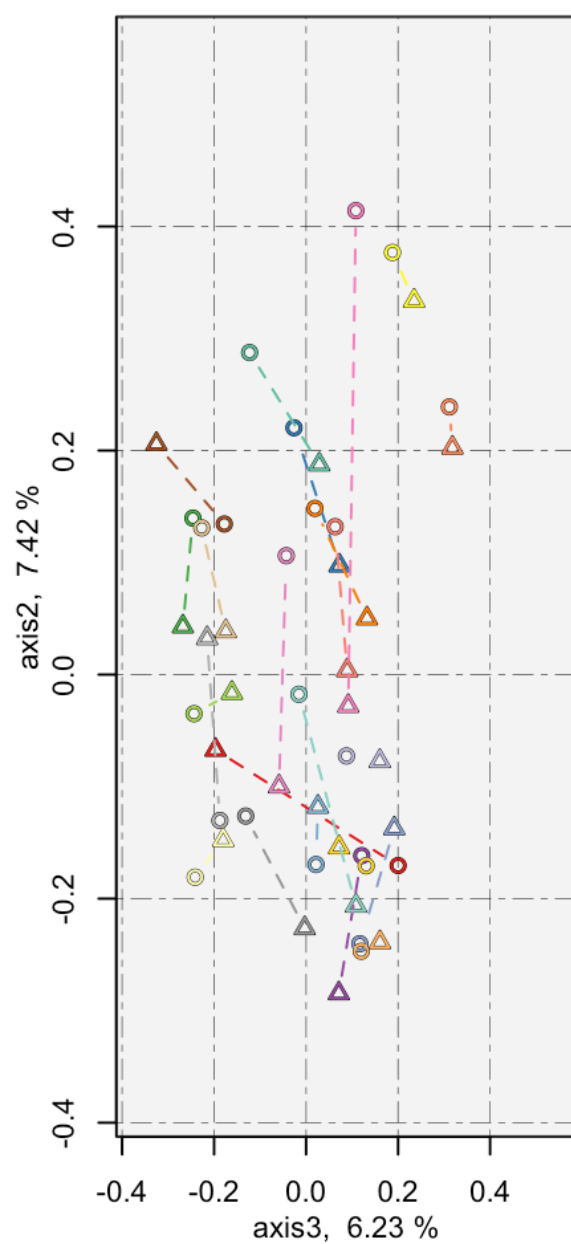
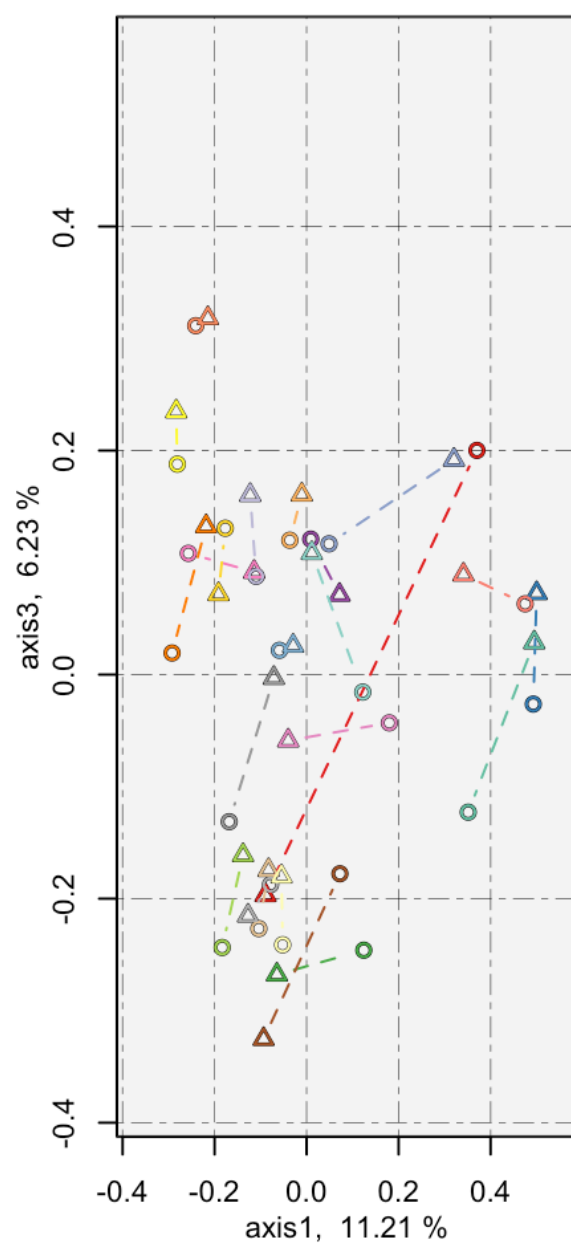
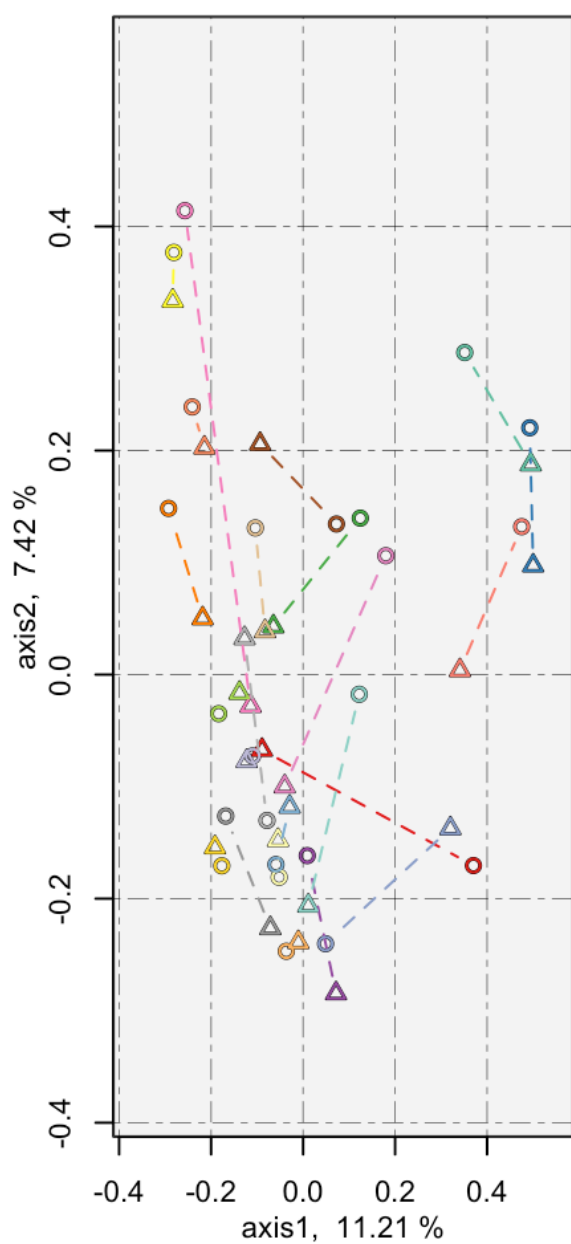
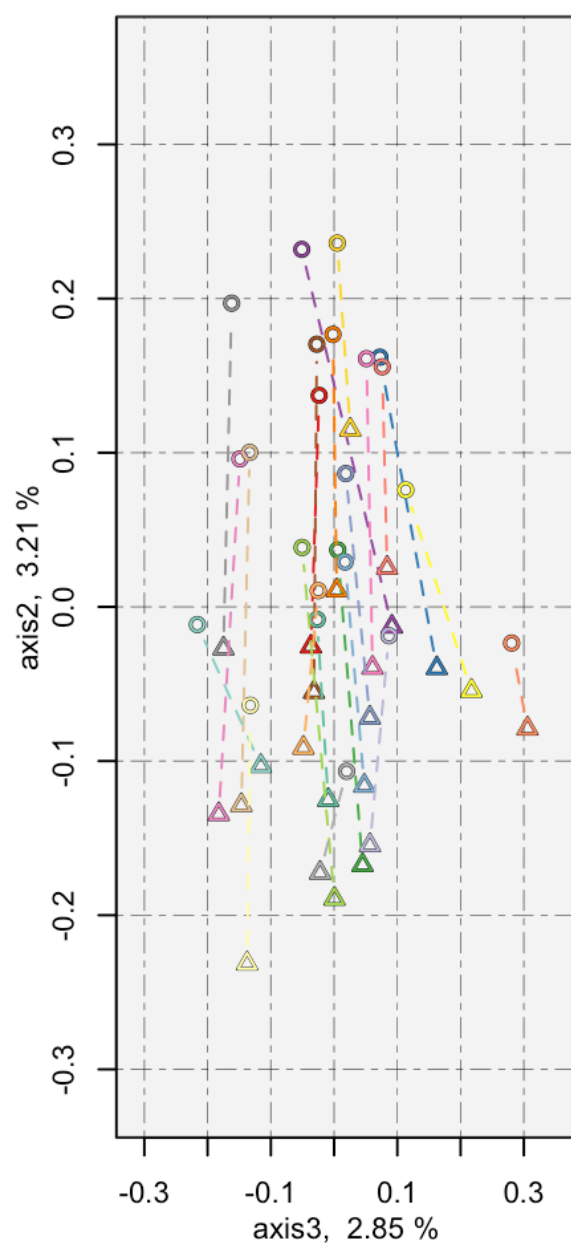
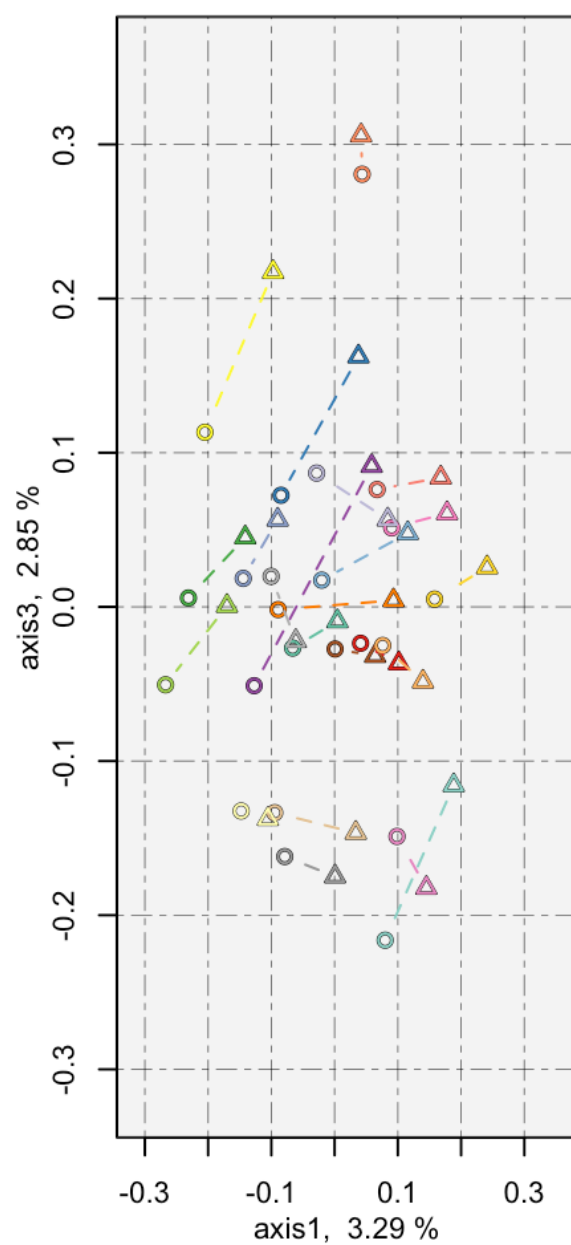
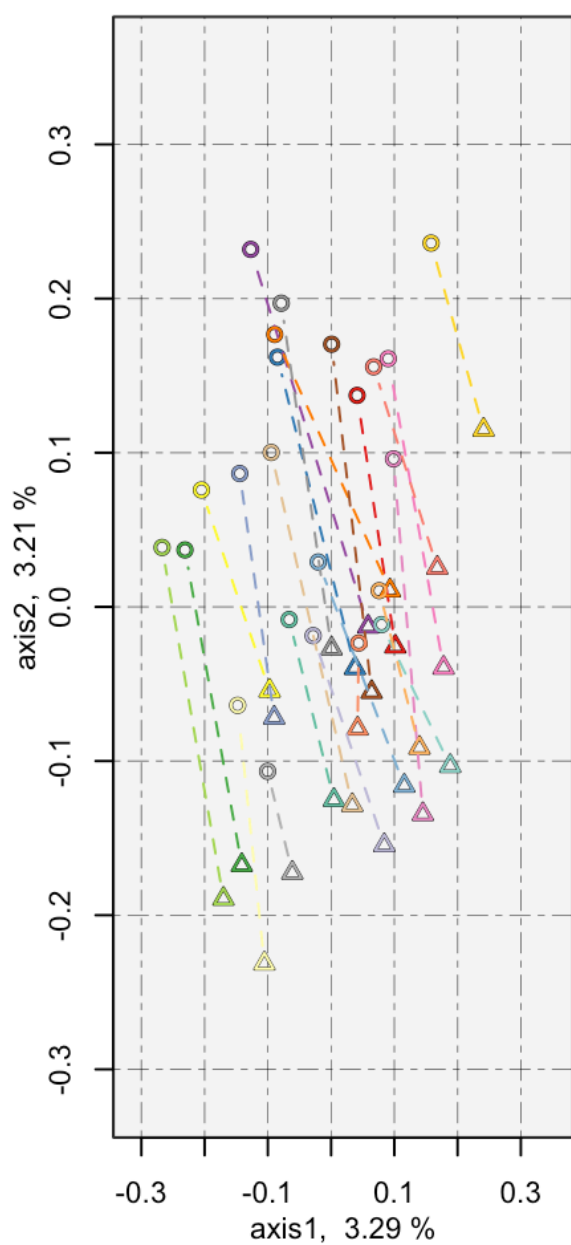
## quartz_off_screen
##                2

```



```
#yue & Clayton PCoA plots
#File names of input data
