

GSB_script

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Data Upload

```
setwd("C:/Users/gaaah/OneDrive/Área de Trabalho/Mestrado/Urochloa/resultados")

#Load main data
db <- readxl::read_xlsx("dbgeral2.xlsx", col_names = TRUE, col_types = NULL, na = "")

#Replace values
db$Depth[db$Depth == "40"] <- "30"

#Convert variables to factor
db$Depth <- as.factor(db$Depth)
db$Genotype <- as.factor(db$Genotype)

#Remove rows
dbc <- db[-c(49:60),]

#Load correlation data
dbc <- readxl::read_xlsx("dbp.xlsx", col_names = TRUE, col_types = NULL, na = "")

#Convert variables to factor
dbc$Depth <- as.factor(dbc$Depth)
dbc$Genotype <- as.factor(dbc$Genotype)

#Remove rows
dbc$10c <- dbc$20c <- dbc$30c <- NULL
```

Process Data for Depth = 10

```
#Subset main data
res1 <- db[(db$Depth == "10"),]
db10 <- as.data.frame(res1)
db10$Depth = NULL
db10$Sample_ID = NULL
db10$Genotype <- as.factor(db10$Genotype)

#Remove rows
db10c <- db10[-c(17:20),]
```

```

#Subset correlation data
res1 <- dbcor[(dbcor$Depth == "10"),]
dbcor10 <- as.data.frame(res1)
dbcor10$Depth = NULL
dbcor10$Sample_ID = NULL
dbcor10$Genotype <- as.factor(dbcor10$Genotype)

#Remove rows
dbcor10c <- dbcor10[-c(17:20),]

```

Process Data for Depth = 20

```

#Subset main data
res2 <- db[(db$Depth == "20"),]
db20 <- as.data.frame(res2)
db20$Depth = NULL
db20$Sample_ID = NULL
db20$Genotype <- as.factor(db20$Genotype)

#Remove rows
db20c <- db20[-c(17:20),]

#Subset correlation data
res2 <- dbcor[(dbcor$Depth == "20"),]
dbcor20 <- as.data.frame(res2)
dbcor20$Depth = NULL
dbcor20$Sample_ID = NULL
dbcor20$Genotype <- as.factor(dbcor20$Genotype)

#Remove rows
dbcor20c <- dbcor20[-c(17:20),]

```

Process Data for Depth = 30

```

#Subset main data
res3 <- db[(db$Depth == "30"),]
db30 <- as.data.frame(res3)
db30$Depth = NULL
db30$Sample_ID = NULL
db30$Genotype <- as.factor(db30$Genotype)

#Remove rows
db30c <- db30[-c(17:20),]

#Subset correlation data
res3=(dbcor[(dbcor$Depth=="40"),])
dbcor30 <- as.data.frame(res3)
dbcor30$Depth = NULL
dbcor30$Sample_ID = NULL

```

```
dbcor30$Genotype <- as.factor(dbc30$Genotype)
```

```
#Remove rows
```

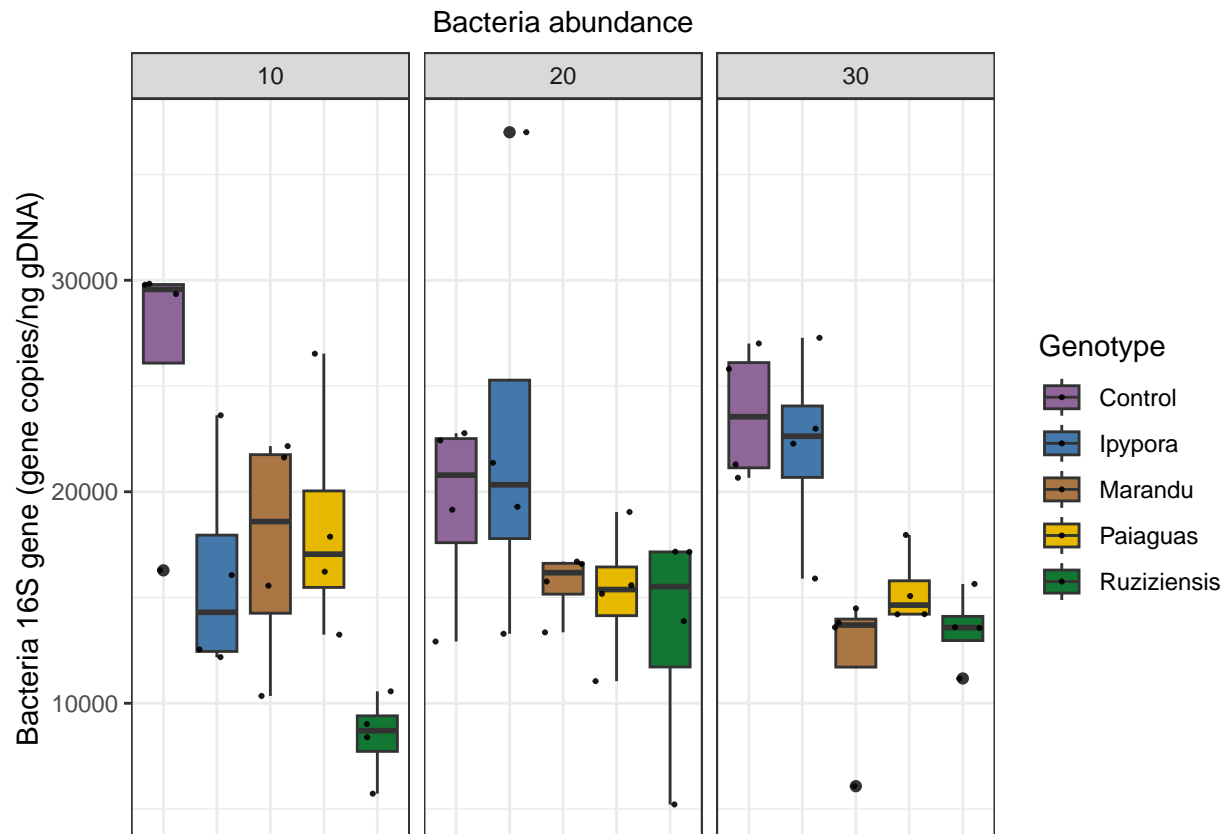
```
dbcor30c = dbcor30[-c(17:20),]
```

Plotting Graphs

qPCR genes quantification

Bacteria 16S

```
bbac <- db %>%  
  #filter(!(Genotype %in% c("Ipyora", "Control"))) %>%  
  ggplot(aes(x=Genotype, y=bac2, fill=Genotype)) +  
  geom_boxplot() +  
  facet_grid(.~Depth, scales="free", space="free_x") +  
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))+  
  geom_jitter(color="black", size=0.4, alpha=0.9) +  
  theme_bw() +  
  theme(axis.title.x=element_blank(),  
        axis.text.x=element_blank(),  
        axis.ticks.x=element_blank()) +  
  theme(legend.position="right", plot.title = element_text(size=11)) +  
  ggtitle("Bacteria abundance") +  
  xlab("Genotypes") +  
  ylab("Bacteria 16S gene (gene copies/ng gDNA)") +  
  ggeasy::easy_center_title() +  
  ggeasy::easy_adjust_legend("center")  
bbac
```

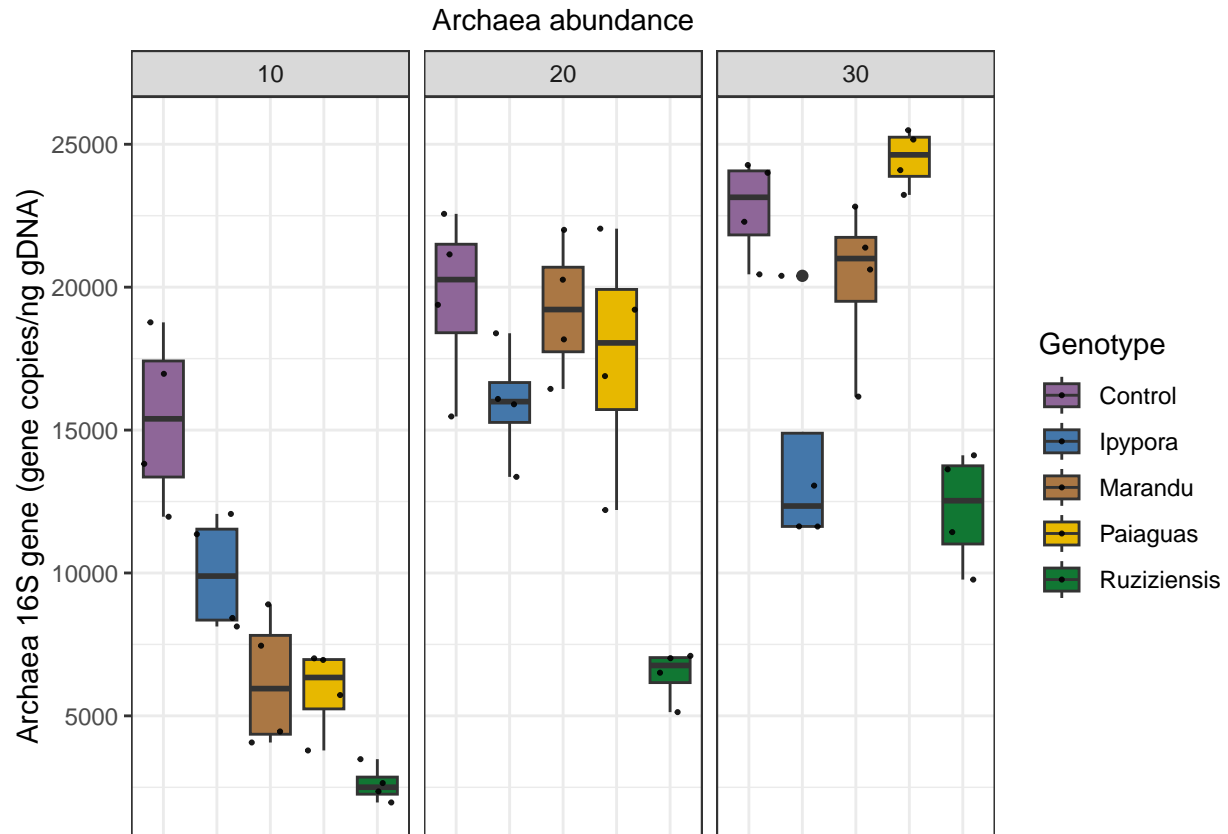


Archaea 16S

```

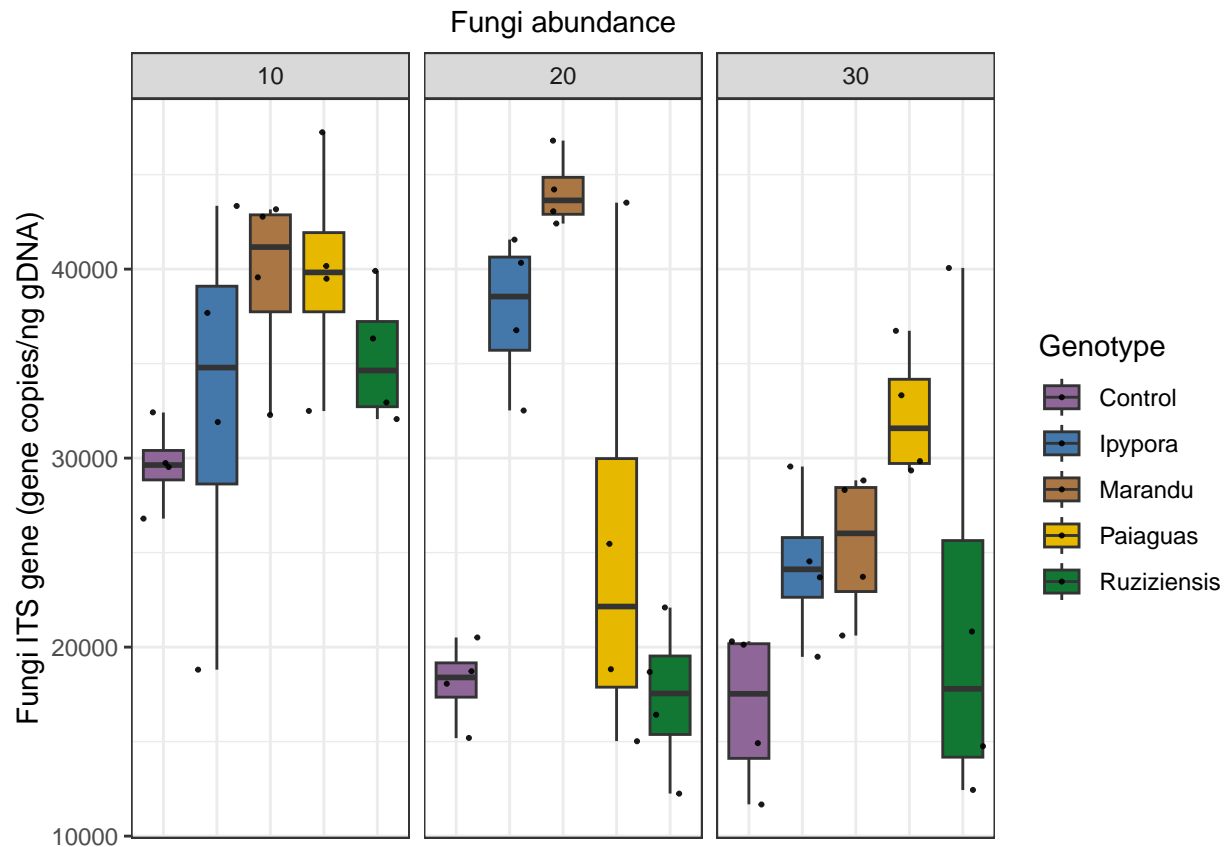
barc <- db %>%
  ggplot(aes(x=Genotype, y=archaea, fill=Genotype)) +
  geom_boxplot() +
  facet_grid(.~Depth, scales="free", space="free_x") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))+
  geom_jitter(color="black", size=0.4, alpha=0.9) +
  theme_bw() +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x=element_blank()) +
  theme(legend.position="right", plot.title = element_text(size=11)) +
  ggtitle("Archaea abundance") +
  xlab("Genotypes") +
  ylab("Archaea 16S gene (gene copies/ng gDNA)") +
  ggeasy::easy_center_title() +
  ggeasy::easy_adjust_legend("center")
barc

