Henry County Mitigation Project 2013: Microbial Community Charactorization

Ariane L. Peralta, Mario E. Muscarella, Jeffrey W. Matthews

Last updated on 14 March, 2017

Project Description:

Initial Setup

```
rm(list=ls())
setwd("~/GitHub/IL_Wetlands/analyses")
se <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}
ci <- function(x, ...){1.96 * sd(x,na.rm = TRUE)}

# Code Dependencies
source("../bin/DiversityFunctions.R")
source("../bin/MothurTools.R")
require("vegan")
require("reshape")
require("ggplot2")
require("nlme")
require("ade4")
require("grid"); require("png")
require("ape"); require("picante")
library("agricolae")

myColors <- c("#448844", "#33CC33", "#CCFF00", "#FFF000", "#FF9933", "#A9A9A9")
names(myColors) <- c("BalledBurlapped", "Bareroot", "Seedling", "Acorn", "Seedbank", "Reference")
```

Import Data Files

Experimental Design File

```
# Import Environmental Data
design <- read.csv("../data/WL.design.csv", row.names = 1)
design$Treatment <- factor(design$Treatment,
                           levels = c("BalledBurlapped", "Bareroot",
                           "Seedling", "Acorn", "Seedbank", "Reference"))
treatments <- design$Treatment
#levels(treatments) == c("BalledBurlapped", "Bareroot", "Seedling", "Acorn",
#                        "Seedbank", "Reference")

# Note: The sample names in the microbial part were incorrect (skipped 72)
#       They are correct in this design file and will be corrected for each below
```

Microbial Data

```
# Import OTU data
# Import Raw Data
WLdata.in <- read.otu("../data/WL.final.shared")

# Remove Mock Community
WLdata.in2 <- WLdata.in[which(rownames(WLdata.in) != "Mock"), ]

# Correct Sample IDs
all.equal(rownames(WLdata.in2), rownames(design))
```

```
## [1] "19 string mismatches"
```

```
rownames(WLdata.in2) <- rownames(design)

# Remove OTUs with less than two occurences across all sites
WLdata <- WLdata.in2[, which(colSums(WLdata.in2) >= 2)]

# Make Presence Absence Matrix
WLdataPA <- (WLdata > 0) * 1

# Make Relative Abundence Matrices
WLdataREL <- WLdata
for(i in 1:dim(WLdata)[1]){
  WLdataREL[i,] <- WLdata[i,]/sum(WLdata[i,])
}

# Log Transform Relative Abundances
WLdataREL.log <- decostand(WLdataREL, method="log")
```

```
## Warning: non-integer data: divided by smallest positive value
```

```
# Import Taxonomy File
#WL.tax <- read.tax(taxonomy = "../data/WL.final.0.03.taxonomy",
#                   format = "rdp", tax.levels = 6, col.tax = 3)
```

Plant Data

```
WL.plant <- read.csv("../data/IL_Wetlands_Plants/IL_Wetlands_HC_Plants.csv",
                     row.names = 1)

# The plant data is already relativized
# But it might be useful to have it as PA

WL.plant.PA <- (WL.plant > 0) * 1
```

Phylogenetic Tree and UniFrac Distance Matrix

```
#WL.phylo <- read.tree("../data/WL.bac.renamed.tree")

WL.unifrac.raw <- read.delim("../data/WL.bac.tree1.weighted.phylip.dist",
```

```
                              header = F, skip = 1, row.names = 1)
WL.unifrac <- WL.unifrac.raw[which(row.names(WL.unifrac.raw) %in%
                                   row.names(WLdata.in)),
                             which(row.names(WL.unifrac.raw) %in%
                                   row.names(WLdata.in))]
rownames(WL.unifrac) <- rownames(WLdata.in2)
WL.unifrac.dist <- as.dist(WL.unifrac, upper = T, diag = T)
```

Notes: Phylogenetic Analysis

```
# The following was done outside of R
python ../bin/name_change.py WL.final.0.03.rep.fasta WL.final.0.03.rep.rename.fasta

FastTree -gtr -nt -gamma -fastest WL.final.0.03.rep.rename.fasta > WL.bac.tree

Output:
ML-NNI round 11: LogLk = -1017514.896 NNIs 4825 max delta 3.21 Time 626.69 (final)
Optimize all lengths: LogLk = -1017490.876 Time 645.88
Gamma(20) LogLk = -1017848.611 alpha = 2.130 rescaling lengths by 1.471
Total time: 733.31 seconds Unique: 56413/56413
Bad splits: 16/56410 Worst delta-LogLk 3.347

# Weighted UniFrac was done using Mothur
This caused an error because of the names. Mothur actually crashed

FastTree -gtr -nt -gamma -fastest WL.final.0.03.rep.fasta > WL.bac.tree

ML-NNI round 12: LogLk = -1017096.874 NNIs 4848 max delta 3.23
Time 633.03 (final)x delta 3.226)
Optimize all lengths: LogLk = -1017090.230 Time 650.74
Gamma(20) LogLk = -1017412.647 alpha = 2.058 rescaling lengths by 1.484
Total time: 735.70 seconds Unique: 56413/56413
Bad splits: 13/56410 Worst delta-LogLk 1.738

Mothur (v 1.38)
unifrac.weighted(tree=WL.bac.tree, count=WL.final.rep.count_table, distance=square)

Output File Names:
WL.bac.treewsummary
WL.bac.tree1.weighted.phylip.dist
```

Soil Data

```
WL.soil <- read.csv("../data/WL_plant_soil.csv")
row.names(WL.soil) <- WL.soil$Sample_Code

WL.soil$Treatment <- factor(WL.soil$Treatment,
                            levels = c("BalledBurlapped", "Bareroot",
                            "Seedling", "Acorn", "Seedbank", "Reference"))
```

Simple Hypothesis Testing

```
# Check that Bac and Plant data have same structure as design
all.equal(row.names(WLdataREL), row.names(design))
```

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

```
all.equal(row.names(WL.unifrac), row.names(design))
```

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

```
all.equal(row.names(WL.plant), row.names(design))
```

```
## [1] "90 string mismatches"
```

```
  # Same info seem to be inside so I'm going to rename the plant data
row.names(WL.plant) <- row.names(design)

# PERMANOVA
WL.bac.adonis <- adonis(WLdataREL ~ treatments, method = "bray", perm=999)
WL.bac.adonis
```

```
##
## Call:
## adonis(formula = WLdataREL ~ treatments, permutations = 999,      method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##            Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## treatments  5    0.4235 0.084706  1.8661 0.09997  0.003 **
## Residuals  84    3.8130 0.045392         0.90003
## Total      89    4.2365                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Odd sites in bacterial composition data (explore more)
odd.sites <- c("HC_A3_ss1_S11", "HC_A3_ss2_S12", "HC_A3_ss3_S13",
               "HC_C3_ss5_S45", "HC_D2_ss3_S53", "HC_E1_ss1_S61")

WLdataREL.2 <- WLdataREL[setdiff(rownames(WLdataREL), odd.sites), ]
treatments.2 <- design[setdiff(rownames(design), odd.sites), ]$Treatment

WL.bac.adonis <- adonis(WLdataREL.2 ~ treatments.2, method = "bray", perm=999)
WL.bac.adonis
```

```
##
## Call:
## adonis(formula = WLdataREL.2 ~ treatments.2, permutations = 999,      method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
```

```
##              Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## treatments.2  5   0.34744 0.069488  2.3362 0.13025  0.001 ***
## Residuals    78   2.31999 0.029743         0.86975
## Total        83   2.66743                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
WL.unifrac.adonis <- adonis(WL.unifrac.dist ~ treatments, perm = 999)
WL.unifrac.adonis
```

```
##
## Call:
## adonis(formula = WL.unifrac.dist ~ treatments, permutations = 999)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##            Df SumsOfSqs   MeanSqs F.Model      R2 Pr(>F)
## treatments  5   0.05081 0.0101624  1.6563 0.08974  0.068 .
## Residuals  84   0.51538 0.0061355         0.91026
## Total      89   0.56619                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
WL.plant.adonis <- adonis(WL.plant ~ treatments, method = "bray", perm=999)
WL.plant.adonis
```

```
##
## Call:
## adonis(formula = WL.plant ~ treatments, permutations = 999, method = "bray")
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##            Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## treatments  5   10.3524 2.07047  18.608 0.52553  0.001 ***
## Residuals  84    9.3466 0.11127         0.47447
## Total      89   19.6990                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Simper Analysis for Species Responses
#WL.bac.simper <- simper(WLdataREL, group = treatments, permutations = 999)
#bac.sum <- summary(WL.bac.simper, ordered = T)

WL.plant.simper <- simper(WL.plant, group = treatments, permutations = 999)
plant.sum <- summary(WL.plant.simper, ordered = T)
#plant.sum
```

