########identifying FOL-altered genus in cultivar D72#############

library(DESeq2); library(dplyr); library(reshape2); library (ggplot2); library(ggpubr); library (ggrepel)

design\_filter\_16s<-read.table("design\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_filter\_16s<- read.table("tax\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s<-read.table("otu\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s <- as.matrix(otu\_filter\_16s) #rownames (otu\_filter\_16s); #colnames (otu\_filter\_16s)

dim(otu\_filter\_16s)

#####Genus abundance#####

myBtaxa <- tax\_filter\_16s

otu\_its\_RA <- otu\_filter\_16s

myBtaxa$Genus.full <- paste(myBtaxa$Phylum, myBtaxa$Class, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Order, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Family, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Genus, sep=",")

myBtaxa$ OTUnames <- rownames(myBtaxa)

not.unass <- rownames(myBtaxa)[ myBtaxa$Genus!="unassigned" ]

for(i in not.unass){

myBtaxa [rownames(myBtaxa)[ myBtaxa $ OTUnames ==paste(i)], ]$Genus.full <- myBtaxa [rownames(myBtaxa)==paste(i),]$Genus }

PHYLAnames\_its <- names(sort(table(myBtaxa[,"Genus.full"]), decr=T))

length(PHYLAnames\_its)

## Preparation of matrix with relative abundance by class

y <- NULL

otunames <- rownames(otu\_its\_RA)

for (i in PHYLAnames\_its){

x <- array(colSums(otu\_its\_RA [rownames(myBtaxa)[which(myBtaxa$Genus.full == paste(i))],,drop=FALSE]))

y <- rbind(y,x) }

## Create matrix

rownames(y) <- paste(PHYLAnames\_its)

colnames(y) <- paste(colnames(otu\_its\_RA))

CLASS\_mat\_its <- y

#head(CLASS\_mat\_its)

colSums(CLASS\_mat\_its)

CLASS\_mat\_its\_mean <- sort(apply(CLASS\_mat\_its,1,mean),decr=T)

CLASS\_mat\_its <- CLASS\_mat\_its[names(CLASS\_mat\_its\_mean),]

dim(CLASS\_mat\_its)

##############################

design <- design\_filter\_16s; otu.data <- CLASS\_mat\_its

library (dplyr)

design <- **filter**(design, Cultivar=="D72")

design.FC <- **filter**(design, FOL %in% c("Control", "Sys")); otu.data.FC <- otu.data[, rownames(design.FC)]

otu.data.FC <- otu.data.FC [rowSums(otu.data.FC)>0, ]

CK.rep=4; Trt.rep=4; BM=50

otu.data <- as.data.frame(otu.data.FC)

Mydesign <- data.frame(Trt = factor(c(rep(c('control'), CK.rep), rep(c('treat'), Trt.rep)), levels = c('control', 'treat')))

dds <- DESeqDataSetFromMatrix( countData = otu.data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'treat', 'control'))

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

tt\_enrich$Genus <- rownames(tt\_enrich)

forMA <- tt\_enrich

forMA <- mutate(forMA, signif = ifelse(padj <= 0.01, "T", "F"))

C\_enrich <- filter(forMA, log2FoldChange > 0 & baseMean>BM & padj<0.01) ; dim (C\_enrich) # Enriched

CK\_enrich <- filter(forMA, log2FoldChange < 0 & baseMean>BM & padj<0.01) ; dim (CK\_enrich) # Depleted

cs <- c(rownames(CK\_enrich), rownames(C\_enrich) )

cs.T.En <- rownames(C\_enrich); cs.T.De <- rownames(CK\_enrich)

## define colors

forMA$col <- "dimgrey"

forMA [which(rownames(forMA) %in% rownames(CK\_enrich) ), ]$col <- "darkorange"

forMA [which(rownames(forMA) %in% rownames(C\_enrich) ), ]$col <- "dodgerblue"