```



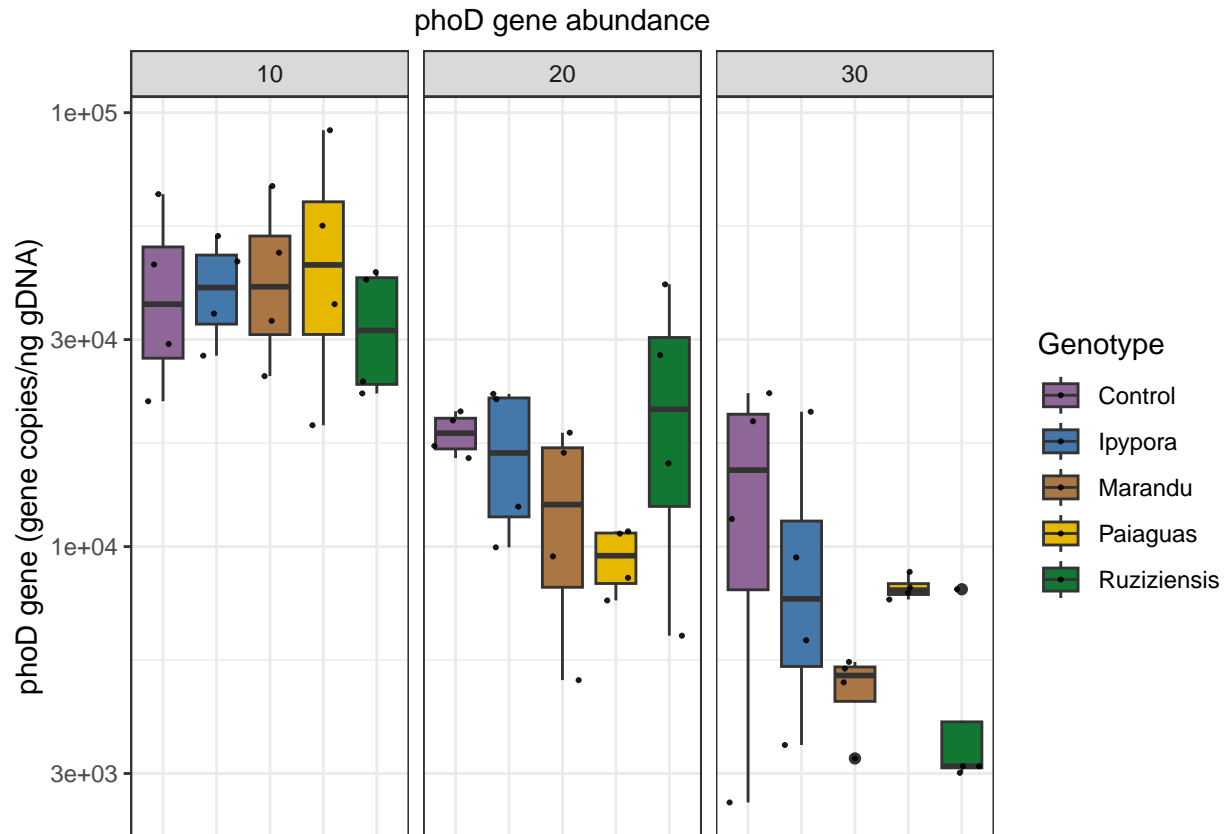
Fungi ITS

```
bits <- db %>%
  ggplot(aes(x=Genotype, y=ITS, fill=Genotype)) +
  geom_boxplot() +
  facet_grid(.~Depth, scales="free", space="free_x") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))+
  geom_jitter(color="black", size=0.4, alpha=0.9) +
  theme_bw() +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x=element_blank()) +
  theme(legend.position="right", plot.title = element_text(size=11)) +
  ggtitle("Fungi abundance") +
  xlab("Genotypes") +
  ylab("Fungi ITS gene (gene copies/ng gDNA)") +
  ggeasy::easy_center_title() +
  ggeasy::easy_adjust_legend("center")
bits
```



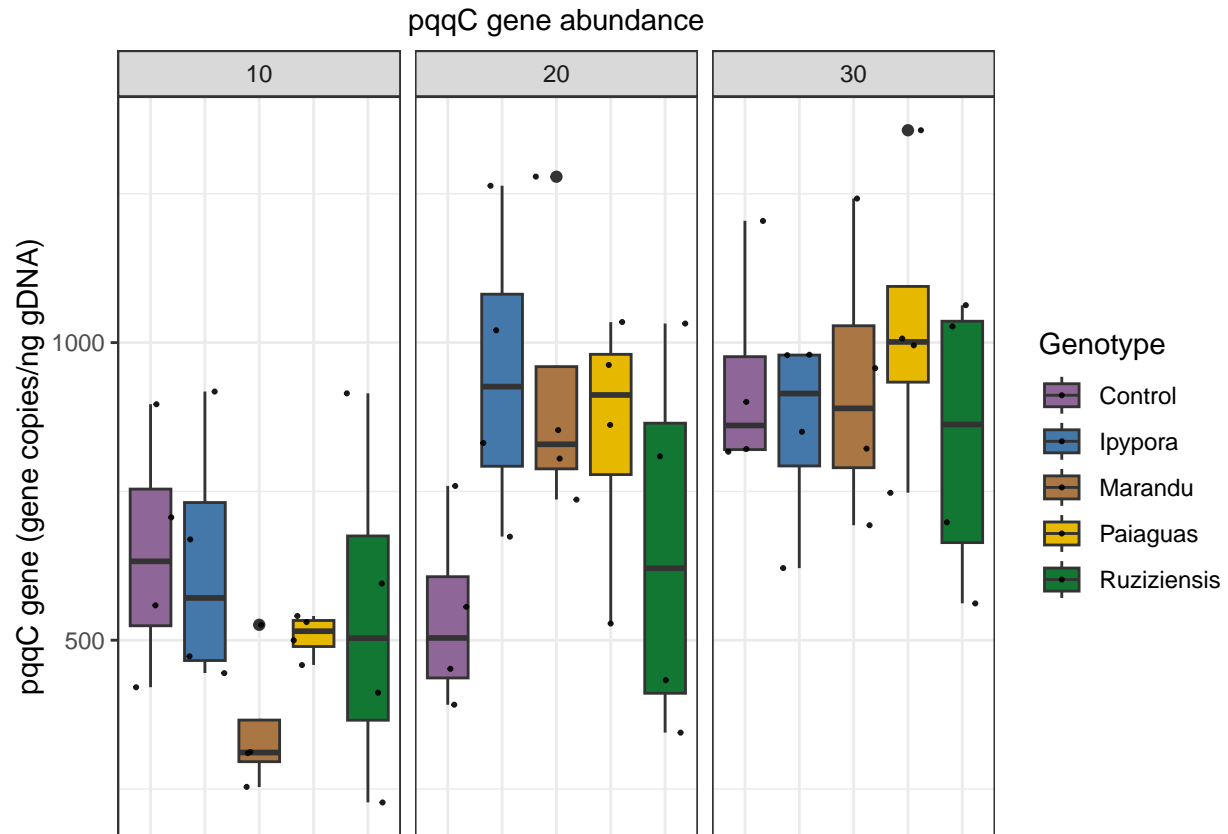
Alkaline phosphatase production by phoD gene

```
bphod <- db %>%
  ggplot(aes(x=Genotype, y=phoD, fill=Genotype)) +
  geom_boxplot() +
  facet_grid(.~Depth, scales="free", space="free_x") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))+
  geom_jitter(color="black", size=0.4, alpha=0.9) +
  theme_bw() +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x=element_blank()) +
  theme(legend.position="right", plot.title = element_text(size=11)) +
  ggtitle("phoD gene abundance") +
  xlab("Genotypes") +
  ylab("phoD gene (gene copies/ng gDNA)") +
  scale_y_log10() +
  ggeasy::easy_center_title() +
  ggeasy::easy_adjust_legend("center")
bphod
```



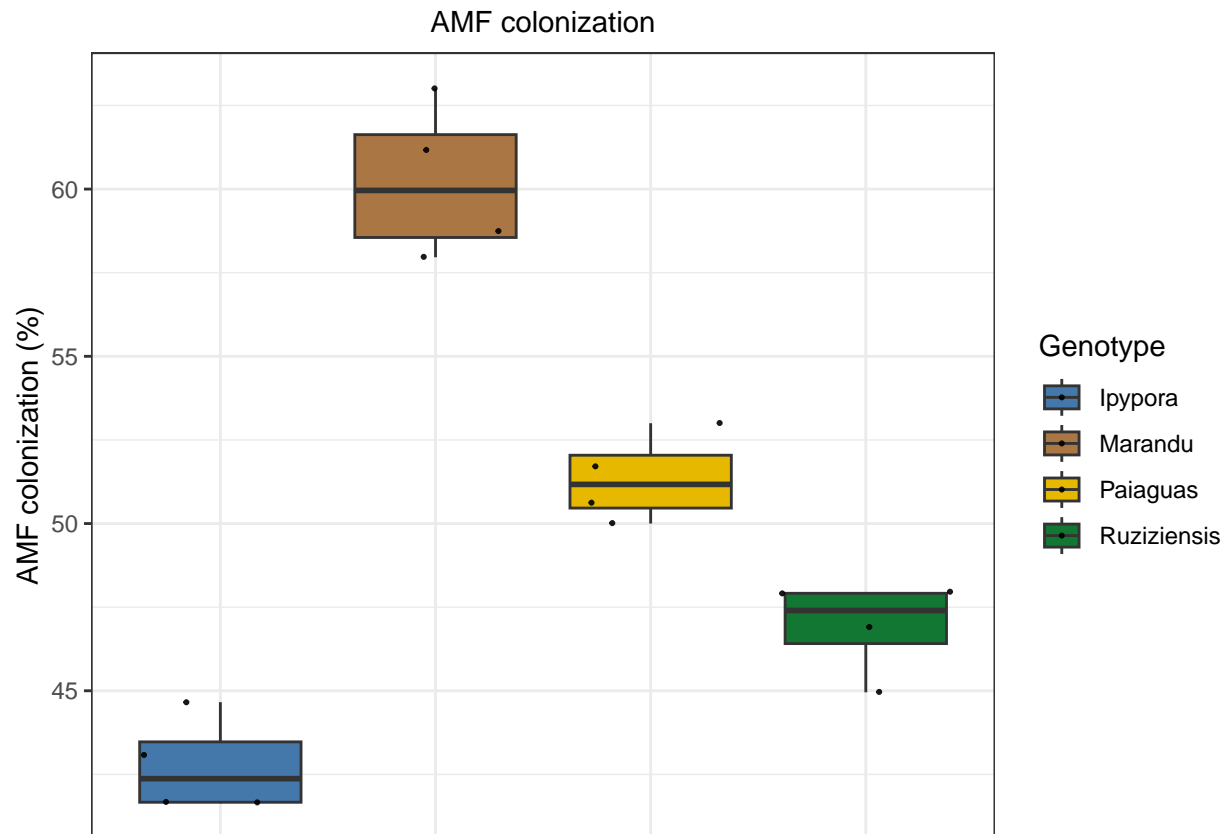
P solubilization via pqqC

```
bpqqc <- db %>%
  ggplot(aes(x=Genotype, y=pqqc3, fill=Genotype)) +
  geom_boxplot() +
  facet_grid(.~Depth, scales="free", space="free_x") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))+
  geom_jitter(color="black", size=0.4, alpha=0.9) +
  theme_bw() +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x=element_blank()) +
  theme(legend.position="right", plot.title = element_text(size=11)) +
  ggtitle("pqqC gene abundance") +
  xlab("Genotypes") +
  ylab("pqqC gene (gene copies/ng gDNA)") +
  ggeasy::easy_center_title() +
  ggeasy::easy_adjust_legend("center")
bpqqc
```



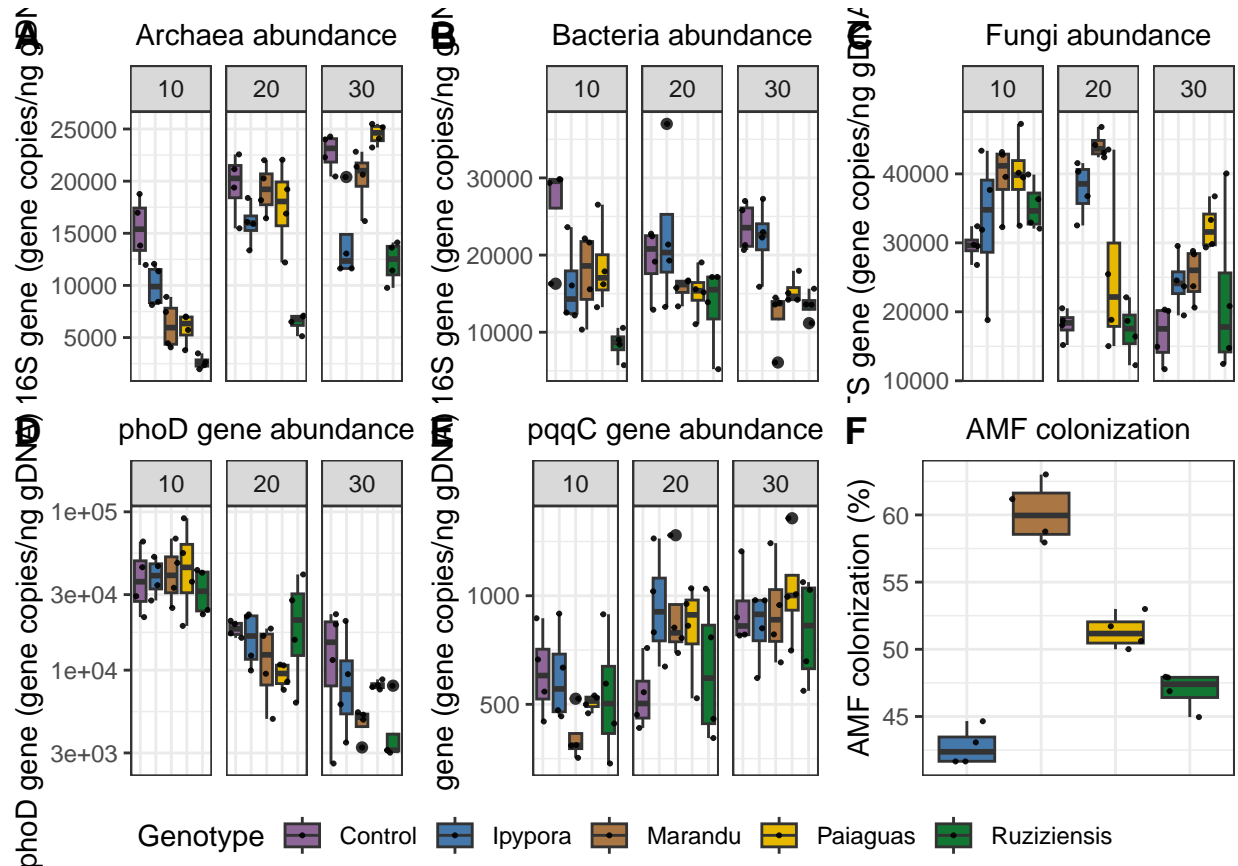
AMF colonization

```
bfma <- db10c %>%
  ggplot(aes(x=Genotype, y=colonization, fill=Genotype)) +
  geom_boxplot() +
  scale_fill_manual(values=c("#4477AA", "#AA7744", "#E7B800", "#117733"))+
  geom_jitter(color="black", size=0.4, alpha=0.9) +
  theme_bw() +
  theme(axis.title.x=element_blank(),
        axis.text.x=element_blank(),
        axis.ticks.x=element_blank()) +
  theme(legend.position="right", plot.title = element_text(size=11)) +
  ggtitle("AMF colonization") +
  xlab("Genotypes") +
  ylab("AMF colonization (%)")+
  ggeasy::easy_center_title() +
  ggeasy::easy_adjust_legend("center")
bfma
```

