setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Manuscript/Nat Com/Revised/SP\_mOTU3\_JP") **#SampleIDに注意ここではMETAFをIDとして解析**

Meta <- read.csv("Meta.csv", header = TRUE, na.strings = c(NA, ''), row.names=1)

Meta <- rename(Meta, Group = UC\_CD)

#rownames\_to\_column(Meta, "METAF") -> Meta #β Diversityの時は実行しない。alphaの時は実行する。

METADATA=sample\_data(Meta, errorIfNULL = TRUE)

**#SP(mOTU3)**

#Shannon

SP\_input <- read.csv("SP\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

SP\_input2 <- SP\_input[, -which (colnames(SP\_input) %in% c("ID", "IBD", "UC", "CD"))]

DATA=otu\_table(SP\_input2, taxa\_are\_rows = FALSE)

Shannon\_SP<-diversity(SP\_input2,index="**shannon**",base=2) %>% as.data.frame() #simpson, invsimpsonにかえられる。

colnames(Shannon\_SP)[1] <- "Shannon"

rownames\_to\_column(Shannon\_SP, "METAF") -> Shannon\_SP

Shannon\_SP <- full\_join(Shannon\_SP, Meta, by = "METAF")

ggplot(Shannon\_SP, aes(Group, Shannon, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Shannon for species") +

scale\_y\_continuous(limits = c(0, 8.5)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 8, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 7.8, yend = 7.8) +

annotate("text", x = 2, y = 8.5, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 8.3, yend = 8.3)+

annotate("text", x = 2.5, y = 7.7, label = "P = **0.007**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 7.5, yend = 7.5)+

guides(fill=guide\_legend(title=NULL))

#Simpson

SP\_input <- read.csv("SP\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

SP\_input2 <- SP\_input[, -which (colnames(SP\_input) %in% c("ID", "IBD", "UC", "CD"))]

DATA=otu\_table(SP\_input2, taxa\_are\_rows = FALSE)

Simpson\_SP<-diversity(SP\_input2,index="**simpson**",base=2) %>% as.data.frame() #simpson, invsimpsonにかえられる。

colnames(Simpson\_SP)[1] <- "Simpson"

rownames\_to\_column(Simpson\_SP, "METAF") -> Simpson\_SP

Simpson\_SP <- full\_join(Simpson\_SP, Meta, by = "METAF")

ggplot(Simpson\_SP, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Simpson for species") +

scale\_y\_continuous(limits = c(0, 1.3)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 1.15, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 1.1, yend = 1.1) +

annotate("text", x = 2, y = 1.25, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 1.2, yend = 1.2)+

annotate("text", x = 2.5, y = 1.1, label = "P = **0.004**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 1.05, yend = 1.05)+

guides(fill=guide\_legend(title=NULL))

#Invese Simpson

SP\_input <- read.csv("SP\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

SP\_input2 <- SP\_input[, -which (colnames(SP\_input) %in% c("ID", "IBD", "UC", "CD"))]

DATA=otu\_table(SP\_input2, taxa\_are\_rows = FALSE)

Simpson\_SP<-diversity(SP\_input2,index="inv**simpson**",base=2) %>% as.data.frame() #simpson, invsimpsonにかえられる。

colnames(Simpson\_SP)[1] <- "Simpson"

rownames\_to\_column(Simpson\_SP, "METAF") -> Simpson\_SP

Simpson\_SP <- full\_join(Simpson\_SP, Meta, by = "METAF")

ggplot(Simpson\_SP, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Inverse Simpson for species") +

scale\_y\_continuous(limits = c(0, 100)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 79, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 76, yend = 76) +

annotate("text", x = 2, y = 85, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 82, yend = 82)+

annotate("text", x = 2.5, y = 75, label = "P = **0.004**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 72, yend = 72)+

guides(fill=guide\_legend(title=NULL))

#beta-diversity

adonis2(SP\_input2~Group, data=Meta, permutation=9999, method="bray") #obtain p value for PERMANOVA #data=MetaとなっておりMetaからgroupを探し出してくれる。

data\_phylo<-phyloseq(DATA, METADATA)

bc=ordinate(data\_phylo, method = "MDS", distane = "bray") #NMDSなど選べる

plot\_ordination(data\_phylo, bc, color= "Group")+

geom\_point(size=3)+

stat\_ellipse() +

labs(x = "MDS1", y = "MDS2") +

scale\_color\_manual(values = c("royalblue","#84919e","red"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.line = element\_line(colour = "black", linewidth= 0.7, linetype = "solid"),

legend.background = element\_blank(),

legend.key = element\_blank(),

legend.box.background = element\_rect(colour = "black"),

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

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

panel.background = element\_blank(),

text = element\_text(size = 20))+

guides(fill=guide\_legend(title=NULL))+

annotate("text", x = 0.4, y = -0.35, label = "P = 1e-04", size = 8, fontface = "plain")