Beta Diversity Analysis

Bray Curtis Ordination (PCoA)

```
png(filename="../figures/WL.bac.PCoA.png",
    width = 1200, height = 800, res = 96*2)
# Create Distance Matrix
sampleREL.dist <- vegdist(WLdataREL, method="bray")

# Principal Coordinates Analysis
WL_pcoa <- cmdscale(sampleREL.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

# Remove Odd Sites
odd.sites <- row.names(WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ])
odd.sbys <- WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ]
mean(rowSums((WLdataREL > 0) * 1))
```

```
## [1] 3605.033
```

```
rowSums((odd.sbys > 0) * 1)
```

```
## HC_A3_ss1_S11 HC_A3_ss2_S12 HC_A3_ss3_S13 HC_C3_ss5_S45 HC_D2_ss3_S53
##          3907          3415          3883          3340          3696
## HC_E1_ss1_S61
##          3673
```

```
WLdataREL.2 <- WLdataREL[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
design2 <- design[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
treatments <- design2$Treatment

# Create Distance Matrix
sampleREL.dist2 <- vegdist(WLdataREL.2, method="bray")

# Principal Coordinates Analysis
WL_pcoa <- cmdscale(sampleREL.dist2, k=2, eig=TRUE, add=FALSE)
# Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
# eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1 <- round(WL_pcoa$eig[1] / sum(WL_pcoa$eig), 3) * 100
explainvar2 <- round(WL_pcoa$eig[2] / sum(WL_pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2)

# Plot
points <- cbind(as.data.frame(WL_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids, variable ~ treatments, sd)

cent.dataframe <- t(data.frame(rbind(centroids[1,-1], centroids[2,-1],
                             centroids.sd[1,-1],centroids.sd[2,-1])))
colnames(cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
```

```r
cent.treats <- rownames(cent.dataframe)

# Define Plot Parameters
par(mar = c(5, 5.5, 1, 1) + 0.1)
layout(matrix(1:2, 1, 2), widths = c(4,2))

plot(cent.dataframe[,1], cent.dataframe[,2], type = 'n', las = 1,
     xlim = c(-0.1, 0.1), ylim = c(-0.1, 0.1),
     xaxt = "n", xlab = "", yaxt = "n", ylab="")

abline(h = 0, lty = 3, col = "gray")
abline(v = 0, lty = 3, col = "gray")

arrows(x0 = cent.dataframe[,1],
       y1 = cent.dataframe[,2] - cent.dataframe[,4],
       y0 = cent.dataframe[,2] + cent.dataframe[,4],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
arrows(y0 = cent.dataframe[,2],
       x1 = cent.dataframe[,1] - cent.dataframe[,3],
       x0 = cent.dataframe[,1] + cent.dataframe[,3],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
points(cent.dataframe[,1], cent.dataframe[,2],
       cex = 2.5, bg = myColors, col = "black", pch = 22, lwd = 2)

# text(cent.dataframe[,1], cent.dataframe[,2], rownames(cent.dataframe))

axis(side = 1, labels = T, las = 1, lwd.ticks = 2)
axis(side = 2, labels = T, las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)

mtext(paste("PCoA 1 (", explainvar1, "%)", sep = ""), side = 1, line = 3, cex = 1.5)
mtext(paste("PCoA 2 (", explainvar2, "%)", sep = ""), side = 2, line = 3.5, cex = 1.5)

box(lwd = 2)

par(mar = c(5, 0, 1, 1) + 0.1)
plot.new()

legend(0, 1, legend = levels(treatments), pt.bg = myColors, col = "black",
       pch = 22, cex = 0.9, bty = 'n', inset = c(0.1, 0.05),
       y.intersp = 1.25)


# Close Plot Device
```

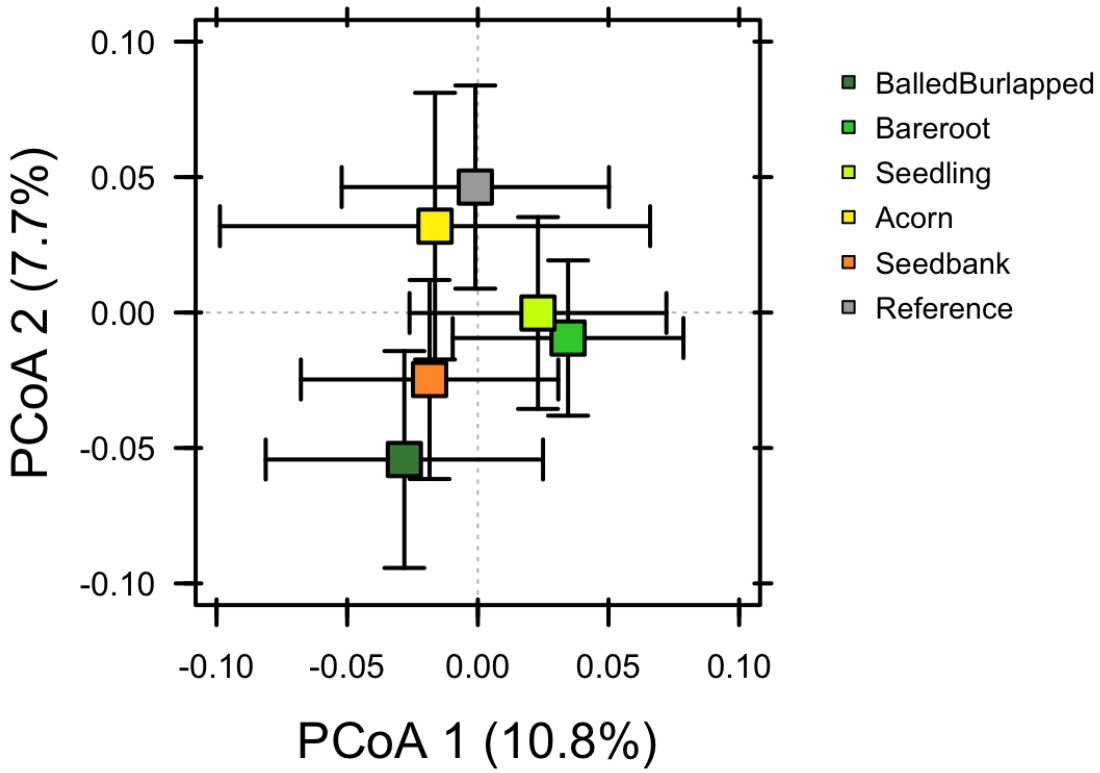
```
dev.off()
```

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

```
graphics.off()
```

Show Plot

```
img <- readPNG("../figures/WL.bac.PcoA.png")
grid.raster(img)
```



Plot: Microbial Phylogenetic Ordination

```
png(filename="../figures/WL.unifrac.PCoA.png",
    width = 1200, height = 800, res = 96*2)

# Principal Coordinates Analysis
WL_pcoa <- cmdscale(WL.unifrac.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

# Remove Odd Sites
odd.sites <- row.names(WL.unifrac[c(abs(WL_pcoa$points[, 1]) > 0.1), ])
```

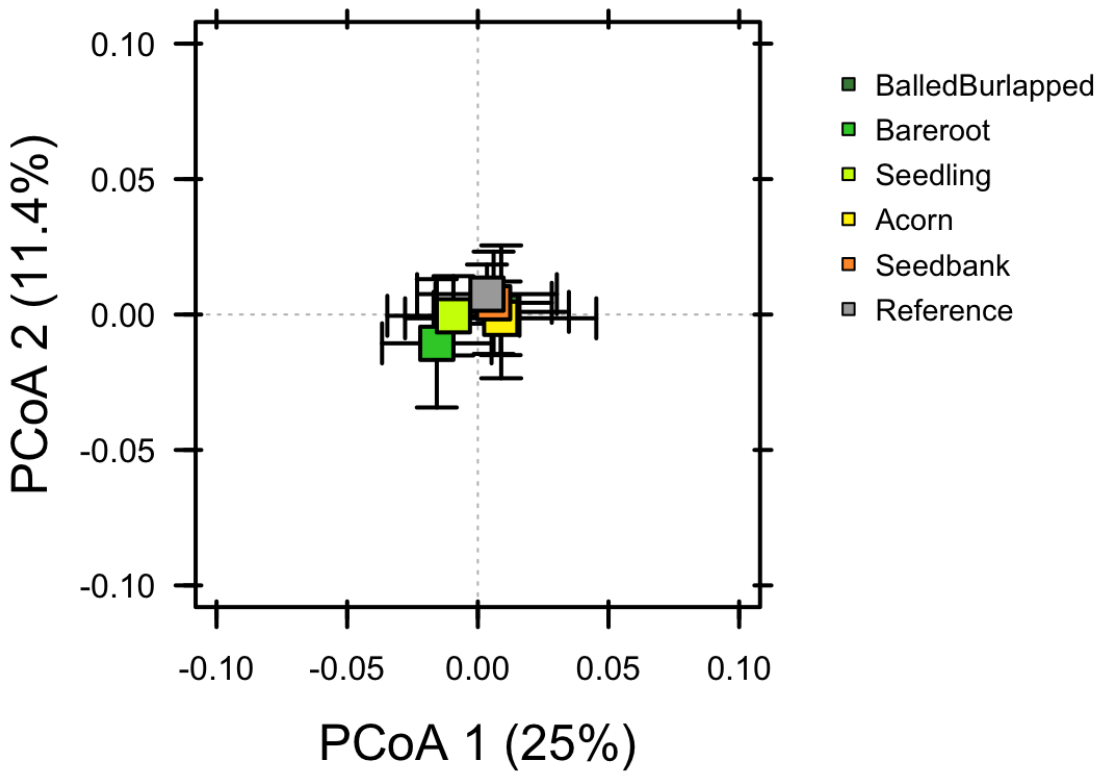
```r
# Create Distance Matrix W/O Odd Sites
WL.unifrac.2 <- WL.unifrac[-c(which(row.names(WL.unifrac) %in% odd.sites)),
                           -c(which(row.names(WL.unifrac) %in% odd.sites))]
WL.unifrac.dist2 <- as.dist(WL.unifrac.2, upper = T, diag = T)

treatments <- design[-c(which(row.names(design) %in% odd.sites)), ]$Treatment


# Principal Coordinates Analysis
WL_pcoa <- cmdscale(WL.unifrac.dist2, k=2, eig=TRUE, add=FALSE)
# Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
# eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1 <- round(WL_pcoa$eig[1] / sum(WL_pcoa$eig), 3) * 100
explainvar2 <- round(WL_pcoa$eig[2] / sum(WL_pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2)

# Plot
points <- cbind(as.data.frame(WL_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids, variable ~ treatments, sd)

cent.dataframe <- t(data.frame(rbind(centroids[1,-1], centroids[2,-1],
                               centroids.sd[1,-1],centroids.sd[2,-1])))
colnames(cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
cent.treats <- rownames(cent.dataframe)

# Define Plot Parameters
par(mar = c(5, 5.5, 1, 1) + 0.1)
layout(matrix(1:2, 1, 2), widths = c(4,2))

plot(cent.dataframe[,1], cent.dataframe[,2], type = 'n', las = 1,
     xlim = c(-0.1, 0.1), ylim = c(-0.1, 0.1),
     xaxt = "n", xlab = "", yaxt = "n", ylab="")

abline(h = 0, lty = 3, col = "gray")
abline(v = 0, lty = 3, col = "gray")

arrows(x0 = cent.dataframe[,1],
       y1 = cent.dataframe[,2] - cent.dataframe[,4],
       y0 = cent.dataframe[,2] + cent.dataframe[,4],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
arrows(y0 = cent.dataframe[,2],
       x1 = cent.dataframe[,1] - cent.dataframe[,3],
       x0 = cent.dataframe[,1] + cent.dataframe[,3],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
points(cent.dataframe[,1], cent.dataframe[,2],
       cex = 2.5, bg = myColors, col = "black", pch = 22, lwd = 2)
```

```
#text(cent.dataframe[,1], cent.dataframe[,2], rownames(cent.dataframe))

axis(side = 1, labels = T, las = 1, lwd.ticks = 2)
axis(side = 2, labels = T, las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)

mtext(paste("PCoA 1 (", explainvar1, "%)", sep = ""), side = 1, line = 3, cex = 1.5)
mtext(paste("PCoA 2 (", explainvar2, "%)", sep = ""), side = 2, line = 3.5, cex = 1.5)

box(lwd = 2)

par(mar = c(5, 0, 1, 1) + 0.1)
plot.new()

legend(0, 1, legend = cent.treats, pt.bg = myColors, col = "black",
       pch = 22, cex = 0.9, bty = 'n', inset = c(0.1, 0.05),
       y.intersp = 1.25)


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

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