Plotting boxplots all together

```
ggarrange(barc, bbac, bits, bphod, bpqqc, bfma,
  labels = c("A", "B", "C", "D", "E", "F"),
  common.legend = TRUE,
  legend = "bottom",
  ncol = 3, nrow = 2)
```



Correlation plots

pqqC gene

```

pixpqq<-ggplot(db, aes(pqqc3, NaOH)) +
  geom_point(size = 4) +
  #facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se = T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 1200, label.y = 80) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 1200, label.y = 75, size = 4) +
  theme_bw()+
  theme(legend.position="right", plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  ylab("Inorganic P (mg/Kg)") +
  xlab("pqqC gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pixpqq

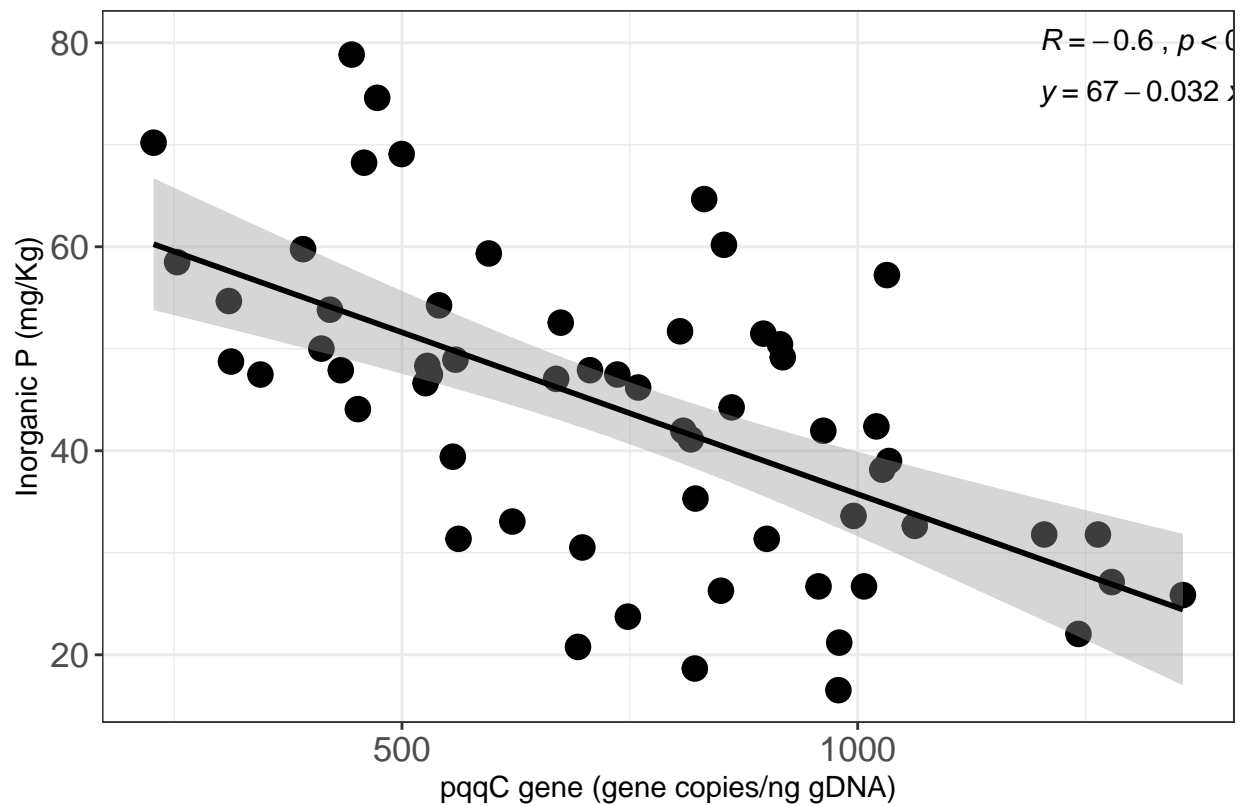
```

```

## Warning: The dot-dot notation ('..r.label..') was deprecated in ggplot2 3.4.0.
## i Please use 'after_stat(r.label)' instead.

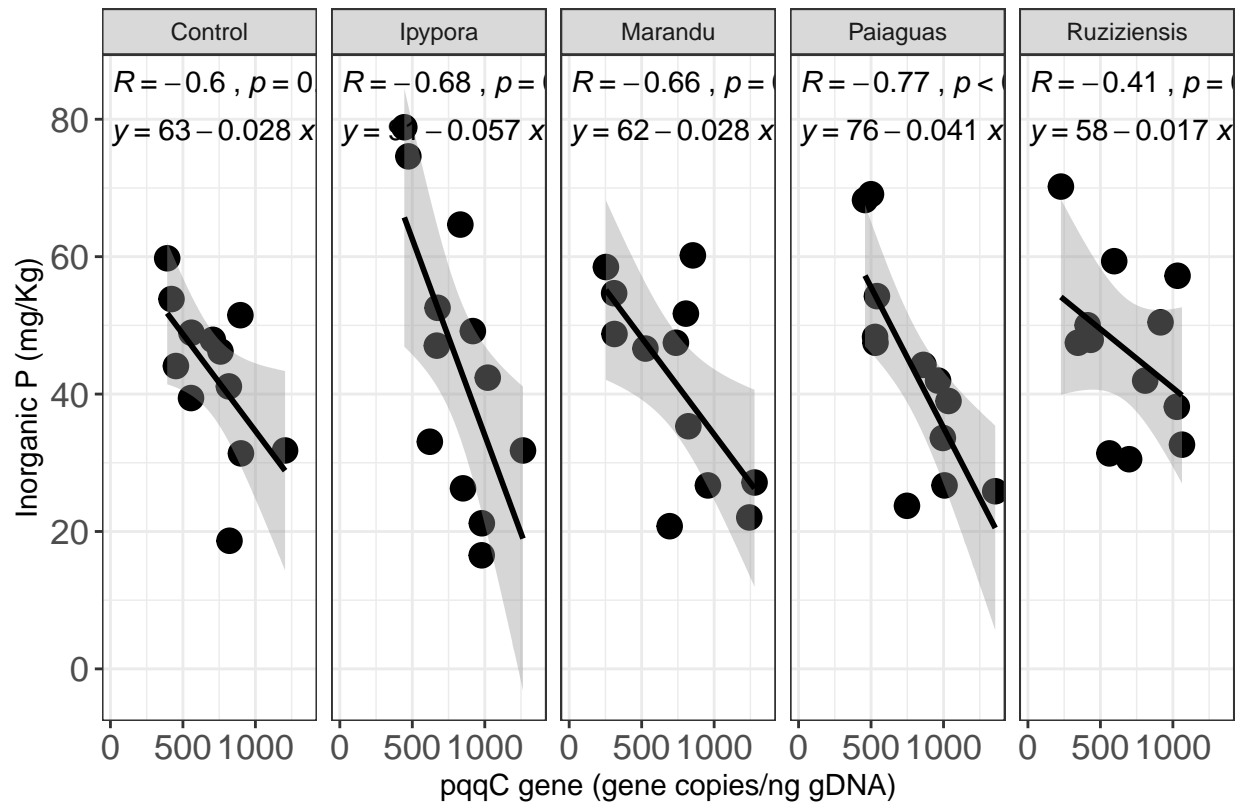
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



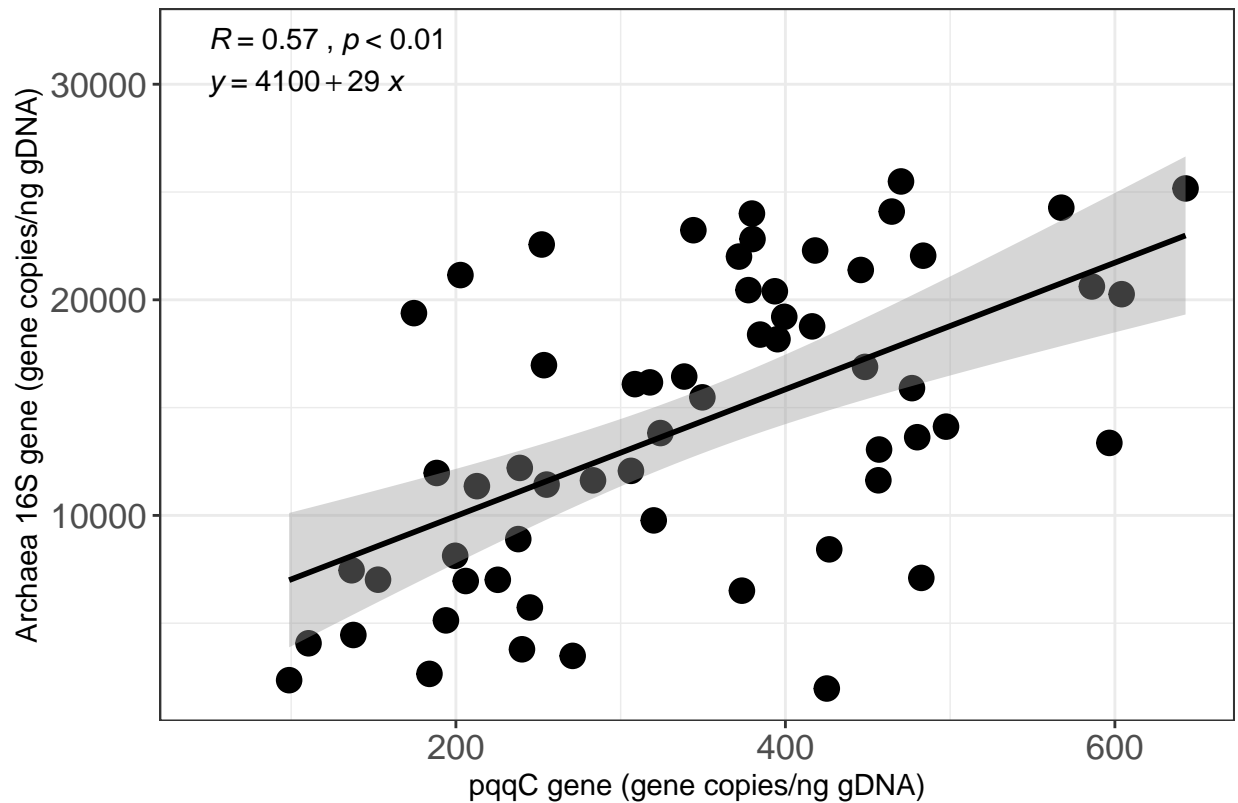
```
pixpqq2<-ggplot(db, aes(pqqc3, NaOH1)) +
  geom_point(size = 4) +
  facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~`,`~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 10, label.y = 85) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 10, label.y = 78, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  ylab("Inorganic P (mg/Kg)") +
  xlab("pqqC gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pixpqq2
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



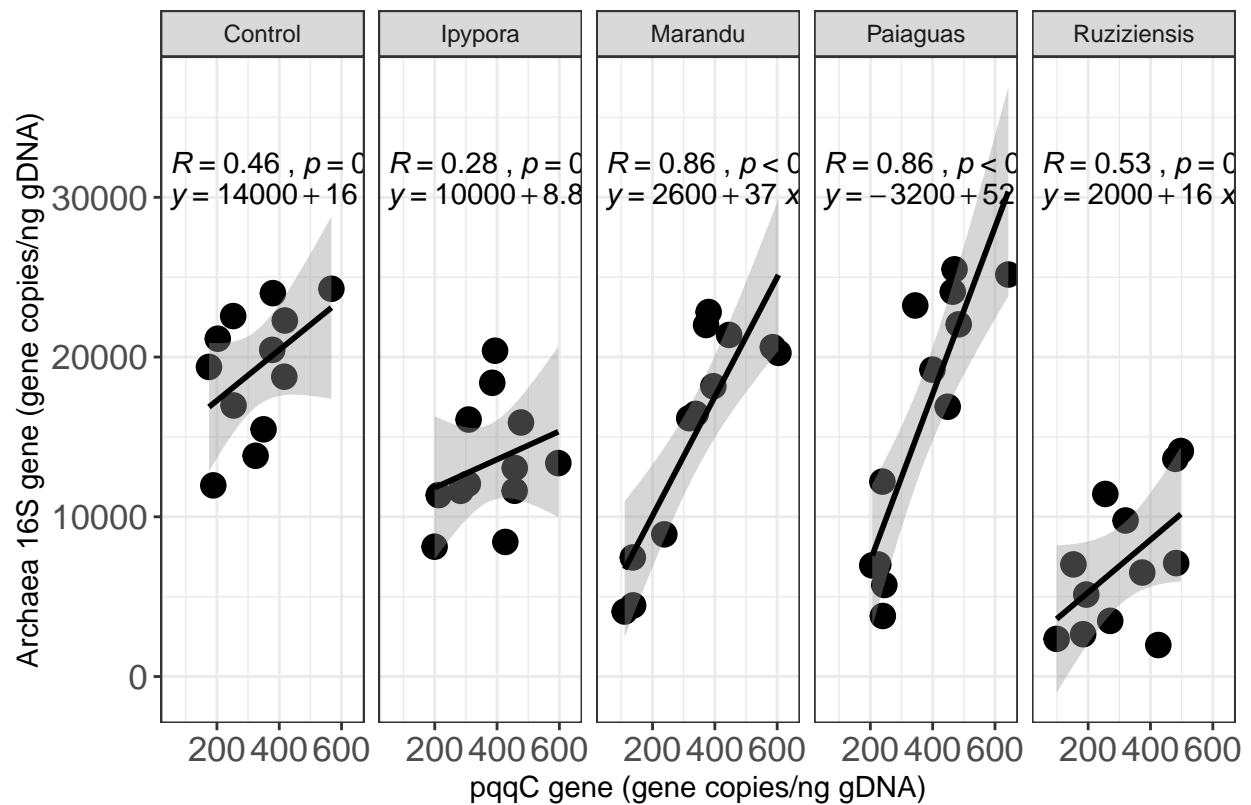
```
arcxpqq<-ggplot(db, aes(pqqc2, archaea)) +
  geom_point(size = 4) +
  #facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 50, label.y = 32000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 50, label.y = 30000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  ylab("Archaea 16S gene (gene copies/ng gDNA)") +
  xlab("pqqC gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
arcxpqq
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
arcxpqq2<-ggplot(db, aes(pqqc2, archaea)) +
  geom_point(size = 4) +
  facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~~,~~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 50, label.y = 32000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 50, label.y = 30000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  ylab("Archaea 16S gene (gene copies/ng gDNA)") +
  xlab("pqqC gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
arcxpqq2
```

