Resuscitation of microbial seed banks

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Objective

Analysis of plant and microbial data from soils exposed to recombinantly produced resuscitation promoting factor (Rpf)

Set working environment and load packages

```r
# Clear and set working directory
rm(list = ls())
setwd("~/GitHub/BrassicaRpf/data")

# Require and/or install packages
package.list <- c('vegan', 'nlme' ,'data.table', 'plyr', 'pander', 'reshape', 'grid', 'png', 'car', 'bbr
for (package in package.list) {
  if (!require(package, character.only=T, quietly=T)) {
    install.packages(package)
    library(package, character.only=T)
  } }

# Load sem function
sem <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}

# Load t-test custom functions #
ttest <- function(reg, coefnum, val){
  co <- coef(summary(reg))
  tstat <- (co[coefnum,1]-val)/co[coefnum,2]
  pstat <- 2 * pt(abs(tstat), reg$df.residual, lower.tail = FALSE)
  return(list = c(t = tstat, df = reg$df.residual, p =  pstat))
}
```

1) Rpf effects on Brassica rapa traits

```r
# Load plant trait data
plant.data <- read.delim("~/GitHub/BrassicaRpf/data/plantTrait.txt", sep = ",", header = TRUE)
# Remove soil sterilization treatment from dataset #
plant.data <- subset(plant.data, soil == "Live")

# Subseting data for relevant information
data.sub <- subset(plant.data, select=(c(Treatment, abiomass, bbiomass)))
# Combining the above and belowground biomass #
data.m <- melt(data.sub)
```

```
## Using Treatment as id variables
```

```
# Above-ground shoot biomass
# Selecting shoot biomass data for plotting #
shoot.rpf <- data.m[ which(data.m$Treatment == "Rpf+" & data.m$variable == "abiomass"),]
shoot.con <- data.m[ which(data.m$Treatment == "Rpf-" & data.m$variable == "abiomass"),]
# Generating shoot biomass mean and sem table #
shoot.mean <- aggregate(plant.data$abiomass ~ Treatment, plant.data, mean)
shoot.sem <- aggregate(plant.data$abiomass ~ Treatment, plant.data, sem)
shoot.sem.LL <- shoot.mean[2] + shoot.sem[2]
shoot.sem.UL <- shoot.mean[2] - shoot.sem[2]
shoot.table <- data.frame(shoot.mean[1], shoot.mean[2], shoot.sem[2],
          shoot.sem.LL[1], shoot.sem.UL[1])
colnames(shoot.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
shoot.table <- shoot.table[order(shoot.table$mean),]

# Below-ground root biomass
# Selecting root biomass data for plotting #
root.rpf <- data.m[ which(data.m$Treatment == "Rpf+" & data.m$variable == "bbiomass"),]
root.con <- data.m[ which(data.m$Treatment == "Rpf-" & data.m$variable == "bbiomass"),]
# Generating root biomass  mean and sem table #
root.mean <- aggregate(plant.data$bbiomass ~ Treatment, plant.data, mean)
root.sem <- aggregate(plant.data$bbiomass ~ Treatment, plant.data, sem)
root.sem.LL <- root.mean[2] + root.sem[2]
root.sem.UL <- root.mean[2] - root.sem[2]
root.table <- data.frame(root.mean[1], root.mean[2], root.sem[2],
          root.sem.LL[1], root.sem.UL[1])
colnames(root.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
root.table <- root.table[order(root.table$mean),]

# Flower number per plant
# Flower count data for plotting #
flower.rpf <- plant.data[ which(plant.data$Treatment == "Rpf+"),]
flower.con <- plant.data[ which(plant.data$Treatment == "Rpf-"),]
# Generate flower count data table #
flower.mean <- aggregate(plant.data$flower.count ~ Treatment, plant.data, mean)
flower.sem <- aggregate(plant.data$flower.count ~ Treatment, plant.data, sem)
flower.sem.LL <- flower.mean[2] + flower.sem[2]
flower.sem.UL <- flower.mean[2] - flower.sem[2]
flower.table <- data.frame(flower.mean[1], flower.mean[2], flower.sem[2],
          flower.sem.LL[1], flower.sem.UL[1])
colnames(flower.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
flower.table <- flower.table[order(flower.table$mean),]


# Generating 3 panel figure showing Rpf effects on plant traits #
png(filename="../figures/Figure2-PlantTraits.png",
    width = 1200, height = 800, res = 96*2)
par(oma=c(7,3,7,1), mar=c(2,3,3,3.5), mfrow=c(1,3))

# Plotting first panel for shoot biomass #
shoot.fig <- plot(jitter(rep(1, length(shoot.con$value)), amount = 0.1), shoot.con$value,
      ylim = c(0, 1.5), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(shoot.rpf$value)), amount = 0.1), shoot.rpf$value, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean value point #
points(1, mean(shoot.con$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(shoot.rpf$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y-axis label #
mtext(expression('Shoot biomass (g)'), side = 2,
      outer = FALSE, cex = 1.25, line = 2.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("0", "0.5", "1.0", "1.5"), at = c(0, 0.5, 1.0, 1.5))
axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at=c(0, 0.5, 1.0, 1.5), labels = F, tck = -0.05)
axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Adding SEM bars #
arrows(x0 = c(2,1), y0 = shoot.table$mean, y1 = shoot.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(2,1), y0 = shoot.table$mean, y1 = shoot.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label #
text(0.65,1.45 ,labels="A", col="black", cex=2)

# p-value label
mtext(text = expression(italic("P")~" = 0.017") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 8"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)

# Plotting second panel for root biomass #
root.fig <- plot(jitter(rep(1, length(root.con$value)), amount = 0.1), root.con$value,
      ylim = c(0, 1.5), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(root.rpf$value)), amount = 0.1), root.rpf$value, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean circle #
points(1, mean(root.con$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
```

```
points(2, mean(root.rpf$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y-axis label #
mtext(expression('Root biomass (g)'), side = 2,
      outer = FALSE, cex = 1.25, line = 2.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("0", "0.5", "1.0", "1.5"), at = c(0, 0.5, 1.0, 1.5))
axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at=c(0, 0.5, 1.0, 1.5), labels = F, tck = -0.05)
axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Adding SEM bars #
arrows(x0 = c(2,1), y0 = root.table$mean, y1 = root.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(2,1), y0 = root.table$mean, y1 = root.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label
text(0.65,1.45 ,labels="B", col="black", cex=2)

# p-value label
mtext(text = expression(italic("P")~" = 0.049") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 8"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)

# Plotting third panel for flower count #
flowercount.fig <- plot(jitter(rep(1, length(flower.con$flower.count)), amount = 0.1), flower.con$flowe
      ylim = c(0, 15), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(flower.rpf$flower.count)), amount = 0.1), flower.rpf$flower.count, pch = 21
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

points(1, mean(flower.con$flower.count), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(flower.rpf$flower.count), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis label #
mtext(expression('Flower number'), side = 2,
      outer = FALSE, cex = 1.25, line = 2.5, adj = 0.5)
```

```
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("0", "5", "10", "15"), at = c(0, 5, 10, 15))
axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at=c(0, 5, 10, 15), labels = F, tck = -0.05)
axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))
axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Add SEM bars
arrows(x0 = c(2,1), y0 = flower.table$mean, y1 = flower.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(2,1), y0 = flower.table$mean, y1 = flower.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label #
text(0.65,14.5 ,labels="C", col="black", cex=2)

# p-value label
mtext(text = expression(italic("P")~" = 0.097") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 8"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)


# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Figure2-PlantTraits.png")
grid.raster(img)


# Statistical test: Two sample t-test of Rpf effect on plant traits
# Total plant biomass
total.ttest <- t.test(total.biomass ~ Treatment, data = plant.data)
total.ttest   # Significant: t = 2.8365, df = 9.2015, p = 0.0191
```

```
##
##  Welch Two Sample t-test
##
## data:  total.biomass by Treatment
## t = 2.8365, df = 9.2015, p-value = 0.0191
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.133024 1.163976
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##           1.942375           1.293875
```

```
# Root biomass
root.ttest <- t.test(bbiomass ~ Treatment, data = plant.data)
root.ttest    # Significant: t = 2.3017, df = 8.3705, p = 0.04895
```

```
##
##  Welch Two Sample t-test
##
## data:  bbiomass by Treatment
## t = 2.3017, df = 8.3705, p-value = 0.04895
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.002854404 0.979395596
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##           0.951000           0.459875
```

```
# Shoot biomass
shoot.ttest <- t.test(abiomass ~ Treatment, data = plant.data)
shoot.ttest   # Significant: t = 2.704, df = 14, p = 0.01712
```

```
##
##  Welch Two Sample t-test
##
## data:  abiomass by Treatment
## t = 2.704, df = 14, p-value = 0.01712
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  0.04596519 0.39853481
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##            1.05625            0.83400
```

```
# Flower number
flower.ttest <- t.test(flower.count ~ Treatment, data = plant.data)
flower.ttest  # Marginally significant: t = 1.7954, df = 12.288, p = 0.09721
```

```
##
##  Welch Two Sample t-test
##
## data:  flower.count by Treatment
## t = 1.7954, df = 12.288, p-value = 0.09721
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.6838463  7.1838463
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##              10.00               6.75
```

```
# Seed number
seed.ttest <- t.test(seed.count ~ Treatment, data = plant.data)
seed.ttest    # Non-significant: t = 0.83156, df = 12.983, p = 0.4207
```

```
##
##  Welch Two Sample t-test
##
## data:  seed.count by Treatment
## t = 0.83156, df = 12.983, p-value = 0.4207
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -25.3735  57.1235
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##             55.250             39.375
```

```
# Shoot:Root ratio
SRratio.ttest <- t.test(shoot.root ~ Treatment, data = plant.data)
SRratio.ttest # Non-significant: t = -1.2033, df = 12.903, p = 0.2505
```

```
##
##  Welch Two Sample t-test
##
## data:  shoot.root by Treatment
## t = -1.2033, df = 12.903, p-value = 0.2505
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.4725964  0.4195267
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##           1.582071           2.108606
```

```
# Specific leaf area (SLA)
sla.ttest <- t.test(SLA ~ Treatment, data = plant.data)
sla.ttest     # Non-significant: t = 0.80287, df = 9.1288, p = 0.4424
```

```
##
##  Welch Two Sample t-test
##
## data:  SLA by Treatment
## t = 0.80287, df = 9.1288, p-value = 0.4424
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -96.05961 202.11318
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##           247.3297           194.3029
```

```
# Shoot height
height.ttest <- t.test(height ~ Treatment, data = plant.data)
height.ttest  # Non-significant: t = 1.1102, df = 13.384, p = 0.2865
```