axes_file_name <- "colonoscopy.makecontigsfile.trim.contigs.good.unique.good.filter.unique.precluster.pick.pick.an.unique_list.0.03.pick.thetayc.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.thetayc.0.03.lt.ave.pcoa.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)
```

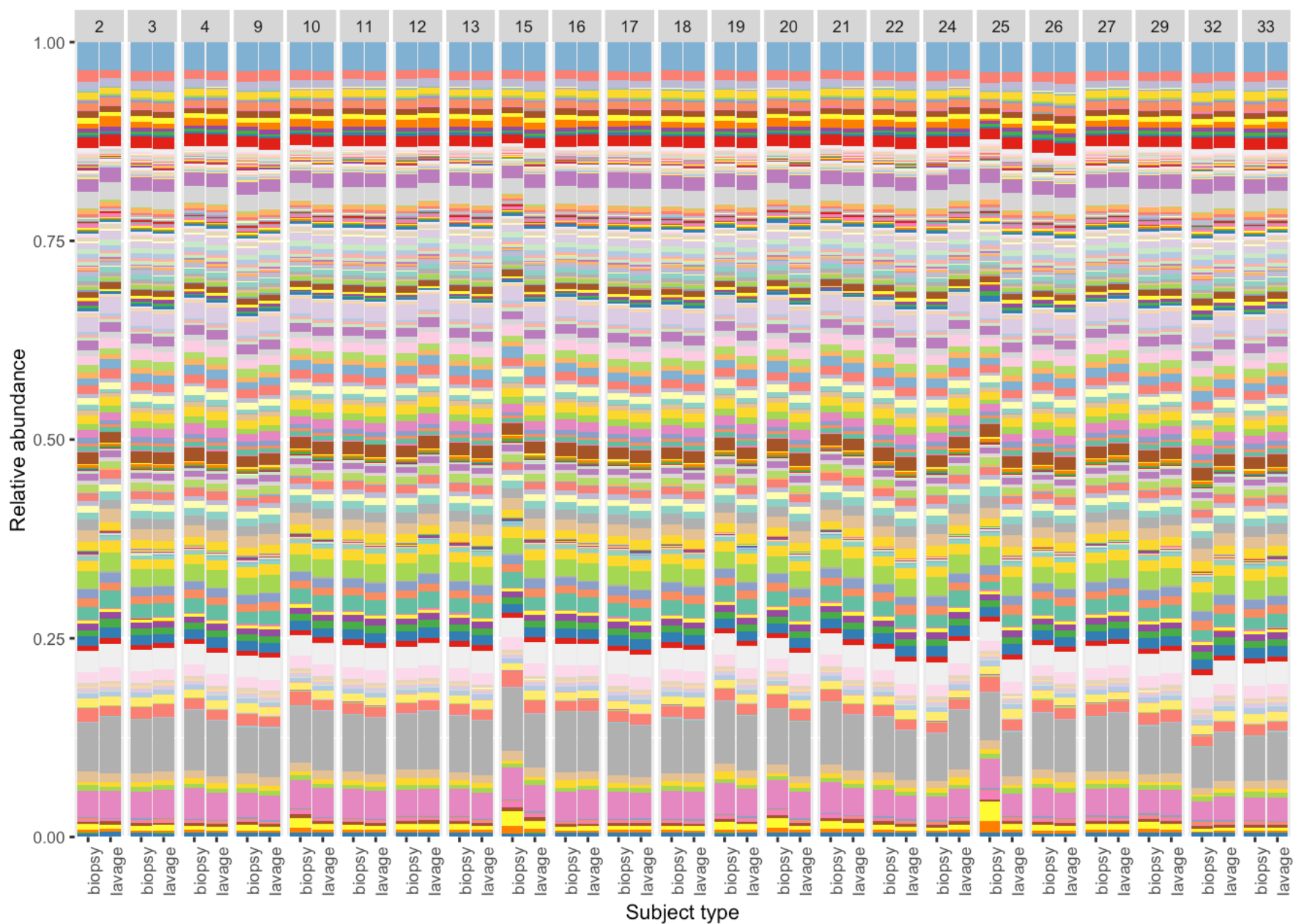
```
## quartz_off_screen
##                2
```