```
graphics.off()
```

Show Plot



Plot: Plant Principal Coordinates Ordination

```
png(filename="../figures/WL.plant.PCoA.png",
    width = 1200, height = 800, res = 96*2)
# Create Distance Matrix
treatments <- design$Treatment
samplePlant.dist <- vegdist(WL.plant, method="bray")

# Principal Coordinates Analysis
WLplant_pcoa <- cmdscale(samplePlant.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

# # Remove Odd Sites
# odd.sites <- row.names(WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ])
# odd.sbys <- WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ]
# mean(rowSums((WLdataREL > 0) * 1))
# rowSums((odd.sbys > 0) * 1)
# WLdataREL.2 <- WLdataREL[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
# design2 <- design[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
# treatments <- design2$Treatment

# Create Distance Matrix
```

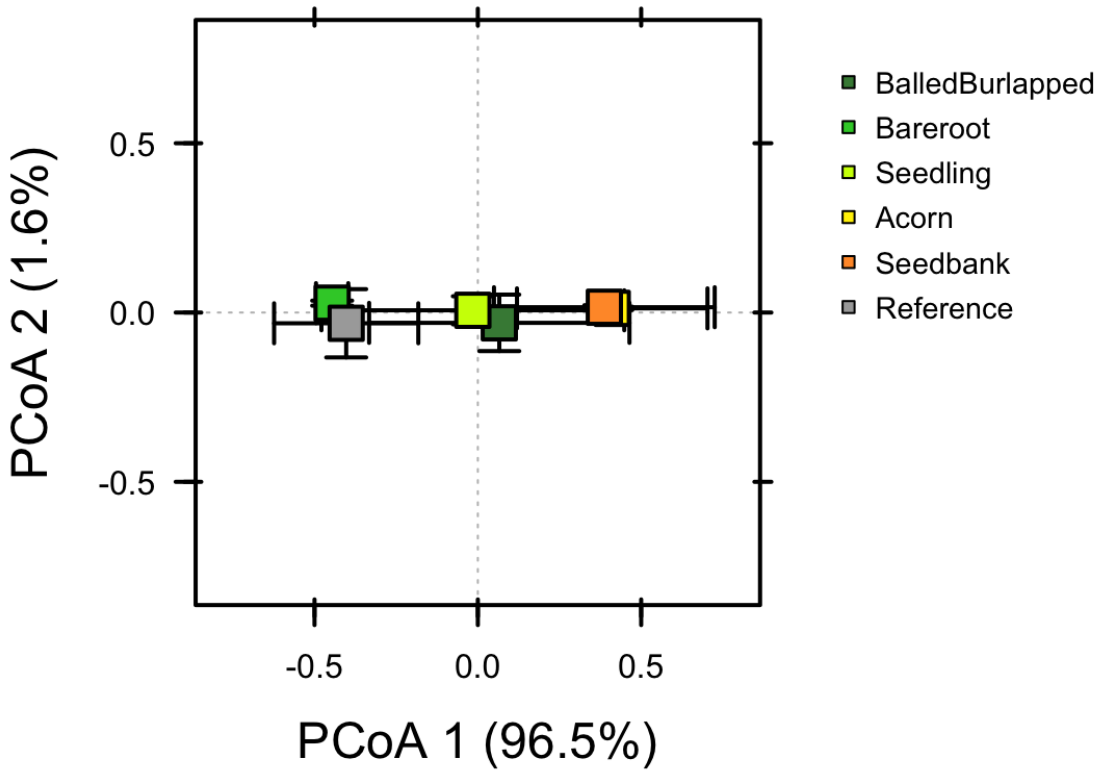
```r
#sampleREL.dist2 <- vegdist(WLdataREL.2, method="bray")

# Principal Coordinates Analysis
#WL_pcoa <- cmdscale(sampleREL.dist2, k=2, eig=TRUE, add=FALSE)
# Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
# eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1 <- round(WLplant_pcoa$eig[1] / sum(WLplant_pcoa$eig), 3) * 100
explainvar2 <- round(WLplant_pcoa$eig[2] / sum(WLplant_pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2)

# Plot
points <- cbind(as.data.frame(WLplant_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids, variable ~ treatments, sd)

cent.dataframe <- t(data.frame(rbind(centroids[1,-1], centroids[2,-1],
                              centroids.sd[1,-1],centroids.sd[2,-1])))
colnames(cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
cent.treats <- rownames(cent.dataframe)

# Define Plot Parameters
par(mar = c(5, 5.5, 1, 1) + 0.1)
layout(matrix(1:2, 1, 2), widths = c(4,2))

plot(cent.dataframe[,1], cent.dataframe[,2], type = 'n', las = 1,
     xlim = c(-0.8, 0.8), ylim = c(-0.8, 0.8),
     xaxt = "n", xlab = "", yaxt = "n", ylab="")

abline(h = 0, lty = 3, col = "gray")
abline(v = 0, lty = 3, col = "gray")

arrows(x0 = cent.dataframe[,1],
       y1 = cent.dataframe[,2] - cent.dataframe[,4],
       y0 = cent.dataframe[,2] + cent.dataframe[,4],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
arrows(y0 = cent.dataframe[,2],
       x1 = cent.dataframe[,1] - cent.dataframe[,3],
       x0 = cent.dataframe[,1] + cent.dataframe[,3],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
points(cent.dataframe[,1], cent.dataframe[,2],
       cex = 2.5, bg = myColors, col = "black", pch = 22, lwd = 2)

axis(side = 1, labels = T, las = 1, lwd.ticks = 2)
axis(side = 2, labels = T, las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
```

```r
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)

mtext(paste("PCoA 1 (", explainvar1, "%)", sep = ""), side = 1, line = 3, cex = 1.5)
mtext(paste("PCoA 2 (", explainvar2, "%)", sep = ""), side = 2, line = 3.5, cex = 1.5)

box(lwd = 2)

par(mar = c(5, 0, 1, 1) + 0.1)
plot.new()

legend(0, 1, legend = cent.treats, pt.bg = myColors, col = "black",
       pch = 22, cex = 0.9, bty = 'n', inset = c(0.1, 0.05),
       y.intersp = 1.25)


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

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

```r
graphics.off()
```

Show Plot



Joint Plot: Microbial and Plant Bray Curtis Ordination (PCoA)

```
png(filename="../figures/WL.PCoA.png",
    width = 1800, height = 800, res = 96*2)

layout(matrix(1:3, 1, 3, byrow = T), widths = c(4, 4, 1.5))

# Define Plot Parameters
par(mar = c(5, 6, 1, 2), oma = c(1, 1, 1, 1))

# Create Distance Matrix
sampleREL.dist <- vegdist(WLdataREL, method="bray")

# Bacterial Principal Coordinates Analysis
WL_pcoa <- cmdscale(sampleREL.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

# Remove Odd Sites
odd.sites <- row.names(WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ])
odd.sbys <- WLdataREL[c(abs(WL_pcoa$points[, 1]) > 0.3), ]
mean(rowSums((WLdataREL > 0) * 1))
```

```
## [1] 3605.033
```

```r
rowSums((odd.sbys > 0) * 1)
```

```
## HC_A3_ss1_S11 HC_A3_ss2_S12 HC_A3_ss3_S13 HC_C3_ss5_S45 HC_D2_ss3_S53
##          3907          3415          3883          3340          3696
## HC_E1_ss1_S61
##          3673
```

```r
WLdataREL.2 <- WLdataREL[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
design2 <- design[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
treatments <- design2$Treatment

# Create Distance Matrix
sampleREL.dist2 <- vegdist(WLdataREL.2, method="bray")

# Principal Coordinates Analysis
WL_pcoa <- cmdscale(sampleREL.dist2, k=2, eig=TRUE, add=FALSE)
# Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
# eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1 <- round(WL_pcoa$eig[1] / sum(WL_pcoa$eig), 3) * 100
explainvar2 <- round(WL_pcoa$eig[2] / sum(WL_pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2)


points <- cbind(as.data.frame(WL_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids, variable ~ treatments, sd)

cent.dataframe <- t(data.frame(rbind(centroids[1,-1], centroids[2,-1],
                             centroids.sd[1,-1],centroids.sd[2,-1])))
colnames(cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
cent.treats <- rownames(cent.dataframe)

# Bacterial Plot
plot(cent.dataframe[,1], cent.dataframe[,2], type = 'n', las = 1,
     xlim = c(-0.1, 0.1), ylim = c(-0.1, 0.1),
     xaxt = "n", xlab = "", yaxt = "n", ylab="")

abline(h = 0, lty = 3, col = "gray")
abline(v = 0, lty = 3, col = "gray")

arrows(x0 = cent.dataframe[,1],
       y1 = cent.dataframe[,2] - cent.dataframe[,4],
       y0 = cent.dataframe[,2] + cent.dataframe[,4],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
arrows(y0 = cent.dataframe[,2],
       x1 = cent.dataframe[,1] - cent.dataframe[,3],
       x0 = cent.dataframe[,1] + cent.dataframe[,3],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
points(cent.dataframe[,1], cent.dataframe[,2],
```

```
       cex = 2.5, bg = myColors, col = "black", pch = 22, lwd = 2)

range.x <- par("usr")[2] - par("usr")[1]
range.y <- par("usr")[4] - par("usr")[3]
text(par("usr")[1] + 0.13 * range.x, par("usr")[4] - 0.05 * range.y, "Bacteria")
# text(cent.dataframe[,1], cent.dataframe[,2], rownames(cent.dataframe))

axis(side = 1, labels = T, las = 1, lwd.ticks = 2)
axis(side = 2, labels = T, las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)

mtext(paste("PCoA 1 (", explainvar1, "%)", sep = ""), side = 1,
      line = 3.5, cex = 1.5)
mtext(paste("PCoA 2 (", explainvar2, "%)", sep = ""), side = 2,
      line = 3.5, cex = 1.5)

box(lwd = 2)

# Plant PcoA
# Create Distance Matrix
treatments <- design$Treatment
samplePlant.dist <- vegdist(WL.plant, method="bray")

# Principal Coordinates Analysis
WLplant_pcoa <- cmdscale(samplePlant.dist, k=3, eig=TRUE, add=FALSE)
  # Classical (Metric) Multidimensional Scaling; returns PCoA coordinates
  # eig=TRUE returns eigenvalues; k = # of dimensions to calculate

explainvar1 <- round(WLplant_pcoa$eig[1] / sum(WLplant_pcoa$eig), 3) * 100
explainvar2 <- round(WLplant_pcoa$eig[2] / sum(WLplant_pcoa$eig), 3) * 100
sum.eig <- sum(explainvar1, explainvar2)

# Plot
points <- cbind(as.data.frame(WLplant_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids, variable ~ treatments, sd)

cent.dataframe <- t(data.frame(rbind(centroids[1,-1], centroids[2,-1],
                             centroids.sd[1,-1],centroids.sd[2,-1])))
colnames(cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
cent.treats <- rownames(cent.dataframe)

# Initiate Plot
plot(cent.dataframe[,1], cent.dataframe[,2], type = 'n', las = 1,
```