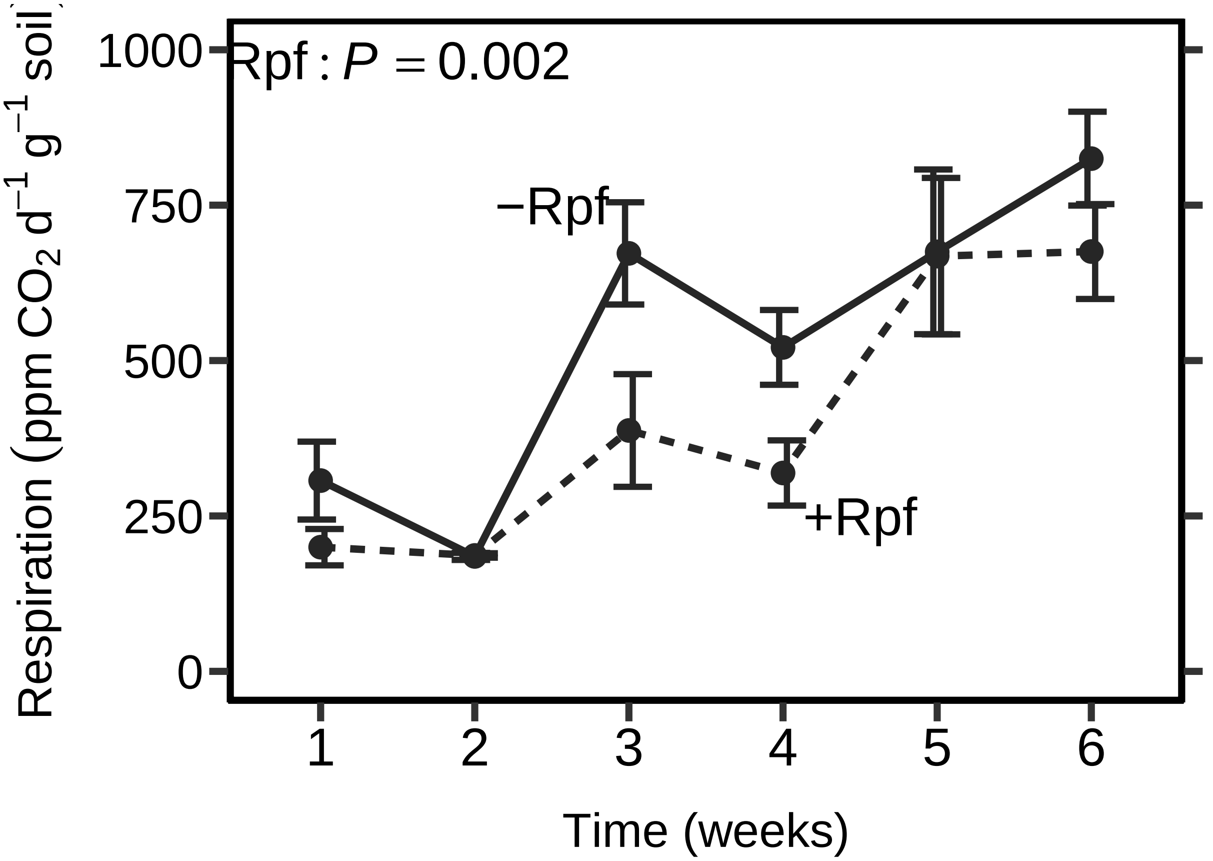
```
##
##  Welch Two Sample t-test
##
## data:  height by Treatment
## t = 1.1102, df = 13.384, p-value = 0.2865
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -3.643533 11.393533
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##            30.2125            26.3375
```

2a) Rpf effects on soil microbial CO2 respiration

```r
# Load soil respirtation data #
CO2 <- read.csv("~/GitHub/BrassicaRpf/data/GCH_CO2.txt", sep = ",", header = TRUE)

# Subsetting relevant data from CO2 dataset #
CO2.sub <- subset(CO2, plant=="present" & hour =="24" & soil=="live", select=c(Treatment, soil, Week.1,
# Change column names #
colnames(CO2.sub) <- c("Treatment", "soil", "1","2","3","4","5","6")
# Melt dataset into three columns of treatment, soil and weeks for anaylsis
CO2.m <- melt(CO2.sub)
```

```
## Using Treatment, soil as id variables
```

```r
# Change variable column name to week
colnames(CO2.m) <- c("Treatment", "soil", "Week", "value")
# Set week as factor
CO2.m$Week <- as.factor(CO2.m$Week)

# Standardizing the soil respiration values by 24 hours
CO2.m$StdCO2 <- (CO2.m$value)/24


# Calculating CO2 respiration mean and sem table #
CO2.means.sem <- ddply(CO2.m, c("Treatment", "Week"), summarise,
                   mean=mean(StdCO2), sem=sd(StdCO2)/sqrt(length(StdCO2)))
CO2.means.sem <- transform(CO2.means.sem, lower=mean-sem, upper=mean+sem)

# The errorbars overlapped, so use position_dodge to move them horizontally
pd <- position_dodge(0)


# Generate figure 3 for Rpf effects on soil respiration #
co <- ggplot(CO2.means.sem, aes(x=Week, y=mean, colour=Treatment, group=Treatment)) +
      geom_errorbar(aes(ymax=upper, ymin=lower), position=position_dodge(0.1),
                data=CO2.means.sem, width = 0.5, size=1.1) +
      geom_line(aes(linetype=Treatment) ,position=pd, size=1.3) +
      geom_point(aes(shape=Treatment), position=pd, size=4) +
      scale_shape_manual(values=c(16, 16)) +
      xlab("Time (weeks)") +
      ylab(expression(~Respiration~(ppm~CO[2]~d^-1~g^-1~soil)))

co + scale_y_continuous(limits = c(0, 1000), breaks = seq(0, 1000, 250),
                     sec.axis = sec_axis(~ . * 1, labels = c(" "," "," ", " ", " "))) +
     theme_classic() +
     theme(axis.text.y=element_text(colour = "black", size = 18),
          axis.text.x=element_text(colour = "black", size = 20),
          axis.ticks = element_line(size = 1.25),
          axis.ticks.length = unit(.25, "cm"),
          axis.title.y = element_text(size = 18, colour = "black", margin = margin(0,10,0,0)),
          axis.title.x = element_text(size = 18, colour = "black", margin = margin(15,10,0,10)),
          panel.border = element_rect(linetype = "solid", colour = "black", size = 2, fill = NA),
          legend.position="none") +
    scale_color_manual(values=c('gray15','gray15')) +
  annotate("text", x = 2.5, y = 750, label = "-Rpf", cex=7) +
  annotate("text", x = 4.5, y = 250, label = "+Rpf", cex=7) +
  #annotate("text", x = 1.5, y = 975, label = "Rpf: P = 0.002", cex=7) +
  annotate("text", x = 1.5, y = 975, label = "Rpf: italic(P)==0.002", parse = TRUE, cex=7) +
  #annotate("text", x = 0.925, y = 900, label = "n = 8", cex=7)



ggsave("../figures/Figure3-Soilrespiration.png", width = 20, height = 15, units = "cm")
```



```
# Statistical tests: Rpf effects on soil CO2 respiration
# RM-ANOVA
CO2.rm <- lme(StdCO2 ~ Week*Treatment, random = ~ 1 | soil,
              correlation = corAR1(form = ~1 | soil),
              data = CO2.m)
# Make ANOVA table #
set.caption("RMANOVA for soil CO2 respiration")
pander(anova(CO2.rm))
```

Table 1: RMANOVA for soil CO2 respiration

	numDF	denDF	F-value	p-value
(Intercept)	1	84	57.47	4.161e-11
Week	5	84	21.6	7.772e-14
Treatment	1	84	9.676	0.00255

	numDF	denDF	F-value	p-value
Week:Treatment	5	84	1.217	0.3084

```
    # Significant effect of week: F5,84 = 21.6, p < 0.000
    # Significant effect of Rpf: F1,84 = 9.676, p = 0.00255
    # Non-significant interaction: F5,84 = 1.217, p = 0.3084
```

