Perenniality (and/or) management drives soil biological communities and functions

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Project Description: Fill out

Initial Setup

Import Files

Environmental Data

Nematode Data

Microbial Data

Diversity Metrics - Hypothesis Testing

```
# Rarefy Abundances (min abundance is 88033. We are sampling to 80000)
min(rowSums(otus))
```

```
## [1] 88033
```

```
PWESdata.r <- rrarefy(otus, 80000)

# Fisher's Alpha
fisher <- fisher.alpha(PWESdata.r)

# Species Richness
richness <- rowSums((PWESdata.r >= 1))

# Shannon Diversity
shannon <- diversity(PWESdata.r, "shannon")

# Simpson's Evenness
simp.even <- apply(PWESdata.r, 1, simp_even)

#Pielou's evenness
J <- shannon/log(specnumber(PWESdata.r[,-c(1:1)]))

#combined richness, diversity, evenness
diversity <- cbind(design,richness,shannon,simp.even,J)
```

Diversity Metrics - Hypothesis Testing

```
# First check the order
length(design$Management) == length(fisher)
```

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

```
all.equal(gsub("-", "", rownames(design)), names(fisher))
```

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

```
library(lme4)
```

```
## Warning: package 'lme4' was built under R version 3.4.4
```

```
## Loading required package: Matrix
```

```
##
## Attaching package: 'Matrix'
```

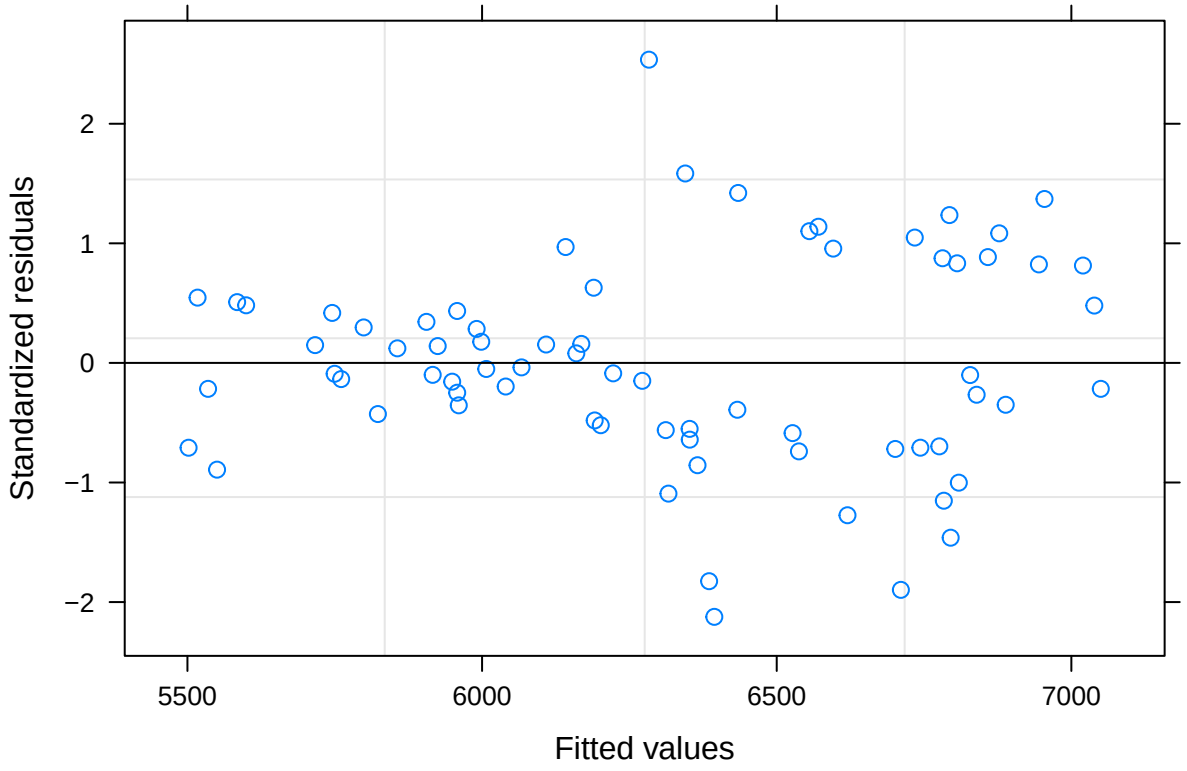
```
## The following object is masked from 'package:tidyr':
##
##     expand
```

```
## The following object is masked from 'package:reshape':
##
##     expand
```

```
##
## Attaching package: 'lme4'
```

```
## The following object is masked from 'package:nlme':
##
##     lmList
```

```
richness.lm <- lme(richness ~ Date*Management*Plant, random = ~1|Block, data = diversity)
plot(richness.lm)
```




```
richness.lm
```

