Lingering land use legacies after different wetland restoration strategies

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Project Description: Anthropogenic legacy effects often occur as a consequence of land use change and can leave behind long-lasting changes to ecosystem structure and function. These physical, chemical, and biological changes can linger and interact with contemporary management to impact restoration outcomes. In the present study, we evaluated how restoration strategy can overcome the lingering effects of land-use in wetlands.

Initial Setup

```r
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

```r
# 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
```

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

Soil Factors

```r
# 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
```

```r
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
```

```r
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
```

```r
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
```

```r
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
```

```
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
```

```
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
```

```
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)

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

```
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))
#
```

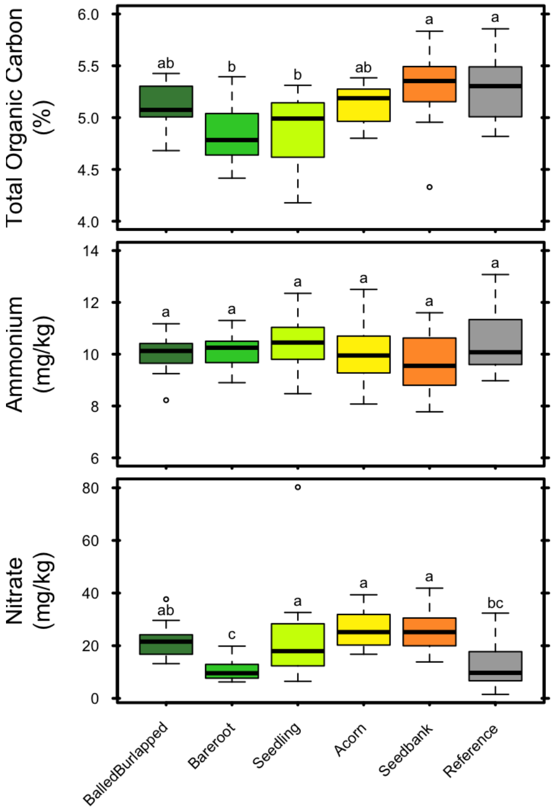
```
#   # Axes with Tick Marks
#   axis(side = 1, labels = F, tck = -0.01, lwd = 2)
#   axis(side = 2, labels = T, tck = -0.02, lwd = 2, las = 1)
#   axis(side = 4, labels = F, tck = -0.02, lwd = 2)
#   axis(side = 2, labels = F, tck = 0.01, lwd = 2)
#   axis(side = 4, labels = F, tck = 0.01, lwd = 2)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

```r
        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
```

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

Show Plot



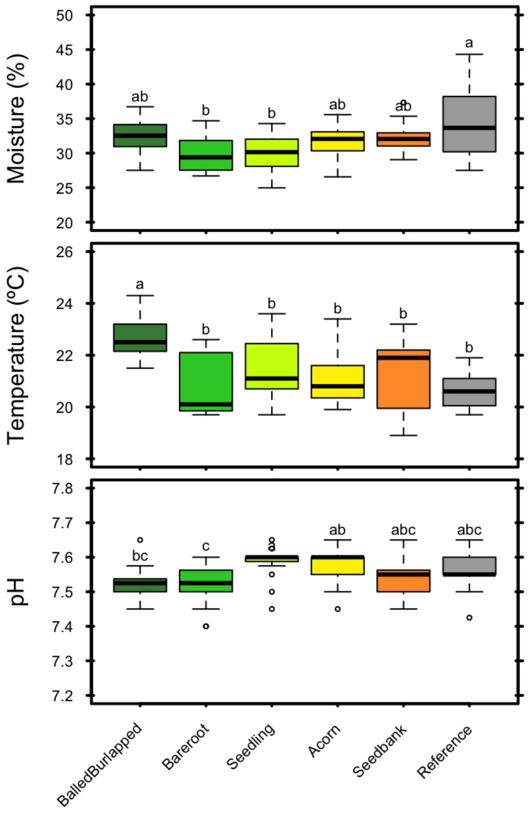
Plot: WL Soil Phys