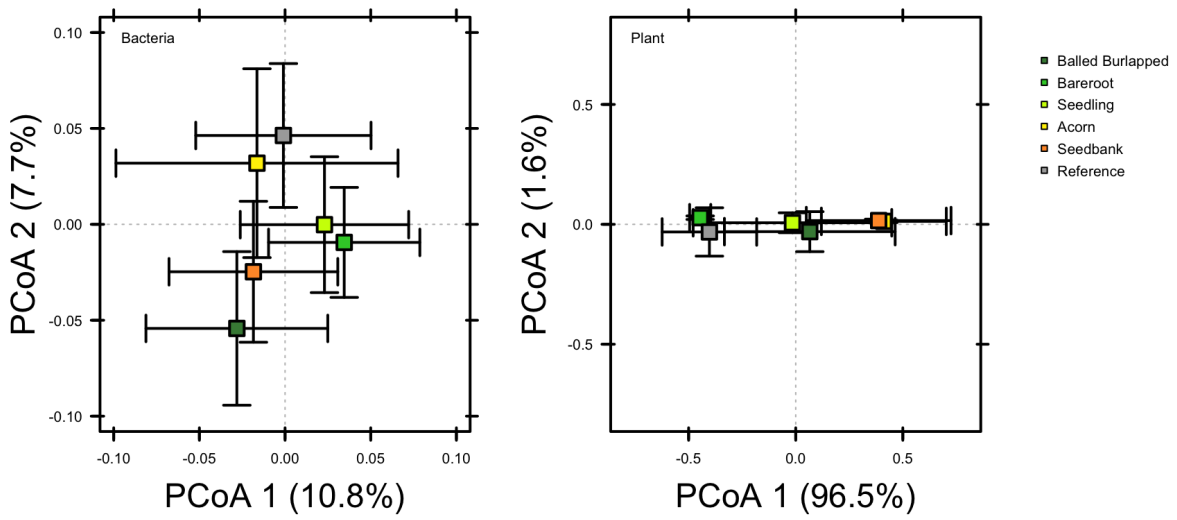
```
     xlim = c(-0.8, 0.8), ylim = c(-0.8, 0.8),
     xaxt = "n", xlab = "", yaxt = "n", ylab="")

abline(h = 0, lty = 3, col = "gray")
abline(v = 0, lty = 3, col = "gray")

arrows(x0 = cent.dataframe[,1],
       y1 = cent.dataframe[,2] - cent.dataframe[,4],
       y0 = cent.dataframe[,2] + cent.dataframe[,4],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
arrows(y0 = cent.dataframe[,2],
       x1 = cent.dataframe[,1] - cent.dataframe[,3],
       x0 = cent.dataframe[,1] + cent.dataframe[,3],
       angle = 90,
       length=0.1, lwd = 2, code = 3)
points(cent.dataframe[,1], cent.dataframe[,2],
       cex = 2.5, bg = myColors, col = "black", pch = 22, lwd = 2)

range.x <- par("usr")[2] - par("usr")[1]
range.y <- par("usr")[4] - par("usr")[3]
text(par("usr")[1] + 0.1 * range.x, par("usr")[4] - 0.05 * range.y, "Plant")

axis(side = 1, labels = T, las = 1, lwd.ticks = 2)
axis(side = 2, labels = T, las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1)
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1)

mtext(paste("PCoA 1 (", explainvar1, "%)", sep = ""), side = 1,
      line = 3.5, cex = 1.5)
mtext(paste("PCoA 2 (", explainvar2, "%)", sep = ""), side = 2,
      line = 3.5, cex = 1.5)

box(lwd = 2)

par(mar = c(5, 0, 1, 0) + 0.5)
plot.new()

legend(0, 1, legend = c("Balled Burlapped", cent.treats[-1]), pt.bg = myColors, col = "black",
       pch = 22, cex = 1, bty = 'n', inset = c(0.1, 0.05),
       y.intersp = 1.25)


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

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

```
graphics.off()
```

Show Plot

```
img <- readPNG("../figures/WL.PcoA.png")
grid.raster(img)
```



Mantel Tests

Similarity between microbe and plant communities

```
# Name check
all.equal(rownames(WL.plant), rownames(WLdataREL))
```

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

```
# Remove Odd Sites from Bac Community
bac.comm <- WLdataREL[-which(rownames(WLdataREL) %in% odd.sites),]
plant.comm <- WL.plant[-which(rownames(WL.plant) %in% odd.sites), ]

# Remove Bare Treatment
bare.sites <- rownames(design[design$Treatment == "Bareroot", ])
bac.comm <- bac.comm[-which(rownames(bac.comm) %in% bare.sites),]
plant.comm <- plant.comm[-which(rownames(plant.comm) %in% bare.sites), ]

all.equal(rownames(bac.comm), rownames(plant.comm))
```

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

```
#rowSums(plant.comm)

dist.bac <- vegdist(bac.comm, method = "bray")
dist.plant <- vegdist(plant.comm, method = "bray")
```

```
mantel.rtest(dist.bac, dist.plant)
```

```
## Warning in is.euclid(m2): Zero distance(s)
```

```
## Monte-Carlo test
## Observation: 0.1130565
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 99 replicates
## Simulated p-value: 0.02
```

```
WL.plant_points <- cmdscale(dist.plant, k=3, eig=TRUE, add=FALSE)$points

dbRDA <- dbrda(bac.comm ~ WL.plant_points, distance = "bray") #does not work
anova(dbRDA) #does not work
```

```
## Permutation test for dbrda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = bac.comm ~ WL.plant_points, distance = "bray")
##          Df SumOfSqs      F Pr(>F)
## Model     3  0.14382 1.5438  0.002 **
## Residual 65  2.01847
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbRDA)
```

```
## $r.squared
## [1] 0.06651357
##
## $adj.r.squared
## [1] 0.02342958
```

Similarity between microbes and soil

```
# Remove Odd Sites from Bac Community
bac.comm <- WLdataREL[-which(rownames(WLdataREL) %in% odd.sites),]
plant.comm <- WL.plant[-which(rownames(WL.plant) %in% odd.sites), ]

# Remove Odd Sites from Soil
WL.soil2 <- WL.soil[-which(rownames(WLdataREL) %in% odd.sites),]

WL.soil.phys <- WL.soil2[, which(colnames(WL.soil2) %in%
                                   c("Moisture", "Temp", "pH"))]
WL.soil.nuts <- WL.soil2[, which(colnames(WL.soil2) %in%
                                   c("TOC", "TN", "OM", "NH4.N", "NO3.N"))]
WL.soil.all <- WL.soil2[, which(colnames(WL.soil2) %in%
                                c("Moisture", "Temp", "pH", "TOC", "TN",
                                  "OM", "NH4.N", "NO3.N"))]

all.equal(grep("[0-9][0-9]$", rownames(WL.soil2)),
          grep("S*$", rownames(bac.comm)))
```

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

```
dist.bac <- vegdist(bac.comm, method = "bray")
dist.plant <- vegdist(plant.comm, method = "bray")
dist.nuts <- vegdist(WL.soil.nuts, method = "euclidean")
dist.phys <- vegdist(WL.soil.phys, method = "euclidean")
dist.soil <- vegdist(WL.soil.all, method = "euclidean")


mantel.rtest(dist.bac, dist.plant)
```

```
## Warning in is.euclid(m2): Zero distance(s)
```

```
## Monte-Carlo test
## Observation: 0.09849538
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 99 replicates
## Simulated p-value: 0.01
```

```
mantel.rtest(dist.bac, dist.nuts)
```

```
## Monte-Carlo test
## Observation: -0.04139427
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.nuts)
## Based on 99 replicates
## Simulated p-value: 0.64
```

```
mantel.rtest(dist.bac, dist.phys)
```

```
## Monte-Carlo test
## Observation: 0.1637996
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.phys)
## Based on 99 replicates
## Simulated p-value: 0.01
```

```
mantel.rtest(dist.bac, dist.soil)
```

```
## Monte-Carlo test
## Observation: -0.01610846
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.soil)
## Based on 99 replicates
## Simulated p-value: 0.56
```

```
mantel.rtest(dist.plant, dist.nuts)
```

```
## Warning in is.euclid(m1): Zero distance(s)
```

```
## Monte-Carlo test
## Observation: 0.265419
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 99 replicates
## Simulated p-value: 0.01
```

```
mantel.rtest(dist.plant, dist.phys)
```

```
## Warning in is.euclid(m1): Zero distance(s)
```

```
## Monte-Carlo test
## Observation: 0.04439773
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
```

```
## Based on 99 replicates
## Simulated p-value: 0.01
```

```
mantel.rtest(dist.plant, dist.soil)
```

```
## Warning in is.euclid(m1): Zero distance(s)
```

```
## Monte-Carlo test
## Observation: 0.2481641
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 99 replicates
## Simulated p-value: 0.01
```

Soil Factors