#主座標分析において軸の向きに意味はないの。

#RIchness

SP\_input <- read.csv("SP\_IBD\_num.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data SP\_inout\_numのみRICHNESSをいれている。

SP\_input2 <- SP\_input[, -which (colnames(SP\_input) %in% c("ID", "IBD", "UC", "CD"))]

rownames\_to\_column(SP\_input2, "METAF") -> SP\_input2

Richness\_SP <- full\_join(SP\_input2, Meta, by = "METAF")

ggplot(Richness\_SP, aes(Group, Richness, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Richness for species") +

scale\_y\_continuous(limits = c(0, 600)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 570, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 555, yend = 555) +

annotate("text", x = 2, y = 600, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 585, yend = 585)+

annotate("text", x = 2.5, y = 540, label = "P = **0.007**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 525, yend = 525)+

guides(fill=guide\_legend(title=NULL))

**#ARG**

#Shannon

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Figure用解析/Bacteriome\_all\_results\_for\_pval/Diversity/Microbiome\_diversity")

Meta <- read.csv("Meta.csv", header = TRUE, na.strings = c(NA, ''), row.names=1)

Meta <- rename(Meta, Group = UC\_CD)

METADATA=sample\_data(Meta, errorIfNULL = TRUE)

ARG\_input <- read.csv("ARG\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

ARG\_input2 <- ARG\_input[, -which (colnames(ARG\_input) %in% c("IBD\_HC2", "IBD\_1", "UC", "Crohn"))]

DATA=otu\_table(ARG\_input2, taxa\_are\_rows = FALSE)

Shannon\_ARG<-diversity(ARG\_input2,index="shannon",base=2) %>% as.data.frame()

colnames(Shannon\_ARG)[1] <- "Shannon"

Shannon\_ARG <- cbind(Shannon\_ARG, Meta)

ggplot(Shannon\_ARG, aes(Group, Shannon, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Shannon for ARG") +

scale\_y\_continuous(limits = c(0, 8)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

guides(fill=guide\_legend(title=NULL))

#simpson

ARG\_input <- read.csv("ARG\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

ARG\_input2 <- ARG\_input[, -which (colnames(ARG\_input) %in% c("IBD\_HC2", "IBD\_1", "UC", "Crohn"))]

DATA=otu\_table(ARG\_input2, taxa\_are\_rows = FALSE)

Simpson\_ARG<-diversity(ARG\_input2,index="simpson",base=2) %>% as.data.frame()

colnames(Simpson\_ARG)[1] <- "Simpson"

Simpson\_ARG <- cbind(Simpson\_ARG, Meta)

ggplot(Simpson\_ARG, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Simpson for ARG") +

scale\_y\_continuous(limits = c(0, 1)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

guides(fill=guide\_legend(title=NULL))

#Inverse simpson

ARG\_input <- read.csv("ARG\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

ARG\_input2 <- ARG\_input[, -which (colnames(ARG\_input) %in% c("IBD\_HC2", "IBD\_1", "UC", "Crohn"))]

DATA=otu\_table(ARG\_input2, taxa\_are\_rows = FALSE)

Simpson\_ARG<-diversity(ARG\_input2,index="invsimpson",base=2) %>% as.data.frame()

colnames(Simpson\_ARG)[1] <- "Simpson"

Simpson\_ARG <- cbind(Simpson\_ARG, Meta)

ggplot(Simpson\_ARG, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Inverse Simpson for ARG") +

scale\_y\_continuous(limits = c(0, 100)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

guides(fill=guide\_legend(title=NULL))

#beta-diversity

adonis2(ARG\_input2~Group, data=Meta, permutation=9999, method="bray") #obtain p value for PERMANOVA #data=MetaとなっておりMetaからgroupを探し出してくれる。

data\_phylo<-phyloseq(DATA, METADATA)

bc=ordinate(data\_phylo, method = "MDS", distane = "bray") #NMDSなど選べる

plot\_ordination(data\_phylo, bc, color= "Group")+

geom\_point(size=3)+

stat\_ellipse() +

labs(x = "MDS1", y = "MDS2") +

scale\_color\_manual(values = c("royalblue","#84919e","red"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.line = element\_line(colour = "black", linewidth= 0.7, linetype = "solid"),

legend.background = element\_blank(),

legend.key = element\_blank(),

legend.box.background = element\_rect(colour = "black"),

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

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

panel.background = element\_blank(),

text = element\_text(size = 20))+

guides(fill=guide\_legend(title=NULL))+

annotate("text", x = 0.4, y = -0.35, label = "P = 1e-04", size = 8, fontface = "plain")

#RIchness

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Figure用解析/Bacteriome\_all\_results\_for\_pval/Diversity/Microbiome\_diversity")