```r
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

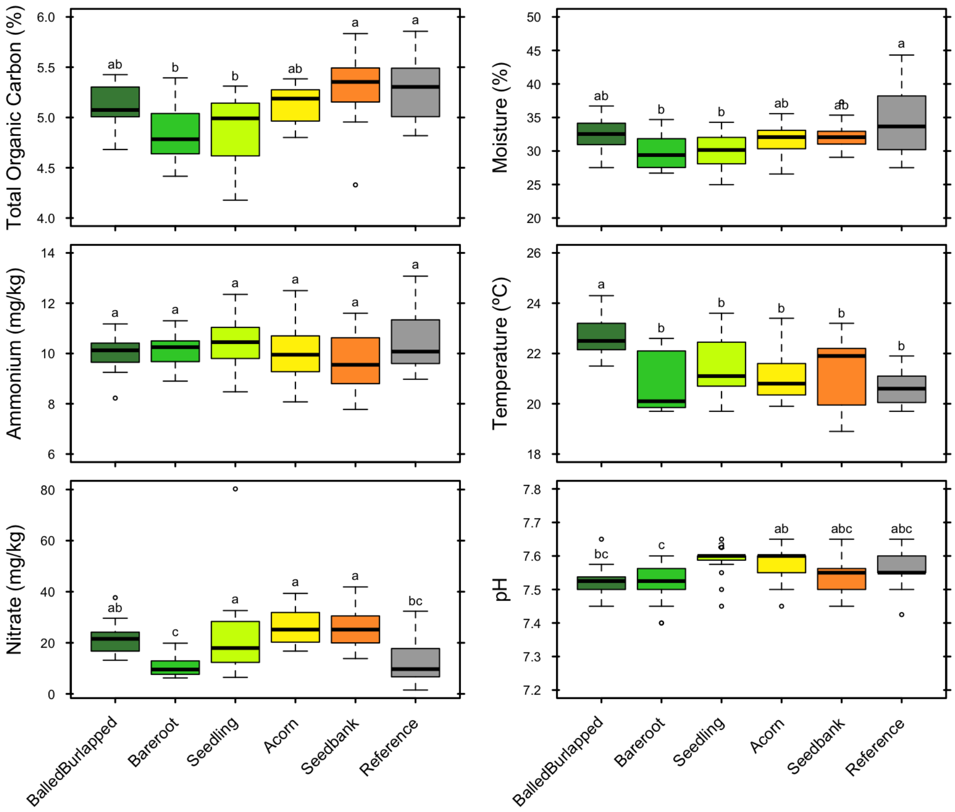
```
png(filename="../figures/WL.soil.png",
    width = 1600, height = 1400, res = 96*2)

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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)

  # 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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)

  # 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
```

```
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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)

  # 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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)

  # 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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)


  # 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 = 1.5)
  axis(side = 2, labels = T, tck = -0.02, lwd = 1.5, las = 1)
  axis(side = 4, labels = F, tck = -0.02, lwd = 1.5)
  axis(side = 2, labels = F, tck = 0.01, lwd = 1.5)
  axis(side = 4, labels = F, tck = 0.01, lwd = 1.5)
  box(lwd = 1.5)

  # 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



Simple Community 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  0.004 **
## 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

odd.sites
```

```
## [1] "HC_A3_ss1_S11" "HC_A3_ss2_S12" "HC_A3_ss3_S13" "HC_C3_ss5_S45"
## [5] "HC_D2_ss3_S53" "HC_E1_ss1_S61"
```

```
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  0.046 *
## 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.unifrac.dist2 <- as.matrix(WL.unifrac.dist)[setdiff(labels(WL.unifrac.dist),
                              odd.sites), setdiff(labels(WL.unifrac.dist),
                              odd.sites)]

WL.unifrac.adonis <- adonis(as.dist(WL.unifrac.dist2) ~ treatments.2, perm = 999)
WL.unifrac.adonis
```

```
##
## Call:
## adonis(formula = as.dist(WL.unifrac.dist2) ~ treatments.2, permutations = 999)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##              Df SumsOfSqs   MeanSqs F.Model      R2 Pr(>F)
## treatments.2  5  0.036942 0.0073883  2.6616 0.14575  0.001 ***
## Residuals    78  0.216521 0.0027759         0.85425
## Total        83  0.253462                   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)


# Which plants influenced plant communities between trts
#WL.plant.simper <- simper(WL.plant, group = treatments, permutations = 999)
#plant.sum <- summary(WL.plant.simper, ordered = T)
#plant.sum
```