```
# Organize Data
WL.trts <- WL.soil[, "Treatment"]
WL.soil.phys <- WL.soil[, which(colnames(WL.soil) %in% c("Moisture",
                                                         "Temp", "pH"))]
WL.soil.nuts <- WL.soil[, which(colnames(WL.soil) %in% c("TOC", "TN",
                                                         "OM", "NH4.N", "NO3.N"))]

# Linear Models
moisture.lm <- lm(Moisture ~ Treatment, data=WL.soil)
anova(moisture.lm)
```

```
## Analysis of Variance Table
##
## Response: Moisture
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment  5 255.28  51.055  4.8689 0.0005844 ***
## Residuals 84 880.83  10.486
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(moisture.lm)
```

```
##
## Call:
## lm(formula = Moisture ~ Treatment, data = WL.soil)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -7.0653 -2.0458 -0.0647  1.8072  9.7347
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)        32.50467    0.83610  38.876   <2e-16 ***
## TreatmentBareroot  -2.68733    1.18243  -2.273   0.0256 *
## TreatmentSeedling  -2.82333    1.18243  -2.388   0.0192 *
## TreatmentAcorn     -0.78333    1.18243  -0.662   0.5095
## TreatmentSeedbank  -0.07667    1.18243  -0.065   0.9485
## TreatmentReference  2.07067    1.18243   1.751   0.0836 .
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.238 on 84 degrees of freedom
## Multiple R-squared:  0.2247, Adjusted R-squared:  0.1785
## F-statistic: 4.869 on 5 and 84 DF,  p-value: 0.0005844
```

```
HSD <- HSD.test(moisture.lm,"Treatment", console=TRUE)
```

```
##
## Study: moisture.lm ~ "Treatment"
##
## HSD Test for Moisture
##
## Mean Square Error:  10.48604
##
## Treatment,  means
##
##                 Moisture      std  r   Min   Max
## Acorn           31.72133 2.359243 15 26.57 35.57
## BalledBurlapped 32.50467 2.623563 15 27.52 36.71
## Bareroot        29.81733 2.715018 15 26.70 34.69
## Reference       34.57533 5.581024 15 27.51 44.31
## Seedbank        32.42800 2.095302 15 29.06 37.33
## Seedling        29.68133 2.749122 15 24.98 34.28
##
## alpha: 0.05 ; Df Error: 84
## Critical Value of Studentized Range: 4.124617
##
## Honestly Significant Difference: 3.448607
##
## Means with the same letter are not significantly different.
##
## Groups, Treatments and means
## a     Reference           34.58
## ab    BalledBurlapped     32.5
## ab    Seedbank            32.43
## ab    Acorn               31.72
## b     Bareroot            29.82
## b     Seedling            29.68
```

```
pH.lm <- lm(pH ~ Treatment, data=WL.soil)
anova(pH.lm)
```

```
## Analysis of Variance Table
##
## Response: pH
##           Df   Sum Sq  Mean Sq F value   Pr(>F)
## Treatment  5 0.055259 0.011052  3.7149 0.004362 **
## Residuals 84 0.249896 0.002975
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(pH.lm)
```

```
##
## Call:
```

```
## lm(formula = pH ~ Treatment, data = WL.soil)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.135000 -0.024792  0.008333  0.025625  0.125833
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)         7.52417    0.01408 534.274  < 2e-16 ***
## TreatmentBareroot  -0.00750    0.01992  -0.377  0.70744
## TreatmentSeedling   0.05917    0.01992   2.971  0.00387 **
## TreatmentAcorn      0.05083    0.01992   2.552  0.01251 *
## TreatmentSeedbank   0.01750    0.01992   0.879  0.38208
## TreatmentReference  0.03583    0.01992   1.799  0.07558 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.05454 on 84 degrees of freedom
## Multiple R-squared:  0.1811, Adjusted R-squared:  0.1323
## F-statistic: 3.715 on 5 and 84 DF,  p-value: 0.004362
```

```
HSD <- HSD.test(pH.lm,"Treatment", console=TRUE)
```

```
##
## Study: pH.lm ~ "Treatment"
##
## HSD Test for pH
##
## Mean Square Error:  0.00297495
##
## Treatment,  means
##
##                       pH        std  r   Min  Max
## Acorn           7.575000 0.05088502 15 7.450 7.65
## BalledBurlapped 7.524167 0.04735567 15 7.450 7.65
## Bareroot        7.516667 0.06315137 15 7.400 7.60
## Reference       7.560000 0.05809475 15 7.425 7.65
## Seedbank        7.541667 0.05643159 15 7.450 7.65
## Seedling        7.583333 0.04970149 15 7.450 7.65
##
## alpha: 0.05 ; Df Error: 84
## Critical Value of Studentized Range: 4.124617
##
## Honestly Significant Difference: 0.05808685
##
## Means with the same letter are not significantly different.
##
## Groups, Treatments and means
## a     Seedling            7.583
## ab    Acorn               7.575
## abc   Reference           7.56
## abc   Seedbank            7.542
## bc    BalledBurlapped     7.524
## c     Bareroot            7.517
```

```r
temp.lm <- lm(Temp ~ Treatment, data=WL.soil)
anova(temp.lm)
```

```
## Analysis of Variance Table
##
## Response: Temp
##           Df  Sum Sq Mean Sq F value    Pr(>F)
## Treatment  5  42.674  8.5348  7.1405 1.308e-05 ***
## Residuals 84 100.403  1.1953
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
summary(temp.lm)
```

```
##
## Call:
## lm(formula = Temp ~ Treatment, data = WL.soil)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.4133 -0.7467 -0.2133  0.7867  2.3200
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         22.7133     0.2823  80.463  < 2e-16 ***
## TreatmentBareroot   -1.9667     0.3992  -4.926 4.15e-06 ***
## TreatmentSeedling   -1.2000     0.3992  -3.006  0.00349 **
## TreatmentAcorn      -1.6333     0.3992  -4.091 9.80e-05 ***
## TreatmentSeedbank   -1.4000     0.3992  -3.507  0.00073 ***
## TreatmentReference  -2.0867     0.3992  -5.227 1.24e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.093 on 84 degrees of freedom
## Multiple R-squared:  0.2983, Adjusted R-squared:  0.2565
## F-statistic:  7.14 on 5 and 84 DF,  p-value: 1.308e-05
```

```r
HSD <- HSD.test(temp.lm,"Treatment", console=TRUE)
```

```
##
## Study: temp.lm ~ "Treatment"
##
## HSD Test for Temp
##
## Mean Square Error:  1.19527
##
## Treatment,  means
##
##                      Temp       std  r  Min  Max
## Acorn           21.08000 0.9630309 15 19.9 23.4
## BalledBurlapped 22.71333 0.7818172 15 21.5 24.3
## Bareroot        20.74667 1.2374321 15 19.7 22.6
## Reference       20.62667 0.6943308 15 19.7 21.9
## Seedbank        21.31333 1.3314153 15 18.9 23.2
## Seedling        21.51333 1.3590263 15 19.7 23.6
```

```
##
## alpha: 0.05 ; Df Error: 84
## Critical Value of Studentized Range: 4.124617
##
## Honestly Significant Difference: 1.164316
##
## Means with the same letter are not significantly different.
##
## Groups, Treatments and means
## a     BalledBurlapped     22.71
## b     Seedling            21.51
## b     Seedbank            21.31
## b     Acorn               21.08
## b     Bareroot            20.75
## b     Reference           20.63
```

```
TOC.lm <- lm(TOC ~ Treatment, data=WL.soil)
anova(TOC.lm)
```

```
## Analysis of Variance Table
##
## Response: TOC
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment  5 2.5744 0.51488  5.8751 0.0001054 ***
## Residuals 84 7.3615 0.08764
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(TOC.lm)
```

```
##
## Call:
## lm(formula = TOC ~ Treatment, data = WL.soil)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.9659 -0.1943  0.0587  0.2202  0.5948
##
## Coefficients:
##                     Estimate Std. Error t value Pr(>|t|)
## (Intercept)         5.116667   0.076436  66.941   <2e-16 ***
## TreatmentBareroot  -0.258933   0.108097  -2.395   0.0188 *
## TreatmentSeedling  -0.236300   0.108097  -2.186   0.0316 *
## TreatmentAcorn      0.007333   0.108097   0.068   0.9461
## TreatmentSeedbank   0.178767   0.108097   1.654   0.1019
## TreatmentReference  0.145567   0.108097   1.347   0.1817
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.296 on 84 degrees of freedom
## Multiple R-squared:  0.2591, Adjusted R-squared:  0.215
## F-statistic: 5.875 on 5 and 84 DF,  p-value: 0.0001054
```

```
HSD <- HSD.test(TOC.lm,"Treatment", console=TRUE)
```

```
##
```

```
## Study: TOC.lm ~ "Treatment"
##
## HSD Test for TOC
##
## Mean Square Error:  0.08763679
##
## Treatment,  means
##
##                      TOC       std  r    Min   Max
## Acorn           5.124000 0.2037942 15 4.8010 5.384
## BalledBurlapped 5.116667 0.2281678 15 4.6815 5.426
## Bareroot        4.857733 0.3032293 15 4.4155 5.394
## Reference       5.262233 0.2957362 15 4.8190 5.857
## Seedbank        5.295433 0.3555548 15 4.3295 5.834
## Seedling        4.880367 0.3555291 15 4.1775 5.312
##
## alpha: 0.05 ; Df Error: 84
## Critical Value of Studentized Range: 4.124617
##
## Honestly Significant Difference: 0.315269
##
## Means with the same letter are not significantly different.
##
## Groups, Treatments and means
## a     Seedbank            5.295
## a     Reference           5.262
## ab    Acorn               5.124
## ab    BalledBurlapped     5.117
## b     Seedling            4.88
## b     Bareroot            4.858
```

```
NH4.lm <- lm(NH4.N ~ Treatment, data=WL.soil)
anova(NH4.lm)
```

```
## Analysis of Variance Table
##
## Response: NH4.N
##           Df Sum Sq Mean Sq F value Pr(>F)
## Treatment  5  8.607  1.7213  1.4738  0.207
## Residuals 84 98.108  1.1680
```