Meta <- read.csv("Meta.csv", header = TRUE, na.strings = c(NA, ''), row.names=1)

Meta <- rename(Meta, Group = UC\_CD)

rownames\_to\_column(Meta, "ID") -> Meta

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Manuscript/Nat Com/Revised/MaAsLin/ARG")

SP\_input <- read.csv("ARG.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data ARG\_numがあるところにデータをとりにいった。

rownames\_to\_column(SP\_input, "ID") -> SP\_input2

Richness\_SP <- full\_join(SP\_input2, Meta, by = "ID")

ggplot(Richness\_SP, aes(Group, Number\_of\_ARG, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Richness for ARG") +

scale\_y\_continuous(limits = c(0, 200)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

#annotate("text", x = 1.5, y = 570, label = "P < 0.001", size = 6, fontface = "plain") +

#geom\_segment(x = 1, xend = 2, y = 555, yend = 555) +

#annotate("text", x = 2, y = 600, label = "P < 0.001", size = 6, fontface = "plain")+

#geom\_segment(x = 1, xend = 3, y = 585, yend = 585)+

annotate("text", x = 2.5, y = 175, label = "P = **0.02**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 165, yend = 165)+

guides(fill=guide\_legend(title=NULL))

**#vOTU**

#Shannon

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Figure用解析/Bacteriome\_all\_results\_for\_pval/Diversity/Microbiome\_diversity")

Meta <- read.csv("Meta.csv", header = TRUE, na.strings = c(NA, ''), row.names=1)

Meta <- rename(Meta, Group = UC\_CD)

METADATA=sample\_data(Meta, errorIfNULL = TRUE)

vOTU\_input <- read.csv("vOTU\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

vOTU\_input2 <- vOTU\_input[, -which (colnames(vOTU\_input) %in% c("IBD\_HC2", "UC", "Crohn"))]

DATA=otu\_table(vOTU\_input2, taxa\_are\_rows = FALSE)

Shannon\_vOTU<-diversity(vOTU\_input2,index="shannon",base=2) %>% as.data.frame()

colnames(Shannon\_vOTU)[1] <- "Shannon"

Shannon\_vOTU <- cbind(Shannon\_vOTU, Meta)

ggplot(Shannon\_vOTU, aes(Group, Shannon, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Shannon for vOTU") +

scale\_y\_continuous(limits = c(0, 8.5)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 7.7, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 7.5, yend = 7.5) +

annotate("text", x = 2, y = 8.2, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 8, yend = 8)+

annotate("text", x = 2.5, y = 7.4, label = "P = 0.02", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 7.2, yend = 7.2)+

guides(fill=guide\_legend(title=NULL))

#simpson

vOTU\_input <- read.csv("vOTU\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

vOTU\_input2 <- vOTU\_input[, -which (colnames(vOTU\_input) %in% c("IBD\_HC2", "UC", "Crohn"))]

DATA=otu\_table(vOTU\_input2, taxa\_are\_rows = FALSE)

Simpson\_vOTU<-diversity(vOTU\_input2,index="simpson",base=2) %>% as.data.frame()

colnames(Simpson\_vOTU)[1] <- "Simpson"

Simpson\_vOTU <- cbind(Simpson\_vOTU, Meta)

ggplot(Simpson\_vOTU, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Simpson for vOTU") +

scale\_y\_continuous(limits = c(0, 1.3)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 1.15, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 1.1, yend = 1.1) +

annotate("text", x = 2, y = 1.25, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 1.2, yend = 1.2)+

annotate("text", x = 2.5, y = 1.1, label = "P = **0.02**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 1.05, yend = 1.05)+

guides(fill=guide\_legend(title=NULL))

#Inverse Simpson

vOTU\_input <- read.csv("vOTU\_IBD.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data

vOTU\_input2 <- vOTU\_input[, -which (colnames(vOTU\_input) %in% c("IBD\_HC2", "UC", "Crohn"))]

DATA=otu\_table(vOTU\_input2, taxa\_are\_rows = FALSE)

Simpson\_vOTU<-diversity(vOTU\_input2,index="invsimpson",base=2) %>% as.data.frame()

colnames(Simpson\_vOTU)[1] <- "Simpson"

Simpson\_vOTU <- cbind(Simpson\_vOTU, Meta)

ggplot(Simpson\_vOTU, aes(Group, Simpson, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Inverse Simpson for vOTU") +

scale\_y\_continuous(limits = c(0, 100)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 79, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 76, yend = 76) +

annotate("text", x = 2, y = 85, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 82, yend = 82)+

annotate("text", x = 2.5, y = 75, label = "P = **0.02**", size = 6, fontface = "plain")+

geom\_segment(x = 2, xend = 3, y = 72, yend = 72)+

guides(fill=guide\_legend(title=NULL))