Indicator Species Analysis

```
# Plant Communities
library("labdsv")
```

```
## Loading required package: mgcv
```

```
## This is mgcv 1.8-16. For overview type 'help("mgcv-package")'.
```

```
## Loading required package: MASS
```

```
## Loading required package: cluster
```

```
##
## Attaching package: 'labdsv'
```

```
## The following object is masked from 'package:stats':
##
##     density
```

```
plant.ind <- indval(WL.plant, treatments)
summary(plant.ind)
```

```
##                       cluster indicator_value probability
## BARE                       2          0.3201       0.001
## Phalaris.arundinacea       4          0.3148       0.001
## Lemna.minor                6          0.4000       0.001
## Acer.saccharinum           6          0.2000       0.023
## Bidens.frondosa            6          0.2000       0.032
##
## Sum of probabilities                 =  11.702
##
## Sum of Indicator Values              =  2.51
##
## Sum of Significant Indicator Values  =  1.43
##
## Number of Significant Indicators     =  5
##
## Significant Indicator Distribution
##
## 2 4 6
## 1 1 3
```

```
plant.ind$indval
```

```
##                                BalledBurlapped    Bareroot  Seedling
## Acer.saccharinum                    0.00000000 0.000000000 0.0000000
## Aster.lanceolatus.var..simplex      0.11001710 0.000000000 0.0000000
## BARE                                0.12122001 0.320139686 0.1304718
## Bidens.frondosa                     0.00000000 0.000000000 0.0000000
## Carex.sp.                           0.00000000 0.000000000 0.0000000
## Elymus.virginicus                   0.00000000 0.000000000 0.0000000
## Impatiens.capensis                  0.00000000 0.000000000 0.0000000
## Leersia.virginica                   0.05807388 0.000000000 0.0000000
## Lemna.minor                         0.00000000 0.000000000 0.0000000
## Lycopus.virginicus                  0.00000000 0.000000000 0.0000000
## Morus.alba                          0.05678233 0.000000000 0.0000000
## Persicaria.pensylvanica             0.00000000 0.000000000 0.0000000
## Phalaris.arundinacea                0.16332556 0.006775177 0.1310697
## Physostegia.virginiana              0.00000000 0.000000000 0.0000000
## Pilea.pumila                        0.03255814 0.000000000 0.0000000
## Sicyos.angulatus                    0.00000000 0.066666667 0.0000000
## Stachys.palustris                   0.00000000 0.000000000 0.0000000
## Ulmus.americana                     0.06666667 0.000000000 0.0000000
## Vitis.riparia                       0.06666667 0.000000000 0.0000000
##                                      Acorn   Seedbank   Reference
## Acer.saccharinum               0.000000000 0.00000000 0.200000000
## Aster.lanceolatus.var..simplex 0.000000000 0.00000000 0.156649562
## BARE                           0.004877485 0.01486021 0.262185693
## Bidens.frondosa                0.000000000 0.00000000 0.200000000
## Carex.sp.                      0.000000000 0.00000000 0.066666667
## Elymus.virginicus              0.000000000 0.00000000 0.066666667
## Impatiens.capensis             0.000000000 0.00000000 0.066666667
## Leersia.virginica              0.000000000 0.00000000 0.017185568
## Lemna.minor                    0.000000000 0.00000000 0.400000000
## Lycopus.virginicus             0.000000000 0.00000000 0.066666667
## Morus.alba                     0.000000000 0.00000000 0.009884332
## Persicaria.pensylvanica        0.000000000 0.00000000 0.066666667
## Phalaris.arundinacea           0.314842939 0.30629608 0.007130337
## Physostegia.virginiana         0.000000000 0.00000000 0.066666667
## Pilea.pumila                   0.000000000 0.00000000 0.068217054
## Sicyos.angulatus               0.000000000 0.00000000 0.000000000
## Stachys.palustris              0.000000000 0.00000000 0.133333333
## Ulmus.americana                0.000000000 0.00000000 0.000000000
## Vitis.riparia                  0.000000000 0.00000000 0.000000000
```

```
levels(treatments)
```

```
## [1] "BalledBurlapped" "Bareroot"        "Seedling"        "Acorn"
## [5] "Seedbank"        "Reference"
```

```
plant.groups <- rep(NA, length(treatments))
for (i in 1:length(treatments)){
  if (treatments[i] == "Reference" |
      treatments[i] == "Bareroot"){
    plant.groups[i] <- "Group 1"
  }
  if (treatments[i] == "Seedling" |
      treatments[i] == "BalledBurlapped"){
  plant.groups[i] <- "Group 2"
  }
  if (treatments[i] == "Seedbank" |
      treatments[i]== "Acorn"){
  plant.groups[i] <- "Group 3"
  }
}

plant.ind2 <- indval(WL.plant, plant.groups)
summary(plant.ind2)
```

```
##                       cluster indicator_value probability
## BARE                        1          0.5810       0.001
## Lemna.minor                 1          0.2000       0.002
## Phalaris.arundinacea        3          0.6211       0.001
##
## Sum of probabilities                 =  12.739
##
## Sum of Indicator Values              =  2.14
##
## Sum of Significant Indicator Values  =  1.4
##
## Number of Significant Indicators     =  3
##
## Significant Indicator Distribution
##
## 1 3
## 2 1
```

```
WLdataREL.2 <- WLdataREL.2[, colSums(WLdataREL.2) > 0]
bac.ind <- indval(WLdataREL.2, treatments.2)
summary(bac.ind)
```

```
##
## Sum of probabilities                 =  17130.934
##
## Sum of Indicator Values              =  2897.24
##
## Sum of Significant Indicator Values  =  466.17
##
## Number of Significant Indicators     =  1918
##
## Significant Indicator Distribution
##
##   1   2   3   4   5   6
## 427  89 172 525 242 463
```

```
#bac.ind

WLdataREL.2 <- WLdataREL.2[, colSums(WLdataREL.2) > 0.05]
bac.ind <- indval(WLdataREL.2, treatments.2)
levels(treatments.2)
```

```
## [1] "BalledBurlapped" "Bareroot"        "Seedling"        "Acorn"
## [5] "Seedbank"        "Reference"
```

```r
summary(bac.ind)
```

```