```
summary(NH4.lm)
```

```
##
## Call:
## lm(formula = NH4.N ~ Treatment, data = WL.soil)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.01667 -0.75542 -0.01167  0.67708  2.50833
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)          9.9867     0.2790  35.789   <2e-16 ***
## TreatmentBareroot    0.1283     0.3946   0.325    0.746
```

```
## TreatmentSeedling    0.4750     0.3946   1.204    0.232
## TreatmentAcorn       0.1050     0.3946   0.266    0.791
## TreatmentSeedbank   -0.3583     0.3946  -0.908    0.366
## TreatmentReference   0.5800     0.3946   1.470    0.145
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.081 on 84 degrees of freedom
## Multiple R-squared:  0.08065,    Adjusted R-squared:  0.02593
## F-statistic: 1.474 on 5 and 84 DF,  p-value: 0.207
```

```
NO3.lm <- lm(NO3.N ~ Treatment, data=WL.soil)
anova(NO3.lm)
```

```
## Analysis of Variance Table
##
## Response: NO3.N
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment  5 3316.9  663.37  7.1093 1.376e-05 ***
## Residuals 84 7838.1   93.31
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(NO3.lm)
```

```
##
## Call:
## lm(formula = NO3.N ~ Treatment, data = WL.soil)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -16.400  -5.817  -1.142   5.270  57.375
##
## Coefficients:
##                    Estimate Std. Error t value Pr(>|t|)
## (Intercept)          21.447      2.494   8.599 3.81e-13 ***
## TreatmentBareroot   -10.495      3.527  -2.975  0.00382 **
## TreatmentSeedling     1.428      3.527   0.405  0.68655
## TreatmentAcorn        4.470      3.527   1.267  0.20856
## TreatmentSeedbank     3.968      3.527   1.125  0.26377
## TreatmentReference   -9.504      3.527  -2.694  0.00852 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 9.66 on 84 degrees of freedom
## Multiple R-squared:  0.2973, Adjusted R-squared:  0.2555
## F-statistic: 7.109 on 5 and 84 DF,  p-value: 1.376e-05
```

```
HSD <- HSD.test(NO3.lm,"Treatment", console=TRUE)
```

```
##
## Study: NO3.lm ~ "Treatment"
##
## HSD Test for NO3.N
##
## Mean Square Error:  93.31097
```

```
##
## Treatment,  means
##
##                     NO3.N       std  r    Min    Max
## Acorn           25.91667  7.176535 15 16.750 39.350
## BalledBurlapped 21.44667  6.560344 15 13.175 37.675
## Bareroot        10.95167  4.180081 15  6.250 19.850
## Reference       11.94300  8.227502 15  1.510 32.375
## Seedbank        25.41500  7.739381 15 13.825 41.875
## Seedling        22.87500 17.895869 15  6.475 80.250
##
## alpha: 0.05 ; Df Error: 84
## Critical Value of Studentized Range: 4.124617
##
## Honestly Significant Difference: 10.28737
##
## Means with the same letter are not significantly different.
##
## Groups, Treatments and means
## a     Acorn               25.92
## a     Seedbank            25.42
## a     Seedling            22.88
## ab    BalledBurlapped     21.45
## bc    Reference           11.94
## c     Bareroot            10.95
```

```
WL.phys.mean <- apply(WL.soil.phys, 2,
                      FUN = function(avg) aggregate(avg ~ WL.trts, WL.soil.phys, mean))
WL.phys.sem <- apply(WL.soil.phys, 2,
                     FUN = function(sem) aggregate(sem ~ WL.trts, WL.soil.phys, se))
WL.phys.95 <- apply(WL.soil.phys, 2,
                    FUN = function(ci_95) aggregate(ci_95 ~ WL.trts, WL.soil.phys, ci))

WL.nuts.mean <- apply(WL.soil.nuts, 2,
                      FUN = function(avg) aggregate(avg ~ WL.trts, WL.soil.nuts, mean))
WL.nuts.sem <- apply(WL.soil.nuts, 2,
                     FUN = function(sem) aggregate(sem ~ WL.trts, WL.soil.nuts, se))
WL.nuts.95 <- apply(WL.soil.nuts, 2,
                    FUN = function(ci_95) aggregate(ci_95 ~ WL.trts, WL.soil.nuts, ci))


phys.means.table <- data.frame(trt = WL.phys.mean$Moisture$WL.trts,
                               moisture = WL.phys.mean$Moisture$avg,
                               temp = WL.phys.mean$Temp$avg,
                               pH = WL.phys.mean$pH$avg)
phys.sem.table <- data.frame(trt = WL.phys.sem$Moisture$WL.trts,
                               moisture = WL.phys.sem$Moisture$sem,
                               temp = WL.phys.sem$Temp$sem,
                               pH = WL.phys.sem$pH$sem)
phys.ci.table <- data.frame(trt = WL.phys.95$Moisture$WL.trts,
                               moisture = WL.phys.95$Moisture$ci_95,
                               temp = WL.phys.95$Temp$ci_95,
                               pH = WL.phys.95$pH$ci_95)
```

```r
nuts.means.table <- data.frame(trt = WL.nuts.mean$TOC$WL.trts,
                               toc = WL.nuts.mean$TOC$avg,
                               tn = WL.nuts.mean$TN$avg,
                               om = WL.nuts.mean$OM$avg,
                               nh4 = WL.nuts.mean$NH4.N$avg,
                               no3 = WL.nuts.mean$NO3.N$avg)
nuts.sem.table <- data.frame(trt = WL.nuts.sem$TOC$WL.trts,
                               toc = WL.nuts.sem$TOC$sem,
                               tn = WL.nuts.sem$TN$sem,
                               om = WL.nuts.sem$OM$sem,
                               nh4 = WL.nuts.sem$NH4.N$sem,
                               no3 = WL.nuts.sem$NO3.N$sem)
nuts.ci.table <- data.frame(trt = WL.nuts.95$TOC$WL.trts,
                               toc = WL.nuts.95$TOC$ci_95,
                               tn = WL.nuts.95$TN$ci_95,
                               om = WL.nuts.95$OM$ci_95,
                               nh4 = WL.nuts.95$NH4.N$ci_95,
                               no3 = WL.nuts.95$NO3.N$ci_95)
```

Plot: WL Soil Nuts

```r
png(filename="../figures/soil.chem.png",
    width = 800, height = 1200, res = 96*2)

layout(matrix(1:3, 3, byrow = T))
par(mar = (c(0.5, 5, 0, 1) + 0.1), oma = c(7, 1, 1.5, 1))

# TOC
toc <- boxplot(WL.soil.nuts$TOC ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(4,6))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # Lables
  mtext("Total Organic Carbon\n(%)", side = 2, cex = 1, line = 3.5)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$TOC ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = toc$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)
  #par("usr")
  #text(x = seq_along(levels(WL.trts)), y = 5.5, labels = grp$trt)
```

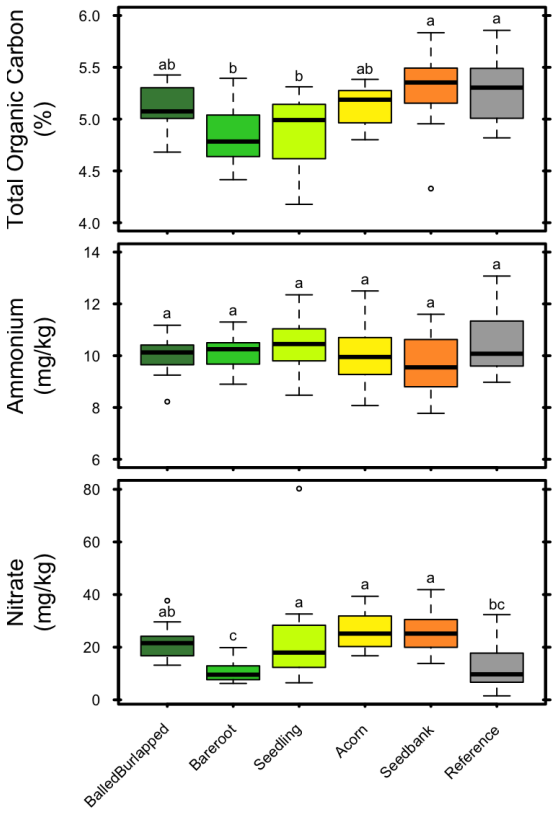
```
# # Total Nitrogen
# boxplot(WL.soil.nuts$TN ~ WL.trts,
#         col = myColors, xaxt = "n",  yaxt = "n",
#         xlab = "", ylab = "", ylim = c(0.25, 0.4))
#
#   # Axes with Tick Marks
#   axis(side = 1, labels = F, tck = -0.01, lwd = 2)
#   axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
#   axis(side = 4, labels = F, tck = -0.02, lwd = 2)
#   axis(side = 2, labels = F, tck = 0.01, lwd = 2)
#   axis(side = 4, labels = F, tck = 0.01, lwd = 2)
#   box(lwd = 2)
#
#   # Lables
#   mtext("Total Nitrogen\n(?)", side = 2, cex = 1, line = 3.5)

# # Organic Matter
# boxplot(WL.soil.nuts$OM ~ WL.trts,
#         col = myColors, xaxt = "n",  yaxt = "n",
#         xlab = "", ylab = "", ylim = c(7, 11))
#
#   # Axes with Tick Marks
#   axis(side = 1, labels = F, tck = -0.01, lwd = 2)
#   axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
#   axis(side = 4, labels = F, tck = -0.02, lwd = 2)
#   axis(side = 2, labels = F, tck = 0.01, lwd = 2)
#   axis(side = 4, labels = F, tck = 0.01, lwd = 2)
#   box(lwd = 2)
#
#   # Lables
#   mtext("Organic Matter\n(?)", side = 2, cex = 1, line = 3.5)

# Ammonium
nh4 <- boxplot(WL.soil.nuts$NH4.N ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(6, 14))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$NH4.N ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = nh4$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Labels
```

```
  mtext("Ammonium\n(mg/kg)", side = 2, cex = 1, line = 3.5)

# Nitrate
no3 <- boxplot(WL.soil.nuts$NO3.N ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "")

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$NO3.N ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = no3$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Lables
  mtext("Nitrate\n(mg/kg)", side = 2, cex = 1, line = 3.5)

# Plot X labs at default X position
par(xpd = NA)
text(x = seq_along(levels(WL.trts)),
     y = par("usr")[3] - 0.1 * (par("usr")[4] - par("usr")[3]),
     srt = 45, adj = 1, labels = levels(WL.trts), xpd=NA)