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))
#Slect colour palette for plot
colset <- rep (c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set
3"), brewer.pal(9, "Pastell")), 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 diveristy 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_diveristy_measure, value, 2:(ncol(ALPHA_DATA)+1))
#Reorder measures
alpha_long$Alpha_diveristy_measure_f <- factor(alpha_long$Alpha_diveristy_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_diveristy_measure_f, value)
colnames(mann_whitney_data)[13:16] <- c("KEGG", "Chao", "ShannonWeiner", "InvSimpson")
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)
```

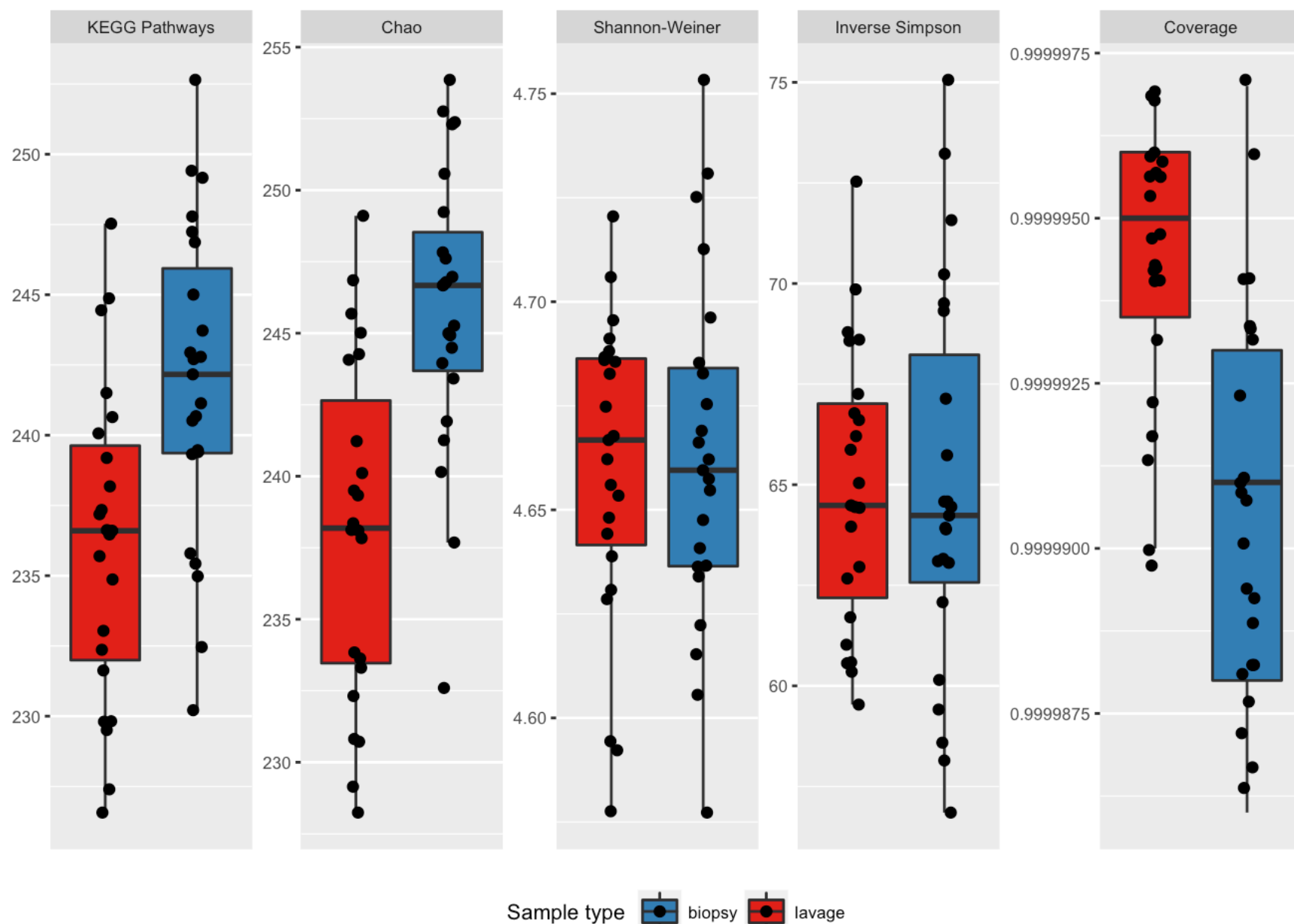
```