##            cluster indicator_value probability
## Otu000173       1          0.2498       0.001
## Otu000199       1          0.2439       0.001
## Otu000180       1          0.2410       0.001
## Otu000070       1          0.2372       0.009
## Otu000047       1          0.2257       0.029
## Otu000296       1          0.2249       0.009
## Otu000178       1          0.2228       0.010
## Otu000244       1          0.2187       0.004
## Otu000283       1          0.2179       0.039
## Otu000133       1          0.2161       0.001
## Otu000104       1          0.2148       0.004
## Otu000092       1          0.2141       0.001
## Otu000192       1          0.2134       0.026
## Otu000057       1          0.2126       0.002
## Otu000090       1          0.2118       0.003
## Otu000089       1          0.2105       0.003
## Otu000170       1          0.2105       0.045
## Otu000020       1          0.2095       0.001
## Otu000204       1          0.2086       0.004
## Otu000097       1          0.2065       0.001
## Otu000260       1          0.2046       0.012
## Otu000049       1          0.2005       0.011
## Otu000258       1          0.1992       0.035
## Otu000105       1          0.1986       0.048
## Otu000014       1          0.1949       0.001
## Otu000005       1          0.1938       0.001
## Otu000021       1          0.1936       0.008
## Otu000100       1          0.1931       0.048
## Otu000037       1          0.1910       0.009
## Otu000033       1          0.1909       0.049
## Otu000008       1          0.1794       0.018
## Otu000030       2          0.2209       0.002
## Otu000189       2          0.2206       0.001
## Otu000207       2          0.2151       0.011
## Otu000056       2          0.2119       0.007
## Otu000211       2          0.2107       0.042
## Otu000177       2          0.2082       0.040
## Otu000205       2          0.2072       0.021
## Otu000222       2          0.2058       0.047
## Otu000140       2          0.2053       0.013
## Otu000144       2          0.2035       0.022
## Otu000143       2          0.1982       0.037
## Otu000059       2          0.1938       0.018
## Otu000044       2          0.1920       0.005
## Otu000093       2          0.1890       0.047
## Otu000026       2          0.1824       0.050
## Otu000193       3          0.2910       0.014
## Otu000058       3          0.2382       0.001
## Otu000186       3          0.2335       0.044
## Otu000220       3          0.1986       0.030
## Otu000078       3          0.1965       0.006
## Otu000040       3          0.1951       0.003
## Otu000119       4          0.2595       0.036
## Otu000125       4          0.2389       0.001
## Otu000221       4          0.2298       0.003
## Otu000067       4          0.2295       0.010
## Otu000212       4          0.2272       0.001
## Otu000181       4          0.2181       0.006
## Otu000096       4          0.2178       0.003
## Otu000266       4          0.2178       0.014
## Otu000188       4          0.2110       0.015
## Otu000128       4          0.2080       0.017
## Otu000122       4          0.2049       0.034
## Otu000230       4          0.1999       0.021
## Otu000130       4          0.1977       0.010
## Otu000017       4          0.1932       0.043
## Otu000066       4          0.1918       0.037
## Otu000110       4          0.1914       0.046
## Otu000237       5          0.2656       0.027
## Otu000279       5          0.2631       0.001
## Otu000201       5          0.2576       0.001
## Otu000268       5          0.2473       0.001
## Otu000274       5          0.2435       0.006
## Otu000016       5          0.2295       0.003
## Otu000155       5          0.2259       0.006
## Otu000142       5          0.2246       0.004
## Otu000242       5          0.2239       0.001
## Otu000120       5          0.2219       0.003
## Otu000206       5          0.2204       0.031
## Otu000061       5          0.2154       0.001
## Otu000129       5          0.2101       0.002
## Otu000138       5          0.2019       0.011
## Otu000084       5          0.1992       0.009
## Otu000074       5          0.1947       0.008
## Otu000029       5          0.1946       0.020
## Otu000041       5          0.1944       0.031
## Otu000046       5          0.1932       0.038
## Otu000080       5          0.1843       0.047
## Otu000200       6          0.2881       0.018
## Otu000224       6          0.2607       0.034
## Otu000232       6          0.2550       0.010
## Otu000275       6          0.2332       0.013
## Otu000117       6          0.2260       0.011
## Otu000082       6          0.2157       0.005
## Otu000055       6          0.2130       0.001
## Otu000183       6          0.2093       0.017
## Otu000087       6          0.2081       0.003
## Otu000111       6          0.2034       0.029
## Otu000031       6          0.1927       0.014
##
## Sum of probabilities                 =  65.352
##
## Sum of Indicator Values              =  55.48
##
## Sum of Significant Indicator Values  =  21.32
```

```
##
## Number of Significant Indicators     =  99
##
## Significant Indicator Distribution
##
##  1  2  3  4  5  6
## 31 15  6 16 20 11
```

```r
inds <- which(bac.ind$pval <= 0.05)
bac.indicators <- as.data.frame(matrix(NA, nrow = length(inds), ncol = 4))
colnames(bac.indicators) <- c("OTU", "Cluster", "IndVal", "Prob")

bac.indicators$OTU <- names(inds)
bac.indicators$Cluster <- bac.ind$maxcls[inds]
bac.indicators$IndVal <- bac.ind$indcls[inds]
bac.indicators$Prob <- bac.ind$pval[inds]

ind.tax <- WL.tax[which(as.character(WL.tax$OTU) %in% bac.indicators$OTU), ]
ind.tax <- ind.tax[match(ind.tax$OTU, bac.indicators$OTU), ]

indicator.bac <- cbind(bac.indicators, ind.tax[, -c(1)])

indicator.bac <- indicator.bac[order(as.numeric(indicator.bac$Cluster)), ]

table(indicator.bac$Cluster)
```

```
##
##  1  2  3  4  5  6
## 31 15  6 16 20 11
```

```r
table(indicator.bac$Phylum)
```

```
##
##         Acidobacteria        Actinobacteria Bacteria_unclassified
##                    18                     4                    17
##         Bacteroidetes           Chloroflexi            Firmicutes
##                     6                     2                     4
##        Planctomycetes        Proteobacteria       Verrucomicrobia
##                     2                    39                     7
```

```r
table(indicator.bac$Cluster)
```

```
##
##  1  2  3  4  5  6
## 31 15  6 16 20 11
```

```r
levels(treatments.2)
```

```
## [1] "BalledBurlapped" "Bareroot"        "Seedling"        "Acorn"
## [5] "Seedbank"        "Reference"
```