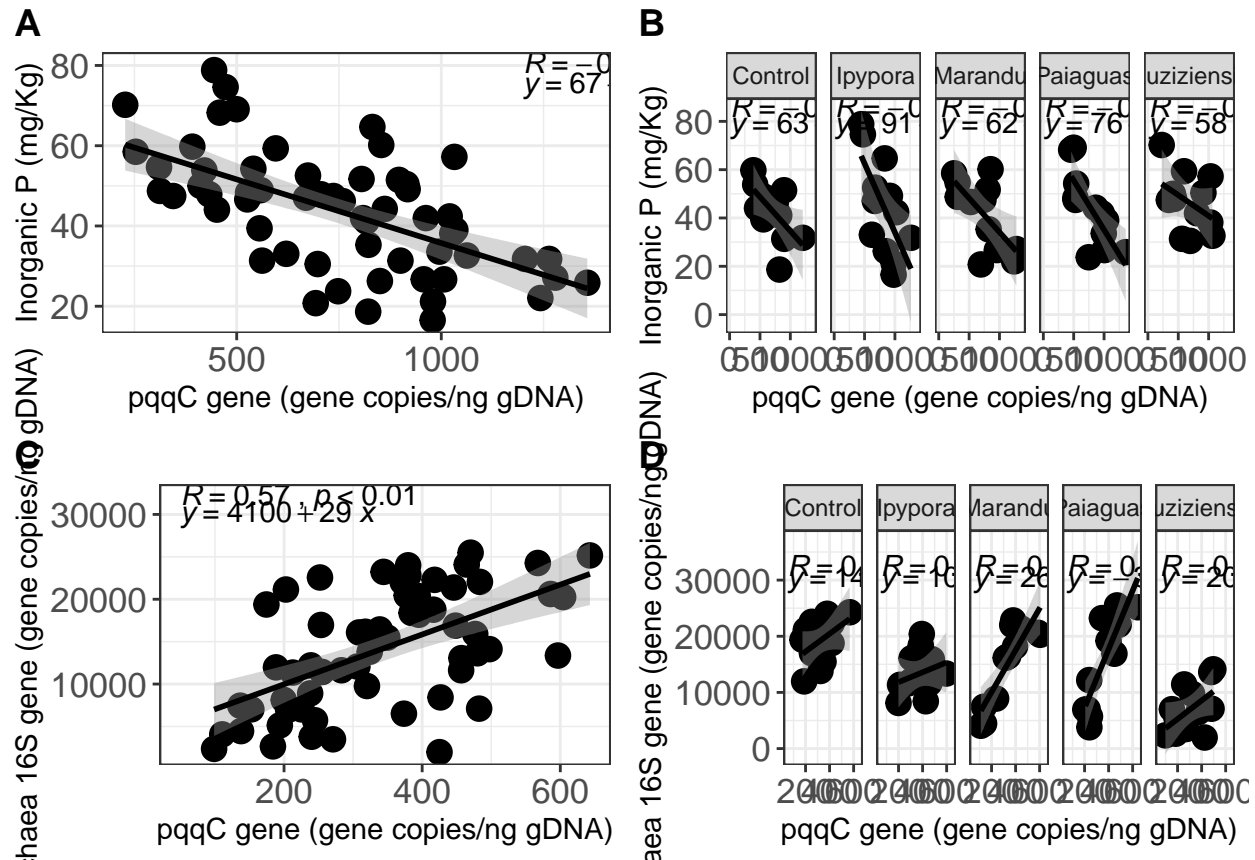
```
## 'geom_smooth()' using formula = 'y ~ x'
```



Plotting all together

```
ggarrange(pixpqq, pixpqq2, arcxpqq, arcxpqq2,
  labels = c("A", "B", "C", "D"),
  common.legend = TRUE,
  legend = "none",
  ncol = 2, nrow = 2)
```

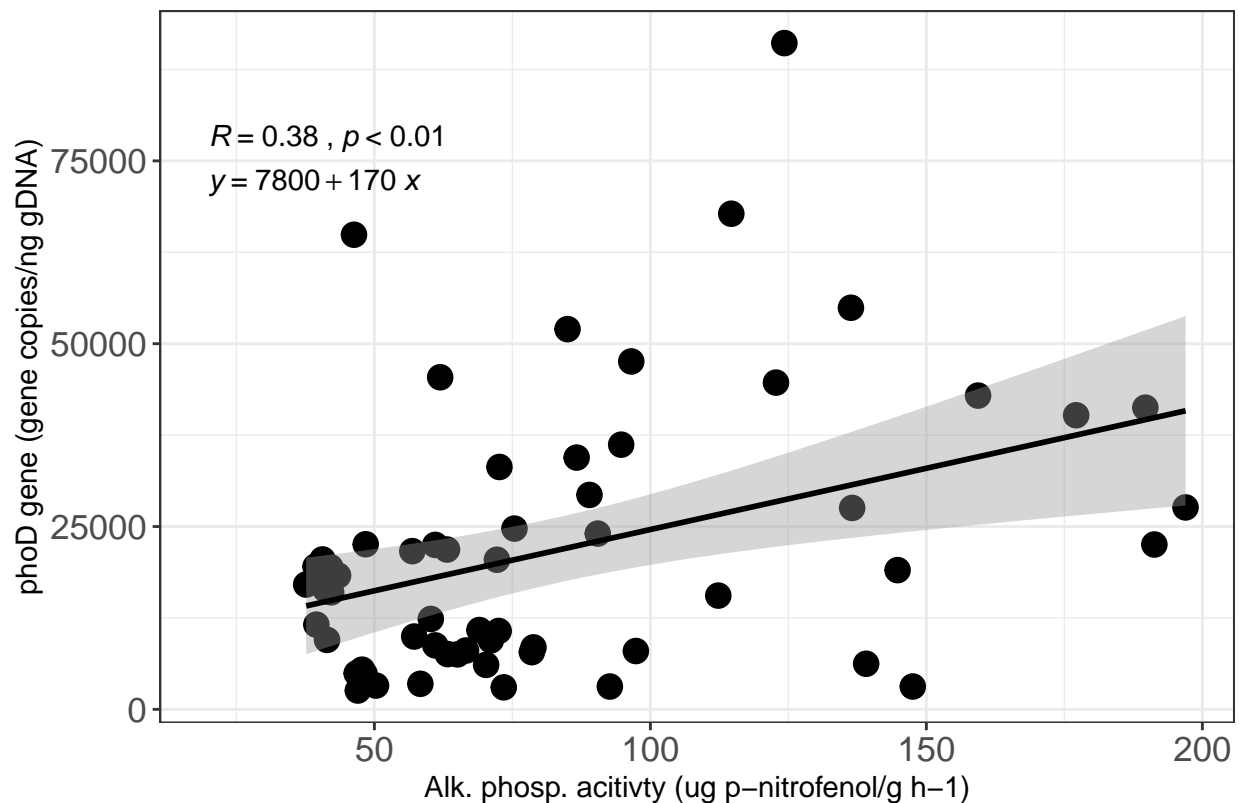
```
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
```



phoD gene

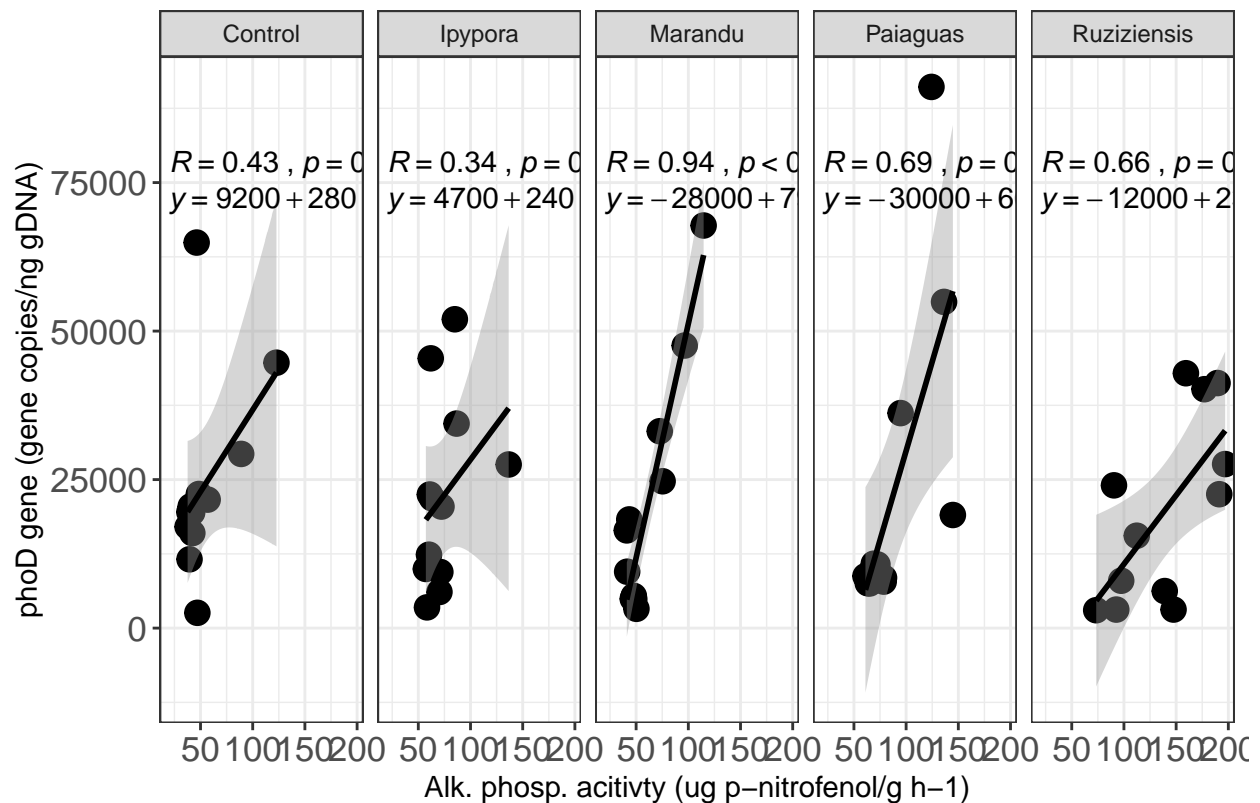
```
pdxfal<-ggplot(db, aes(fosfalk, phoD)) +
  geom_point(size = 4) +
  #facet_wrap(dbc$Genotype, nrow = 1)+
  geom_smooth(method = lm, se = T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 20, label.y = 78000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 20, label.y = 72000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  xlab("Alk. phosp. acitivity (ug p-nitrofenol/g h-1)") +
  ylab("phoD gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pdxfal
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



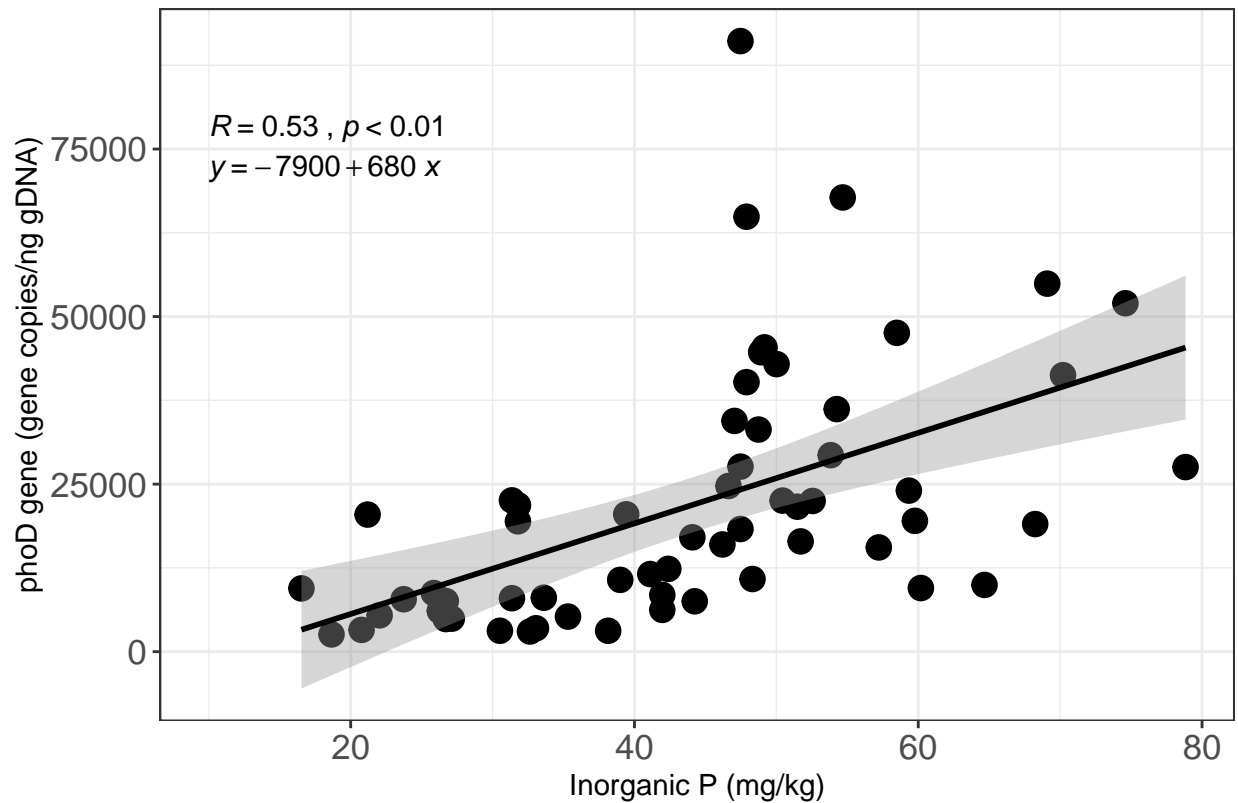
```
pdxfal2<-ggplot(db, aes(fosfalk, phoD)) +
  geom_point(size = 4) +
  facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 20, label.y = 78000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 20, label.y = 72000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  xlab("Alk. phosp. activity (ug p-nitrofenol/g h-1)") +
  ylab("phoD gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pdxfal2
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