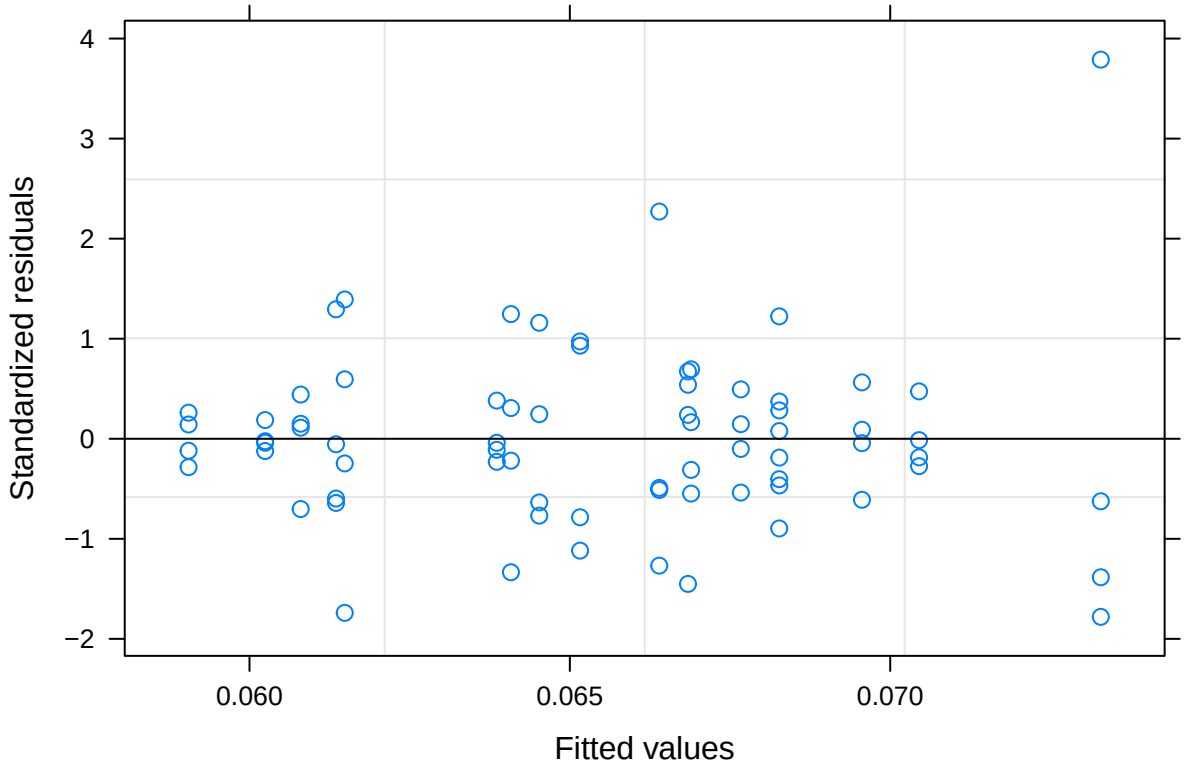
```
## Linear mixed-effects model fit by REML
##   Data: diversity
##   Log-restricted-likelihood: -436.8028
##   Fixed: richness ~ Date * Management * Plant
##                                       (Intercept)
##                                           5591.75
##                                       DateJun-12
##                                            422.75
##                                       DateJun-13
##                                            809.75
##                                  ManagementLowN
##                                             15.25
##                                ManagementOrganic
##                                            455.75
##                             PlantIntWheatgrass
##                                            414.25
##                        DateJun-12:ManagementLowN
##                                            343.25
##                        DateJun-13:ManagementLowN
##                                            468.25
##                     DateJun-12:ManagementOrganic
##                                           -191.00
##                     DateJun-13:ManagementOrganic
##                                              8.50
##                   DateJun-12:PlantIntWheatgrass
##                                            198.75
##                   DateJun-13:PlantIntWheatgrass
##                                           -617.25
```

```
##               ManagementLowN:PlantIntWheatgrass
##                                         -214.75
##            ManagementOrganic:PlantIntWheatgrass
##                                         -548.75
##    DateJun-12:ManagementLowN:PlantIntWheatgrass
##                                         -329.25
##    DateJun-13:ManagementLowN:PlantIntWheatgrass
##                                          428.75
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                                          457.00
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                                          677.00
##
## Random effects:
##  Formula: ~1 | Block
##         (Intercept) Residual
## StdDev:    150.0442 613.1673
##
## Number of Observations: 72
## Number of Groups: 4
```

```
anova(richness.lm)
```

```
##                       numDF denDF  F-value p-value
## (Intercept)               1    51 3667.446  <.0001
## Date                      2    51   12.006  0.0001
## Management                2    51    1.563  0.2193
## Plant                     1    51    1.185  0.2815
## Date:Management           4    51    0.674  0.6133
## Date:Plant                2    51    0.958  0.3905
## Management:Plant          2    51    0.166  0.8479
## Date:Management:Plant     4    51    0.377  0.8240
```

```
evenness.lm <- lme(simp.even ~ Date*Management*Plant, random = ~1|Block, data = diversity)
plot(evenness.lm)
```




```
evenness.lm
```