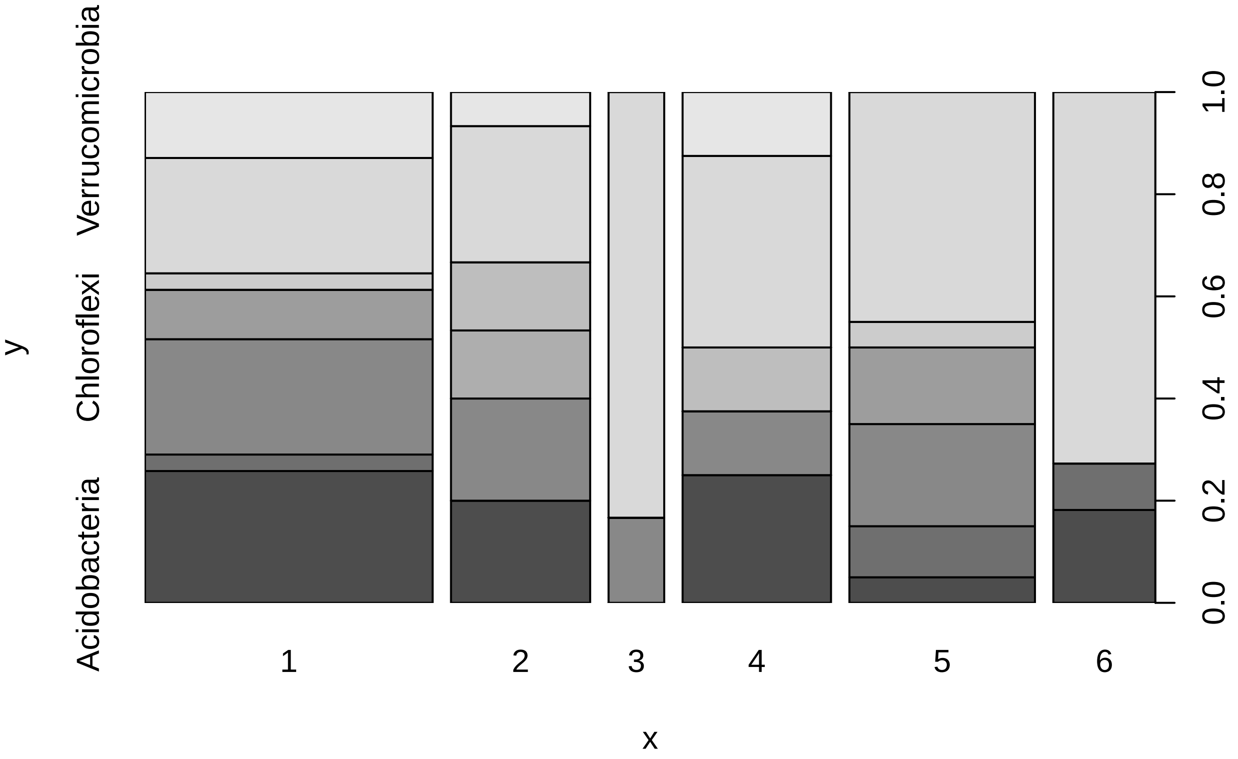
```r
# Export Bacteria Indicator Table
write.table(indicator.bac, "../data/BacterialIndicators.txt",
            sep="\t", row.names = F, quote = F)

split(indicator.bac$Phylum, indicator.bac$Cluster)
```

```
## $`1`
##  [1] "Acidobacteria"         "Proteobacteria"
##  [3] "Acidobacteria"         "Acidobacteria"
##  [5] "Verrucomicrobia"       "Bacteroidetes"
##  [7] "Proteobacteria"        "Proteobacteria"
##  [9] "Bacteria_unclassified" "Bacteroidetes"
## [11] "Bacteria_unclassified" "Acidobacteria"
## [13] "Bacteria_unclassified" "Verrucomicrobia"
## [15] "Verrucomicrobia"       "Proteobacteria"
## [17] "Bacteria_unclassified" "Acidobacteria"
## [19] "Acidobacteria"         "Bacteria_unclassified"
## [21] "Bacteroidetes"         "Proteobacteria"
## [23] "Planctomycetes"        "Verrucomicrobia"
## [25] "Proteobacteria"        "Bacteria_unclassified"
## [27] "Proteobacteria"        "Actinobacteria"
## [29] "Bacteria_unclassified" "Acidobacteria"
## [31] "Acidobacteria"
##
## $`2`
##  [1] "Acidobacteria"         "Proteobacteria"
##  [3] "Proteobacteria"        "Acidobacteria"
##  [5] "Bacteria_unclassified" "Firmicutes"
##  [7] "Bacteria_unclassified" "Proteobacteria"
##  [9] "Chloroflexi"           "Verrucomicrobia"
## [11] "Bacteria_unclassified" "Acidobacteria"
## [13] "Proteobacteria"        "Chloroflexi"
## [15] "Firmicutes"
##
## $`3`
## [1] "Bacteria_unclassified" "Proteobacteria"        "Proteobacteria"
## [4] "Proteobacteria"        "Proteobacteria"        "Proteobacteria"
##
## $`4`
##  [1] "Proteobacteria"        "Proteobacteria"
##  [3] "Acidobacteria"         "Verrucomicrobia"
##  [5] "Proteobacteria"        "Proteobacteria"
##  [7] "Acidobacteria"         "Proteobacteria"
##  [9] "Firmicutes"            "Firmicutes"
## [11] "Bacteria_unclassified" "Proteobacteria"
## [13] "Acidobacteria"         "Bacteria_unclassified"
## [15] "Verrucomicrobia"       "Acidobacteria"
##
## $`5`
##  [1] "Proteobacteria"        "Actinobacteria"
##  [3] "Proteobacteria"        "Bacteria_unclassified"
##  [5] "Proteobacteria"        "Bacteria_unclassified"
##  [7] "Proteobacteria"        "Actinobacteria"
##  [9] "Bacteroidetes"         "Bacteria_unclassified"
## [11] "Proteobacteria"        "Bacteroidetes"
## [13] "Proteobacteria"        "Proteobacteria"
## [15] "Acidobacteria"         "Proteobacteria"
## [17] "Planctomycetes"        "Bacteria_unclassified"
## [19] "Proteobacteria"        "Bacteroidetes"
##
## $`6`
##  [1] "Actinobacteria" "Acidobacteria"  "Proteobacteria" "Proteobacteria"
##  [5] "Proteobacteria" "Acidobacteria"  "Proteobacteria" "Proteobacteria"
##  [9] "Proteobacteria" "Proteobacteria" "Proteobacteria"
```

```
split(indicator.bac$Class, indicator.bac$Cluster)
```

```