2b) Effect of Rpf on soil microbial abundance

```
# Load microbial qPCR abundance data #
abundance <- read.csv("~/GitHub/BrassicaRpf/data/qPCR.txt", sep = ",", header = TRUE)
# remove week 1 data #
abundance <- subset(abundance, Week == "6")
# Reset data frame index #
rownames(abundance) <- NULL

# Standardizing gene copy abundances by soil amount #
abundance$stdGeneCopy <- (abundance$GeneCopy)/(abundance$Soil)
# Log10 transforming standardized gene copy number #
abundance$log10stdGeneCopy <- log10(abundance$stdGeneCopy)

# Splitting abundance dataset by fungi and bacteria #
data.sub.bac <- subset(abundance, Gene == "rRNA")
data.sub.fun <- subset(abundance, Gene == "ITS")

# Calculating fungi to bacteria ratios #
gca.B <- data.sub.bac$stdGeneCopy
gca.F <- data.sub.fun$stdGeneCopy
abundance$FB <- (gca.F/gca.B)
ratioFB <- abundance[1:18, ]
ratioFB <- subset(ratioFB, select=(c(Treatment, Week, FB)))

# Subsetting abundance dataset
data.sub <- subset(abundance, select=(c(Treatment, Gene, stdGeneCopy)))
# Melting the dataset
data.m <- melt(data.sub)
```

```
## Using Treatment, Gene as id variables
```

```
# Bacterial abundance data for plotting #
bac.rpf <- data.m[ which(data.m$Treatment == "Rpf+" & data.m$Gene == "rRNA"),]
bac.con <- data.m[ which(data.m$Treatment == "Rpf-" & data.m$Gene == "rRNA"),]
# Bacterial abundance mean and sem table #
bac.mean <- aggregate(data.sub.bac$stdGeneCopy ~ Treatment, data.sub.bac, mean)
bac.sem <- aggregate(data.sub.bac$stdGeneCopy ~ Treatment, data.sub.bac, sem)
bac.sem.LL <- bac.mean[2] - bac.sem[2]
bac.sem.UL <- bac.mean[2] + bac.sem[2]
bac.table <- data.frame(bac.mean[1], bac.mean[2], bac.sem[2],
          bac.sem.LL[1], bac.sem.UL[1])
colnames(bac.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
bac.table <- bac.table[order(bac.table$mean),]
```

```
# Fungal abundance data for plotting #
fun.rpf <- data.m[ which(data.m$Treatment == "Rpf+" & data.m$Gene == "ITS"),]
fun.con <- data.m[ which(data.m$Treatment == "Rpf-" & data.m$Gene == "ITS"),]
# Fungal abundance mean and sem table #
fun.mean <- aggregate(data.sub.fun$stdGeneCopy ~ Treatment, data.sub.fun, mean)
fun.sem <- aggregate(data.sub.fun$stdGeneCopy ~ Treatment, data.sub.fun, sem)
fun.sem.LL <- fun.mean[2] - fun.sem[2]
fun.sem.UL <- fun.mean[2] + fun.sem[2]
fun.table <- data.frame(fun.mean[1], fun.mean[2], fun.sem[2],
          fun.sem.LL[1], fun.sem.UL[1])
colnames(fun.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
fun.table <- fun.table[order(fun.table$mean),]

# Fungi: Bacteria ratio data for plotting #
FB.rpf <- ratioFB[ which(ratioFB$Treatment == "Rpf+"),]
FB.con <- ratioFB[ which(ratioFB$Treatment == "Rpf-"),]
# FB: Bacteria ratio mean and sem #
FB.mean <- aggregate(ratioFB$FB ~ Treatment, ratioFB, mean)
FB.sem <- aggregate(ratioFB$FB ~ Treatment, ratioFB, sem)
FB.sem.LL <- FB.mean[2] + FB.sem[2]
FB.sem.UL <- FB.mean[2] - FB.sem[2]
FB.table <- data.frame(FB.mean[1], FB.mean[2], FB.sem[2],
          FB.sem.LL[1], FB.sem.UL[1])
colnames(FB.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
FB.table <- FB.table[order(FB.table$mean),]


# Generating 3 panel figure for Rpf effects on soil microbial abundance
# Plotting microbial abundance and ratio figure #
png(filename="../figures/Figure4-MicrobialAbundanceRatio.png",
    width = 1200, height = 800, res = 96*2)
par(oma=c(7,3,7,1), mar=c(2,3,3,4), mfrow=c(1,3))

# Panel 1: Rpf effects on bacterial abundance #
abun.bac.fig <- plot(jitter(rep(1, length(bac.con$value)), amount = 0.1), bac.con$value,
      ylim = c(0, 2.4E6), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(bac.rpf$value)), amount = 0.1), bac.rpf$value, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data pointfor each treatment #
points(1, mean(bac.con$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(bac.rpf$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis labels
mtext(expression('16S rRNA gene copy/ g soil'), side = 2,
      outer = FALSE, cex = 1, line = 3.5, adj = 0.5)
```

```
# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0E0", "8.0E5", "1.6E6", "2.4E6"),
     at = c(0.0E0, 8.0E5, 1.6E6, 2.4E6))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0E0, 8.0E5, 1.6E6, 2.4E6), labels = F, tck = -0.05)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Adding SEM #
arrows(x0 = c(2,1), y0 = bac.table$mean, y1 = bac.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(2,1), y0 = bac.table$mean, y1 = bac.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label #
text(0.7,2.3E6 ,labels="A", col="black", cex=2)

# P-value label
mtext(text = expression(italic("P")~" = 0.016") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 9"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)

# Panel 2: Rpf effects on fungal abundance #
abun.fun.fig <- plot(jitter(rep(1, length(fun.con$value)), amount = 0.1), fun.con$value,
      ylim = c(0, 1.5E6), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(fun.rpf$value)), amount = 0.1), fun.rpf$value, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data pointfor each treatment #
points(1, mean(fun.con$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(fun.rpf$value), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis labels
mtext(expression('ITS gene copy / g soil'), side = 2,
      outer = FALSE, cex = 1, line = 3.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0E0", "5.0E5", "1.0E6", "1.5E6"),
     at = c(0.0E0, 5.0E5, 1.0E6, 1.5E6))
```

```
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0E0, 5.0E5, 1.0E6, 1.5E6), labels = F, tck = -0.05)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Adding SEM #
arrows(x0 = c(1,2), y0 = fun.table$mean, y1 = fun.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(1,2), y0 = fun.table$mean, y1 = fun.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label #
text(0.7,1.45E6 ,labels="B", col="black", cex=2)

# p-value
mtext(text = expression(italic("P")~" = 0.007") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 9"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)

# Panel 3: Rpf effects on fungal abundance #
FB.fig <- plot(jitter(rep(1, length(FB.con$FB)), amount = 0.1), FB.con$FB,
      ylim = c(0, 1.5), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(FB.rpf$FB)), amount = 0.1), FB.rpf$FB, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data pointfor each treatment #
points(1, mean(FB.con$FB), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(FB.rpf$FB), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis labels
mtext(expression('Fungal : bacterial ratio'), side = 2,
      outer = FALSE, cex = 1, line = 2.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0", "0.5", "1.0", "1.5"),
     at = c(0.0, 0.5, 1.0, 1.5))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0, 0.5, 1.0, 1.5), labels = F, tck = -0.05)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
```

```r
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.05)

# Adding SEM #
arrows(x0 = c(1,2), y0 = FB.table$mean, y1 = FB.table$LCI, angle = 90,
       length = 0.15, lwd = 2)
arrows(x0 = c(1,2), y0 = FB.table$mean, y1 = FB.table$UCI, angle = 90,
       length=0.15, lwd = 2)

# Panel label #
text(0.7,1.45,labels="C", col="black", cex=2)

# p-value
mtext(text = expression(italic("P")~" = 0.018") , side =3, line = -1.2, adj = 0.925, col="black", cex=0
# Sample number label
#mtext(text = expression(italic("n")~" = 9"), side = 3, line = -2.2, adj = 0.925, col="black", cex=0.8)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```r
graphics.off()

# Show Plot
img <- readPNG("../figures/Figure4-MicrobialAbundanceRatio.png")
grid.raster(img)


# Statistical tests: 2 sample t-test for Rpf effects on microbial abundance
# Bacterial abundance
bac.ttest <- t.test(stdGeneCopy ~ Treatment, data = data.sub.bac)
bac.ttest    # Significant: t = 2.7069, df = 14.777, p = 0.0164
```

```
##
##  Welch Two Sample t-test
##
## data:  stdGeneCopy by Treatment
## t = 2.7069, df = 14.777, p-value = 0.0164
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  104946.7 887208.7
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##            1683463            1187385
```

```r
# Fungal abundance
fun.ttest <- t.test(stdGeneCopy ~ Treatment, data = data.sub.fun)
fun.ttest    # Significant: t = -3.2719, df = 11.701, p = 0.00689
```

```
##
##  Welch Two Sample t-test
```

```
##
## data:  stdGeneCopy by Treatment
## t = -3.2719, df = 11.701, p-value = 0.00689
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -636711.6 -126817.7
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##           236624.1           618388.7
```

```
# F:B ratio
FB.ttest <- t.test(FB ~ Treatment, data = ratioFB)
FB.ttest     # Significant: t= -2.8454, df = 9.4476, p = 0.01835
```

```
##
##  Welch Two Sample t-test
##
## data:  FB by Treatment
## t = -2.8454, df = 9.4476, p-value = 0.01835
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.80465024 -0.09474171
## sample estimates:
## mean in group Rpf- mean in group Rpf+
##          0.1582796          0.6079756
```

4) Effect of Rpf and metabolic status on soil bacterial community structure

Set up work environment and load packages

```
source("~/GitHub/BrassicaRpf/bin/DiversityFunctions.R")
source("~/GitHub/BrassicaRpf/bin/MothurTools.R")
source("~/GitHub/BrassicaRpf/bin/phylodiversity2.R")

# Run All: Select if all section are to be re-run
run.all <- TRUE

# Load files
  # design = general design file for experiment
  # shared = OTU table from mothur with sequence similarity clustering
  # tax    = Taxonomy for 97% similarity OTUs

design <- "~/GitHub/BrassicaRpf/data/Brassica.design.txt"
shared <- "~/GitHub/BrassicaRpf/mothur/output/Brassica.bac.final.shared"
tax <- "~/GitHub/BrassicaRpf/mothur/output/Brassica.bac.final.0.03.taxonomy"

# Import Design
design <- read.delim(design, header=T, row.names=1)
# Import Shared Files
OTU <- read.otu(shared = shared, cutoff = "0.03") # 97% similarity
# Import Taxonomy
```

```
OTU.tax <- read.tax(taxonomy = tax, format = "rdp")
# Import Phylogenetic tree
OTU.tre <- read.tree("../phylo/Brassica.bac.rename.tree.2")
```

