#######Plotting beta diversity plot, all sample together#########

library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel); library (dplyr); library(edgeR)

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)

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)

##### TMM normalize 16S counts for whole community beta diversity analysis #####

## Apply TMM normalization to entire 16S data set and create phyloseq objects for later analysis

group\_16s <- design\_filter\_16s$Trt

edgeR\_16s<- DGEList(counts= otu\_filter\_16s,

group=design\_filter\_16s$Trt,

genes=tax\_filter\_16s)

edgeR\_16s <- calcNormFactors(edgeR\_16s)

otu\_norm\_16s <- **cpm**(edgeR\_16s, normalized.lib.sizes=T,log=F)

**write.table**(otu\_norm\_16s, file="otu\_norm\_16s.txt", sep="\t",quote=F)

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

design <- design\_filter\_16s; otu.data <- otu\_norm\_16s

distance.w <- vegdist(**decostand**(t(otu.data), "hellinger"), method = 'bray')

res.w <- **pcoa**(distance.w)

res.v.w <- res.w$vectors

site.w <- data.frame(x= res.v.w[,1], y= res.v.w[,2], FOL=design$FOL,

Trt=design$Trt, Cultivar = design$ Cultivar )

site.w$Trt <- as.factor(site.w$Trt)

site.w$ Cultivar <- as.factor(site.w$ Cultivar)

site.w$sample <- rownames(site.w)

B.PCoA.Bray <- ggplot(data=site.w, aes(x, y, colour = Trt))+geom\_point(size=4) + #, shape= Cultivar

geom\_text\_repel(data=site.w, aes(x, y, label=sample), colour="black", size=5) +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" ")) +

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

**stat\_ellipse**(aes(group=Cultivar, fill= Cultivar), type="norm", alpha=0.2, level=0.95, geom="polygon", color=NA) +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" "))+

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

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() )

B.PCoA.Bray

## Perform PERMANVOA

design$Cultivar <- as.factor (design$Cultivar); adonis2(distance.w ~ Cultivar, data=design, permutations=9999)

#######Plotting beta diversity plot, each cultivar#########

library(vegan); library(ape); library(ggpubr); library(ggplot2); library (ggrepel); library (dplyr); library(edgeR)

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)

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)

##### TMM normalize 16S counts for whole community beta diversity analysis #####

## Apply TMM normalization to entire 16S data set and create phyloseq objects for later analysis

group\_16s <- design\_filter\_16s$Trt

edgeR\_16s<- DGEList(counts= otu\_filter\_16s,

group=design\_filter\_16s$Trt,

genes=tax\_filter\_16s)

edgeR\_16s <- calcNormFactors(edgeR\_16s)

otu\_norm\_16s <- **cpm**(edgeR\_16s, normalized.lib.sizes=T,log=F)

**write.table**(otu\_norm\_16s, file="otu\_norm\_16s.txt", sep="\t",quote=F)

###############Cultivar D72###############################

design <- design\_filter\_16s; otu.data <- otu\_norm\_16s

library (dplyr); design <- **filter**(design, Cultivar=="D72"); otu.data <- otu.data[, rownames(design)]

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

distance.w <- vegdist(**decostand**(t(otu.data), "hellinger"), method = 'bray')

res.w <- **pcoa**(distance.w)

res.v.w <- res.w$vectors

site.w <- data.frame(x= res.v.w[,1], y= res.v.w[,2], FOL=design$FOL,

Trt=design$Trt, Ino= design$ Inoculated )

site.w$Trt <- as.factor(site.w$Trt)

site.w$ Ino <- as.factor(site.w$ Ino)

site.w$sample <- rownames(site.w)

#############Plotting####################

B.PCoA.Bray.D72 <- ggplot(data=site.w, aes(x, y, colour = FOL))+geom\_point(size=4, shape=16) + #, shape=Trt

geom\_text\_repel(data=site.w, aes(x, y, label=sample), colour="black", size=5) +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" ")) +

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

**stat\_ellipse**(aes(group=FOL, fill=FOL), type="norm", alpha=0.2, level=0.75, geom="polygon", color=NA) +

scale\_color\_manual( values= c("darkorange2", "dodgerblue", "forestgreen"), labels=c("Fol", "Sys", "Control") ) +

scale\_fill\_manual(values= c("darkorange2", "dodgerblue", "forestgreen"), guide="none") +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" "))+

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

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() )

B.PCoA.Bray.D72

## Perform PERMANVOA

design$Trt <- as.factor (design$Trt); adonis2(distance.w ~ Trt, data=design, permutations=9999)

###############Cultivar Z19###############################

design <- design\_filter\_16s; otu.data <- otu\_norm\_16s

library (dplyr); design <- **filter**(design, Cultivar=="Z19"); otu.data <- otu.data[, rownames(design)]

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

distance.w <- vegdist(**decostand**(t(otu.data), "hellinger"), method = 'bray')

res.w <- **pcoa**(distance.w)

res.v.w <- res.w$vectors

site.w <- data.frame(x= res.v.w[,1], y= res.v.w[,2], FOL=design$FOL,

Trt=design$Trt, Ino= design$ Inoculated )

site.w$Trt <- as.factor(site.w$Trt)

site.w$ Ino <- as.factor(site.w$ Ino)

site.w$sample <- rownames(site.w)

#############Plotting####################

B.PCoA.Bray.Z19 <- ggplot(data=site.w, aes(x, y, colour = FOL))+geom\_point(size=4, shape=16) + #, shape=Trt

geom\_text\_repel(data=site.w, aes(x, y, label=sample), colour="black", size=5) +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" ")) +

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

**stat\_ellipse**(aes(group=FOL, fill=FOL), type="norm", alpha=0.2, level=0.75, geom="polygon", color=NA) +

scale\_color\_manual( values= c("darkorange2", "dodgerblue", "forestgreen"), labels=c("Fol", "Sys", "Control") ) +

scale\_fill\_manual(values= c("darkorange2", "dodgerblue", "forestgreen"), guide="none") +

xlab(paste("PCoA1", paste("(",round(res.w$values[1,2]\*100,2),"%",")",sep=""),sep=" "))+

ylab(paste("PCoA2", paste("(",round(res.w$values[2,2]\*100,2),"%",")",sep=""),sep=" ")) +

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() )

B.PCoA.Bray.Z19

## Perform PERMANVOA

design$Trt <- as.factor (design$Trt); adonis2(distance.w ~ Trt, data=design, permutations=9999)

**ggarrange**(B.PCoA.Bray.D72, B.PCoA.Bray.Z19, ncol=2, nrow=1, align="hv", legend="none" )