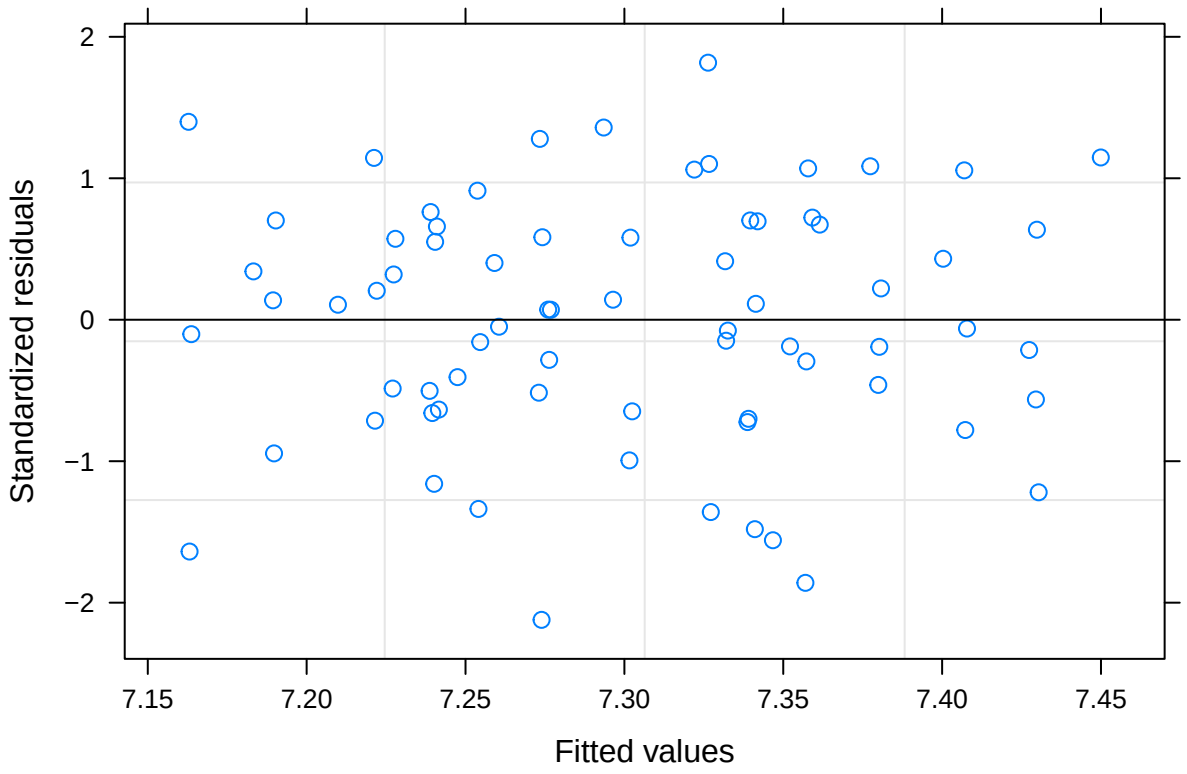
```
## Linear mixed-effects model fit by REML
##   Data: diversity
##   Log-restricted-likelihood: 174.9303
##   Fixed: simp.even ~ Date * Management * Plant
##                                       (Intercept)
##                                      0.0682681756
##                                        DateJun-12
##                                     -0.0041872288
##                                        DateJun-13
##                                     -0.0080242091
##                                    ManagementLowN
##                                     -0.0005996527
##                                  ManagementOrganic
##                                     -0.0074689250
##                                PlantIntWheatgrass
##                                      0.0021828734
##                           DateJun-12:ManagementLowN
##                                     -0.0019945595
##                           DateJun-13:ManagementLowN
##                                     -0.0005947314
##                         DateJun-12:ManagementOrganic
##                                      0.0102804458
##                         DateJun-13:ManagementOrganic
##                                      0.0117479299
##                       DateJun-12:PlantIntWheatgrass
##                                     -0.0049130188
##                       DateJun-13:PlantIntWheatgrass
##                                      0.0108598799
```

```
##               ManagementLowN:PlantIntWheatgrass
##                                   -0.0030074396
##            ManagementOrganic:PlantIntWheatgrass
##                                    0.0052879697
##    DateJun-12:ManagementLowN:PlantIntWheatgrass
##                                    0.0106465964
##    DateJun-13:ManagementLowN:PlantIntWheatgrass
##                                   -0.0052254685
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                                   -0.0042905318
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                                   -0.0132942031
##
## Random effects:
##  Formula: ~1 | Block
##          (Intercept)    Residual
## StdDev: 3.811975e-07 0.007525653
##
## Number of Observations: 72
## Number of Groups: 4
```

```
anova(evenness.lm)
```

```
##                       numDF denDF  F-value p-value
## (Intercept)               1    51 5446.651  <.0001
## Date                      2    51    0.887  0.4182
## Management                2    51    0.505  0.6066
## Plant                     1    51    4.059  0.0492
## Date:Management           4    51    1.059  0.3864
## Date:Plant                2    51    1.514  0.2298
## Management:Plant          2    51    0.038  0.9626
## Date:Management:Plant     4    51    0.985  0.4241
```

```
shannon.lm <- lme(shannon ~ Date*Management*Plant, random = ~1|Block, data = diversity)
plot(shannon.lm)
```




```
shannon.lm
```

```
## Linear mixed-effects model fit by REML
##   Data: diversity
##   Log-restricted-likelihood: 41.76216
##   Fixed: shannon ~ Date * Management * Plant
##                                       (Intercept)
##                                        7.19482826
##                                         DateJun-12
##                                        0.04927266
##                                         DateJun-13
##                                        0.08364624
##                                     ManagementLowN
##                                       -0.02659003
##                                  ManagementOrganic
##                                        0.03177059
##                                PlantIntWheatgrass
##                                        0.08658297
##                           DateJun-12:ManagementLowN
##                                        0.04165487
##                           DateJun-13:ManagementLowN
##                                        0.09457473
##                        DateJun-12:ManagementOrganic
##                                        0.06123283
##                        DateJun-13:ManagementOrganic
##                                        0.07506181
##                      DateJun-12:PlantIntWheatgrass
##                                       -0.02369395
##                      DateJun-13:PlantIntWheatgrass
##                                       -0.03332540
```

```
##               ManagementLowN:PlantIntWheatgrass
##                                      -0.02234943
##            ManagementOrganic:PlantIntWheatgrass
##                                      -0.06765307
##    DateJun-12:ManagementLowN:PlantIntWheatgrass
##                                       0.04443869
##    DateJun-13:ManagementLowN:PlantIntWheatgrass
##                                       0.03500324
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                                       0.03003099
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                                       0.06400551
##
## Random effects:
##  Formula: ~1 | Block
##         (Intercept)   Residual
## StdDev:  0.01620766 0.08744605
##
## Number of Observations: 72
## Number of Groups: 4
```

```
anova(shannon.lm)
```

```
##                       numDF denDF   F-value p-value
## (Intercept)               1    51 310009.13  <.0001
## Date                      2    51     15.59  <.0001
## Management                2    51      2.81  0.0693
## Plant                     1    51      7.61  0.0080
## Date:Management           4    51      1.09  0.3692
## Date:Plant                2    51      0.00  0.9995
## Management:Plant          2    51      0.39  0.6801
## Date:Management:Plant     4    51      0.09  0.9852
```

```
#calculate mean and se
library(reshape2)
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following object is masked from 'package:nlme':
##
##     collapse
```

```
## The following object is masked from 'package:reshape':
##
##     rename
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
diversity.1 <- subset(diversity, Year == 2013)
dim(diversity.1)
```