Soil bacteria beta and alpha diversity

```
### Sequence Coverage
# Remove OTUs with less than two occurences across all sites #
OTU <- OTU[, which(colSums(OTU) >= 2)]

# Remove mock community #
OTU <- OTU[1:20, ]

# Determine coverage of sequences #
cov.seqs <- count.groups(OTU)
cov.mean <- mean(cov.seqs) # 160,871
cov.sem <- sem(cov.seqs) # 16,095.38
cov.min <- min(cov.seqs) # 79,797
total.seqs <- sum(cov.seqs) # 3,217,419

# Good's coverage
goods.c <- function(x = ""){
              1 - (apply(OTU, 1, function(x){sum(x == 1)}) / rowSums(x))
}

goods.c.Brassica <- goods.c(OTU)
mean.good.c <- mean(goods.c.Brassica) # 0.984  Good mean coverage
min.good.c <- min(goods.c.Brassica) # 0.967  Good lowest coverage

### Alpha diversity
# Resampling code to estimate alpha diversity (used if run.all = T)
if (run.all == TRUE){
  rich <- round(richness.iter(input = OTU, size = 1000,
                              iters = 100, shared = "FALSE"), 3)
  even <- round(evenness.iter(input = OTU, size = 1000,
                              iters = 100, shared = "FALSE",
                              method = "simp_even"), 3)
  rare <- rarefy(OTU, 1000, se = FALSE, MARGIN = 1)
  # Write output to files
  write.table(rich, "../data/rich.txt", sep = "\t",
              col.names = T, row.names = T)
  write.table(even, "../data/even.txt", sep = "\t",
              col.names = T, row.names = T)
}

# Read in alpha diversity files from above
rich2 <- read.table("../data/rich.txt", sep = "\t")
even2 <- read.table("../data/even.txt", sep = "\t")

# Merge data to design and calculate mean and sem per sample
rich.data <- merge(design, rich2, by = "row.names")
row.names(rich.data) <- rich.data$Row.names
```

```
rich.data <- rich.data[sort(row.names(rich.data)), ]
rich.mean <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, mean, na.rm = TRUE),3)
rich.sem <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, sem, na.rm = TRUE), 3)

even.data <- merge(design, even2, by = "row.names")
row.names(even.data) <- even.data$Row.names
even.data <- even.data[sort(row.names(even.data)), ]
even.mean <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, mean, na.rm = TRUE),3)
even.sem <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, sem, na.rm = TRUE),4)

# Make new dataframe merging design file and mean diversity
Brassica.div <- data.frame(design[sort(row.names(design)), ], rich.mean, even.mean)

# Take averages of technial reps
rich.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, rich = mean(rich.mean))
even.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, even = mean(even.mean))

# Reshape data
rich.2 <- reshape(rich.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")
even.2 <- reshape(even.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")


## Statistical test: One-way ANOVA of Rpf and metabolic status
# Soil bacterial richness
trans <- Brassica.div[ which(Brassica.div$type == 'cDNA'), ]
gDNA <- Brassica.div[ which(Brassica.div$type == 'DNA'), ]

trans.aov <- aov(rich.mean ~ treatment, trans)
summary(trans.aov)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## treatment    1     81      81   0.032  0.862
## Residuals    8  20161    2520
```

```
gDNA.aov <- aov(rich.mean ~ treatment, gDNA)
summary(gDNA.aov)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## treatment    1   1718    1718   0.572  0.471
## Residuals    8  24038    3005
```

```
rich.anova.c <- aov(rich.mean ~ treatment*type, Brassica.div)
summary(rich.anova.c)
```

```
##                Df Sum Sq Mean Sq F value Pr(>F)
## treatment       1    527   526.6   0.191  0.668
## type            1      6     5.8   0.002  0.964
## treatment:type  1   1273  1272.8   0.461  0.507
## Residuals      16  44199  2762.4
```

```
# Soil bacterial evenness
even.anova.c <- aov(even.mean ~ treatment*type, Brassica.div)
summary(even.anova.c)
```

```
##                Df  Sum Sq  Mean Sq F value Pr(>F)
## treatment       1 0.00058 0.000583   0.052  0.823
## type            1 0.00792 0.007920   0.704  0.414
## treatment:type  1 0.00006 0.000058   0.005  0.944
## Residuals      16 0.18012 0.011257
```

```
# Alpha diversity: Wilcox Signed Rank Test to account for paired design
# Richness
rich.wilcox <- wilcox.test(Brassica.div$rich.mean[which(Brassica.div$treatment == 'Rpf-')], Brassica.di
rich.wilcox   # No effect of rpf
```

```
##
##  Wilcoxon signed rank test
##
## data:  Brassica.div$rich.mean[which(Brassica.div$treatment == "Rpf-")] and Brassica.div$rich.mean[wh
## V = 28, p-value = 1
## alternative hypothesis: true location shift is not equal to 0
```

```
# Evenness
even.wilcox <- wilcox.test(Brassica.div$even.mean[which(Brassica.div$treatment == 'Rpf-')], Brassica.di
even.wilcox   # No effect of rpf
```

```
##
##  Wilcoxon signed rank test
##
## data:  Brassica.div$even.mean[which(Brassica.div$treatment == "Rpf-")] and Brassica.div$even.mean[wh
## V = 28, p-value = 1
## alternative hypothesis: true location shift is not equal to 0
```

```
### Beta Diversity
# Make presence-absence matrix
OTU.PA <- (OTU > 0) * 1

# Make relative abundence matrix
OTU.REL <- OTU
for (i in 1:dim(OTU)[1]){
  OTU.REL[i,] <- OTU[i,]/sum(OTU[i,])
  }

# Log-transform relative abundances
OTU.REL.log <- decostand(OTU, method="log")

# Generate sample distance matrix from log-transformed relative abundance of OTU
Brassica.bc.dis <- vegdist(OTU.REL.log, method = "bray", binary = "FALSE")
Brassica.dis.mean <- mean(Brassica.bc.dis)

# Principal Coordinates Analysis (PCoA)
Brassica.PCoA <- cmdscale(Brassica.bc.dis, eig = TRUE, k = 3)
explainvar1 <- round(Brassica.PCoA$eig[1] / sum(Brassica.PCoA$eig), 3) * 100
explainvar2 <- round(Brassica.PCoA$eig[2] / sum(Brassica.PCoA$eig), 3) * 100
explainvar3 <- round(Brassica.PCoA$eig[3] / sum(Brassica.PCoA$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# OTU Scores
otu.scores <- t(cor(Brassica.PCoA$points, OTU.REL))
otu.scores <- as.matrix(otu.scores)[,1:2]
```

```r
otu.scores <- otu.scores[abs(otu.scores[,1]) > 0.7|abs(otu.scores[,2]) > 0.7,]

# Average BC Distance Between Treatments
Brassica.bc.dis.m <- as.matrix(Brassica.bc.dis)
all.equal(row.names(Brassica.div), rownames(Brassica.bc.dis.m))
```

```
## [1] TRUE
```

```r
treatment.div <- unique(Brassica.div$treatment)
treatment.dis <- rep(NA, length(treatment.div))
for(i in 1:length(treatment.div)){
  temp <- row.names(Brassica.div[Brassica.div$treatment == treatment.div[i], ])
  treatment.dis[i] <- Brassica.bc.dis.m[temp[1], temp[2]]
}

mean(treatment.dis)
```

```
## [1] 0.4775868
```

```r
# Plot figure -- Supplement for all bacteria ordination
png(filename="../figures/Suppl.Fig4.Bacteria.png",
    width = 1800, height = 800, res = 96*2)

layout(matrix(1:3, 1, 3), widths = c(20, 9, 2.5))

par(mar = c(7, 10, 1, 0) + 0.5)

plot(Brassica.PCoA$points[ ,1], Brassica.PCoA$points[ ,2],
     ylim = c(-0.4, 0.4), xlim = c(-0.5, 0.4),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""), line = 5,
     #xlab = "", ylab = "", xaxt = "n", yaxt = "n",
     pch = 22, cex = 2.0, type = "n", cex.lab = 2.5, cex.axis = 3,
     axes = FALSE)
```

```
## Warning in plot.window(...): "line" is not a graphical parameter

## Warning in plot.xy(xy, type, ...): "line" is not a graphical parameter
```

```r
# Add Axes
axis(side = 1, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 2, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 3, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
axis(side = 4, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
abline(h = 0, v = 0, lty = 3)

box(lwd = 2)

# Subset data
all.equal(row.names(Brassica.PCoA$points), rownames(Brassica.div))
```

```
## [1] TRUE
```

```r
Brassica.points <- data.frame(Brassica.PCoA$points, Brassica.div)

# Active community
Brassica.active.rpf <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.active.no <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf-"), ]
# Total community
Brassica.total.rpf <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.total.no <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf-"), ]

# Add points
# Active community Rpf+
points(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Active community Rpf-
points(Brassica.active.no[ ,1], Brassica.active.no[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)
# Total community Rpf+
points(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Total community Rpf-
points(Brassica.total.no[ ,1], Brassica.total.no[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)

# Add ellipses
# Active Rpf+
ordiellipse(cbind(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2]), Brassica.active.rpf$treatment, ki
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Active Rpf-
ordiellipse(cbind(Brassica.active.no[ ,1], Brassica.active.no[ ,2]), Brassica.active.no$treatment, kind=
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf+
ordiellipse(cbind(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2]), Brassica.total.rpf$treatment, kind=
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf-
ordiellipse(cbind(Brassica.total.no[ ,1], Brassica.total.no[ ,2]), Brassica.total.no$treatment, kind="s
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)

# Add Legend Outside
par(mar = c(4, 0, 5, 1) + 0.5)
plot.new()
legend(0, 1, c("Active, -Rpf", "Active, +Rpf", "Total, -Rpf", "Total, +Rpf"),
       pch = c(21, 21, 24, 24),
       col = c("Black", "Black", "Black", "Black"),
       pt.bg = c("lightgrey", "grey15", "lightgrey", "grey15"),
       bty = "n", y.intersp = 1, pt.cex = 3.2, cex = 2, lwd= 2, lty =  NA)

# Sample number label
mtext(text = expression(italic("n")~" = 5"), line = 1, adj = -0.65, col="black", cex=1.5)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```r
graphics.off()

# Show Plot
img <- readPNG("../figures/Suppl.Fig4.Bacteria.png")
grid.raster(img)

# Statistical test
# Add factor for pot number to account of paired/match design of study
Brassica.div$pot <- c(1,2,3,4,5,6,7,8,9,10,1,2,3,4,5,6,7,8,9,10)
Brassica.div$pot <- as.factor(Brassica.div$pot) #unranked order

# PERMANOVA to test Rpf and metabolic status effects on soil bacterial community structure
all.bray.permanova <- adonis(OTU.REL.log ~ type + treatment + pot +
                          type*treatment , data = Brassica.div,
                         method = "bray", binary = FALSE, permutations = 999)
all.bray.permanova
```

```
##
## Call:
## adonis(formula = OTU.REL.log ~ type + treatment + pot + type *      treatment, data = Brassica.div, p
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## type             1   0.54671 0.54671  7.2402 0.22571  0.001 ***
## treatment        1   0.14742 0.14742  1.9523 0.06086  0.032 *
## pot              8   1.02737 0.12842  1.7007 0.42415  0.001 ***
## type:treatment   1   0.09661 0.09661  1.2794 0.03988  0.168
## Residuals        8   0.60409 0.07551         0.24940
## Total           19   2.42219                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Indentify indicator species
#spe.corr <- add.spec.scores(Brassica.PCoA, OTU.REL.log,
#                            method = "cor.scores")$cproj
#corrcut <- 0.7 # User defined cutoff
#imp.spp <- spe.corr[abs(spe.corr[, 1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]
#imp.spp
#imp.otu <- as.vector(rownames(imp.spp))
#imp.otu

#imp.spp.lst <- OTU.tax[ which(OTU.tax$OTU == imp.otu | OTU.tax$OTU == "Otu00014" | OTU.tax$OTU == "Otu
#imp.spp.lst
```