# order for plotting colors

forMA$ord[forMA$col=="dimgrey"] <- 1; forMA$ord[forMA$col=="darkorange"] <- 2; forMA$ord[forMA$col=="dodgerblue"] <- 3

forMA <- forMA[sort(forMA$ord, ind=T, decr=F) $ ix, ]

## define pch

forMA$pch <- 1 ; forMA [which(rownames(forMA) %in% cs ), ] $pch <- 16

## define size

forMA$size <- 1; forMA [which(rownames(forMA) %in% cs ), ]$size <- 1.5

forMA$label <- NA

forMA $ OTUnames <- rownames(forMA)

for(i in cs){ forMA [rownames(forMA)[ forMA $ OTUnames ==paste(i)], ]$label <- forMA [rownames(forMA)==paste(i),]$Genus}

**write.table**(forMA, 'forMA.D72.Genus.txt', col.names = NA, sep = '\t', quote = FALSE)

xrng <- range(log(forMA$baseMean)) ; yrng <- range(forMA$log2FoldChange)

MA.Sys.D72 <- ggplot(data=forMA, aes(x= log(baseMean), y= log2FoldChange, colour=col))+

geom\_point(aes(shape= factor(pch), size=size) )+

scale\_color\_manual(values=c("forestgreen", "dimgrey", "dodgerblue"), guide="none" )+

scale\_shape\_manual(values = c(1, 16) , guide="none")+

scale\_size\_continuous(range = c(0.9, 1.4), guide="none")+

labs(x="Average abundance (log(BM))", y="log2 fold change")+

**geom\_text\_repel**(data=forMA, aes(x=log(baseMean), y= log2FoldChange, label=label), colour="black", size=3) +

annotate("text",label=paste("CK enriched: ", format(length(rownames(CK\_enrich))), sep=""), color="forestgreen",

x=xrng[2]-abs(xrng[1])\*0.5, y=yrng[1], size=4, vjust="inward", hjust="inward")+

annotate("text", label=paste("C enriched: ", format(length(rownames(C\_enrich))), sep=""), color= "dodgerblue",

x=xrng[2]-abs(xrng[1])\*0.5, y=yrng[2], size=4, vjust="inward", hjust="inward")+

theme\_bw()+

theme(panel.grid=element\_blank(),

axis.text=element\_text(size=10, color="black"), axis.title=element\_text(size=12),

legend.background=element\_rect(fill=NA, linewidth=0.6, linetype="dashed", colour ="grey20"),

legend.text=element\_text(size=12) )

MA.Sys.D72

########identifying FOL-altered genus in cultivar D72#############

design\_filter\_16s<-read.table("design\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_filter\_16s<- read.table("tax\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s<-read.table("otu\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s <- as.matrix(otu\_filter\_16s) #rownames (otu\_filter\_16s); #colnames (otu\_filter\_16s)

dim(otu\_filter\_16s)

#####Genus abundance#####

myBtaxa <- tax\_filter\_16s

otu\_its\_RA <- otu\_filter\_16s

myBtaxa$Genus.full <- paste(myBtaxa$Phylum, myBtaxa$Class, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Order, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Family, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Genus, sep=",")

myBtaxa$ OTUnames <- rownames(myBtaxa)

not.unass <- rownames(myBtaxa)[ myBtaxa$Genus!="unassigned" ]

for(i in not.unass){

myBtaxa [rownames(myBtaxa)[ myBtaxa $ OTUnames ==paste(i)], ]$Genus.full <- myBtaxa [rownames(myBtaxa)==paste(i),]$Genus }

PHYLAnames\_its <- names(sort(table(myBtaxa[,"Genus.full"]), decr=T))

length(PHYLAnames\_its)

## Preparation of matrix with relative abundance by class

y <- NULL

otunames <- rownames(otu\_its\_RA)

for (i in PHYLAnames\_its){

x <- array(colSums(otu\_its\_RA [rownames(myBtaxa)[which(myBtaxa$Genus.full == paste(i))],,drop=FALSE]))

y <- rbind(y,x) }

## Create matrix

rownames(y) <- paste(PHYLAnames\_its)

colnames(y) <- paste(colnames(otu\_its\_RA))