## $`1`
##  [1] "Acidobacteria_Gp6"            "Alphaproteobacteria"
##  [3] "Acidobacteria_Gp6"            "Acidobacteria_Gp6"
##  [5] "Subdivision3"                 "Sphingobacteria"
##  [7] "Alphaproteobacteria"          "Gammaproteobacteria"
##  [9] "Bacteria_unclassified"        "Sphingobacteria"
## [11] "Bacteria_unclassified"        "Acidobacteria_Gp5"
## [13] "Bacteria_unclassified"        "Subdivision3"
## [15] "Spartobacteria"               "Proteobacteria_unclassified"
## [17] "Bacteria_unclassified"        "Acidobacteria_Gp4"
## [19] "Acidobacteria_Gp6"            "Bacteria_unclassified"
## [21] "Flavobacteria"                "Deltaproteobacteria"
## [23] "Planctomycetacia"             "Verrucomicrobia_unclassified"
## [25] "Gammaproteobacteria"          "Bacteria_unclassified"
## [27] "Gammaproteobacteria"          "Actinobacteria"
## [29] "Bacteria_unclassified"        "Acidobacteria_Gp17"
## [31] "Acidobacteria_Gp22"
##
## $`2`
##  [1] "Acidobacteria_Gp6"        "Alphaproteobacteria"
##  [3] "Alphaproteobacteria"      "Acidobacteria_Gp6"
##  [5] "Bacteria_unclassified"    "Bacilli"
##  [7] "Bacteria_unclassified"    "Alphaproteobacteria"
##  [9] "Chloroflexi_unclassified" "Subdivision3"
## [11] "Bacteria_unclassified"    "Acidobacteria_Gp4"
## [13] "Deltaproteobacteria"      "Chloroflexi_unclassified"
## [15] "Bacilli"
##
## $`3`
## [1] "Bacteria_unclassified"       "Deltaproteobacteria"
## [3] "Deltaproteobacteria"         "Proteobacteria_unclassified"
## [5] "Proteobacteria_unclassified" "Betaproteobacteria"
##
## $`4`
##  [1] "Betaproteobacteria"      "Alphaproteobacteria"
##  [3] "Acidobacteria_Gp4"       "Spartobacteria"
##  [5] "Betaproteobacteria"      "Gammaproteobacteria"
##  [7] "Acidobacteria_Gp4"       "Alphaproteobacteria"
##  [9] "Bacilli"                 "Firmicutes_unclassified"
## [11] "Bacteria_unclassified"   "Deltaproteobacteria"
## [13] "Acidobacteria_Gp3"       "Bacteria_unclassified"
## [15] "Subdivision3"            "Acidobacteria_Gp25"
##
## $`5`
##  [1] "Deltaproteobacteria"         "Actinobacteria"
##  [3] "Alphaproteobacteria"         "Bacteria_unclassified"
##  [5] "Deltaproteobacteria"         "Bacteria_unclassified"
##  [7] "Betaproteobacteria"          "Actinobacteria"
##  [9] "Sphingobacteria"             "Bacteria_unclassified"
## [11] "Proteobacteria_unclassified" "Sphingobacteria"
## [13] "Deltaproteobacteria"         "Deltaproteobacteria"
## [15] "Acidobacteria_Gp22"          "Gammaproteobacteria"
## [17] "Planctomycetacia"            "Bacteria_unclassified"
## [19] "Deltaproteobacteria"         "Flavobacteria"
##
## $`6`
##  [1] "Actinobacteria"              "Acidobacteria_Gp10"
##  [3] "Alphaproteobacteria"         "Betaproteobacteria"
##  [5] "Deltaproteobacteria"         "Acidobacteria_Gp7"
##  [7] "Alphaproteobacteria"         "Betaproteobacteria"
##  [9] "Betaproteobacteria"          "Proteobacteria_unclassified"
## [11] "Proteobacteria_unclassified"
```

```
plot(as.factor(indicator.bac$Cluster), as.factor(indicator.bac$Phylum))
```