Gram positive bacteria alpha and beta- diversity

```r
# Subset Gram positive OTU from taxonomy file
GramPositive.OTU.tax <- OTU.tax[ which(OTU.tax$Phylum == 'Actinobacteria' | OTU.tax$Phylum == 'Firmicute
# Subset OTU table to contain only Gram positive bacteria
GramPositive.OTU <- match(GramPositive.OTU.tax$OTU, colnames(OTU))
GramPositive.OTU <- sort(c(GramPositive.OTU-1, GramPositive.OTU))
Gpos.OTU <- OTU[, GramPositive.OTU]

# Remove OTUs with less than two occurences across all sites #
Gpos.OTU <- Gpos.OTU[, which(colSums(Gpos.OTU) >= 2)]

# Remove mock community #
Gpos.OTU <- Gpos.OTU[1:20, ]

### Alpha diversity
# Resampling code to estimate alpha diversity (used if run.all = T)
if (run.all == TRUE){
  rich <- round(richness.iter(input = Gpos.OTU, size = 1000,
                              iters = 100, shared = "FALSE"), 3)
  even <- round(evenness.iter(input = Gpos.OTU, size = 1000,
                              iters = 100, shared = "FALSE",
                              method = "simp_even"), 3)
  rare <- rarefy(Gpos.OTU, 1000, se = FALSE, MARGIN = 1)
  # Write output to files
  write.table(rich, "../data/rich.txt", sep = "\t",
              col.names = T, row.names = T)
  write.table(even, "../data/even.txt", sep = "\t",
              col.names = T, row.names = T)
}

# Read in alpha diversity files from above
rich2 <- read.table("../data/rich.txt", sep = "\t")
even2 <- read.table("../data/even.txt", sep = "\t")

# Merge data to design and calculate mean and sem per sample
rich.data <- merge(design, rich2, by = "row.names")
row.names(rich.data) <- rich.data$Row.names
rich.data <- rich.data[sort(row.names(rich.data)), ]
rich.mean <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, mean, na.rm = TRUE),3)
rich.sem <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, sem, na.rm = TRUE), 3)

even.data <- merge(design, even2, by = "row.names")
row.names(even.data) <- even.data$Row.names
even.data <- even.data[sort(row.names(even.data)), ]
even.mean <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, mean, na.rm = TRUE),3)
even.sem <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, sem, na.rm = TRUE),4)

# Make new dataframe merging design file and mean diversity
Brassica.div <- data.frame(design[sort(row.names(design)), ], rich.mean, even.mean)

# Take averages of technial reps
rich.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, rich = mean(rich.mean))
even.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, even = mean(even.mean))

# Reshape data
rich.2 <- reshape(rich.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")

even.2 <- reshape(even.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")


## Statistical test: two-way ANOVA of Rpf and metabolic status
# Soil bacterial richness
rich.anova.c <- aov(rich.mean ~ type + treatment + treatment*type, Brassica.div)
summary(rich.anova.c)
```

```
##                 Df Sum Sq Mean Sq F value Pr(>F)
## type             1    850   849.7   0.744  0.401
## treatment        1     33    33.5   0.029  0.866
## type:treatment   1    296   295.7   0.259  0.618
## Residuals       16  18276  1142.2
```

```
# Soil bacterial evenness
even.anova.c <- aov(even.mean ~ type + treatment + treatment*type, Brassica.div)
summary(even.anova.c)
```

```
##                 Df   Sum Sq  Mean Sq F value  Pr(>F)
## type             1 0.027602 0.027602  14.436 0.00157 **
## treatment        1 0.001674 0.001674   0.876 0.36329
## type:treatment   1 0.001566 0.001566   0.819 0.37883
## Residuals       16 0.030594 0.001912
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
### Beta Diversity
# Make presence-absence matrix
Gpos.OTU.PA <- (Gpos.OTU > 0) * 1

# Make relative abundence matrix
Gpos.OTU.REL <- Gpos.OTU
for (i in 1:dim(Gpos.OTU)[1]){
  Gpos.OTU.REL[i,] <- Gpos.OTU[i,]/sum(Gpos.OTU[i,])
  }

# Log-transform relative abundances
Gpos.OTU.REL.log <- decostand(Gpos.OTU, method="log")

Brassica.bc.dis <- vegdist(Gpos.OTU.REL.log, method = "bray", binary = "FALSE")
Brassica.dis.mean <- mean(Brassica.bc.dis)

# Principal Coordinates Analysis (PCoA)
Brassica.PCoA <- cmdscale(Brassica.bc.dis, eig = TRUE, k = 3)
explainvar1 <- round(Brassica.PCoA$eig[1] / sum(Brassica.PCoA$eig), 3) * 100
explainvar2 <- round(Brassica.PCoA$eig[2] / sum(Brassica.PCoA$eig), 3) * 100
explainvar3 <- round(Brassica.PCoA$eig[3] / sum(Brassica.PCoA$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Gpos.OTU Scores
Gpos.OTU.scores <- t(cor(Brassica.PCoA$points, Gpos.OTU.REL))
Gpos.OTU.scores <- as.matrix(Gpos.OTU.scores)[,1:2]
Gpos.OTU.scores <- Gpos.OTU.scores[abs(Gpos.OTU.scores[,1]) > 0.7|abs(Gpos.OTU.scores[,2]) > 0.7,]

# Average BC Distance Between Treatments
Brassica.bc.dis.m <- as.matrix(Brassica.bc.dis)
all.equal(row.names(Brassica.div), rownames(Brassica.bc.dis.m))
```

```
## [1] TRUE
```

```
treatment.div <- unique(Brassica.div$treatment)
treatment.dis <- rep(NA, length(treatment.div))
for(i in 1:length(treatment.div)){
  temp <- row.names(Brassica.div[Brassica.div$treatment == treatment.div[i], ])
  treatment.dis[i] <- Brassica.bc.dis.m[temp[1], temp[2]]
}

mean(treatment.dis)
```

```
## [1] 0.464032
```

```
# Plot figure # Gram positive ordination
png(filename="../figures/Suppl.Fig3.GramPositive.png",
    width = 1800, height = 800, res = 96*2)

layout(matrix(1:3, 1, 3), widths = c(20, 9, 2.5))

par(mar = c(7, 10, 1, 0) + 0.5)

plot(Brassica.PCoA$points[ ,1], Brassica.PCoA$points[ ,2],
     ylim = c(-0.4, 0.4), xlim = c(-0.5, 0.4),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""), line = 5,
     #xlab = "", ylab = "", xaxt = "n", yaxt = "n",
     pch = 22, cex = 2.0, type = "n", cex.lab = 2.5, cex.axis = 3,
     axes = FALSE)
```

```
## Warning in plot.window(...): "line" is not a graphical parameter

## Warning in plot.xy(xy, type, ...): "line" is not a graphical parameter
```

```
# Add Axes
axis(side = 1, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 2, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 3, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
axis(side = 4, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
abline(h = 0, v = 0, lty = 3)

box(lwd = 2)

# Subset data
all.equal(row.names(Brassica.PCoA$points), rownames(Brassica.div))
```

```
## [1] TRUE
```

```
Brassica.points <- data.frame(Brassica.PCoA$points, Brassica.div)

# Active community
Brassica.active.rpf <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.active.no <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf-"), ]
# Total community
Brassica.total.rpf <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.total.no <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf-"), ]

# Add points
# Active community Rpf+
points(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Active community Rpf-
points(Brassica.active.no[ ,1], Brassica.active.no[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)
# Total community Rpf+
points(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Total community Rpf-
points(Brassica.total.no[ ,1], Brassica.total.no[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)

# Add elipcses
# Active Rpf+
ordiellipse(cbind(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2]), Brassica.active.rpf$treatment, ki
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Active Rpf-
ordiellipse(cbind(Brassica.active.no[ ,1], Brassica.active.no[ ,2]), Brassica.active.no$treatment, kind
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf+
ordiellipse(cbind(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2]), Brassica.total.rpf$treatment, kind
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf-
ordiellipse(cbind(Brassica.total.no[ ,1], Brassica.total.no[ ,2]), Brassica.total.no$treatment, kind="s
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)


# Add Legend Outside
par(mar = c(4, 0, 5, 1) + 0.5)
plot.new()
legend(0, 1, c("Active, -Rpf", "Active, +Rpf", "Total, -Rpf", "Total, +Rpf"),
       pch = c(21, 21, 24, 24),
       col = c("Black", "Black", "Black", "Black"),
       pt.bg = c("lightgrey", "grey15", "lightgrey", "grey15"),
       bty = "n", y.intersp = 1, pt.cex = 3.2, cex = 2, lwd= 2, lty =  NA)

# Sample number label
mtext(text = expression(italic("n")~" = 5"), line = 1, adj = -0.65, col="black", cex=1.5)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Suppl.Fig3.GramPositive.png")
grid.raster(img)

# Factoring pot number for match/pair design
Brassica.div$pot <- c(1,2,3,4,5,6,7,8,9,10,1,2,3,4,5,6,7,8,9,10)
Brassica.div$pot <- as.factor(Brassica.div$pot)

## Statistical test: PERMANOVA to test Rpf and metabolic status effects on gram positive bacteria compo
gpos.bray.permanova <- adonis(Gpos.OTU.REL ~ type + treatment + pot +
                           type*treatment, data = Brassica.div,
                         method = "bray", binary = FALSE, permutations = 999)
gpos.bray.permanova
```

```
##
## Call:
## adonis(formula = Gpos.OTU.REL ~ type + treatment + pot + type *      treatment, data = Brassica.div,
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## type             1   0.70147 0.70147 13.4025 0.27823  0.001 ***
## treatment        1   0.18241 0.18241  3.4851 0.07235  0.002 **
## pot              8   1.12593 0.14074  2.6890 0.44659  0.001 ***
## type:treatment   1   0.09265 0.09265  1.7702 0.03675  0.075 .
## Residuals        8   0.41871 0.05234         0.16608
## Total           19   2.52117                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Indentify indicator species
#spe.corr <- add.spec.scores(Brassica.PCoA, Gpos.OTU.REL,
#                              method = "cor.scores")$cproj
#corrcut <- 0.94 # User defined cutoff
#imp.spp <- spe.corr[abs(spe.corr[, 1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]
#imp.spp
#imp.otu <- as.vector(rownames(imp.spp))
#imp.otu

#imp.spp.lst <- OTU.tax[ which(OTU.tax$OTU == imp.otu), ]
#imp.spp.lst
```