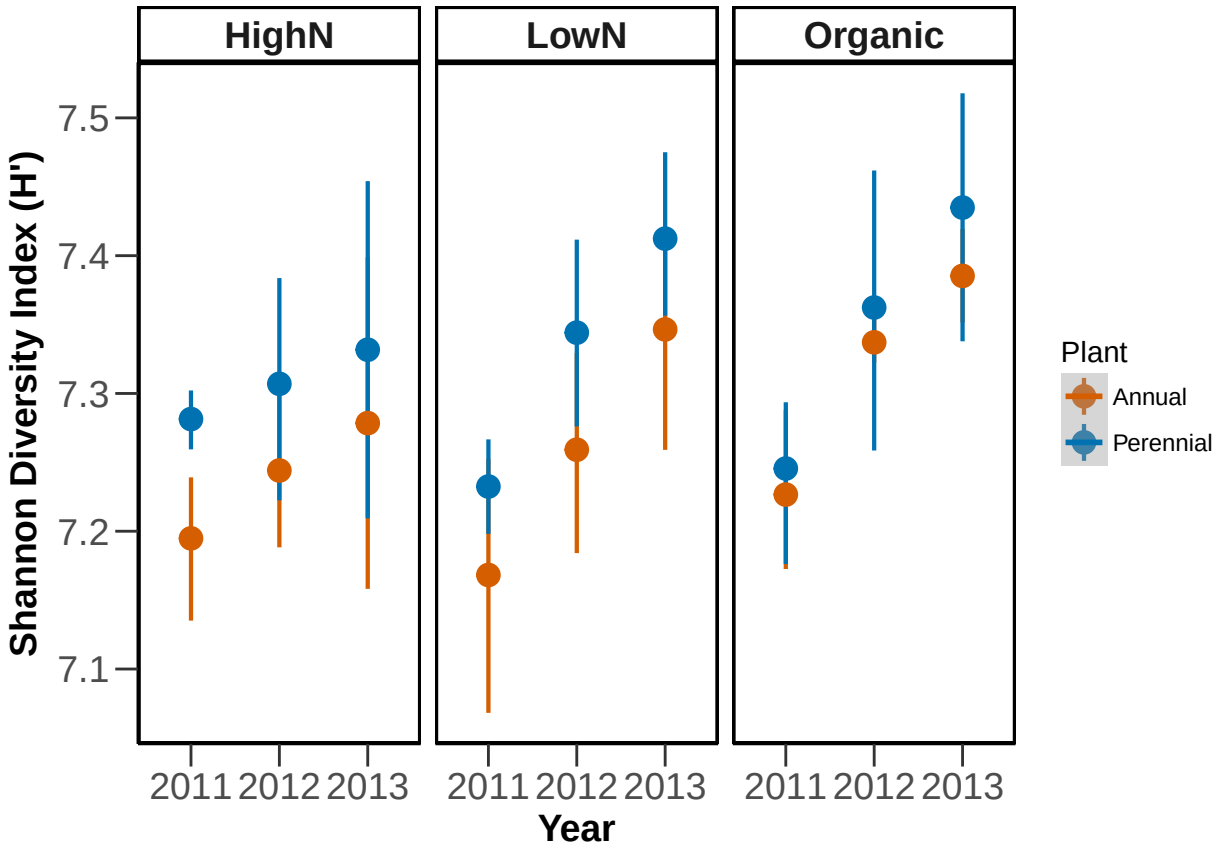
```
## [1] 24 13
```

```
summary <- diversity.1 %>% group_by(Management, Plant) %>% summarise(mean.richness=mean(richness), se.ri
print(summary)
```

```
## # A tibble: 6 x 6
## # Groups:   Management [?]
##   Management Plant       mean.richness se.richness mean.shannon se.shannon
##   <fct>      <fct>               <dbl>       <dbl>        <dbl>      <dbl>
## 1 HighN      Annual               6402         534         7.28     0.0753
## 2 HighN      IntWheatgr~          6198         191         7.33     0.0718
## 3 LowN       Annual               6885         367         7.35     0.0473
## 4 LowN       IntWheatgr~          6896         151         7.41     0.0330
## 5 Organic    Annual               6866         345         7.39     0.0215
## 6 Organic    IntWheatgr~          6791         362         7.43     0.0510
```

