library (readr); library(edgeR); library(phyloseq); library (vegan); library(Hmisc); library (ggplot2); library(ggpubr); library ([gdata](http://127.0.0.1:24319/library/gdata/html/upperTriangle.html)); library (stringr); source("CorrDF.R"); library(reshape2); 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)

Enriched.EHB <- read\_rds ("Enriched.EHB.rds"); length(Enriched.EHB)

Depleted.EHB <- read\_rds ("Depleted.EHB.rds"); length(Depleted.EHB)

# csO <- **setdiff**(Depleted.W, Depleted.C) ; length (csO)

csO <- Enriched.EHB

csO <- Depleted.EHB

csOTUs.tax <- tax\_rarefy\_16s [csO, ]

csOTUs.tax <-csOTUs.tax [order(csOTUs.tax[,2], csOTUs.tax[,3], csOTUs.tax[,6]), ]

csOTUs <- rownames (csOTUs.tax)

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

otu\_16s\_RA <- t(t(otu\_rarefy\_16s)/colSums(otu\_rarefy\_16s))\*100; colSums(otu\_16s\_RA)

otu\_RA <- as.data.frame(t(otu\_16s\_RA [csOTUs, ])); range(otu\_RA)

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

#################处理平均值###########

Sam.Rep=5

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

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

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

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

t=t+ Sam.Rep }

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

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

otu\_RA <- as.data.frame(otu\_RA\_AVE)

otu\_RA$Phylum <- tax\_rarefy\_16s[csOTUs, ]$Phylum

otu\_RA$Class <- tax\_rarefy\_16s[csOTUs, ]$Class

otu\_RA$Order <- tax\_rarefy\_16s[csOTUs, ]$Order

otu\_RA$Family <- tax\_rarefy\_16s[csOTUs, ]$Family

otu\_RA$Genus <- tax\_rarefy\_16s[csOTUs, ]$Genus

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

otu\_RA$id <- paste(otu\_RA $Genus, otu\_RA$id, sep="; ")

otu\_RA$id <- paste(otu\_RA $Family, otu\_RA $id, sep="; g:")

otu\_RA$id <- paste(otu\_RA $Order, otu\_RA $id, sep="; f:")

otu\_RA$id <- paste(otu\_RA $Class, otu\_RA $id, sep="; o:")

otu\_RA$id <- paste(otu\_RA $Phylum, otu\_RA $id, sep="; c:")

otu\_RA <- otu\_RA [order(otu\_RA [, c("id")], decreasing = T), ] #排序, decreasing = T

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

otu\_RA <- otu\_RA [ , !colnames(otu\_RA) %in% c("Phylum", "Class", "Order", "Family", "Genus", "id")]

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

csO <- colnames(otu\_RA)

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

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

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

measure.vars=c(csO),

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

range(otu\_RA.long$ Abundance)

#otu\_RA.long$ csOTU <- gsub("b", "", otu\_RA.long$ csOTU)

p.RA.dot<-ggplot(otu\_RA.long, aes(x=factor(Trt, levels=c('N', 'Y')), y=csO ))+

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

scale\_size\_continuous(name="RA (%)", range = c(0.5, 6), breaks=c(0.05, 0.1, 0.25, 0.5, 1.0)) +

theme\_bw()+

theme(axis.text=element\_text(size=6, 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),

# axis.text.y=element\_blank(), axis.ticks.y= element\_blank (),

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

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

p.RA.dot