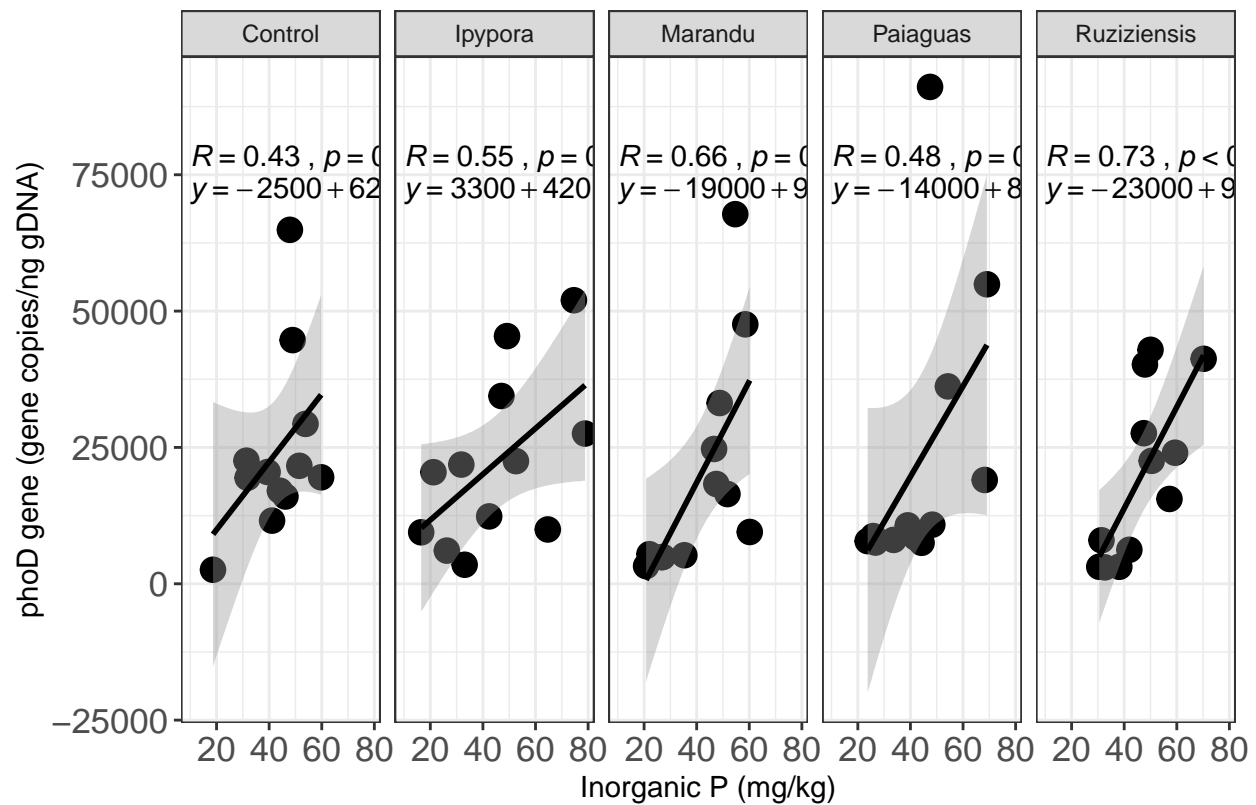
```
pdxpi<-ggplot(db, aes(NaOH, phoD)) +
  geom_point(size = 4) +
  #facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 10, label.y = 78000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 10, label.y = 72000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  xlab("Inorganic P (mg/kg)") +
  ylab("phoD gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pdxpi
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
pdxpi2<-ggplot(db, aes(NaOH_i, phoD)) +
  geom_point(size = 4) +
  facet_wrap(db$Genotype, nrow = 1)+
  geom_smooth(method = lm, se=T, color = "black") +
  stat_cor(aes(label = paste(..r.label.., ..p.label.., sep = "~~", "~")),
    r.accuracy = 0.01,
    p.accuracy = 0.01, size = 4,
    label.x = 10, label.y = 78000) +
  stat_regline_equation(aes(label = ..eq.label..),
    label.x = 10, label.y = 72000, size = 4) +
  theme_bw()+
  theme(legend.position="right",plot.title = element_text(size=11), axis.text.y=element_text(size = 13))
  ggtitle("") +
  xlab("Inorganic P (mg/kg)") +
  ylab("phoD gene (gene copies/ng gDNA)") +
  scale_fill_manual(values=c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"))
pdxpi2
```

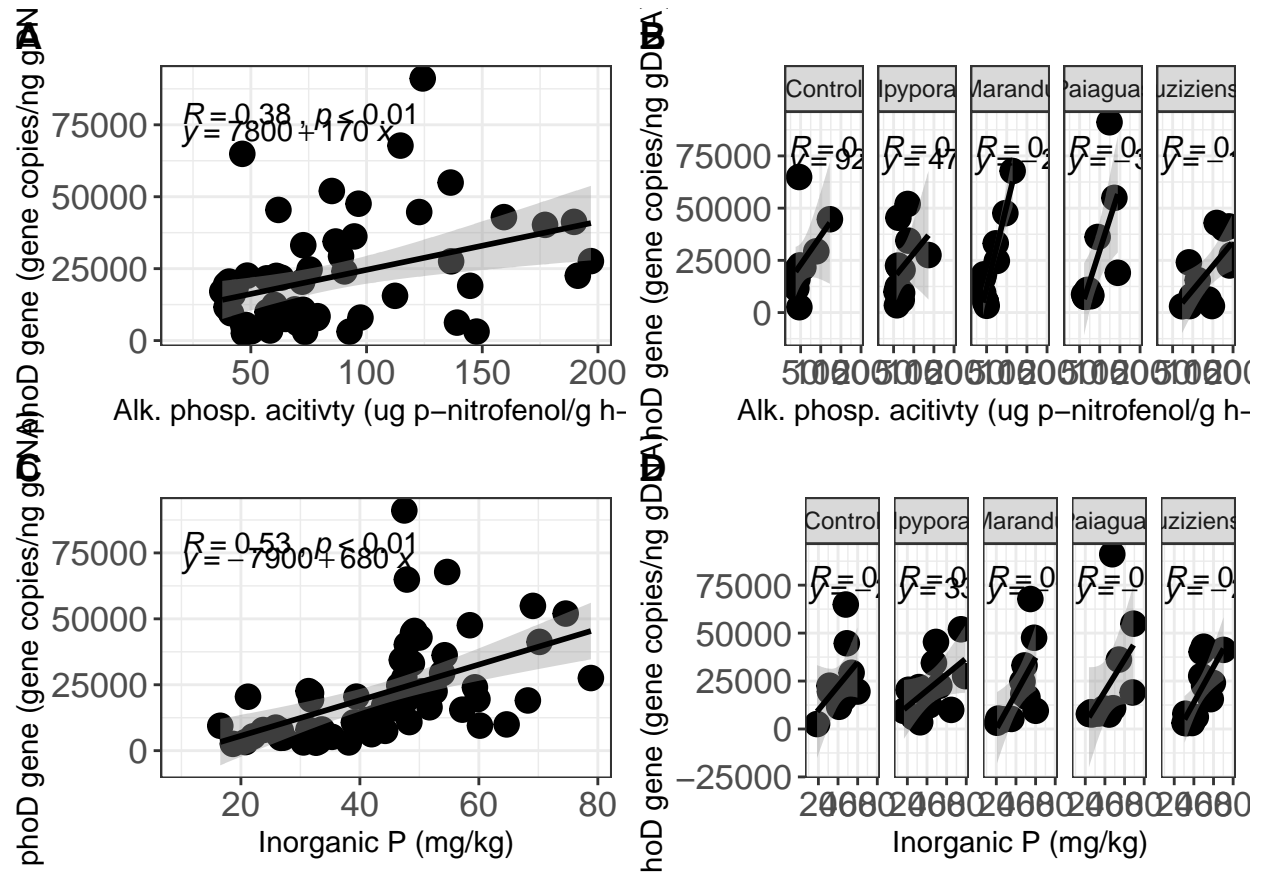
```
## 'geom_smooth()' using formula = 'y ~ x'
```



Plotting all together

```
ggarrange(pdxfal, pdxfal2, pdxpi, pdxpi2,
  labels = c("A", "B", "C", "D"),
  common.legend = TRUE,
  legend = "none",
  ncol = 2, nrow = 2)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
## 'geom_smooth()' using formula = 'y ~ x'
```



Statistical analysis

AMF colonization

```
stpqq <- lm(colonization ~ Genotype, data = db10c)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

```
##      Ipypora      Marandu      Paiaguas Ruziziensis
##      "a"         "b"         "c"         "d"
```

Bacteria 16S

```
#10cm
stpqq <- lm(bac2 ~ Genotype, data = db10)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

##	Control	Ipypora	Marandu	Paiaguas	Ruziziensis
##	"a"	"ab"	"ab"	"ab"	"b"

```
#30cm
stpqq <- lm(bac2 ~ Genotype, data = db30)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

##	Control	Ipypora	Marandu	Paiaguas	Ruziziensis
##	"a"	"ab"	"c"	"bc"	"c"

Archaea 16S

```
#10cm
stpqq <- lm(archea ~ Genotype, data = db10)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

##	Control	Ipypora	Marandu	Paiaguas	Ruziziensis
##	"a"	"b"	"bc"	"bc"	"c"

```
#20cm
stpqq <- lm(archea ~ Genotype, data = db20)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

##	Control	Ipypora	Marandu	Paiaguas	Ruziziensis
##	"a"	"a"	"a"	"a"	"b"

```
#30cm
stpqq <- lm(archea ~ Genotype, data = db30)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

##	Control	Ipypora	Marandu	Paiaguas	Ruziziensis
##	"a"	"b"	"a"	"a"	"b"

Fungi ITS

```
#20cm
stpqq <- lm(ITS ~ Genotype, data = db20)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