CLASS\_mat\_its <- y

colSums(CLASS\_mat\_its)

CLASS\_mat\_its\_mean <- sort(apply(CLASS\_mat\_its,1,mean),decr=T)

CLASS\_mat\_its <- CLASS\_mat\_its[names(CLASS\_mat\_its\_mean),]

dim(CLASS\_mat\_its)

##############################

design <- design\_filter\_16s; otu.data <- CLASS\_mat\_its

library (dplyr)

design <- **filter**(design, Cultivar=="Z19")

design.FC <- **filter**(design, FOL %in% c("Control", "Sys")); otu.data.FC <- otu.data[, rownames(design.FC)]

otu.data.FC <- otu.data.FC [rowSums(otu.data.FC)>0, ]

CK.rep=4; Trt.rep=4; BM=50

otu.data <- as.data.frame(otu.data.FC) ; CK.rep=4; Trt.rep=4

Mydesign <- data.frame(Trt = factor(c(rep(c('control'), CK.rep), rep(c('treat'), Trt.rep)), levels = c('control', 'treat')))

dds <- DESeqDataSetFromMatrix( countData = otu.data, colData = Mydesign , design= ~Trt )

dds1 <- DESeq(dds, fitType = 'mean', minReplicatesForReplace = 7, parallel = F)

res <- results(dds1, contrast = c('Trt', 'treat', 'control'))

res1 <- data.frame(res, stringsAsFactors = FALSE, check.names = FALSE)

tt\_enrich <- as.data.frame(res1)

tt\_enrich$Genus <- rownames(tt\_enrich)

forMA <- tt\_enrich

forMA <- mutate(forMA, signif = ifelse(padj <= 0.01, "T", "F"))

C\_enrich <- filter(forMA, log2FoldChange > 0 & baseMean>BM & padj<0.01) ; dim (C\_enrich) # Enriched

CK\_enrich <- filter(forMA, log2FoldChange < 0 & baseMean>BM & padj<0.01) ; dim (CK\_enrich) # Depleted

cs <- c(rownames(CK\_enrich), rownames(C\_enrich) )

cs.T.En <- rownames(C\_enrich); cs.T.De <- rownames(CK\_enrich)

## define colors

forMA$col <- "dimgrey"

forMA [which(rownames(forMA) %in% rownames(CK\_enrich) ), ]$col <- "darkorange"

forMA [which(rownames(forMA) %in% rownames(C\_enrich) ), ]$col <- "dodgerblue"

# order for plotting colors

forMA$ord[forMA$col=="dimgrey"] <- 1; forMA$ord[forMA$col=="darkorange"] <- 2; forMA$ord[forMA$col=="dodgerblue"] <- 3

forMA <- forMA[sort(forMA$ord, ind=T, decr=F) $ ix, ]

## define pch

forMA$pch <- 1 ; forMA [which(rownames(forMA) %in% cs ), ] $pch <- 16

## define size

forMA$size <- 1; forMA [which(rownames(forMA) %in% cs ), ]$size <- 1.5

forMA$label <- NA

forMA $ OTUnames <- rownames(forMA)

for(i in cs){ forMA [rownames(forMA)[ forMA $ OTUnames ==paste(i)], ]$label <- forMA [rownames(forMA)==paste(i),]$Genus}

**write.table**(forMA, 'forMA.Z19.Genus.txt', col.names = NA, sep = '\t', quote = FALSE)

xrng <- range(log(forMA$baseMean)) ; yrng <- range(forMA$log2FoldChange)

