library (readr); library(edgeR); library(phyloseq); library (ggplot2); library(ggpubr); library (ggrepel)

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

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

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

otu\_filter\_16s<-read.table("otu\_filter\_16s.DDS.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)

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

design\_filter\_16s $RA <- otu\_16s\_RA[c("bOTU\_3304"), ]

pOTU.1 <-**ggplot**(design\_filter\_16s, aes(x= **factor**(Trt, levels=c('Control', 'DDS')), y=RA, fill=Trt)) +

**stat\_summary**(fun=mean, position=position\_dodge(), color="transparent", width=0.70, size=0, geom="bar")+

**stat\_summary**(fun.data=mean\_se, geom="errorbar", position=position\_dodge(0.70),

width=0.40, size=0.75, colour="black") +

scale\_fill\_manual(values = c("dodgerblue", "darkorange")) +

scale\_y\_continuous (limits=c(0, 0.9), expand=c(0, 0.0001)) +

labs(x=NULL, y="OTU3304 (RA %)") +

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=9, color="black"), axis.title=element\_text(size=12) )

pOTU.1

data = design\_filter\_16s

(sum(data[rownames(data)[data$Trt=="DDS"],]$RA)-sum(data[rownames(data)[data$Trt=="Control"], ]$RA))/sum(data[rownames(data)[data$Trt=="Control"], ]$ RA)\*100

**stats::t.test**(data $ RA ~ data $Trt, p.adj="BH", var.equal=F) #Welch’s T test

design\_filter\_16s $RA <- otu\_16s\_RA[c("bOTU\_4955"), ]

pOTU.2 <-**ggplot**(design\_filter\_16s, aes(x= **factor**(Trt, levels=c('Control', 'DDS')), y=RA, fill=Trt)) +

**stat\_summary**(fun=mean, position=position\_dodge(), color="transparent", width=0.70, size=0, geom="bar")+

**stat\_summary**(fun.data=mean\_se, geom="errorbar", position=position\_dodge(0.70),

width=0.40, size=0.75, colour="black") +

scale\_fill\_manual(values = c("dodgerblue", "darkorange")) +

scale\_y\_continuous (limits=c(0, 0.55), expand=c(0, 0.0001)) +

labs(x=NULL, y="OTU4955 (RA %)") +

theme\_bw() +

theme(panel.grid=element\_blank(), axis.text=element\_text(size=9, color="black"), axis.title=element\_text(size=12) )

pOTU.2

data = design\_filter\_16s

(sum(data[rownames(data)[data$Trt=="DDS"],]$RA)-sum(data[rownames(data)[data$Trt=="Control"], ]$RA))/sum(data[rownames(data)[data$Trt=="Control"], ]$ RA)\*100

**stats::t.test**(data $ RA ~ data $Trt, p.adj="BH", var.equal=F) #Welch’s T test

**ggarrange**(pOTU.1, pOTU.2, ncol=2, nrow=1, align="hv", legend="none" )