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
```

```r
  # 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
```

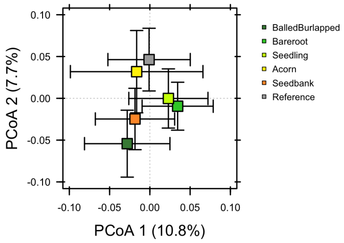
```r
WLdataREL.2 <- WLdataREL[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
design2 <- design[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
treatments <- design2$Treatment

# 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)

# 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 = 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



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), ])


# 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")
```

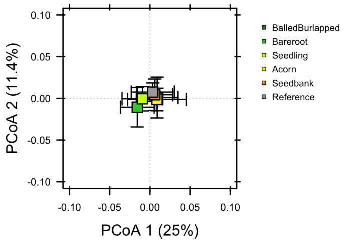
```r
cent.treats <- rownames(cent.dataframe)

# 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 = 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
#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)
```

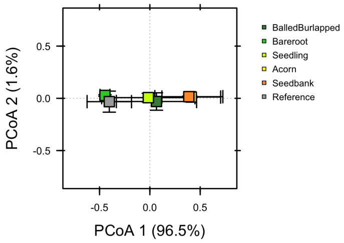
```
# Plot
points <- cbind(as.data.frame(WLplant_pcoa$points), treatments)
L.centroids <- melt(points, id="treatments", measure.vars = c("V1", "V2"))
centroids <- cast(L.centroids, variable ~ treatments, mean)
centroids.se <- cast(L.centroids, variable ~ treatments, se)
centroids.sd <- cast(L.centroids,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

```r
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)

```r
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))

# 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)
```

```r
# Bacterial Principal Coordinates Analysis
# Create Distance Matrix
sampleREL.dist <- vegdist(WLdataREL, method="bray")
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
```

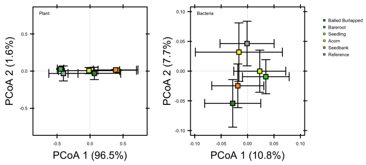
```r
WLdataREL.2 <- WLdataREL[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
design2 <- design[c(abs(WL_pcoa$points[, 1]) < 0.3), ]
treatments <- design2$Treatment

# 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)

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
```

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

Show Plot



Mantel Tests

Similarity between microbe and plant communities

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

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

```r
# 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
```

```r
#rowSums(plant.comm)

bac.comm <- bac.comm[, colSums(bac.comm) > 0]
plant.comm <- plant.comm[, colSums(plant.comm) > 0]

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

mantel.rtest(dist.bac, dist.plant, nrepet = 999)
```

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

```
## Monte-Carlo test
## Observation: 0.09849538
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.001
```

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

```
dbRDA <- dbrda(bac.comm ~ WL.plant_points[, 1], distance = "bray")
anova(dbRDA)
```

```
## Permutation test for dbrda under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = bac.comm ~ WL.plant_points[, 1], distance = "bray")
##          Df SumOfSqs      F Pr(>F)
## Model     1  0.09609 3.0644  0.001 ***
## Residual 82  2.57134
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dbRDA)
```

```
## $r.squared
## [1] 0.03602451
##
## $adj.r.squared
## [1] 0.02426871
```

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 Zero Sum Columns
bac.comm <- bac.comm[, colSums(bac.comm) > 0]
plant.comm <- plant.comm[, colSums(plant.comm) > 0]

# 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")
```

```r
mantel.rtest(dist.bac, dist.plant, nrepet = 999)
```

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

```
## Monte-Carlo test
## Observation: 0.09849538
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.001
```

```r
mantel.rtest(dist.bac, dist.nuts, nrepet = 999)
```

```
## Monte-Carlo test
## Observation: -0.04139427
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.nuts, nrepet = 999)
## Based on 999 replicates
## Simulated p-value: 0.677
```

```r
mantel.rtest(dist.bac, dist.phys, nrepet = 999)
```

```
## Monte-Carlo test
## Observation: 0.1637996
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.phys, nrepet = 999)
## Based on 999 replicates
## Simulated p-value: 0.023
```

```r
mantel.rtest(dist.bac, dist.soil, nrepet = 999)
```

```
## Monte-Carlo test
## Observation: -0.01610846
## Call: mantel.rtest(m1 = dist.bac, m2 = dist.soil, nrepet = 999)
## Based on 999 replicates
## Simulated p-value: 0.535
```

```r
mantel.rtest(dist.plant, dist.nuts, nrepet = 999)
```

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

```
## Monte-Carlo test
## Observation: 0.265419
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.001
```

```r
mantel.rtest(dist.plant, dist.phys, nrepet = 999)
```

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

```
## Monte-Carlo test
## Observation: 0.04439773
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.013
```

```r
mantel.rtest(dist.plant, dist.soil, nrepet = 999)
```

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

```
## Monte-Carlo test
## Observation: 0.2481641
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
## Based on 999 replicates
## Simulated p-value: 0.001
```

Distance Based RDA Models

```r
# Nutrients
chem.dist <- dist(apply(WL.soil.nuts, 2, scale),
                  method = "euclidean")
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

# Physical Factors
phys.dist <- dist(apply(WL.soil.phys, 2, scale),
                  method = "euclidean")
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

# Bac dbRDA
dbRDA.c <- dbrda(bac.comm ~ chem.pcoa$points[,1], distance = "bray")
anova(dbRDA.c, 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 = bac.comm ~ chem.pcoa$points[, 1], distance = "bray")
##                       Df SumOfSqs      F Pr(>F)
## chem.pcoa$points[, 1]  1  0.08661 2.7518  0.001 ***
## Residual              82  2.58082
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
RsquareAdj(dbRDA.c)
```

```
## $r.squared
## [1] 0.03246874
##
## $adj.r.squared
## [1] 0.02066957
```

```r
dbRDA.p <- dbrda(bac.comm ~ 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 = bac.comm ~ phys.pcoa$points[, 1], distance = "bray")
##          Df SumOfSqs     F Pr(>F)    
## Model     1  0.07508 2.375  0.001 ***
## Residual 82  2.59235                 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
## $r.squared
## [1] 0.02814833
## 
## $adj.r.squared
## [1] 0.01629648
```

```
dbRDA.cp <- dbrda(bac.comm ~ chem.pcoa$points[,1] + phys.pcoa$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 = bac.comm ~ chem.pcoa$points[, 1] + phys.pcoa$points[, 1], distance = "bray", a
##                       Df SumOfSqs      F Pr(>F)    
## chem.pcoa$points[, 1]  1  0.08661 2.7721  0.001 ***
## phys.pcoa$points[, 1]  1  0.05019 1.6064  0.013 *  
## Residual              81  2.53063                  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
## $r.squared
## [1] 0.0512842
## 
## $adj.r.squared
## [1] 0.02785911
```

```
dbRDA.P.cp <- dbrda(plant.comm ~ 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 = plant.comm ~ phys.pcoa$points[, 1] * chem.pcoa$points[, 1], distance = "bray"
##                                             Df SumOfSqs       F Pr(>F)   
## phys.pcoa$points[, 1]                        1   0.2752  1.1953  0.250   
## chem.pcoa$points[, 1]                        1   3.0843 13.3955  0.002 **
## phys.pcoa$points[, 1]:chem.pcoa$points[, 1]  1   1.0717  4.6545  0.022 * 
## Residual                                    80  18.4197                  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
## $r.squared
## [1] 0.1939162
## 
## $adj.r.squared
## [1] 0.163688
```