MA.Sys.ZZ <- ggplot(data=forMA, aes(x= log(baseMean), y= log2FoldChange, colour=col))+

geom\_point(aes(shape= factor(pch), size=size) )+

scale\_color\_manual(values=c("forestgreen", "dimgrey", "dodgerblue"), guide="none" )+

scale\_shape\_manual(values = c(1, 16) , guide="none")+

scale\_size\_continuous(range = c(0.9, 1.4), guide="none")+

labs(x="Average abundance (log(BM))", y="log2 fold change")+

**geom\_text\_repel**(data=forMA, aes(x=log(baseMean), y= log2FoldChange, label=label), colour="black", size=3) +

annotate("text",label=paste("CK enriched: ", format(length(rownames(CK\_enrich))), sep=""), color="forestgreen",

x=xrng[2]-abs(xrng[1])\*0.5, y=yrng[1], size=4, vjust="inward", hjust="inward")+

annotate("text", label=paste("C enriched: ", format(length(rownames(C\_enrich))), sep=""), color= "dodgerblue",

x=xrng[2]-abs(xrng[1])\*0.5, y=yrng[2], size=4, vjust="inward", hjust="inward")+

theme\_bw()+

theme(panel.grid=element\_blank(),

axis.text=element\_text(size=10, color="black"), axis.title=element\_text(size=12),

legend.background=element\_rect(fill=NA, linewidth=0.6, linetype="dashed", colour ="grey20"),

legend.text=element\_text(size=12) )

MA.Sys.ZZ

#####Plotting log2FC values for FOL-altered genus ######

library (ggplot2); library(ggpubr); library (ggrepel)

design\_rarefy\_16s<-read.table("design\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_rarefy\_16s<- read.table("tax\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s<- read.table("otu\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s <- as.matrix(otu\_rarefy\_16s) #rownames (otu\_rarefy\_16s); #colnames (otu\_rarefy\_16s)

forMA.D72<-read.table("forMA.D72.Genus.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

forMA.Z19<-read.table("forMA.Z19.Genus.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

Pct <- 0.01; BM=50

library(dplyr)

D72.Sys\_En <- filter(forMA.D72, log2FoldChange>0&baseMean>BM & padj< Pct); dim (D72.Sys\_En)

D72.Sys\_De <- filter(forMA.D72, log2FoldChange<0&baseMean>BM & padj< Pct); dim (D72.Sys\_De)

Z19.Sys\_En <- filter(forMA.Z19, log2FoldChange>0&baseMean>BM & padj< Pct); dim (Z19.Sys\_En)

Z19.Sys\_De <- filter(forMA.Z19, log2FoldChange<0&baseMean>BM & padj< Pct); dim (Z19.Sys\_De)

Sys\_En = union( rownames(D72.Sys\_En), rownames(Z19.Sys\_En) )

Sys\_De = union( rownames(D72.Sys\_De), rownames(Z19.Sys\_De) )

De.En = intersect(Sys\_En, Sys\_De) # tax\_filter\_16s [intersect(Sys\_En, Sys\_De), ]

De.En.All = union(Sys\_En, Sys\_De)

Group.En = setdiff(Sys\_En, De.En) # tax\_filter\_16s [Group.En, ]

Group.De = setdiff(Sys\_De, De.En)

############################################################

design\_filter\_16s<-read.table("design\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_filter\_16s<- read.table("tax\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_rarefy\_16s<- read.table("tax\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s<-read.table("otu\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s<-read.table("otu\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s <- as.matrix(otu\_filter\_16s) #rownames (otu\_filter\_16s); #colnames (otu\_filter\_16s)

dim(otu\_filter\_16s)

#####Genus abundance#####

myBtaxa <- tax\_filter\_16s

# otu\_its\_RA <- t(t(myBotu)/colSums(myBotu))\*100

otu\_its\_RA <- otu\_filter\_16s

## Get names of bacteria phyla present (use 'labels' as this specifies class within Proteobacteria)

myBtaxa$Genus.full <- paste(myBtaxa$Phylum, myBtaxa$Class, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Order, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Family, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Genus, sep=",")

myBtaxa$ OTUnames <- rownames(myBtaxa)

not.unass <- rownames(myBtaxa)[ myBtaxa$Genus!="unassigned" ]

for(i in not.unass){

myBtaxa [rownames(myBtaxa)[ myBtaxa $ OTUnames ==paste(i)], ]$Genus.full <- myBtaxa [rownames(myBtaxa)==paste(i),]$Genus }

