library(vegan)

library(ggplot2)

library(ggprism)

library(ggpubr)

setwd('C:/Users/Administrator/Desktop')

df <- read.table("otu.txt",sep="\t",header = T,row.names = 1,check.names = F)

df <-data.frame(t(df))

#环境因子数据

env <- read.table("benv.txt",sep="\t",header = T,row.names = 1,check.names = F)

head(df)

head(env)

#使用vegan包中的cca()函数进行CCA分析

df\_otu\_cca <- cca(df~., env)

#查看CCA结果信息，以 I 型标尺为例，具体见参考文章

df\_otu\_cca.scaling1 <- summary(df\_otu\_cca, scaling = 1)

R2 <- RsquareAdj(df\_otu\_cca)

df\_otu\_cca\_noadj <- R2$r.squared #原R2

df\_otu\_cca\_adj <- R2$adj.r.squared #校正R2

#计算校正 R2 后的约束轴解释率

df\_otu\_cca\_exp\_adj <- df\_otu\_cca\_adj \* df\_otu\_cca$CCA$eig/sum(df\_otu\_cca$CCA$eig)

CCA1 <- paste("CCA1 (",round(df\_otu\_cca\_exp\_adj[1]\*100, 1),"%)")

CCA2 <- paste("CCA2 (",round(df\_otu\_cca\_exp\_adj[2]\*100, 1),"%)")

## 置换检验##

# 所有约束轴的置换检验，即全局检验，基于 999 次置换，详情 ?anova.cca

df\_otu\_cca\_test <- anova.cca(df\_otu\_cca, permutations = 999)

# 各约束轴逐一检验，基于 999 次置换

df\_otu\_cca\_test\_axis <- anova.cca(df\_otu\_cca, by = 'axis', permutations = 999)

# p值校正（Bonferroni为例）

df\_otu\_cca\_test\_axis$`Pr(>F)` <- p.adjust(df\_otu\_cca\_test\_axis$`Pr(>F)`, method = 'bonferroni')

###提取作图数据

df\_otu\_cca\_sites <- data.frame(df\_otu\_cca.scaling1$sites)[1:2]

df\_otu\_cca\_env <- data.frame(df\_otu\_cca.scaling1$biplot)[1:2]

#######添加分组信息

df\_otu\_cca\_sites$samples <- rownames(df\_otu\_cca\_sites)

#读入分组信息

group <- read.table("group.txt", sep='\t', header=T)

#修改列名

colnames(group) <- c("samples","group")

#将绘图数据和分组合并

df\_otu\_cca\_sites <- merge(df\_otu\_cca\_sites,group,by="samples")

color=c("#1597A5","#FFC24B","#FEB3AE","#FF9515","#6193ED") #颜色变量

p1<-ggplot(data=df\_otu\_cca\_sites,aes(x=CCA1,y=CCA2,

color=group))+#指定数据、X轴、Y轴，颜色

theme\_bw()+#主题设置

geom\_point(size=3,shape=16)+#绘制点图并设定大小

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

geom\_vline(xintercept = 0,lty="dashed",color = 'black', size = 0.8)+

geom\_hline(yintercept = 0,lty="dashed",color = 'black', size = 0.8)+#图中虚线

geom\_text(aes(label=samples, y=CCA2+0.1,x=CCA1+0.1, vjust=0),size=3)+#添加数据点的标签

# guides(color=guide\_legend(title=NULL))+#去除图例标题

labs(x=CCA1,y=CCA2)+#将x、y轴标题改为贡献度

stat\_ellipse(data=df\_otu\_cca\_sites,

level=0.95,

linetype = 2,size=0.8,

show.legend = T)+

scale\_color\_manual(values = color) +#点的颜色设置

scale\_fill\_manual(values = c("#1597A5","#FFC24B","#FEB3AE","#7792CC","#FF9515","#6193ED"))+

theme(axis.title.x=element\_text(size=12),#修改X轴标题文本

axis.title.y=element\_text(size=12,angle=90),#修改y轴标题文本

axis.text.y=element\_text(size=10),#修改x轴刻度标签文本

axis.text.x=element\_text(size=10),#修改y轴刻度标签文本

panel.grid=element\_blank())#隐藏网格线

p1

p2<-p1+geom\_segment(data=df\_otu\_cca\_env,aes(x=0,y=0,xend=CCA1\*3,yend=CCA2\*3),

color="red",size=0.8,

arrow=arrow(angle = 35,length=unit(0.3,"cm")))+

geom\_text(data=df\_otu\_cca\_env,aes(x=CCA1,y=CCA2,

label=rownames(df\_otu\_cca\_env)),size=3.5,

color="blue",

hjust=(1-sign(df\_otu\_cca\_env$CCA1))/2,angle=(180/pi)\*atan(df\_otu\_cca\_env$CCA2/df\_otu\_cca\_env$CCA1))+

theme(legend.position = "top")

p2

#描述统计

data<-summary(df\_otu\_cca)

#检验环境因子相关显著性（Monte Carlo permutation test）

df\_permutest <- permutest(df\_otu\_cca,permu=999) # permu=999是表示置换循环的次数

#每个环境因子显著性检验

df\_envfit <- envfit(df\_otu\_cca,env,permu=999)

#数据处理

cor\_data<-data.frame(data$constr.chi/data$tot.chi, data$unconst.chi/data$tot.chi)

cor\_com <- data.frame(tax=colnames(env),r=df\_envfit$vectors$r,p=df\_envfit$vectors$pvals)

cor\_com[1:14,3]=cor\_com[,3]>0.05 # 将p<0.05标记为FALSE，p>0.05标记为TRUE，使用此数据绘制柱形图。

p3 <- ggplot(cor\_com,aes(x =tax, y = r),size=2) +

geom\_bar(aes(fill=tax),stat = 'identity', width = 0.8)+

geom\_text(aes(y = r+0.05, label = ifelse(p==T,"","\*")),size = 5, fontface = "bold") +

labs(x = '', y = '')+

xlab("Environmental factor")+

ylab(expression(r^"2"))+

theme\_prism(palette = "candy\_bright",

base\_fontface = "plain", # 字体样式，可选 bold, plain, italic

base\_family = "serif", # 字体格式，可选 serif, sans, mono, Arial等

base\_size = 16, # 图形的字体大小

base\_line\_size = 0.8, # 坐标轴的粗细

axis\_text\_angle = 45)+ # 可选值有 0，45，90，270

scale\_fill\_prism(palette = "colors")

p3

ggarrange(p2,p3,ncol = 2,align="none",heights = c(1,1),widths = c(1,1))