Actinobacteria alpha and beta- diversity

```
# Subset Gram positive OTU from taxonomy file
Actino.OTU.tax <- OTU.tax[ which(OTU.tax$Phylum == 'Actinobacteria'), ]
```

```r
# Subset OTU table to contain only Actinobacteria
Actino.OTU <- match(Actino.OTU.tax$OTU, colnames(OTU))
Actino.OTU <- sort(c(Actino.OTU-1, Actino.OTU))
Actino.OTU <- OTU[, Actino.OTU]

# Remove OTUs with less than two occurences across all sites #
Actino.OTU <- Actino.OTU[, which(colSums(Actino.OTU) >= 2)]

# Remove mock community #
Actino.OTU <- Actino.OTU[1:20, ]


### Alpha diversity
# Resampling code to estimate alpha diversity (used if run.all = T)
if (run.all == TRUE){
  rich <- round(richness.iter(input = Actino.OTU, size = 1000,
                              iters = 100, shared = "FALSE"), 3)
  even <- round(evenness.iter(input = Actino.OTU, size = 1000,
                              iters = 100, shared = "FALSE",
                              method = "simp_even"), 3)
  rare <- rarefy(Actino.OTU, 1000, se = FALSE, MARGIN = 1)
  # Write output to files
  write.table(rich, "../data/rich.txt", sep = "\t",
              col.names = T, row.names = T)
  write.table(even, "../data/even.txt", sep = "\t",
              col.names = T, row.names = T)
}

# Read in alpha diversity files from above
rich2 <- read.table("../data/rich.txt", sep = "\t")
even2 <- read.table("../data/even.txt", sep = "\t")

# Merge data to design and calculate mean and sem per sample
rich.data <- merge(design, rich2, by = "row.names")
row.names(rich.data) <- rich.data$Row.names
rich.data <- rich.data[sort(row.names(rich.data)), ]
rich.mean <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, mean, na.rm = TRUE),3)
rich.sem <- round(apply(rich.data[5:(4 + dim(rich2)[2])], 1, sem, na.rm = TRUE), 3)

even.data <- merge(design, even2, by = "row.names")
row.names(even.data) <- even.data$Row.names
even.data <- even.data[sort(row.names(even.data)), ]
even.mean <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, mean, na.rm = TRUE),3)
even.sem <- round(apply(even.data[5:(4 + dim(even2)[2])], 1, sem, na.rm = TRUE),4)

# Make new dataframe merging design file and mean diversity
Brassica.div <- data.frame(design[sort(row.names(design)), ], rich.mean, even.mean)

# Take averages of technial reps
rich.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, rich = mean(rich.mean))
even.rep.ave <- ddply(Brassica.div, .(treatment, type, rep), summarize, even = mean(even.mean))

# Reshape data
```

```r
rich.2 <- reshape(rich.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")

even.2 <- reshape(even.rep.ave[,1:4], timevar = "type",
                   idvar = c("treatment", "rep"), direction = "wide")


## Statistical test: two-way ANOVA of Rpf and metabolic status
# Soil bacterial richness
rich.anova.c <- aov(rich.mean ~ type + treatment + treatment*type, Brassica.div)
summary(rich.anova.c)
```

```
##                 Df Sum Sq Mean Sq F value Pr(>F)
## type             1      3     2.5   0.003  0.957
## treatment        1     17    17.2   0.021  0.887
## type:treatment   1    509   509.4   0.619  0.443
## Residuals       16  13173   823.3
```

```r
# One-way ANOVA based on Metabolic status
trans <- Brassica.div[ which(Brassica.div$type == 'cDNA'), ]
gDNA <- Brassica.div[ which(Brassica.div$type == 'DNA'), ]

trans.aov <- aov(rich.mean ~ treatment, trans)
summary(trans.aov)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## treatment    1    170   169.7   0.142  0.716
## Residuals    8   9551  1193.9
```

```r
gDNA.aov <- aov(rich.mean ~ treatment, gDNA)
summary(gDNA.aov)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## treatment    1    357   356.9   0.788  0.401
## Residuals    8   3622   452.8
```

```r
# Soil bacterial evenness
even.anova.c <- aov(even.mean ~ type + treatment + treatment*type, Brassica.div)
summary(even.anova.c)
```

```
##                 Df   Sum Sq  Mean Sq F value Pr(>F)  
## type             1 0.010215 0.010215   5.799 0.0285 *
## treatment        1 0.001345 0.001345   0.763 0.3952  
## type:treatment   1 0.000218 0.000218   0.124 0.7297  
## Residuals       16 0.028184 0.001762                 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
trans.e.aov <- aov(even.mean ~ treatment, trans)
summary(trans.e.aov)
```

```
##             Df   Sum Sq   Mean Sq F value Pr(>F)
## treatment    1 0.000240 0.0002401   0.438  0.527
## Residuals    8 0.004382 0.0005478
```

```r
gDNA.e.aov <- aov(even.mean ~ treatment, gDNA)
summary(gDNA.e.aov)
```

```
##              Df   Sum Sq  Mean Sq F value Pr(>F)
## treatment     1 0.001322 0.001323   0.444  0.524
## Residuals     8 0.023802 0.002975
```

```
### Beta Diversity
# Make presence-absence matrix
Actino.OTU.PA <- (Actino.OTU > 0) * 1

# Make relative abundence matrix
Actino.OTU.REL <- Actino.OTU
for (i in 1:dim(Actino.OTU)[1]){
  Actino.OTU.REL[i,] <- Actino.OTU[i,]/sum(Actino.OTU[i,])
  }

# Log-transform relative abundances
Actino.OTU.REL.log <- decostand(Actino.OTU, method="log")

Brassica.bc.dis <- vegdist(Actino.OTU.REL.log, method = "bray", binary = "FALSE")
Brassica.dis.mean <- mean(Brassica.bc.dis)

# Principal Coordinates Analysis (PCoA)
Brassica.PCoA <- cmdscale(Brassica.bc.dis, eig = TRUE, k = 3)
explainvar1 <- round(Brassica.PCoA$eig[1] / sum(Brassica.PCoA$eig), 3) * 100
explainvar2 <- round(Brassica.PCoA$eig[2] / sum(Brassica.PCoA$eig), 3) * 100
explainvar3 <- round(Brassica.PCoA$eig[3] / sum(Brassica.PCoA$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2, explainvar3)

# Actino.OTU Scores
Actino.OTU.scores <- t(cor(Brassica.PCoA$points, Actino.OTU.REL))
Actino.OTU.scores <- as.matrix(Actino.OTU.scores)[,1:2]
Actino.OTU.scores <- Actino.OTU.scores[abs(Actino.OTU.scores[,1]) > 0.7|abs(Actino.OTU.scores[,2]) > 0.7

# Average BC Distance Between Treatments
Brassica.bc.dis.m <- as.matrix(Brassica.bc.dis)
all.equal(row.names(Brassica.div), rownames(Brassica.bc.dis.m))
```

```
## [1] TRUE
```

```
treatment.div <- unique(Brassica.div$treatment)
treatment.dis <- rep(NA, length(treatment.div))
for(i in 1:length(treatment.div)){
  temp <- row.names(Brassica.div[Brassica.div$treatment == treatment.div[i], ])
  treatment.dis[i] <- Brassica.bc.dis.m[temp[1], temp[2]]
}

mean(treatment.dis)
```

```
## [1] 0.4451541
```

```
# Plot figure #
png(filename="../figures/Figure5-ActinoOrdination.png",
    width = 1800, height = 800, res = 96*2)

layout(matrix(1:3, 1, 3), widths = c(20, 9, 2.5))

par(mar = c(7, 10, 1, 0) + 0.5)
```

```
plot(Brassica.PCoA$points[ ,1], Brassica.PCoA$points[ ,2],
     ylim = c(-0.4, 0.4), xlim = c(-0.5, 0.4),
     xlab = paste("PCoA 1 (", explainvar1, "%)", sep = ""),
     ylab = paste("PCoA 2 (", explainvar2, "%)", sep = ""), line = 5,
     #xlab = "", ylab = "", xaxt = "n", yaxt = "n",
     pch = 22, cex = 2.0, type = "n", cex.lab = 2.5, cex.axis = 3,
     axes = FALSE)
```

```
## Warning in plot.window(...): "line" is not a graphical parameter
```

```
## Warning in plot.xy(xy, type, ...): "line" is not a graphical parameter
```

```
# Add Axes
axis(side = 1, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 2, labels = T, lwd.ticks = 3, cex.axis = 2, las = 1, tck=-0.025)
axis(side = 3, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
axis(side = 4, labels = F, lwd.ticks = 3, cex.axis = 1, las = 1, tck=-0.025)
abline(h = 0, v = 0, lty = 3)

box(lwd = 2)

# Subset data
all.equal(row.names(Brassica.PCoA$points), rownames(Brassica.div))
```

```
## [1] TRUE
```

```
Brassica.points <- data.frame(Brassica.PCoA$points, Brassica.div)

# Active community
Brassica.active.rpf <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.active.no <- Brassica.points[ which(Brassica.points$type == "cDNA" &
                                   Brassica.points$treatment == "Rpf-"), ]
# Total community
Brassica.total.rpf <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf+"), ]
Brassica.total.no <- Brassica.points[ which(Brassica.points$type == "DNA" &
                                   Brassica.points$treatment == "Rpf-"), ]

# Add points
# Active community Rpf+
points(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Active community Rpf-
points(Brassica.active.no[ ,1], Brassica.active.no[ ,2], pch = 21,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)
# Total community Rpf+
points(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "grey15", lwd= 2.5)
# Total community Rpf-
points(Brassica.total.no[ ,1], Brassica.total.no[ ,2], pch = 24,
       cex = 3.5, col = "Black", bg = "lightgrey", lwd= 2.5)

# Add elipcses
# Active Rpf+
ordiellipse(cbind(Brassica.active.rpf[ ,1], Brassica.active.rpf[ ,2]), Brassica.active.rpf$treatment, ki
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Active Rpf-
ordiellipse(cbind(Brassica.active.no[ ,1], Brassica.active.no[ ,2]), Brassica.active.no$treatment, kind=
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf+
ordiellipse(cbind(Brassica.total.rpf[ ,1], Brassica.total.rpf[ ,2]), Brassica.total.rpf$treatment, kind=
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)
# Total Rpf-
ordiellipse(cbind(Brassica.total.no[ ,1], Brassica.total.no[ ,2]), Brassica.total.no$treatment, kind="s
            lwd=2, lty=3, draw="lines", col="black", label=FALSE)

# Add Legend Outside
par(mar = c(4, 0, 5, 1) + 0.5)
plot.new()
legend(0, 1, c("Active, -Rpf", "Active, +Rpf", "Total, -Rpf", "Total, +Rpf"),
       pch = c(21, 21, 24, 24),
       col = c("Black", "Black", "Black", "Black"),
       pt.bg = c("lightgrey", "grey15", "lightgrey", "grey15"),
       bty = "n", y.intersp = 1, pt.cex = 3.2, cex = 2, lwd= 2, lty =  NA)

# Sample number label
# mtext(text = expression(italic("n")~" = 5"), line = 1, adj = -0.65, col="black", cex=1.5)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Figure5-ActinoOrdination.png")
grid.raster(img)


# Adding pot number factor
Brassica.div$pot <- c(1,2,3,4,5,6,7,8,9,10,1,2,3,4,5,6,7,8,9,10)
Brassica.div$pot <- as.factor(Brassica.div$pot)

## Statistical test: PERMANOVA to test Rpf and metabolic status effects on Actinobacteria composition
act.bray.permanova <- adonis(Actino.OTU.REL ~ Brassica.div$type + Brassica.div$treatment +
                               Brassica.div$pot + Brassica.div$type*Brassica.div$treatment, method
                             = "bray", binary = FALSE, permutations = 999)
act.bray.permanova
```

```
##
## Call:
## adonis(formula = Actino.OTU.REL ~ Brassica.div$type + Brassica.div$treatment +      Brassica.div$pot
##
## Permutation: free
## Number of permutations: 999
##
```

```
## Terms added sequentially (first to last)
##
##                                            Df SumsOfSqs MeanSqs F.Model
## Brassica.div$type                           1   0.80455 0.80455 16.0765
## Brassica.div$treatment                      1   0.16252 0.16252  3.2475
## Brassica.div$pot                            8   0.96850 0.12106  2.4191
## Brassica.div$type:Brassica.div$treatment    1   0.09792 0.09792  1.9566
## Residuals                                   8   0.40036 0.05004
## Total                                      19   2.43384
##                                                 R2 Pr(>F)
## Brassica.div$type                          0.33057  0.001 ***
## Brassica.div$treatment                     0.06677  0.004 **
## Brassica.div$pot                           0.39793  0.001 ***
## Brassica.div$type:Brassica.div$treatment 0.04023  0.056 .
## Residuals                                  0.16450
## Total                                      1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Indentify indicator species
spe.corr <- add.spec.scores(Brassica.PCoA, Actino.OTU.REL.log,
                            method = "cor.scores")$cproj
corrcut <- 0.7 # User defined cutoff
imp.spp <- spe.corr[abs(spe.corr[, 1]) >= corrcut | abs(spe.corr[, 2]) >= corrcut, ]
imp.spp <- as.matrix(imp.spp)
imp.spp
```

```
##                 Dim1        Dim2         Dim3
## Otu00041  0.94381916  0.04814077  0.030967097
## Otu00043  0.76747042 -0.40614482  0.155918685
## Otu00088  0.72771554  0.12731720  0.223078014
## Otu00089  0.71206071  0.09387614  0.004679730
## Otu00107  0.96034286 -0.03379011  0.060128607
## Otu00123  0.35679161 -0.74185740 -0.099933755
## Otu00326  0.85921722 -0.02808484  0.061608151
## Otu00333  0.18382962 -0.76512659  0.320588965
## Otu00345  0.74148325 -0.39538094  0.129141400
## Otu00361  0.76285456  0.10517675  0.178617067
## Otu00362  0.72606371 -0.46317397  0.114809959
## Otu00396  0.92868880  0.10510438 -0.058518292
## Otu00444 -0.80940522 -0.12705206 -0.031491239
## Otu00445 -0.02434157 -0.76578469  0.394396102
## Otu00450  0.73826260  0.21670886 -0.315001896
## Otu00584  0.03294415 -0.72248314 -0.636192663
## Otu00588  0.94158759  0.05400797  0.013540349
## Otu00593  0.85116440  0.02216679 -0.090761376
## Otu00651  0.76075683 -0.26257639 -0.435906141
## Otu00689  0.74826755 -0.01220389  0.076093423
## Otu00712  0.89351956  0.02111334  0.105495379
## Otu00715 -0.80969329 -0.38173470  0.141080840
## Otu00723  0.78431568 -0.05579502  0.011597332
## Otu00724 -0.10052924 -0.79285640 -0.437547967
## Otu00739  0.15920435 -0.74623341  0.274755412
## Otu00745  0.79036612 -0.18697157 -0.105621360
## Otu00756  0.85990913  0.11222778  0.126606599
## Otu00759  0.87141381  0.04031918  0.110509999
## Otu00760  0.92321740  0.13203911  0.027538908
## Otu00763  0.92258282  0.06969988 -0.002099196
## Otu00774  0.19395639 -0.72674543 -0.321544522
## Otu00813  0.76841557 -0.20481054  0.026234766
## Otu00825  0.76991638  0.17225716 -0.160550974
## Otu00842 -0.01057316 -0.73557720 -0.350104641
## Otu00856 -0.08636043 -0.71878749 -0.062097879
## Otu00878 -0.19195504 -0.77447642 -0.471994619
## Otu00888  0.84831542  0.17994736 -0.217073986
## Otu00901  0.84731317 -0.10311912  0.004000382
## Otu00922 -0.09193357 -0.81327040  0.261037274
## Otu00932 -0.17259958 -0.74802641 -0.536560909
## Otu00933 -0.24154212 -0.83420833 -0.293960006
## Otu00957  0.76300096  0.03372929 -0.294099078
## Otu00958 -0.08090690 -0.82218252 -0.347714221
## Otu00986  0.03031647 -0.70112091  0.326914240
## Otu01000  0.24591308 -0.74562844  0.321822650
## Otu01013 -0.28227690 -0.72404705 -0.321193807
## Otu01098  0.71368384  0.14422806  0.249789863
## Otu01124  0.86916933  0.09327884 -0.255325138
## Otu01131  0.70155128 -0.30441649 -0.025081632
## Otu01178 -0.71004010 -0.55493149  0.187954145
## Otu01182 -0.75998207 -0.40892933  0.162553824
## Otu01201  0.25618214 -0.76734621  0.156302420
## Otu01201  0.25618214 -0.76734621  0.156302420
## Otu01213  0.12523198 -0.84924422  0.238270116
## Otu01373  0.86926126  0.08107373  0.081135035
## Otu01377  0.02030233 -0.77579802  0.205400064
## Otu01416 -0.15176134 -0.83628086 -0.460432539
## Otu01428  0.79177671 -0.01106019  0.360036165
## Otu01429 -0.21174689 -0.75413767 -0.415809264
## Otu01529 -0.87226912 -0.37098201  0.135003701
## Otu01531 -0.92069802 -0.23560267  0.116830492
## Otu01532  0.76488494 -0.38144633 -0.033365950
## Otu01544 -0.11646615 -0.84234001  0.233543212
## Otu01659  0.82445195 -0.05925605  0.049568086
## Otu01660 -0.49204701 -0.73938048  0.047598442
## Otu01680 -0.85606534 -0.16763737  0.106878296
## Otu01681 -0.45872532 -0.72942543  0.302201702
## Otu01693 -0.32673701 -0.70062192  0.046527733
## Otu01694  0.33203447 -0.76923285  0.116829942
## Otu01704  0.08768080 -0.86309152 -0.059373532
## Otu01726  0.79631757  0.06829790 -0.027216588
## Otu01878 -0.83575449 -0.15159472  0.176659455
## Otu01880 -0.16126686 -0.75017163 -0.329670170
## Otu01890 -0.08675671 -0.78227928  0.238140892
## Otu01896 -0.13140521 -0.79893093 -0.377451958
## Otu01897  0.14028872 -0.71839035  0.373101141
## Otu01903  0.72274109 -0.29922032 -0.267397434
## Otu01903  0.72274109 -0.29922032 -0.267397434
## Otu01904 -0.09219415 -0.79880465 -0.443575302
## Otu01928 -0.03938488 -0.77518163  0.082032167
## Otu01992  0.20854352 -0.77715788  0.173461530
## Otu02036  0.92687369  0.12922367  0.061688230
## Otu02075 -0.86572205 -0.32444381  0.036945765
## Otu02076 -0.37701728 -0.77714025 -0.128797482
## Otu02114 -0.77495267 -0.11988964  0.246189516
## Otu02169  0.79005749 -0.04603300  0.064850610
## Otu02388 -0.70333729 -0.39063688  0.137643387
## Otu02417 -0.20853884 -0.71579693 -0.436059832
## Otu02499 -0.70453786 -0.18444295 -0.326875901
## Otu02520  0.17377413 -0.79189333 -0.272104115
## Otu02566 -0.76649331 -0.17396218  0.076034771
## Otu02567 -0.23776256 -0.80184394 -0.377049771
## Otu02571  0.89485161 -0.02323996  0.116926125
## Otu02653  0.79640750  0.07521834  0.052122250
## Otu02725  0.05411807 -0.76433527 -0.185866812
## Otu02751 -0.04955620 -0.73246241  0.361264799
## Otu02760 -0.22536168 -0.80536498 -0.293029578
## Otu02802  0.01704045 -0.81423645 -0.174957838
## Otu03021 -0.31013801 -0.76229666 -0.170649031
## Otu03157  0.70358076  0.22148167  0.181039797
## Otu03198 -0.75279377 -0.13957898  0.018556493
## Otu03231  0.76918439  0.01075444  0.116768179
## Otu03233  0.72934743  0.08339050  0.009881609
## Otu03277  0.77494079 -0.09218918  0.229586402
## Otu03325 -0.26292237 -0.72625785 -0.408091887
## Otu03328  0.71595568  0.27210352 -0.145862863
## Otu03350  0.07789583 -0.71737784 -0.276124139
## Otu03532  0.73999685 -0.29067449  0.296543422
## Otu03545  0.71008310  0.29124450  0.213096324
## Otu03548  0.76509529  0.16740101 -0.069834111
## Otu03572  0.78869771  0.14218137 -0.168501407
## Otu04357  0.71330667  0.20025483 -0.083480816
## Otu04394  0.80472799  0.02142752  0.093810715
## Otu04397  0.73347732  0.02158065  0.254017812
## Otu04398  0.76764141  0.13158320 -0.047492433
## Otu04735  0.83743813  0.16102724  0.092104639
## Otu05581  0.75723284  0.17083136 -0.172265928
## Otu05925  0.71068597  0.06824637  0.278048352
## Otu05962  0.71406301 -0.08310418  0.083123954
## Otu06174  0.80491581 -0.11868848  0.220953336
## Otu06738  0.77733257 -0.12573453 -0.029234751
## Otu06866  0.27692177 -0.72148358  0.115500871
## Otu07469  0.17085264 -0.71805056  0.008971341
## Otu09869  0.70301475  0.03917627 -0.115245892
## Otu11200  0.72522449 -0.02015755  0.356809823
## Otu11200  0.72522449 -0.02015755  0.356809823
```

```
imp.otu <- as.vector(rownames(imp.spp))
imp.otu
```

```
##   [1] "Otu00041" "Otu00043" "Otu00088" "Otu00089" "Otu00107" "Otu00123"
##   [7] "Otu00326" "Otu00333" "Otu00345" "Otu00361" "Otu00362" "Otu00396"
##  [13] "Otu00444" "Otu00445" "Otu00450" "Otu00584" "Otu00588" "Otu00593"
##  [19] "Otu00651" "Otu00689" "Otu00712" "Otu00715" "Otu00723" "Otu00724"
##  [25] "Otu00739" "Otu00745" "Otu00756" "Otu00759" "Otu00760" "Otu00763"
##  [31] "Otu00774" "Otu00813" "Otu00825" "Otu00842" "Otu00856" "Otu00878"
##  [37] "Otu00888" "Otu00901" "Otu00922" "Otu00932" "Otu00933" "Otu00957"
##  [43] "Otu00958" "Otu00986" "Otu01000" "Otu01013" "Otu01098" "Otu01124"
##  [49] "Otu01131" "Otu01178" "Otu01182" "Otu01201" "Otu01201" "Otu01213"
##  [55] "Otu01373" "Otu01377" "Otu01416" "Otu01428" "Otu01429" "Otu01529"
##  [61] "Otu01531" "Otu01532" "Otu01544" "Otu01659" "Otu01660" "Otu01680"
##  [67] "Otu01681" "Otu01693" "Otu01694" "Otu01704" "Otu01726" "Otu01878"
##  [73] "Otu01880" "Otu01890" "Otu01896" "Otu01897" "Otu01903" "Otu01903"
##  [79] "Otu01904" "Otu01928" "Otu01992" "Otu02036" "Otu02075" "Otu02076"
##  [85] "Otu02114" "Otu02169" "Otu02388" "Otu02417" "Otu02499" "Otu02520"
##  [91] "Otu02566" "Otu02567" "Otu02571" "Otu02653" "Otu02725" "Otu02751"
##  [97] "Otu02760" "Otu02802" "Otu03021" "Otu03157" "Otu03198" "Otu03231"
## [103] "Otu03233" "Otu03277" "Otu03325" "Otu03328" "Otu03350" "Otu03532"
## [109] "Otu03545" "Otu03548" "Otu03572" "Otu04357" "Otu04394" "Otu04397"
## [115] "Otu04398" "Otu04735" "Otu05581" "Otu05925" "Otu05962" "Otu06174"
## [121] "Otu06738" "Otu06866" "Otu07469" "Otu09869" "Otu11200" "Otu11200"
```

```
imp.spp.lst.1 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[1:48]), ]
imp.spp.lst.2 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[48:57]), ]
imp.spp.lst.3 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[57:68]), ]
imp.spp.lst.4 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[69:77]), ]
imp.spp.lst.5 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[78:89]), ]
imp.spp.lst.6 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[90:99]), ]
imp.spp.lst.7 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[100:109]), ]
imp.spp.lst.8 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[110:119]), ]
imp.spp.lst.8 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[120:125]), ]
imp.spp.lst.9 <- OTU.tax[ which(OTU.tax$OTU == imp.otu[126]), ]

imp.spp.lst <- rbind(imp.spp.lst.1, imp.spp.lst.2, imp.spp.lst.3, imp.spp.lst.4, imp.spp.lst.5,
                     imp.spp.lst.6, imp.spp.lst.7, imp.spp.lst.8, imp.spp.lst.9)

fit <- envfit(Brassica.PCoA, Actino.OTU.REL.log, perm = 999)
```