#beta-diversity

adonis2(vOTU\_input2~Group, data=Meta, permutation=9999, method="bray") #obtain p value for PERMANOVA #data=MetaとなっておりMetaからgroupを探し出してくれる。

data\_phylo<-phyloseq(DATA, METADATA)

bc=ordinate(data\_phylo, method = "MDS", distane = "bray") #NMDSなど選べる

plot\_ordination(data\_phylo, bc, color= "Group")+

geom\_point(size=3)+

stat\_ellipse() +

labs(x = "MDS1", y = "MDS2") +

scale\_color\_manual(values = c("royalblue","#84919e","red"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 20),

axis.line = element\_line(colour = "black", linewidth= 0.7, linetype = "solid"),

legend.background = element\_blank(),

legend.key = element\_blank(),

legend.box.background = element\_rect(colour = "black"),

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

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

panel.background = element\_blank(),

text = element\_text(size = 20))+

guides(fill=guide\_legend(title=NULL))+

annotate("text", x = 0.4, y = -0.35, label = "P = 1e-04", size = 8, fontface = "plain")

#RIchness

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Figure用解析/Bacteriome\_all\_results\_for\_pval/Diversity/Microbiome\_diversity")

Meta <- read.csv("Meta.csv", header = TRUE, na.strings = c(NA, ''), row.names=1)

Meta <- rename(Meta, Group = UC\_CD)

rownames\_to\_column(Meta, "ID") -> Meta

setwd("/Users/akiyama/Documents/筑波大学/筑波大学研究/プロジェクト/Microbiome共同研究/Manuscript/Nat Com/Revised/MaAsLin/Phage")

SP\_input <- read.csv("vOTU\_num.csv", header = TRUE, na.strings = c(NA, ''), row.names=1) #abundance data ARG\_numがあるところにデータをとりにいった。

rownames\_to\_column(SP\_input, "ID") -> SP\_input2

Richness\_SP <- full\_join(SP\_input2, Meta, by = "ID")

ggplot(Richness\_SP, aes(Group, Richness, fill = Group)) +

geom\_violin(aes(group = Group), width = 0.5) +

scale\_fill\_manual(values = c("royalblue","#84919e","red"))+

geom\_boxplot(aes(group = Group), fill = "white", width = 0.1) +

theme\_classic() +

labs(x = "", y = "Richness for vOTU") +

scale\_y\_continuous(limits = c(0, 280)) +

scale\_x\_discrete(limit=c("Controls","UC","CD"))+

theme(axis.text.x = element\_text(angle = 0, hjust = 0.5, size = 18),

axis.text.y = element\_text(angle = 0, hjust = 0.5, size = 18),

text = element\_text(size = 18)) +

annotate("text", x = 1.5, y = 230, label = "P < 0.001", size = 6, fontface = "plain") +

geom\_segment(x = 1, xend = 2, y = 220, yend = 220) +

annotate("text", x = 2, y = 260, label = "P < 0.001", size = 6, fontface = "plain")+

geom\_segment(x = 1, xend = 3, y = 250, yend = 250)+

#annotate("text", x = 2.5, y = 180, label = "P = **0.02**", size = 6, fontface = "plain")+

#geom\_segment(x = 2, xend = 3, y = 165, yend = 165)+

guides(fill=guide\_legend(title=NULL))

**#Alpha-diversityのグラフ化**

**#SP**

#Shannon

#P valの求め方。

ggbetweenstats(plot.type = "box", data = Shannon\_SP, x = Group, y = Shannon, type = "nonparametirc", title = "alpha-diversity",

centrality.plotting = TRUE,

centrality.point.args = list(size = 2, color = "darkred"),

centrality.label.args = list(size = 1, nudge\_x = 0.5, segment.linetype = 2, min.segment.length = 0),

ggplot.component = list(theme(text=element\_text(size=10))),

results.subtitle = TRUE)

#ARG

#P valの求め方。

ggbetweenstats(plot.type = "box", data = Shannon\_ARG, x = Group, y = Shannon, type = "nonparametirc", title = "alpha-diversity",

centrality.plotting = TRUE,

centrality.point.args = list(size = 2, color = "darkred"),

centrality.label.args = list(size = 1, nudge\_x = 0.5, segment.linetype = 2, min.segment.length = 0),

ggplot.component = list(theme(text=element\_text(size=10))),

results.subtitle = TRUE)

#vOTU

#P valの求め方。

ggbetweenstats(plot.type = "box", data = Shannon\_vOTU, x = Group, y = Shannon, type = "nonparametirc", title = "alpha-diversity",

centrality.plotting = TRUE,

centrality.point.args = list(size = 2, color = "darkred"),

centrality.label.args = list(size = 1, nudge\_x = 0.5, segment.linetype = 2, min.segment.length = 0),

ggplot.component = list(theme(text=element\_text(size=10))),

results.subtitle = TRUE)