PHYLAnames\_its <- names(sort(table(myBtaxa[,"Genus.full"]), decr=T))

length(PHYLAnames\_its)

## Preparation of matrix with relative abundance by class

y <- NULL

otunames <- rownames(otu\_its\_RA)

for (i in PHYLAnames\_its){

x <- array(colSums(otu\_its\_RA [rownames(myBtaxa)[which(myBtaxa$Genus.full == paste(i))],,drop=FALSE]))

y <- rbind(y,x) }

## Create matrix

rownames(y) <- paste(PHYLAnames\_its)

colnames(y) <- paste(colnames(otu\_its\_RA))

CLASS\_mat\_its <- y

colSums(CLASS\_mat\_its)

CLASS\_mat\_its\_mean <- sort(apply(CLASS\_mat\_its,1,mean),decr=T)

CLASS\_mat\_its <- CLASS\_mat\_its[names(CLASS\_mat\_its\_mean),]

dim(CLASS\_mat\_its)

#####################################################################################

Genus.RA <- as.data.frame(CLASS\_mat\_its)

Genus.RA$RA <- rowSums(Genus.RA)/sum(Genus.RA) \*100

csOTUs.dat <- Genus.RA [De.En.All, ]

range(csOTUs.dat $RA)

csOTUs.dat$D72 <- 0

for (i in rownames(csOTUs.dat)) { csOTUs.dat [rownames(csOTUs.dat)==paste(i),]$D72 <- forMA.D72 [rownames(forMA.D72)==paste(i),]$log2FoldChange }

csOTUs.dat$Z19 <- 0

for (i in rownames(csOTUs.dat)) { csOTUs.dat [rownames(csOTUs.dat)==paste(i),]$Z19 <- forMA.Z19 [rownames(forMA.Z19)==paste(i),]$log2FoldChange }

csOTUs.dat$D72.p <- 0

for (i in rownames(csOTUs.dat)) { csOTUs.dat [rownames(csOTUs.dat)==paste(i),]$D72.p <- forMA.D72 [rownames(forMA.D72)==paste(i),]$padj }

csOTUs.dat$Z19.p <- 0

for (i in rownames(csOTUs.dat)) { csOTUs.dat [rownames(csOTUs.dat)==paste(i),]$Z19.p <- forMA.Z19 [rownames(forMA.Z19)==paste(i),]$padj }

csOTUs.dat [is.na(csOTUs.dat)] <- 1

library(dplyr)

csOTUs.dat <- **mutate**(csOTUs.dat, D72.p = ifelse(D72.p <= 0.01, "S", "NS"))

csOTUs.dat <- **mutate**(csOTUs.dat, Z19.p = ifelse(Z19.p <= 0.01, "S", "NS"))

csOTUs.dat$P <- paste(csOTUs.dat$D72.p, csOTUs.dat$Z19.p, sep="-")

range(csOTUs.dat$RA)

csOTUs.dat$label <- rownames(csOTUs.dat)

PGenus.FC <- ggplot(data= csOTUs.dat , aes(x= Z19, y= D72 ))+

geom\_point(aes( size= RA, color= P ) ) +

scale\_size\_continuous(name="RA (%)", range = c(0, 6), breaks=c(0.05, 0.1, 0.5, 1, 2.5, 5)) +

scale\_color\_manual(values= c("yellowgreen", "turquoise3", "dodgerblue")) + #, guide="none"

geom\_hline(yintercept=0, linetype=3, linewidth=1, color='gray')+

geom\_vline(xintercept=0, linetype=3, linewidth=1, color='gray')+

theme\_bw()+

theme(panel.grid=element\_blank(), plot.title = element\_text(face="bold", hjust = 0.5),

axis.text=element\_text(size=12, color="black"), axis.title=element\_text(size=12), #face="bold"

legend.title=element\_text(size=12), legend.text=element\_text(size=12),

legend.key = element\_blank(), legend.background= element\_blank() )