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

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

```
graphics.off()
```

Show Plot



Plot: WL Soil Phys

```
png(filename="../figures/soil.phy.png",
    width = 800, height = 1200, res = 96*2)


layout(matrix(1:3, 3, byrow = T))
par(mar = (c(0.5, 5, 0, 1) + 0.1), oma = c(7, 1, 1.5, 1))

# Moisture
moisture <- boxplot(WL.soil.phys$Moisture ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(20, 50))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)
```

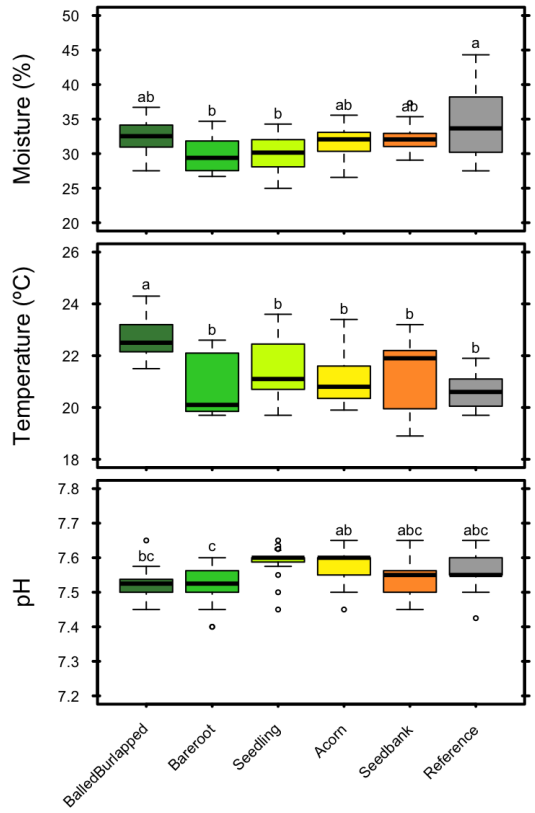
```
  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$Moisture ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = moisture$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Labels
  mtext("Moisture (%)", side = 2, cex = 1, line = 3.5)

# Temperature
temp <- boxplot(WL.soil.phys$Temp ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(18, 26))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)


  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$Temp ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = temp$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Lables
  mtext("Temperature (ºC)", side = 2, cex = 1, line = 3.5)

# pH
  pH <- boxplot(WL.soil.phys$pH ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(7.2, 7.8))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.01, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$pH ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = pH$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)
```

```
  # Lables
  mtext("pH", side = 2, cex = 1, line = 3.5)


# Plot x labs at default x position
par(xpd = NA)
text(x = seq_along(levels(WL.trts)),
     y = par("usr")[3] - 0.1 * (par("usr")[4] - par("usr")[3]),
     srt = 45, adj = 1, labels = levels(WL.trts), xpd=NA)

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

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

```
graphics.off()
```

Show Plot



Joint Plot: Wetland Soil

```
png(filename="../figures/WL.soil.png",
    width = 1600, height = 1400, res = 96*2)

layout(matrix(1:6, 3, byrow = F))
par(mar = (c(1, 4, 0, 2) + 0.1), oma = c(8, 1, 2, 0))

# TOC
toc <- boxplot(WL.soil.nuts$TOC ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(4,6))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # Lables
  mtext("Total Organic Carbon (%)", side = 2, cex = 1, line = 3)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$TOC ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = toc$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

# Ammonium
nh4 <- boxplot(WL.soil.nuts$NH4.N ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(6, 14))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$NH4.N ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = nh4$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Labels
  mtext("Ammonium (mg/kg)", side = 2, cex = 1, line = 3)

# Nitrate
```

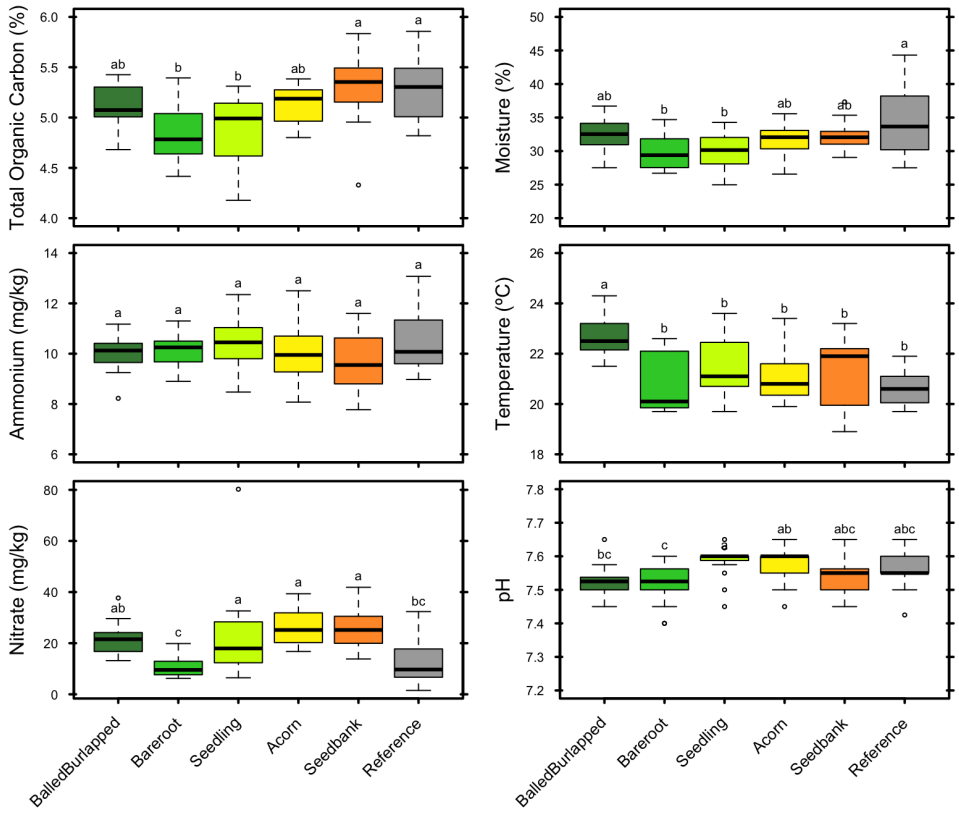
```r
no3 <- boxplot(WL.soil.nuts$NO3.N ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "")

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.nuts$NO3.N ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = no3$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Lables
  mtext("Nitrate (mg/kg)", side = 2, cex = 1, line = 3)

# Plot X labs at default X position
par(xpd = NA)
text(x = seq_along(levels(WL.trts)),
     y = par("usr")[3] - 0.1 * (par("usr")[4] - par("usr")[3]),
     srt = 45, adj = 1, labels = levels(WL.trts), xpd=NA, cex = 1.25)

# Moisture
moisture <- boxplot(WL.soil.phys$Moisture ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(20, 50))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$Moisture ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = moisture$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Labels
  mtext("Moisture (%)", side = 2, cex = 1, line = 3)

# Temperature
temp <- boxplot(WL.soil.phys$Temp ~ WL.trts,
```

```
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(18, 26))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)


  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$Temp ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = temp$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Lables
  mtext("Temperature (ºC)", side = 2, cex = 1, line = 3)

# pH
  pH <- boxplot(WL.soil.phys$pH ~ WL.trts,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "", ylim = c(7.2, 7.8))

  # Axes with Tick Marks
  axis(side = 1, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 2)
  axis(side = 2, labels = F, tck = 0.01, lwd = 2)
  axis(side = 4, labels = F, tck = 0.01, lwd = 2)
  box(lwd = 2)

  # PostHoc Test
  tuk <- HSD.test(aov(WL.soil.phys$pH ~ WL.trts), "WL.trts")
  grp <- tuk$groups[c(match(levels(WL.trts), gsub(" ", "", tuk$groups$trt))), ]
  text(x = seq_along(levels(WL.trts)),
       y = pH$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
       labels = grp$M)

  # Lables
  mtext("pH", side = 2, cex = 1, line = 3)

# Plot X labs at default X position
par(xpd = NA)
text(x = seq_along(levels(WL.trts)),
     y = par("usr")[3] - 0.1 * (par("usr")[4] - par("usr")[3]),
     srt = 45, adj = 1, labels = levels(WL.trts), xpd=NA, cex = 1.25)

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

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

```
graphics.off()
```

Show Plot



Distance Based RDA Models

```
phys.dist <- dist(WL.soil.phys, method = "euclidian")
phys.pcoa <- cmdscale(phys.dist, k = 3, eig = T)

explainvar1 <- round(phys.pcoa$eig[1] / sum(phys.pcoa$eig), 3) * 100
explainvar2 <- round(phys.pcoa$eig[2] / sum(phys.pcoa$eig), 3) * 100

chem.dist <- dist(WL.soil.nuts, method = "euclidian")
chem.pcoa <- cmdscale(chem.dist, k = 3, eig = T)

explainvar1 <- round(chem.pcoa$eig[1] / sum(chem.pcoa$eig), 3) * 100
explainvar2 <- round(chem.pcoa$eig[2] / sum(chem.pcoa$eig), 3) * 100

dbRDA.c <- dbrda(WLdataREL ~ chem.pcoa$points[,1], distance = "bray")
anova(dbRDA.c)
```

```
## Permutation test for dbrda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = WLdataREL ~ chem.pcoa$points[, 1], distance = "bray")
##          Df SumOfSqs      F Pr(>F)
## Model     1   0.0619 1.3051   0.15
## Residual 88   4.1746
```

```
dbRDA.p <- dbrda(WLdataREL ~ phys.pcoa$points[,1], distance = "bray")
anova(dbRDA.p)
```

```
## Permutation test for dbrda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = WLdataREL ~ phys.pcoa$points[, 1], distance = "bray")
##          Df SumOfSqs      F Pr(>F)
## Model     1   0.1226 2.6234  0.025 *
## Residual 88   4.1138
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbRDA.p)
```

```
## $r.squared
## [1] 0.02894872
##
## $adj.r.squared
## [1] 0.01791405
```

```
dbRDA.cp <- dbrda(WLdataREL ~ phys.pcoa$points[,1] * chem.pcoa$points[,1],
              distance = "bray", add = T)
anova(dbRDA.cp, by = "terms", model = "direct")
```

```
## Permutation test for dbrda under direct model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = WLdataREL ~ phys.pcoa$points[, 1] * chem.pcoa$points[, 1], distance = "bray",
##                                            Df SumOfSqs      F Pr(>F)
## phys.pcoa$points[, 1]                       1   0.1226 2.6464  0.020 *
## chem.pcoa$points[, 1]                       1   0.0612 1.3208  0.176
## phys.pcoa$points[, 1]:chem.pcoa$points[, 1]  1   0.0671 1.4488  0.155
## Residual                                   86   3.9855
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbRDA.cp)
```

```
## $r.squared
## [1] 0.05924439
##
## $adj.r.squared
## [1] 0.02642733
```

```
dbRDA.P.cp <- dbrda(WL.plant ~ phys.pcoa$points[,1] * chem.pcoa$points[,1],
               distance = "bray", add = T)
anova(dbRDA.P.cp, by = "terms", model = "direct")
```

```
## Permutation test for dbrda under direct model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = WL.plant ~ phys.pcoa$points[, 1] * chem.pcoa$points[, 1], distance = "bray", a
##                                             Df SumOfSqs       F Pr(>F)
## phys.pcoa$points[, 1]                        1   0.1587  0.7842  0.395
## chem.pcoa$points[, 1]                        1   6.7010 33.1162  0.001 ***
## phys.pcoa$points[, 1]:chem.pcoa$points[, 1]  1   0.1304  0.6444  0.465
## Residual                                    86  17.4018
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbRDA.P.cp)
```

```
## $r.squared
## [1] 0.2865723
##
## $adj.r.squared
## [1] 0.2616853
```