Plot shannon diversity

```
date_1 <- as.factor(diversity$Date)
labels <- c("Jun-11"="2011","Jun-12"="2012", "Jun-13"="2013")

# Graphing Shannon Diversity - Date x Plant over Mgmt
p <- ggplot(diversity, aes(x=Date, y=shannon, color=as.factor(Plant)))+ scale_color_manual(name="Plant"
p1=p+geom_smooth(method="lm")+facet_wrap(~Management)+facet_grid(. ~ Management)
p1 + theme_bw() +
    theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(), axis.line
          =element_line(colour = "black")) +
    theme(axis.title=element_text(vjust=1,size=14,face="bold"),
          axis.text=element_text(size=14), axis.text.x = element_text(vjust=0.65, hjust=0.5,
          size=14), panel.border = element_rect(colour = "black",size=1)) +
    theme(axis.ticks.length=unit(0.3,"cm")) + labs(x = "Year", y = "Shannon Diversity Index (H')") +
    theme(strip.text.x = element_text(size=14, face="bold"), strip.text.y =
          element_text(size=14, face="bold"), strip.background = element_rect(colour="black",
          fill="white", size=1)) +
    scale_x_discrete(breaks=c("Jun-11", "Jun-12", "Jun-13"), labels=c("2011", "2012",
          "2013"))
```




```
ggsave("../figures/shannon.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA, height=NA
```

```
## Saving 6.5 x 4.5 in image
```