PGenus.FC

#####Plotting bubble plots for FOL-altered genus######

library (ggplot2); library(reshape2)

design\_rarefy\_16s<-read.table("design\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_rarefy\_16s<- read.table("tax\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s<- read.table("otu\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s <- as.matrix(otu\_rarefy\_16s) #rownames (otu\_rarefy\_16s); #colnames (otu\_rarefy\_16s)

######################

forMA.D72<-read.table("forMA.D72.Genus1.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

forMA.Z19<-read.table("forMA.Z19.Genus1.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

Pct <- 0.01; BM=50

library(dplyr)

D72.Sys\_En <- filter(forMA.D72, log2FoldChange>0&baseMean>BM & padj< Pct); dim (D72.Sys\_En)

D72.Sys\_De <- filter(forMA.D72, log2FoldChange<0&baseMean>BM & padj< Pct); dim (D72.Sys\_De)

Z19.Sys\_En <- filter(forMA.Z19, log2FoldChange>0&baseMean>BM & padj< Pct); dim (Z19.Sys\_En)

Z19.Sys\_De <- filter(forMA.Z19, log2FoldChange<0&baseMean>BM & padj< Pct); dim (Z19.Sys\_De)

Sys\_En = union( rownames(D72.Sys\_En), rownames(Z19.Sys\_En) )

Sys\_De = union( rownames(D72.Sys\_De), rownames(Z19.Sys\_De) )

De.En = intersect(Sys\_En, Sys\_De) # tax\_filter\_16s [intersect(Sys\_En, Sys\_De), ]

De.En.All = union(Sys\_En, Sys\_De)

Group.En = setdiff(Sys\_En, De.En) # tax\_filter\_16s [Group.En, ]

Group.De = setdiff(Sys\_De, De.En)

csO <-De.En.All

############################################################

design\_filter\_16s<-read.table("design\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_filter\_16s<- read.table("tax\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

tax\_rarefy\_16s<- read.table("tax\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_rarefy\_16s<-read.table("otu\_rarefy\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s<-read.table("otu\_filter\_16s.txt", row.names=1, sep="\t", header=T, blank.lines.skip=F, check.names=F)

otu\_filter\_16s <- as.matrix(otu\_filter\_16s) #rownames (otu\_filter\_16s); #colnames (otu\_filter\_16s)

dim(otu\_filter\_16s)

#####Genus abundance#####

myBtaxa <- tax\_filter\_16s

otu\_its\_RA <- otu\_filter\_16s

myBtaxa$Genus.full <- paste(myBtaxa$Phylum, myBtaxa$Class, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Order, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Family, sep=",")

myBtaxa$Genus.full <- paste(myBtaxa$Genus.full, myBtaxa$Genus, sep=",")

PHYLAnames\_its <- names(sort(table(myBtaxa[,"Genus.full"]), decr=T))

length(PHYLAnames\_its)

# sort(table(myBtaxa[,"Genus"]), decr=T)

## Preparation of matrix with relative abundance by class

y <- NULL

otunames <- rownames(otu\_its\_RA)

for (i in PHYLAnames\_its){

x <- array(colSums(otu\_its\_RA [rownames(myBtaxa)[which(myBtaxa$Genus.full == paste(i))],,drop=FALSE]))

y <- rbind(y,x) }

## Create matrix

rownames(y) <- paste(PHYLAnames\_its)

colnames(y) <- paste(colnames(otu\_its\_RA))

CLASS\_mat\_its <- y

colSums(CLASS\_mat\_its)

CLASS\_mat\_its\_mean <- sort(apply(CLASS\_mat\_its,1,mean),decr=T)

CLASS\_mat\_its <- CLASS\_mat\_its[names(CLASS\_mat\_its\_mean),]

dim(CLASS\_mat\_its)

#####################################################################################