```
##      Control      Ipypora      Marandu      Paiaguas Ruziziensis
##      "a"         "bc"        "b"         "ac"         "a"
```

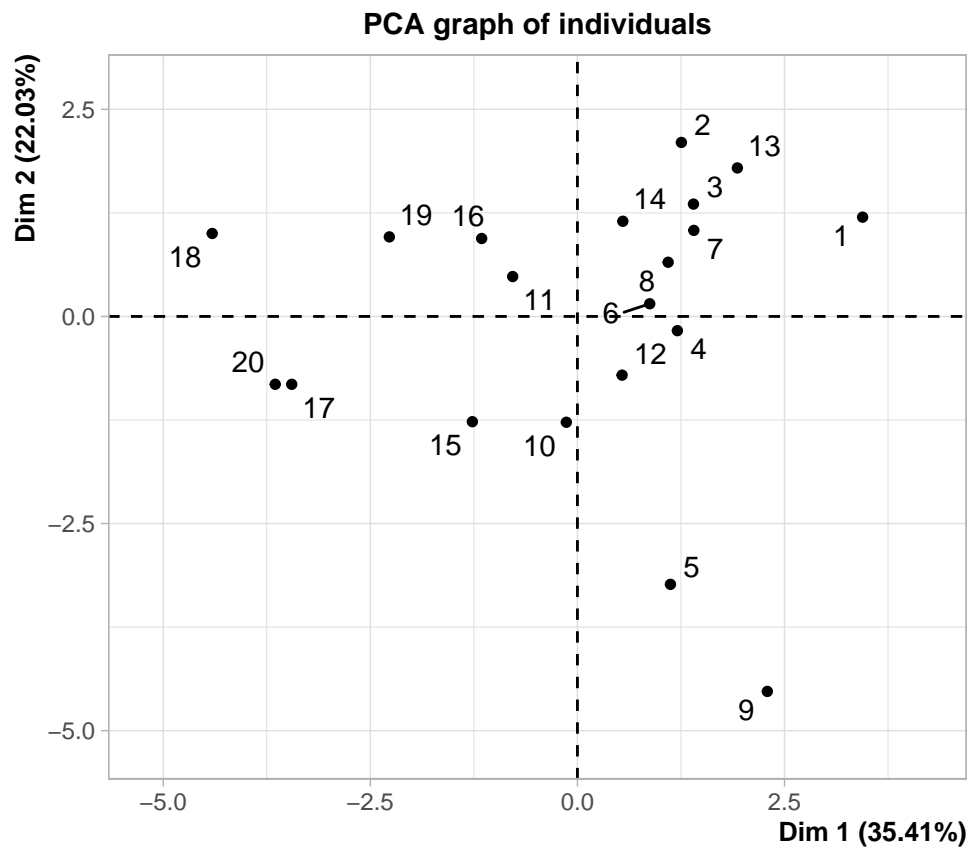
```
#30cm
stpqq <- lm(ITS ~ Genotype, data = db30)
mc <- glht(stpqq, linfct = mcp(Genotype = "Tukey"))
mc_letters <- cld(mc, Letters = letters)
print(mc_letters)
```

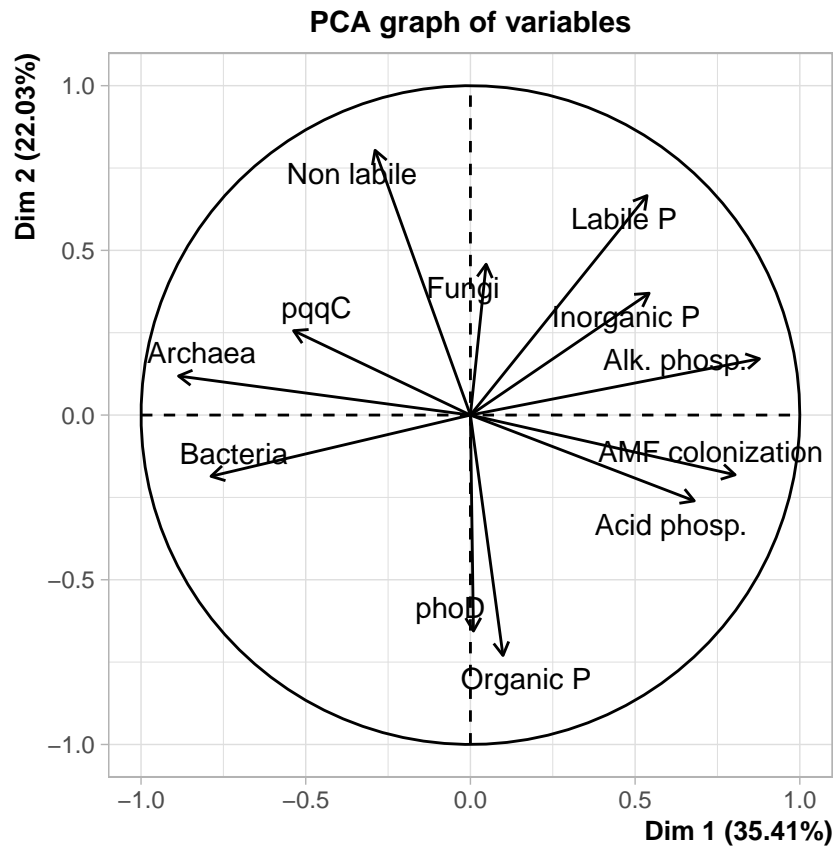
```
##      Control      Ipypora      Marandu      Paiaguas Ruziziensis
##      "a"         "ab"        "ab"        "b"         "ab"
```

Principal Components Analysis (PCA)

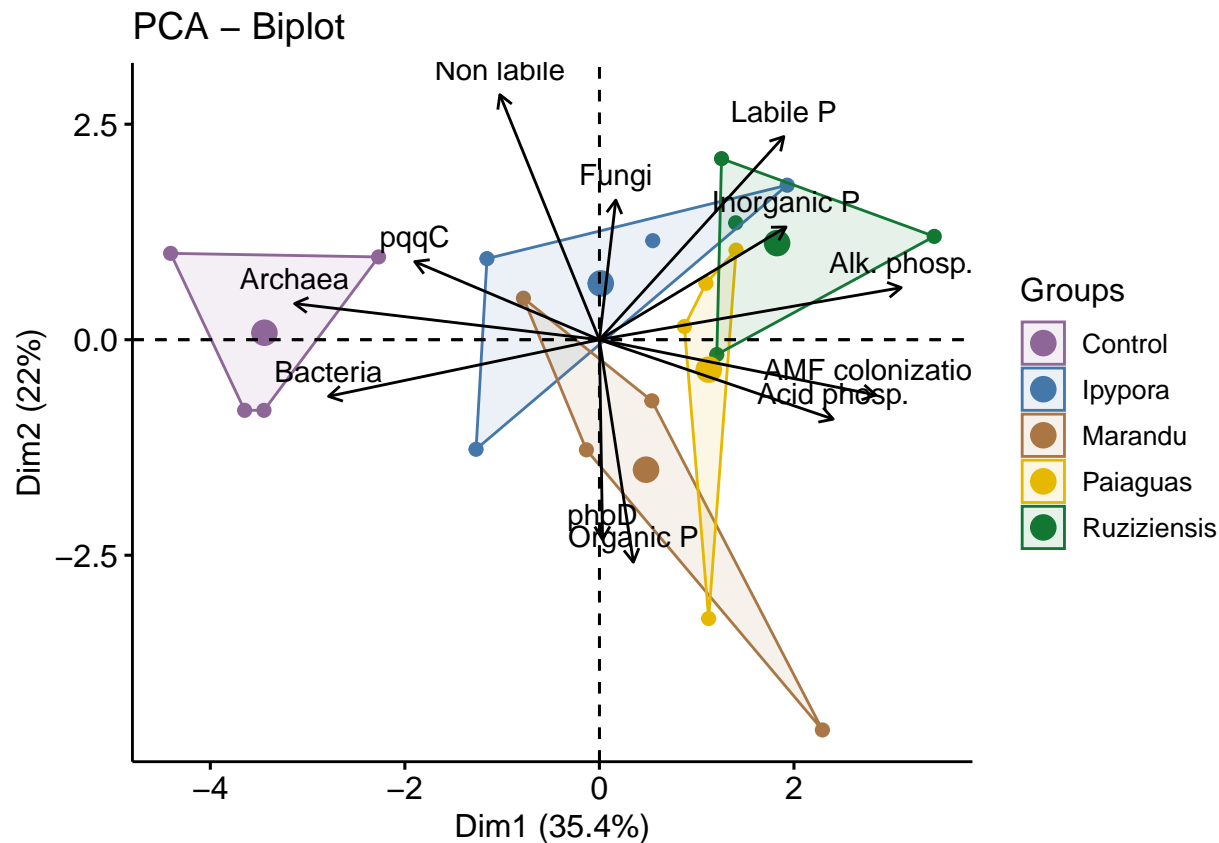
Depth: 0-10cm

```
pca1 <- PCA(scale(dbcor10[,c(4,8,10:11,13,15:17,19:22)]), scale = TRUE)
```





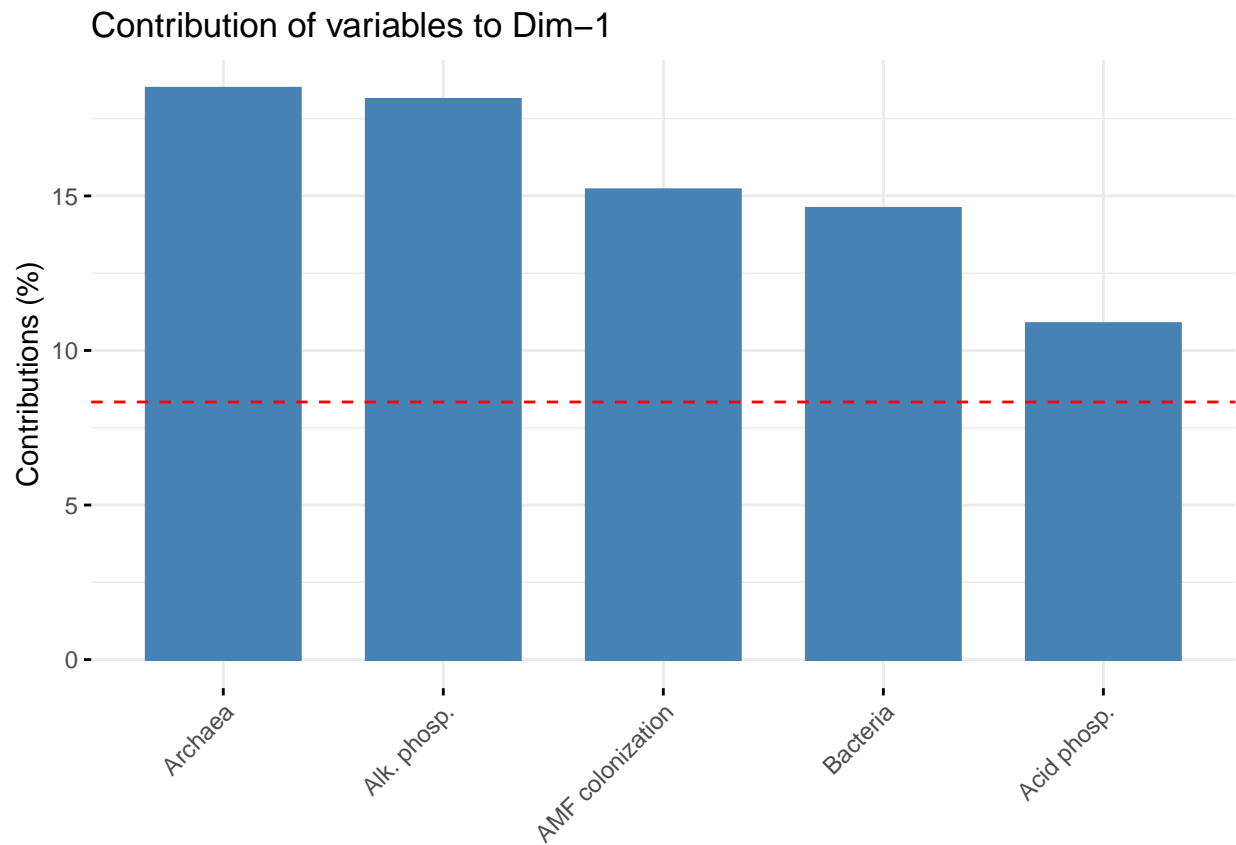
```
fviz_pca_biplot(pca1, habillage = dbcor10$Genotype,
  addEllipses = TRUE,
  pointshape = 19,
  pointsize = 2,
  geom = c("point"),
  ggtheme = theme_pubr(),
  palette = c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"),
  ellipse.type = "convex", col.var = "black")
```



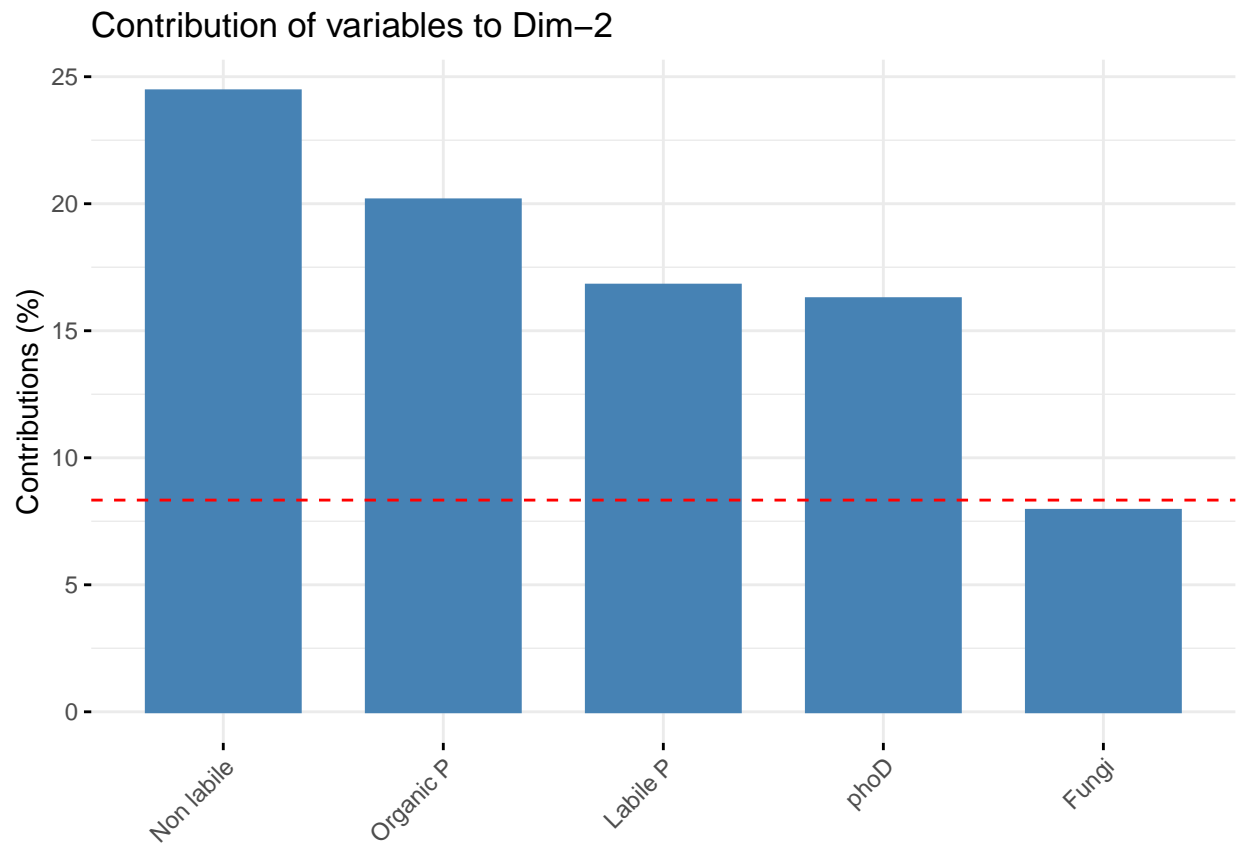
```
factors1 <- as.data.frame(as.factor(dbcor10$Genotype))
dbcor10_2 <- as.data.frame(dbcor10[,c(4,8,10:11,13,15:17,19:22)])
vegan::adonis2(dbcor10_2 ~ factors1[c(1:20)],, permutations = 999, method = "bray")
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## vegan::adonis2(formula = dbcor10_2 ~ factors1[c(1:20), ], permutations = 999, method = "bray")
##          Df SumOfSqs      R2      F Pr(>F)
## factors1[c(1:20), ]  4  0.26398 0.38031 2.3015  0.034 *
## Residual           15  0.43012 0.61969
## Total              19  0.69410 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Contributions of variables to PC1
fviz_contrib(pca1, choice = "var", axes = 1, top = 5)
```

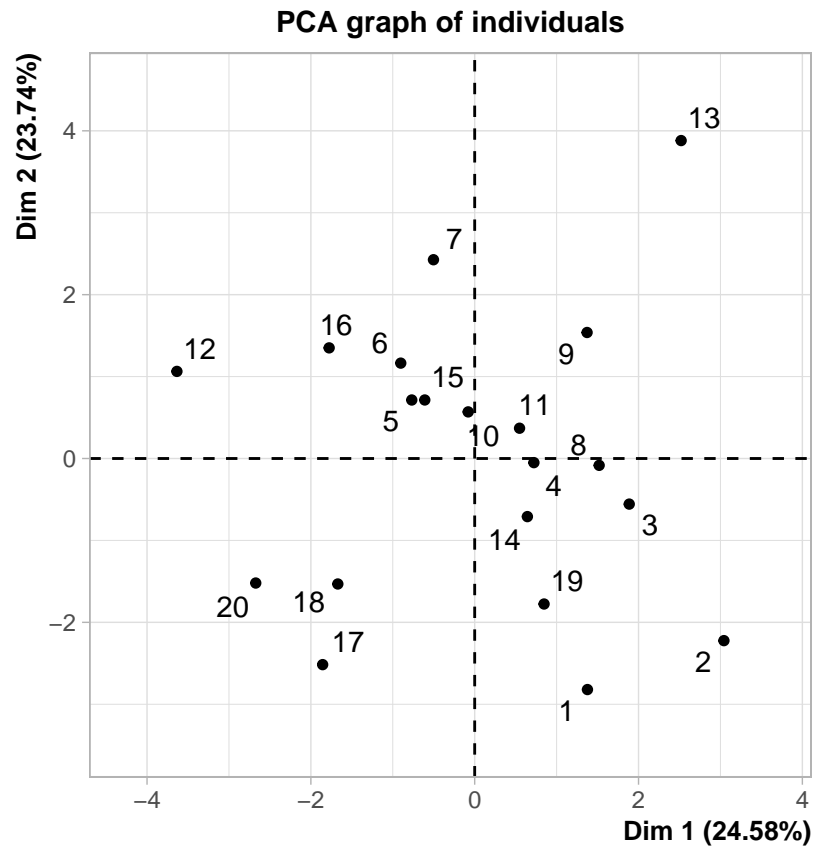



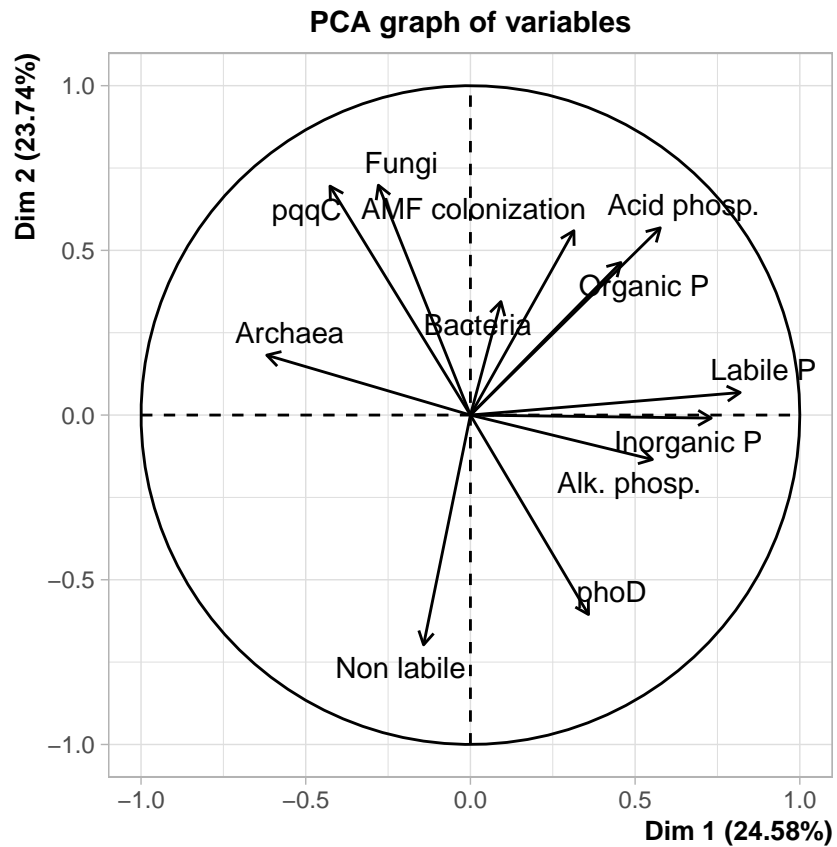
```
# Contributions of variables to PC2  
fviz_contrib(pca1, choice = "var", axes = 2, top = 5)
```



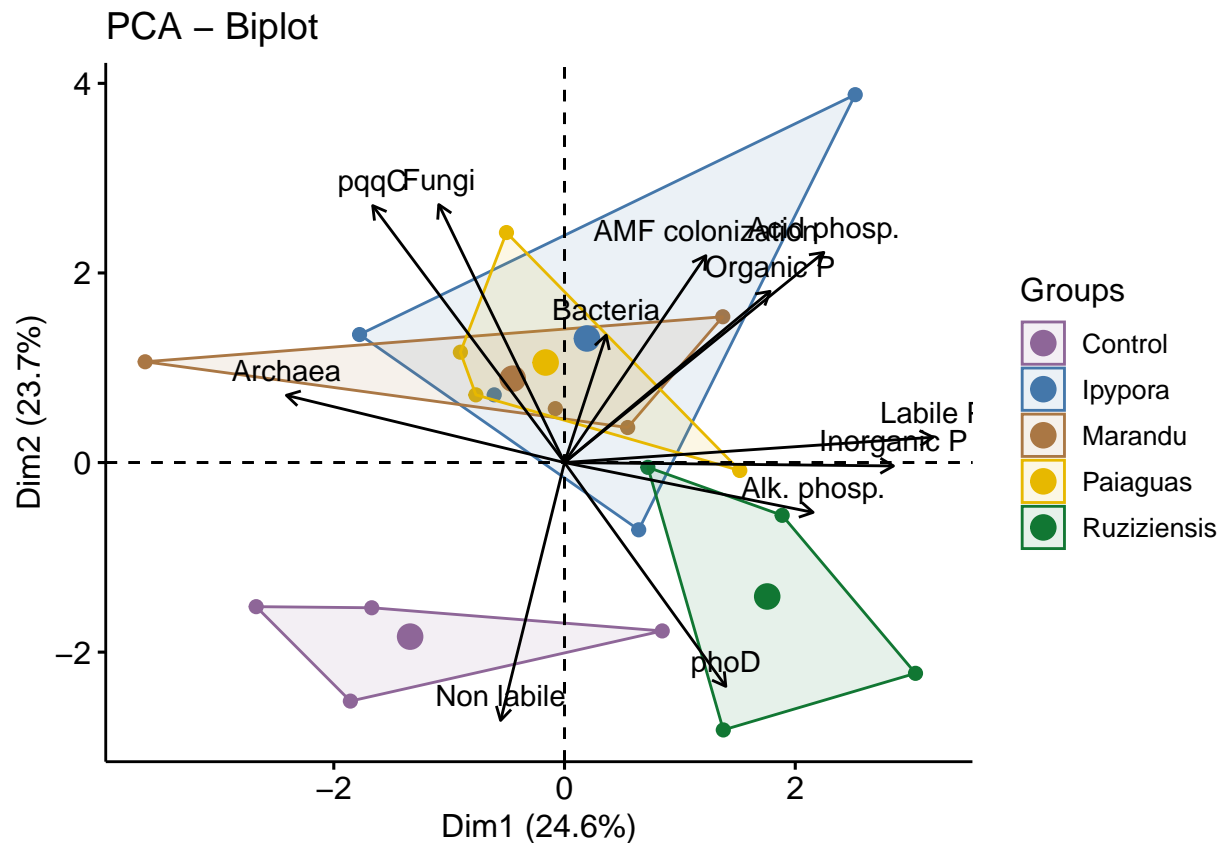
Depth: 10-20cm