6) Supplementary materials

Gram positive: Gram negative ratios

```
# Subsetting
OTU.Rpf <- OTU[c(6:10, 16:20), ]
Gpos.OTU.Rpf <- Gpos.OTU[c(6:10, 16:20), ]

OTU.Con <- OTU[c(1:5, 11:15), ]
Gpos.OTU.Con <- Gpos.OTU[c(1:5, 11:15), ]

# Calculate sum reads for Gram positive
Gram.pos.Rpf <- as.vector(rowSums(Gpos.OTU.Rpf))
All.Rpf <- as.vector(rowSums(OTU.Rpf))
Gram.pos.Con <- as.vector(rowSums(Gpos.OTU.Con))
All.Con <- as.vector(rowSums(OTU.Con))

# Calculate Gram negative read sum
Gram.neg.Rpf <- All.Rpf - Gram.pos.Rpf
Gram.neg.Con <- All.Con - Gram.pos.Con
```

```
# Calculate ratio of Gram postive : Gram negative values
Rpf.Gram.Ratio <- Gram.pos.Rpf / Gram.neg.Rpf  ## points for Rpf+ Ratio
Con.Gram.Ratio <- Gram.pos.Con / Gram.neg.Con  ## points for Rpf- Ratio

# Generate data table for figure
dat <- cbind(Rpf.Gram.Ratio, Con.Gram.Ratio)
dat.m <- melt(dat)
dat.m <- dat.m[, 2:3]
colnames(dat.m) <- c("Treatment", "Ratio")
dat.m$Treatment <- gsub('Rpf.Gram.Ratio', 'Rpf+', dat.m$Treatment)
dat.m$Treatment <- gsub('Con.Gram.Ratio', 'Rpf-', dat.m$Treatment)

# Gram Ratio table #
GramRatio.mean <- aggregate(dat.m$Ratio ~ Treatment, dat.m, mean)
GramRatio.sem <- aggregate(dat.m$Ratio ~ Treatment, dat.m, sem)
GramRatio.sem.LL <- GramRatio.mean[2] + GramRatio.sem[2]
GramRatio.sem.UL <- GramRatio.mean[2] - GramRatio.sem[2]
GramRatio.table <- data.frame(GramRatio.mean[1], GramRatio.mean[2], GramRatio.sem[2],
          GramRatio.sem.LL[1], GramRatio.sem.UL[1])
colnames(GramRatio.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
GramRatio.table <- GramRatio.table[order(GramRatio.table$mean),]


# Plotting Gram Ratio #
png(filename="../figures/Suppl.Fig2a.GramRatio.png",
    width = 800, height = 800, res = 96*2)
par(mar = c(5, 5, 1, 1))

arabid.fig <- plot(jitter(rep(1, length(Con.Gram.Ratio)), amount = 0.1), Con.Gram.Ratio,
      ylim = c(0, 0.2), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(Rpf.Gram.Ratio)), amount = 0.1), Rpf.Gram.Ratio, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data point for each treatment #
points(1, mean(Con.Gram.Ratio), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(Rpf.Gram.Ratio), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis labels
mtext(expression('Gram positive : Gram negative'), side = 2,
      outer = FALSE, cex = 1.5, line = 3.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0", "0.1", "0.2"),
     at = c(0.0, 0.1, 0.2))
```

```
axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0, 0.1, 0.2), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("Rpf-", "Rpf+"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.02)

# Adding SEM #
arrows(x0 = c(2,1), y0 = GramRatio.table$mean, y1 = GramRatio.table$LCI, angle = 90,
       length = 0.25, lwd = 2)
arrows(x0 = c(2,1), y0 = GramRatio.table$mean, y1 = GramRatio.table$UCI, angle = 90,
       length=0.25, lwd = 2)

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Suppl.Fig2a.GramRatio.png")
grid.raster(img)


# t-test for effect of RPf
Gram.Ratio.ttest <- t.test(Con.Gram.Ratio, Rpf.Gram.Ratio, alternative="greater")
Gram.Ratio.ttest  # Non-significant: t = 1.2977, df = 15.203, p = 0.1069
```

```
##
##  Welch Two Sample t-test
##
## data:  Con.Gram.Ratio and Rpf.Gram.Ratio
## t = 1.2977, df = 15.203, p-value = 0.1069
## alternative hypothesis: true difference in means is greater than 0
## 95 percent confidence interval:
##  -0.004341151          Inf
## sample estimates:
##  mean of x  mean of y
## 0.09961739 0.08720269
```