Genus\_RA <- t(t(CLASS\_mat\_its)/colSums(CLASS\_mat\_its))\*100; colSums(Genus\_RA)

Genus\_RA <- as.data.frame(t(Genus\_RA [csO, ])); range(Genus\_RA)

Genus\_RA <- as.data.frame(t(Genus\_RA))

Genus\_RA$id <- rownames (Genus\_RA)

for (i in rownames(Genus\_RA)) {

if (i %in% Group.En ==TRUE)

{ Genus\_RA[rownames(Genus\_RA)==paste(i),]$id <- paste("a", Genus\_RA[rownames(Genus\_RA)==paste(i),]$id, sep=",") }

else

{ Genus\_RA [rownames(Genus\_RA)==paste(i),]$id <- Genus\_RA [rownames(Genus\_RA)==paste(i),]$id } }

for (i in rownames(Genus\_RA)) {

if (i %in% Group.De ==TRUE)

{ Genus\_RA[rownames(Genus\_RA)==paste(i),]$id <- paste("b", Genus\_RA[rownames(Genus\_RA)==paste(i),]$id, sep=",") }

else

{ Genus\_RA [rownames(Genus\_RA)==paste(i),]$id <- Genus\_RA [rownames(Genus\_RA)==paste(i),]$id } }

for (i in rownames(Genus\_RA)) {

if (i %in% De.En ==TRUE)

{ Genus\_RA[rownames(Genus\_RA)==paste(i),]$id <- paste("c", Genus\_RA[rownames(Genus\_RA)==paste(i),]$id, sep=",") }

else

{ Genus\_RA [rownames(Genus\_RA)==paste(i),]$id <- Genus\_RA [rownames(Genus\_RA)==paste(i),]$id } }

Genus\_RA <- Genus\_RA [order(Genus\_RA [, c("id")]), ] #排序

rownames(Genus\_RA) <- Genus\_RA$id

Genus\_RA <- Genus\_RA [ , !colnames(Genus\_RA) %in% c("id")]

Sam.Rep=4

rep\_time <- length(colnames(Genus\_RA))/ Sam.Rep ; t=1; Genus\_RA\_AVE=c()

for (i in 1:rep\_time) { sub\_table= Genus\_RA [,t:(t+ Sam.Rep -1)]

sub\_mean=apply(sub\_table, 1, mean)

Genus\_RA\_AVE=cbind(Genus\_RA\_AVE, sub\_mean)

t=t+ Sam.Rep }

colnames(Genus\_RA\_AVE) <- unique (as.factor(design\_rarefy\_16s$Trt))

##########################################

Genus\_RA <- as.data.frame(t(Genus\_RA\_AVE))

csO <- colnames(Genus\_RA)

Genus\_RA $Trt <- unique (as.factor(design\_rarefy\_16s$Trt))

Genus\_RA $Name <- rownames(Genus\_RA)

otu\_RA.long = melt(Genus\_RA, id.vars=c("Trt", "Name"),

measure.vars=c(csO),

variable.name='csO', value.name='Abundance')

range(otu\_RA.long$ Abundance)

p.RA.dot<-ggplot(otu\_RA.long, aes(x=factor(Trt, levels=c('D72-Control', 'D72-Local', 'D72-Sys', 'Z19-Control', 'Z19-Local', 'Z19-Sys')), y=csO ))+

geom\_point(aes(size= Abundance), colour = "grey15")+

scale\_size\_continuous(name="RA (%)", range = c(0, 5), breaks=c(0.05, 0.5, 1, 2.5, 5, 7.5, 10)) +

theme\_bw()+

theme(axis.text=element\_text(size=5, color="black"), axis.title= element\_blank(),

legend.text=element\_text(size=8), legend.background=element\_rect(fill='NA'),

axis.text.x = element\_text(vjust = 0.5, hjust = 0.5, angle = 90), #axis.text.y = element\_text(size=8),

panel.background = element\_blank(), panel.grid.major=element\_line(colour=NA),

panel.grid.minor=element\_line(colour=NA) )

p.RA.dot