```
pca20 <- PCA(scale(dbcor20[,c(4,8,10:11,13,15:17,19:22)]), scale = TRUE)
```





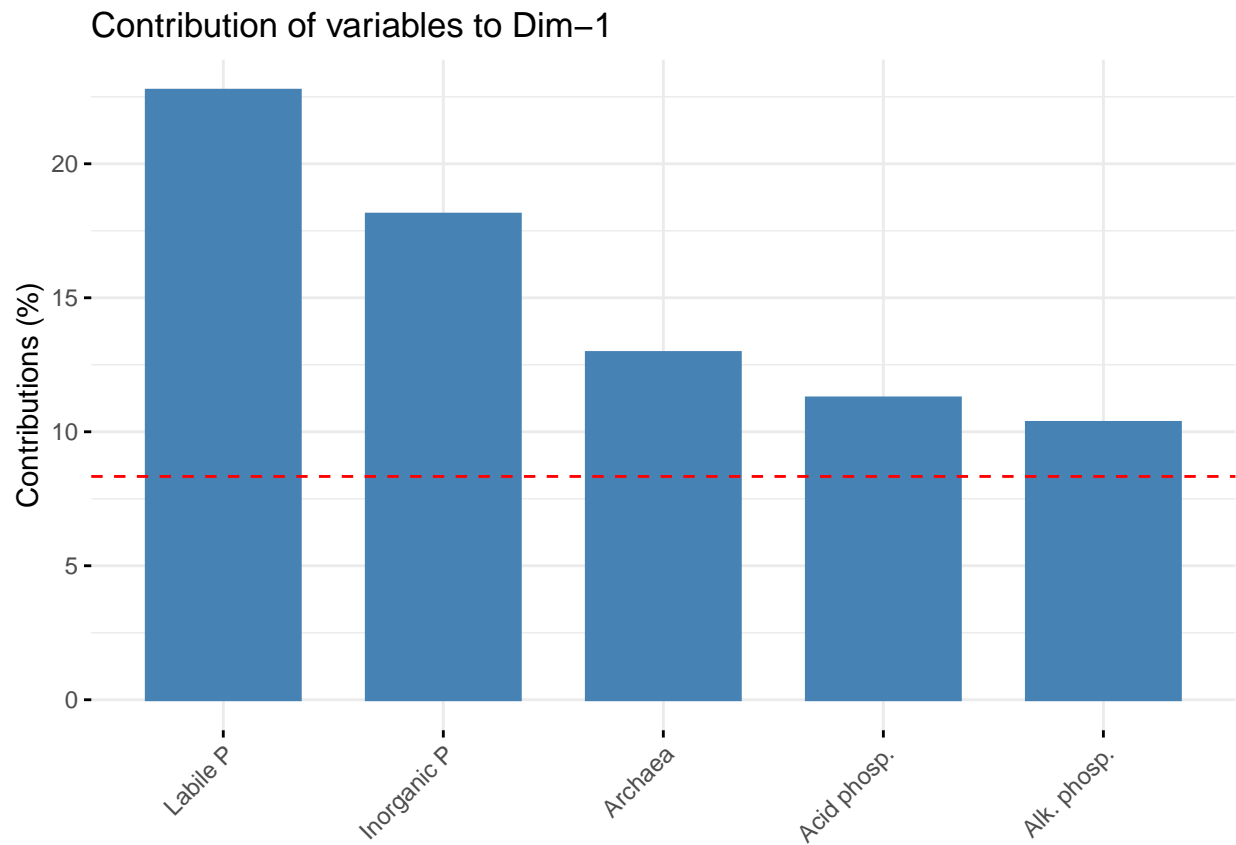
```
fviz_pca_biplot(pca20, habillage = dbcor10$Genotype,
  addEllipses = TRUE,
  pointshape = 19,
  pointsize = 2,
  geom = c("point"),
  ggtheme = theme_pubr(),
  palette = c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"),
  ellipse.type = "convex", col.var = "black")
```



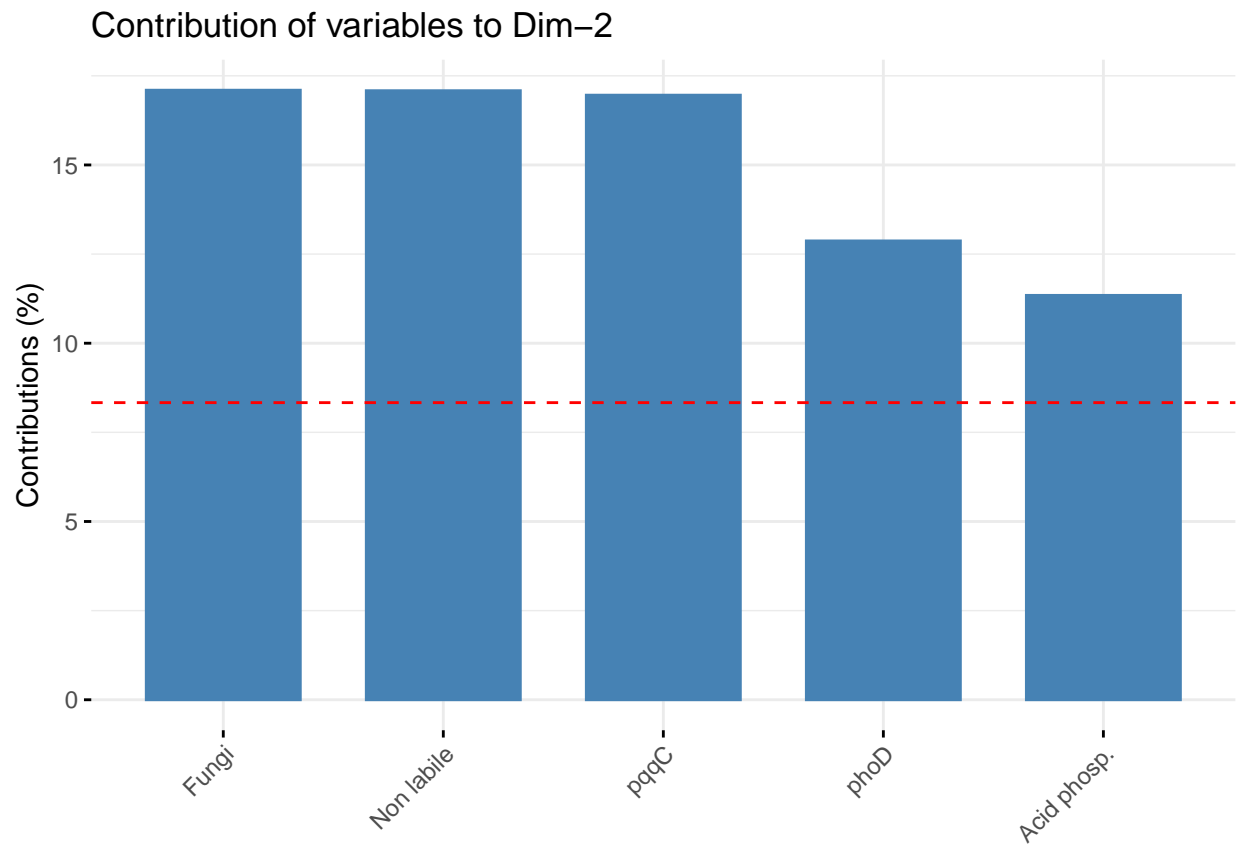
```
factors20 <- as.data.frame(as.factor(dbcor20$Genotype))
dbcor20_2 <- as.data.frame(dbcor20[,c(4,8,10:11,13,15:17,19:22)])
vegan::adonis2(dbcor20_2 ~ factors20[c(1:20)],, permutations = 999, method = "bray") #p = 0.001 ***
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## vegan::adonis2(formula = dbcor20_2 ~ factors20[c(1:20), ], permutations = 999, method = "bray")
##          Df SumOfSqs      R2      F Pr(>F)
## factors20[c(1:20), ]  4  0.33917 0.51115 3.9211 0.001 ***
## Residual             15  0.32437 0.48885
## Total                 19  0.66354 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Contributions of variables to PC1
fviz_contrib(pca20, choice = "var", axes = 1, top = 5)
```

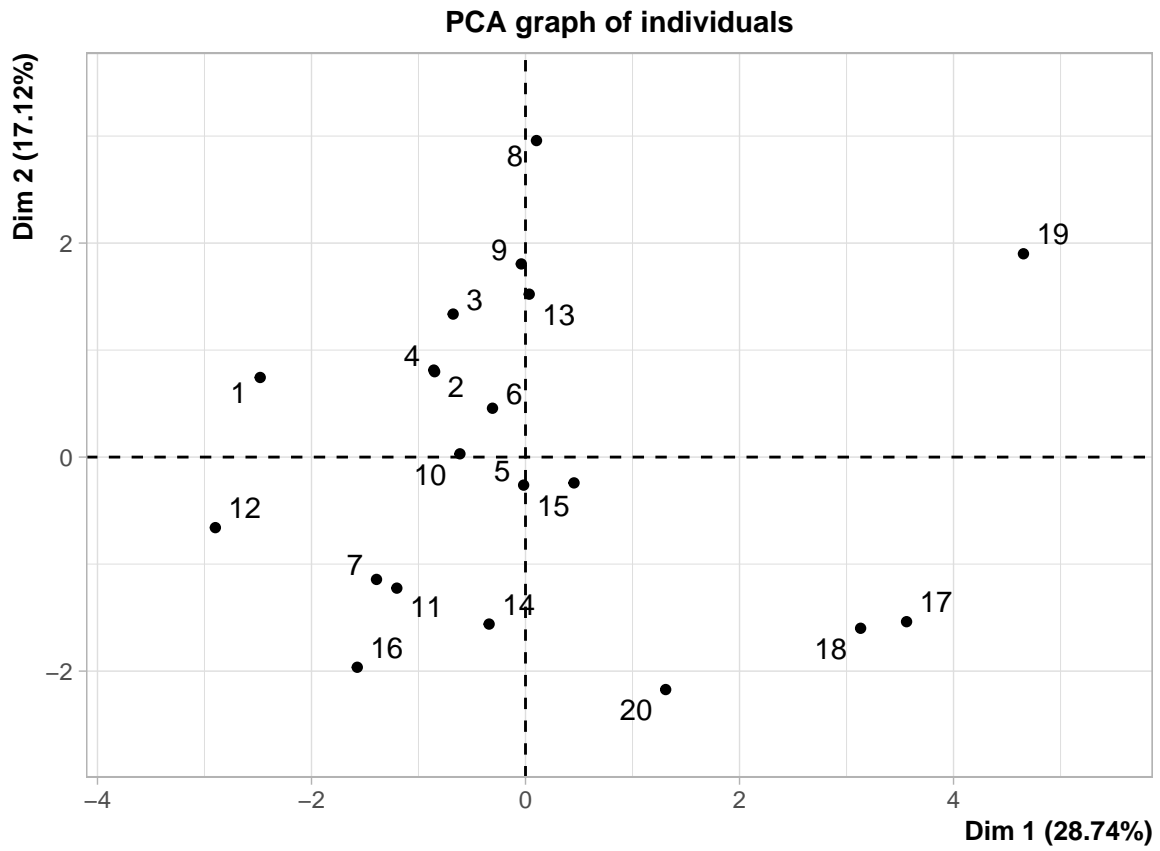


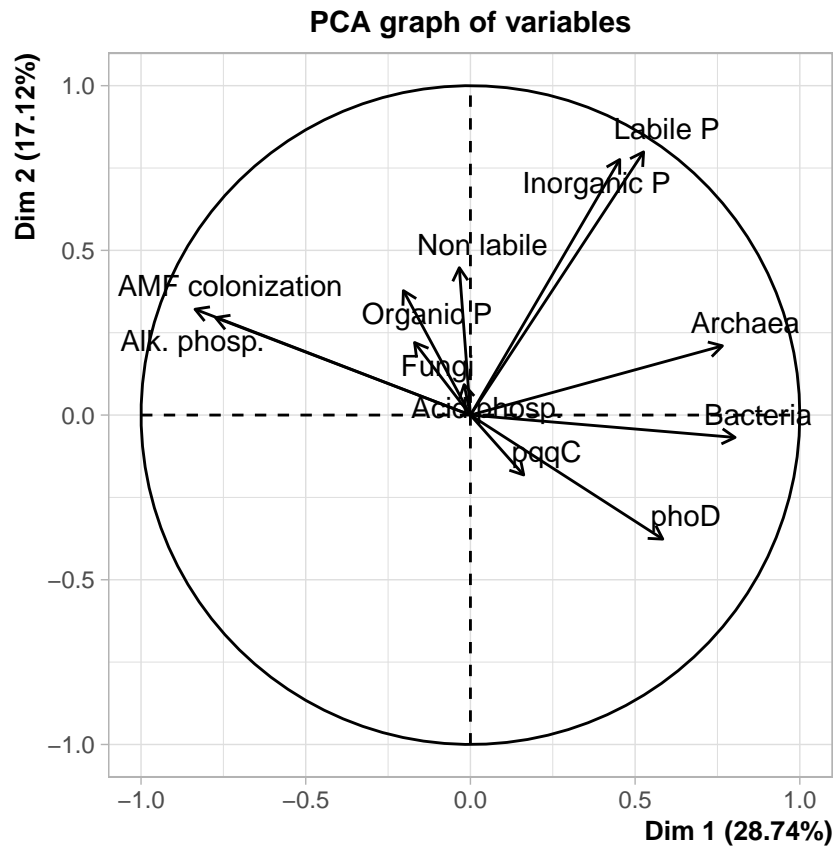
```
# Contributions of variables to PC2  
fviz_contrib(pca20, choice = "var", axes = 2, top = 5)
```



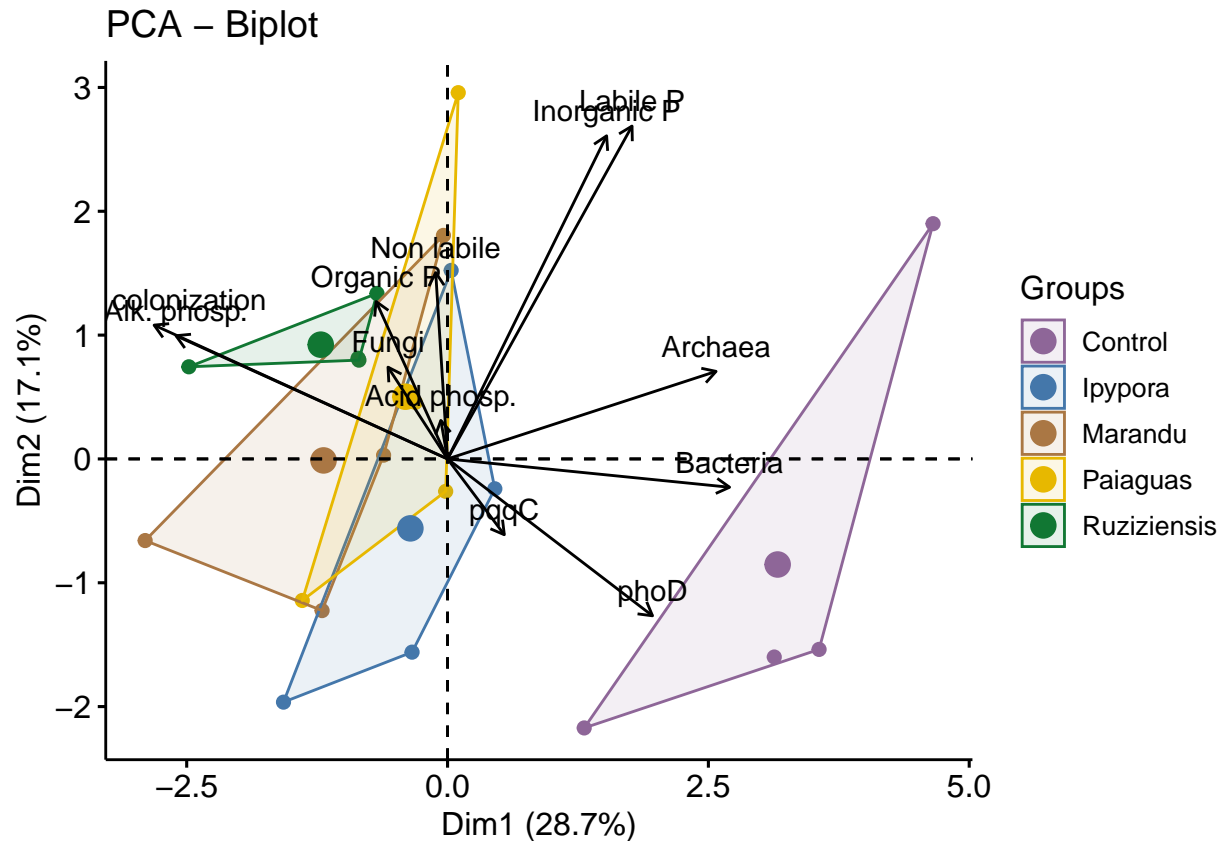
Depth: 20-30cm