PostHoc Tests

```
phys.mod <- lm(phys.pcoa$points[,1] ~ WL.trts)
chem.mod <- lm(chem.pcoa$points[,1] ~ WL.trts)
anova(phys.mod)
```

```
## Analysis of Variance Table
##
## Response: phys.pcoa$points[, 1]
##           Df Sum Sq Mean Sq F value    Pr(>F)
## WL.trts    5 253.58  50.717  4.7956 0.0006631 ***
## Residuals 84 888.37  10.576
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(chem.mod)
```

```
## Analysis of Variance Table
##
## Response: chem.pcoa$points[, 1]
##           Df Sum Sq Mean Sq F value    Pr(>F)
## WL.trts    5 3319.5  663.91  7.1101 1.374e-05 ***
## Residuals 84 7843.5   93.38
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov(phys.mod))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = phys.mod)
##
## $WL.trts
##                                 diff        lwr       upr     p adj
## Bareroot-BalledBurlapped  -2.82924539 -6.2925804 0.6340896 0.1742617
## Seedling-BalledBurlapped  -2.90675244 -6.3700874 0.5565826 0.1520279
## Acorn-BalledBurlapped     -0.90566113 -4.3689961 2.5576739 0.9729566
## Seedbank-BalledBurlapped  -0.18313857 -3.6464736 3.2801964 0.9999874
## Reference-BalledBurlapped  1.90556436 -1.5577706 5.3688994 0.5977984
## Seedling-Bareroot         -0.07750705 -3.5408421 3.3858279 0.9999998
## Acorn-Bareroot             1.92358425 -1.5397507 5.3869192 0.5879116
## Seedbank-Bareroot          2.64610682 -0.8172282 6.1094418 0.2360052
## Reference-Bareroot         4.73480975  1.2714747 8.1981447 0.0019165
## Acorn-Seedling             2.00109131 -1.4622437 5.4644263 0.5453348
## Seedbank-Seedling          2.72361387 -0.7397211 6.1869489 0.2082718
## Reference-Seedling         4.81231680  1.3489818 8.2756518 0.0015298
## Seedbank-Acorn             0.72252257 -2.7408124 4.1858576 0.9901528
## Reference-Acorn            2.81122549 -0.6521095 6.2745605 0.1797575
## Reference-Seedbank         2.08870293 -1.3746321 5.5520379 0.4975542
```

```
TukeyHSD(aov(chem.mod))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = chem.mod)
##
## $WL.trts
##                                 diff        lwr        upr     p adj
## Bareroot-BalledBurlapped  -10.5023475 -20.793269 -0.2114255 0.0427034
## Seedling-BalledBurlapped    1.4097067  -8.881215 11.7006287 0.9986433
## Acorn-BalledBurlapped       4.4662856  -5.824636 14.7572076 0.8025649
## Seedbank-BalledBurlapped    3.9805626  -6.310359 14.2714846 0.8683278
## Reference-BalledBurlapped  -9.5083407 -19.799263  0.7825813 0.0870144
## Seedling-Bareroot          11.9120541   1.621132 22.2029761 0.0137600
## Acorn-Bareroot             14.9686331   4.677711 25.2595550 0.0007832
## Seedbank-Bareroot          14.4829101   4.191988 24.7738321 0.0012758
## Reference-Bareroot          0.9940068  -9.296915 11.2849287 0.9997522
## Acorn-Seedling              3.0565789  -7.234343 13.3475009 0.9534461
## Seedbank-Seedling           2.5708559  -7.720066 12.8617779 0.9778596
## Reference-Seedling        -10.9180474 -21.208969 -0.6271254 0.0310189
## Seedbank-Acorn             -0.4857230 -10.776645  9.8051990 0.9999928
## Reference-Acorn           -13.9746263 -24.265548 -3.6837043 0.0021002
## Reference-Seedbank        -13.4889033 -23.779825 -3.1979814 0.0033404
```

Invasive Species Biomass

```
WL.rc <- data.frame(WL.soil$Treatment, WL.soil$RCGbiomass, WL.soil$Non.RCGbiomass)

WL.rc <- na.omit(WL.rc)
```

```r
WL.rc$ratio <- WL.rc$WL.soil.RCGbiomass / (WL.rc$WL.soil.RCGbiomass + WL.rc$WL.soil.Non.RCGbiomass)



RC.mod <- lm(RCGbiomass ~ Treatment,
             data = WL.soil[which(WL.soil$Treatment != "Reference"), ])
summary(RC.mod)
```

```
##
## Call:
## lm(formula = RCGbiomass ~ Treatment, data = WL.soil[which(WL.soil$Treatment !=
##     "Reference"), ])
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -67.006 -17.864  -0.514   7.307 122.081
##
## Coefficients:
##                   Estimate Std. Error t value Pr(>|t|)
## (Intercept)          8.269      8.113   1.019    0.312
## TreatmentBareroot   -8.190     11.474  -0.714    0.478
## TreatmentSeedling   13.460     11.474   1.173    0.245
## TreatmentAcorn      48.649     11.677   4.166 8.82e-05 ***
## TreatmentSeedbank   58.737     11.474   5.119 2.64e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 31.42 on 69 degrees of freedom
##   (1 observation deleted due to missingness)
## Multiple R-squared:  0.4339, Adjusted R-squared:  0.401
## F-statistic: 13.22 on 4 and 69 DF,  p-value: 4.775e-08
```

```r
anova(RC.mod)
```

```
## Analysis of Variance Table
##
## Response: RCGbiomass
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Treatment  4  52209 13052.2   13.22 4.775e-08 ***
## Residuals 69  68125   987.3
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
TukeyHSD(aov(RC.mod))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = RC.mod)
##
## $Treatment
##                               diff       lwr      upr     p adj
## Bareroot-BalledBurlapped -8.19000 -40.33004 23.95004 0.9526582
## Seedling-BalledBurlapped 13.46000 -18.68004 45.60004 0.7665404
## Acorn-BalledBurlapped    48.64852  15.93959 81.35746 0.0008175
```

```
## Seedbank-BalledBurlapped 58.73667  26.59663 90.87671 0.0000256
## Seedling-Bareroot        21.65000 -10.49004 53.79004 0.3340189
## Acorn-Bareroot           56.83852  24.12959 89.54746 0.0000662
## Seedbank-Bareroot        66.92667  34.78663 99.06671 0.0000016
## Acorn-Seedling           35.18852   2.47959 67.89746 0.0287522
## Seedbank-Seedling        45.27667  13.13663 77.41671 0.0017170
## Seedbank-Acorn           10.08814 -22.62079 42.79708 0.9090114
```

```
tuk <- HSD.test(aov(WL.soil.nuts$TOC ~ WL.trts), "WL.trts")

tuk <- HSD.test(lm(WL.soil.RCGbiomass ~ WL.soil.Treatment,
                   data = WL.rc), "WL.soil.Treatment")
```

Plot: Invasive Species

```
png(filename="../figures/reed.biomass.png",
    width = 1200, height = 800, res = 96*2)

par(mar = (c(6.5,5,1,1) + 0.1))

rcg <- boxplot(WL.rc$WL.soil.RCGbiomass ~ WL.rc$WL.soil.Treatment,
        col = myColors, xaxt = "n",  yaxt = "n",
        xlab = "", ylab = "")

# Lables
mtext("Reed Canary Biomass (g)", side = 2, cex = 1.25, line = 3)

# PostHoc Test
tuk <- HSD.test(lm(WL.soil.RCGbiomass ~ WL.soil.Treatment,
                   data = WL.rc), "WL.soil.Treatment")
grp <- tuk$groups[c(match(gsub(" ", "", tuk$groups$trt),
                          levels(WL.rc$WL.soil.Treatment))), ]
text(x = seq_along(levels(WL.rc$WL.soil.Treatment)),
     y = rcg$stats[5, ] + ((par("usr")[4] - par("usr")[3]) * 0.05),
     labels = grp$M)

# Plot x labs at default x position
text(x = seq_along(levels(WL.rc$WL.soil.Treatment)),
     y = par("usr")[3] - 10, srt = 45, adj = 1,
     labels = levels(WL.rc$WL.soil.Treatment),
     xpd = TRUE)

# Axes with Tick Marks
axis(side = 1, labels = F, tck = -0.01, lwd = 2)
axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
#axis(side = 3, labels = F, tck = -0.02, lwd = 2)
axis(side = 4, labels = F, tck = -0.02, lwd = 2)
#axis(side = 1, labels = F, tck = 0.01, lwd = 2)
axis(side = 2, labels = F, tck = 0.01, lwd = 2)
#axis(side = 3, labels = F, tck = 0.01, lwd = 2)
axis(side = 4, labels = F, tck = 0.01, lwd = 2)

box(lwd = 2)
```

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

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

```
graphics.off()
```

Show Plot