PostHoc Tests

```
phys.mod <- lm(phys.pcoa$points[,1] ~ WL.soil2$Treatment)
chem.mod <- lm(chem.pcoa$points[,1] ~ WL.soil2$Treatment)
anova(phys.mod)
```

```
## Analysis of Variance Table
## 
## Response: phys.pcoa$points[, 1]
##                    Df  Sum Sq Mean Sq F value  Pr(>F)  
## WL.soil2$Treatment  5  14.534  2.9068  2.1447 0.06879 .
## Residuals          78 105.717  1.3554                  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

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

```
## Analysis of Variance Table
## 
## Response: chem.pcoa$points[, 1]
##                    Df Sum Sq Mean Sq F value    Pr(>F)    
## WL.soil2$Treatment  5  73.39  14.678  6.7206 2.959e-05 ***
## Residuals          78 170.35   2.184                      
## ---
## 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)
## 
## $`WL.soil2$Treatment`
##                                 diff         lwr       upr     p adj
## Bareroot-BalledBurlapped   0.9960375 -0.32135712 2.3134322 0.2454950
## Seedling-BalledBurlapped   1.3681678  0.03002546 2.7063100 0.0421120
## Acorn-BalledBurlapped      1.1715954 -0.16654691 2.5097377 0.1203240
## Seedbank-BalledBurlapped   0.6934262 -0.64471608 2.0315685 0.6563663
## Reference-BalledBurlapped  0.8241642 -0.49323048 2.1415588 0.4542536
## Seedling-Bareroot          0.3721302 -0.89190627 1.6361667 0.9547234
## Acorn-Bareroot             0.1755579 -1.08847864 1.4395944 0.9985335
## Seedbank-Bareroot         -0.3026113 -1.56664780 0.9614252 0.9814775
## Reference-Bareroot        -0.1718734 -1.41392493 1.0701782 0.9985591
## Acorn-Seedling            -0.1965724 -1.48221791 1.0890732 0.9976763
## Seedbank-Seedling         -0.6747415 -1.96038707 0.6109040 0.6440150
## Reference-Seedling        -0.5440036 -1.80804009 0.7200329 0.8068369
## Seedbank-Acorn            -0.4781692 -1.76381469 0.8074764 0.8852677
## Reference-Acorn           -0.3474312 -1.61146771 0.9166053 0.9661433
## Reference-Seedbank         0.1307379 -1.13329855 1.3947745 0.9996494
```

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

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = chem.mod)
##
## $`WL.soil2$Treatment`
##                                 diff        lwr        upr     p adj
## Bareroot-BalledBurlapped   1.4379873 -0.2343365  3.1103111 0.1331238
## Seedling-BalledBurlapped   0.8114554 -0.8872059  2.5101166 0.7294419
## Acorn-BalledBurlapped     -0.4487753 -2.1474366  1.2498859 0.9714488
## Seedbank-BalledBurlapped  -1.3101913 -3.0088525  0.3884700 0.2258638
## Reference-BalledBurlapped -0.6494620 -2.3217858  1.0228618 0.8653924
## Seedling-Bareroot         -0.6265319 -2.2311220  0.9780582 0.8627205
## Acorn-Bareroot            -1.8867626 -3.4913527 -0.2821726 0.0118037
## Seedbank-Bareroot         -2.7481786 -4.3527686 -1.1435885 0.0000489
## Reference-Bareroot        -2.0874493 -3.6641313 -0.5107672 0.0029948
## Acorn-Seedling            -1.2602307 -2.8922516  0.3717902 0.2247611
## Seedbank-Seedling         -2.1216467 -3.7536676 -0.4896257 0.0037703
## Reference-Seedling        -1.4609174 -3.0655074  0.1436727 0.0952960
## Seedbank-Acorn            -0.8614159 -2.4934369  0.7706050 0.6384076
## Reference-Acorn           -0.2006866 -1.8052767  1.4039034 0.9991156
## Reference-Seedbank         0.6607293 -0.9438608  2.2653194 0.8340697
```

Invasive Species Biomass

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

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

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
```

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

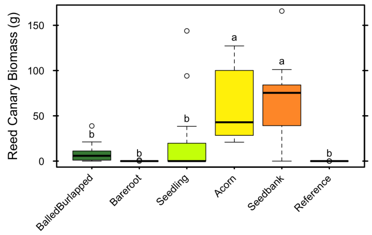
Plot: Invasive Species

```r
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
```

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

Show Plot


Reed Canary Biomass (g)
0
50
100
150
b
b
b
a
a
b
BalledBurlapped
Bareroot
Seedling
Acorn
Seedbank
Reference