```
pca30 <- PCA(scale(dbc30[,c(4,8,10:11,13,15:17,19:22)]), scale = TRUE)
```





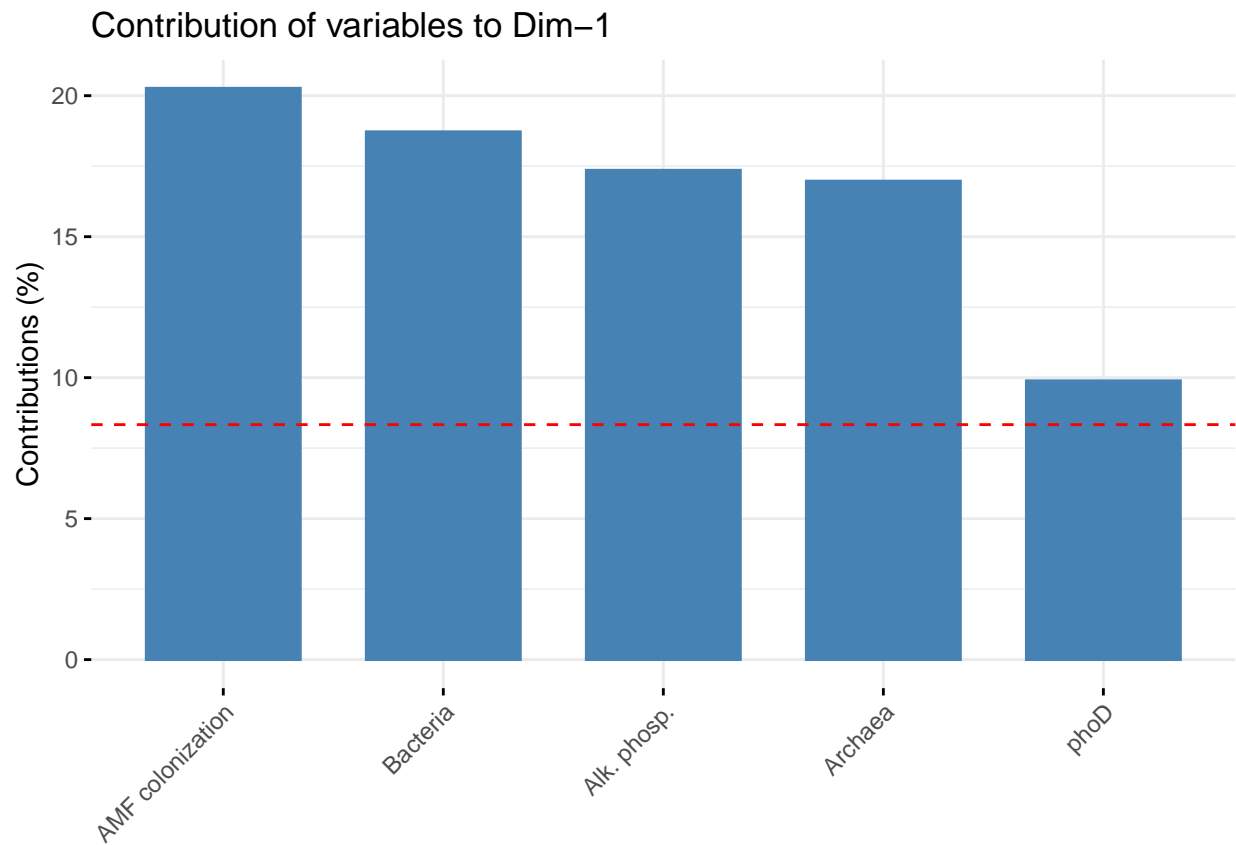
```
fviz_pca_biplot(pca30, habillage = dbcor10$Genotype,
  addEllipses = TRUE,
  pointshape = 19,
  pointsize = 2,
  geom = c("point"),
  ggtheme = theme_pubr(),
  palette = c("#8E6698", "#4477AA", "#AA7744", "#E7B800", "#117733"),
  ellipse.type = "convex", col.var = "black")
```



```
factors30 <- as.data.frame(as.factor(dbcor30$Genotype))
dbc30_2 <- as.data.frame(dbcor30[,c(4,8,10:11,13,15:17,19:22)])
vegan::adonis2(dbc30_2 ~ factors30[c(1:20)], permutations = 999, method = "bray") #p = 0.001 ***
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## vegan::adonis2(formula = dbc30_2 ~ factors30[c(1:20), ], permutations = 999, method = "bray")
##          Df SumOfSqs      R2      F Pr(>F)
## factors30[c(1:20), ]  4  0.31819 0.53673 4.3447 0.001 ***
## Residual             15  0.27463 0.46327
## Total                 19  0.59282 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Contributions of variables to PC1
fviz_contrib(pca30, choice = "var", axes = 1, top = 5)
```



```
# Contributions of variables to PC2  
fviz_contrib(pca30, choice = "var", axes = 2, top = 5)
```