##  
## Wilcoxon rank sum test  
##  
## data: InvSimpson by new.Sample.type  
## W = 253, p-value = 0.8109  
## alternative hypothesis: true location shift is not equal to 0
```

```
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 diveristy 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_w  
rap(~ Alpha_diveristy_measure_f, nrow=1, scales="free") + geom_point(position = po  
sition_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_continuou  
s(name="") + theme_grey(base_size = 8) + theme(legend.position="bottom", legend.ma  
rgin=unit(0,"cm"))  
ggsave(pdf_file_box_plot, g_box, units="mm", height=75, width=170, dpi=300)
```



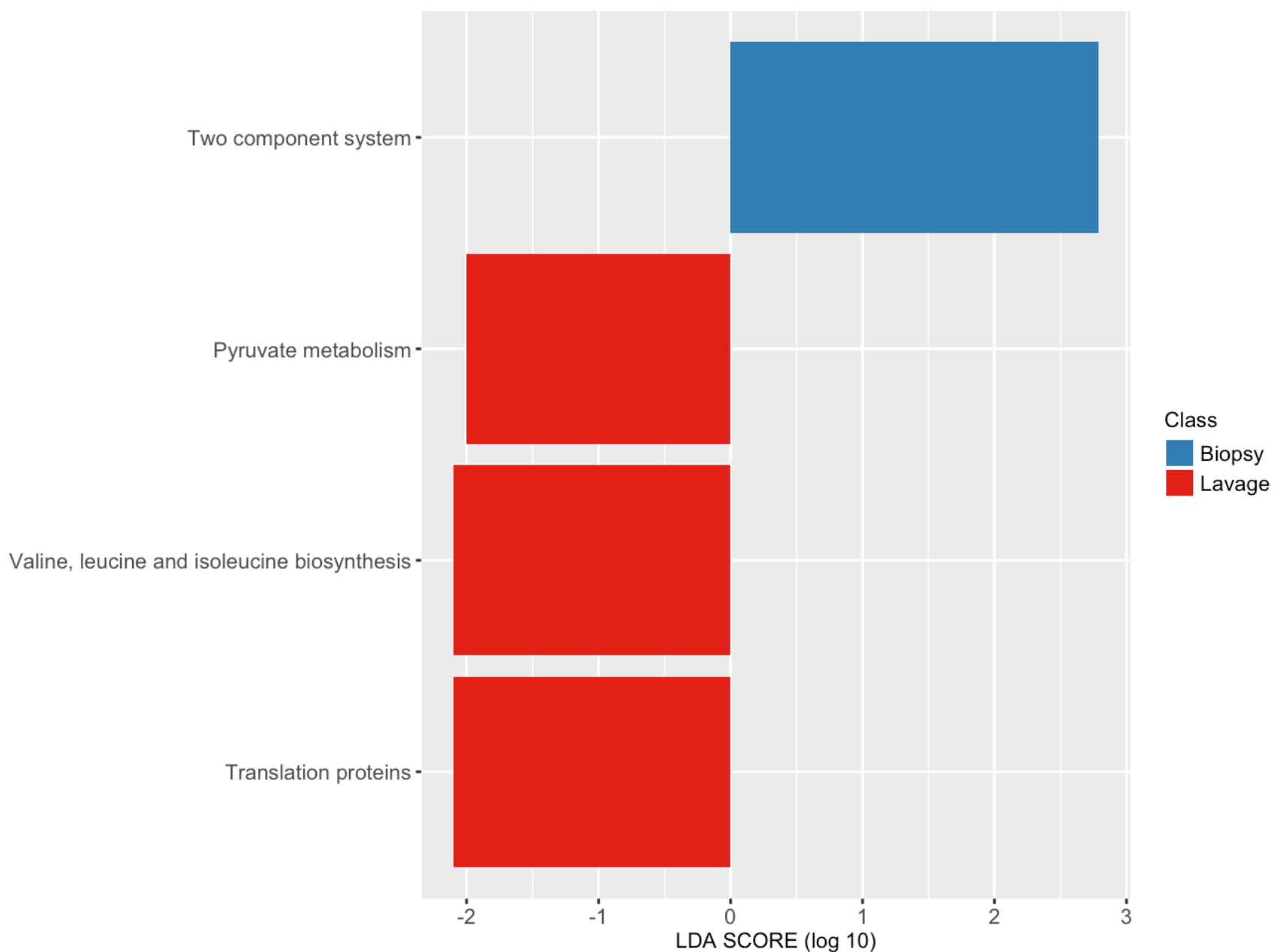
C) LEfSe LDA scores

```
### 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.Class")
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="identity", 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)
#Slect colour palette for plot
colset <- c(brewer.pal(8, "Set1"), brewer.pal(8, "Set2"), brewer.pal(12, "Set3"),
brewer.pal(9, "Pastell"))
#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