Actinobacteria: proprotions

```
# Subsetting based on Rpf treatment
# +Rpf treatment
OTU.Rpf <- OTU[c(6:10, 16:20), ]
Actino.OTU.Rpf <- Actino.OTU[c(6:10, 16:20), ]
# -Rpf treatment
OTU.Con <- OTU[c(1:5, 11:15), ]
Actino.OTU.Con <- Actino.OTU[c(1:5, 11:15), ]
```

```
# Calculate within sample sum reads for Actino and all 16S based on treatment
Actino.Rpf <- as.vector(rowSums(Actino.OTU.Rpf))
All.Rpf <- as.vector(rowSums(OTU.Rpf))
Actino.Con <- as.vector(rowSums(Actino.OTU.Con))
All.Con <- as.vector(rowSums(OTU.Con))

# Calculate Gram negative read sum
#Gram.neg.Rpf <- All.Rpf - Actino.Rpf
#Gram.neg.Con <- All.Con - Actino.Con

# Calculate reads ratio of Actinobacteria : all 16S rRNA
Rpf.Act.Ratio <- Actino.Rpf / All.Rpf
Con.Act.Ratio <- Actino.Con / All.Con

# Generate data table for figure
dat <- cbind(Rpf.Act.Ratio, Con.Act.Ratio)
dat.m <- melt(dat)
dat.m <- dat.m[, 2:3]
colnames(dat.m) <- c("Treatment", "Ratio")
dat.m$Treatment <- gsub('Rpf.Act.Ratio', 'Rpf+', dat.m$Treatment)
dat.m$Treatment <- gsub('Con.Act.Ratio', 'Rpf-', dat.m$Treatment)

# Gram Ratio table #
ActRatio.mean <- aggregate(dat.m$Ratio ~ Treatment, dat.m, mean)
ActRatio.sem <- aggregate(dat.m$Ratio ~ Treatment, dat.m, sem)
ActRatio.sem.LL <- ActRatio.mean[2] + ActRatio.sem[2]
ActRatio.sem.UL <- ActRatio.mean[2] - ActRatio.sem[2]
ActRatio.table <- data.frame(ActRatio.mean[1], ActRatio.mean[2], ActRatio.sem[2],
          ActRatio.sem.LL[1], ActRatio.sem.UL[1])
colnames(ActRatio.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
ActRatio.table <- ActRatio.table[order(ActRatio.table$mean),]


# Plotting Actino Ratio #
png(filename="../figures/Suppl.Fig2.ActinoProportion.png",
    width = 800, height = 800, res = 96*2)
par(mar = c(5, 5, 1, 1))

arabid.fig <- plot(jitter(rep(1, length(Con.Act.Ratio)), amount = 0.1), Con.Act.Ratio,
      ylim = c(0, 0.2), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(Rpf.Act.Ratio)), amount = 0.1), Rpf.Act.Ratio, pch = 21,
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data point for each treatment #
points(1, mean(Con.Act.Ratio), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(Rpf.Act.Ratio), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)
```

```
# Y axis labels
mtext(expression('Proportion Actinobacteria'), side = 2,
      outer = FALSE, cex = 1.5, line = 3.5, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0", "0.1", "0.2"),
     at = c(0.0, 0.1, 0.2))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0, 0.1, 0.2), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.02)

# Adding SEM #
arrows(x0 = c(2,1), y0 = ActRatio.table$mean, y1 = ActRatio.table$LCI, angle = 90,
       length = 0.25, lwd = 2)
arrows(x0 = c(2,1), y0 = ActRatio.table$mean, y1 = ActRatio.table$UCI, angle = 90,
       length=0.25, lwd = 2)

# p-value
mtext(text = expression(italic("P")~" = 0.097") , side =3, line = -1.2, adj = 0.925, col="black", cex=1
# Sample number label
#mtext(text = expression(italic("n")~" = 10"), side = 3, line = -2.2, adj = 0.925, col="black", cex=1.2

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Suppl.Fig2.ActinoProportion.png")
grid.raster(img)


# Statistics
Act.Ratio.ttest <- t.test(Con.Act.Ratio, Rpf.Act.Ratio, alternative="greater")
Act.Ratio.ttest  # Non-significant: t = 1.3691, df = 13.303, p = 0.09682
```

```
##
##  Welch Two Sample t-test
##
## data:  Con.Act.Ratio and Rpf.Act.Ratio
## t = 1.3653, df = 13.669, p-value = 0.09711
## alternative hypothesis: true difference in means is greater than 0
## 95 percent confidence interval:
##  -0.002782881          Inf
## sample estimates:
##  mean of x  mean of y
## 0.06326911 0.05374770
```

Control Arabidopsis experiment

```
# Load dataset
seedling <- read.delim("~/Github/BrassicaRpf/data/seedlingbiomass.txt", sep = ",", head = TRUE)

# Calculate relative biomass #
seedling$Biomass <- (seedling$BiomassPixel)/(seedling$PlatePixel)
seedling$RelativeBiomass <- (seedling$Biomass)/(seedling$Seedlings)*100

# Biomass data points #
seedling.rpf <- seedling[ which(seedling$Treatment == "Rpf+"),]
seedling.con <- seedling[ which(seedling$Treatment == "Rpf-"),]

# Biomass data table #
seedling.mean <- aggregate(seedling$RelativeBiomass ~ Treatment, seedling, mean)
seedling.sem <- aggregate(seedling$RelativeBiomass ~ Treatment, seedling, sem)
seedling.sem.LL <- seedling.mean[2] + seedling.sem[2]
seedling.sem.UL <- seedling.mean[2] - seedling.sem[2]
seedling.table <- data.frame(seedling.mean[1], seedling.mean[2], seedling.sem[2],
          seedling.sem.LL[1], seedling.sem.UL[1])
colnames(seedling.table) <- c("Treatment", "mean", "sem", "LCI", "UCI")
seedling.table <- seedling.table[order(seedling.table$mean),]

# Plotting Arabidopsis biomass #
png(filename="../figures/Suppl.Fig5.Arabidopsis.png",
    width = 800, height = 800, res = 96*2)
par(mar = c(5, 5, 1, 1))

arabid.fig <- plot(jitter(rep(1, length(seedling.con$RelativeBiomass)), amount = 0.1), seedling.con$Rela
      ylim = c(0, 1), xlim = c(0.5, 2.5), pch = 21, col = "lightgrey", bg = "lightgrey", lwd = 3.5,
      cex = 1.7, yaxt = "n", xaxt = "n", cex.lab = 2, cex.axis = 2,
      las = 1, ylab = "", xlab = "")
      box(lwd = 2)
points(jitter(rep(2, length(seedling.rpf$RelativeBiomass)), amount = 0.1), seedling.rpf$RelativeBiomass
       bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# Adding mean data point for each treatment #
points(1, mean(seedling.con$RelativeBiomass), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(seedling.rpf$RelativeBiomass), pch = 21, col = "black",
       bg = "NA", lwd = 2, cex = 2.5)

box(lwd = 2)

# Y axis labels
mtext(expression('Relative Biomass'), side = 2,
      outer = FALSE, cex = 1.5, line = 3.5, adj = 0.5)

# Major Axes
```

```
axis(side = 2, lwd.ticks = 2, cex.axis = 1, las = 1,
     labels = c("0.0", "0.5", "1.0"),
     at = c(0.0, 0.5, 1.0))

axis(side = 4, lwd.ticks = 2, cex.axis = 1, las = 1,
     at=c(0.0, 0.5, 1.0), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 1.25, las = 1,
     labels = c("-Rpf", "+Rpf"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.02)

# Adding SEM #
arrows(x0 = c(2,1), y0 = seedling.table$mean, y1 = seedling.table$LCI, angle = 90,
       length = 0.25, lwd = 2)
arrows(x0 = c(2,1), y0 = seedling.table$mean, y1 = seedling.table$UCI, angle = 90,
       length=0.25, lwd = 2)

# p-value
mtext(text = expression(italic("P")~" = 0.320") , side =3, line = -1.2, adj = 0.925, col="black", cex=1
# Sample number label
#mtext(text = expression(italic("n")~" = 4"), side = 3, line = -2.2, adj = 0.925, col="black", cex=1.25

# Close Plot Device
dev.off()
```

```
## pdf
##   2
```

```
graphics.off()

# Show Plot
img <- readPNG("../figures/Suppl.Fig5.Arabidopsis.png")
grid.raster(img)


# Statistical test: t-test of Rpf effects on Arabdiopsis plant biomass
Arabid.rpf <- seedling[ which(seedling$Treatment == "Rpf+"), ]
Arabid.con <- seedling[ which(seedling$Treatment == "Rpf-"), ]
Arabid.ttest <- t.test(Arabid.rpf$RelativeBiomass, Arabid.con$RelativeBiomass)
Arabid.ttest
```

```
##
##  Welch Two Sample t-test
##
## data:  Arabid.rpf$RelativeBiomass and Arabid.con$RelativeBiomass
## t = -1.1134, df = 4.6148, p-value = 0.3201
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.3878870  0.1575483
## sample estimates:
## mean of x mean of y
## 0.5348800 0.6500494
```

```r
anova <- aov(seedling$RelativeBiomass ~ seedling$Treatment, data = seedling)
summary(anova)
```

```
##                    Df  Sum Sq Mean Sq F value Pr(>F)
## seedling$Treatment  1 0.02274 0.02274   1.214  0.321
## Residuals           5 0.09363 0.01873
```

```r
TukeyHSD(anova)  # Non-significant: p = 0.321
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = seedling$RelativeBiomass ~ seedling$Treatment, data = seedling)
##
## $`seedling$Treatment`
##                 diff        lwr       upr     p adj
## Rpf+-Rpf- -0.1151693 -0.3838394 0.1535007 0.3206918
```