Simple Hypothesis Testing - Microbes

```
#PERMANOVA Date|Plant|Management
new.data <-cbind(design,dataREL)
adonis = adonis(new.data[,-c(1:9)]~Block+Date*Management*Plant, method = "bray", data = new.data, perm=
adonis
```

```
##
## Call:
## adonis(formula = new.data[, -c(1:9)] ~ Block + Date * Management *      Plant, data = new.data, permu
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##                       Df SumsOfSqs  MeanSqs F.Model      R2   Pr(>F)
## Block                  1    0.0783 0.078300  1.4367 0.01795 0.100899
## Date                   2    0.4027 0.201328  3.6942 0.09233 0.000999 ***
## Management             2    0.2698 0.134918  2.4756 0.06188 0.001998 **
## Plant                  1    0.1084 0.108392  1.9889 0.02486 0.032967 *
## Date:Management        4    0.1796 0.044909  0.8241 0.04119 0.870130
```

```
## Date:Plant             2    0.1342 0.067076  1.2308 0.03076 0.156843
## Management:Plant       2    0.1323 0.066139  1.2136 0.03033 0.173826
## Date:Management:Plant  4    0.1673 0.041820  0.7674 0.03836 0.965035
## Residuals             53    2.8884 0.054498         0.66234
## Total                 71    4.3609                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Microbial Ordinations

Principal Coordinates Ordination

Principal Coordinates Ordination -BACTERIA seaparate years

```
new.data <-cbind(design,dataREL)
# PERMANOVA - 2011 only
new.data.2011 <- subset(new.data, Year == 2011)
adonis.2011 = adonis(new.data.2011[,-c(1:9)] ~Block+Management*Plant, method = "bray", data = new.data.
adonis.2011
```

```
##
## Call:
## adonis(formula = new.data.2011[, -c(1:9)] ~ Block + Management *      Plant, data = new.data.2011, p
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##                   Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Block              1   0.04654 0.046544 0.88030 0.03996 0.4975
## Management         2   0.07623 0.038117 0.72092 0.06545 0.8292
## Plant              1   0.04778 0.047775 0.90359 0.04102 0.4476
## Management:Plant   2   0.09531 0.047653 0.90127 0.08183 0.4945
## Residuals         17   0.89884 0.052873         0.77174
## Total             23   1.16469                  1.00000
```

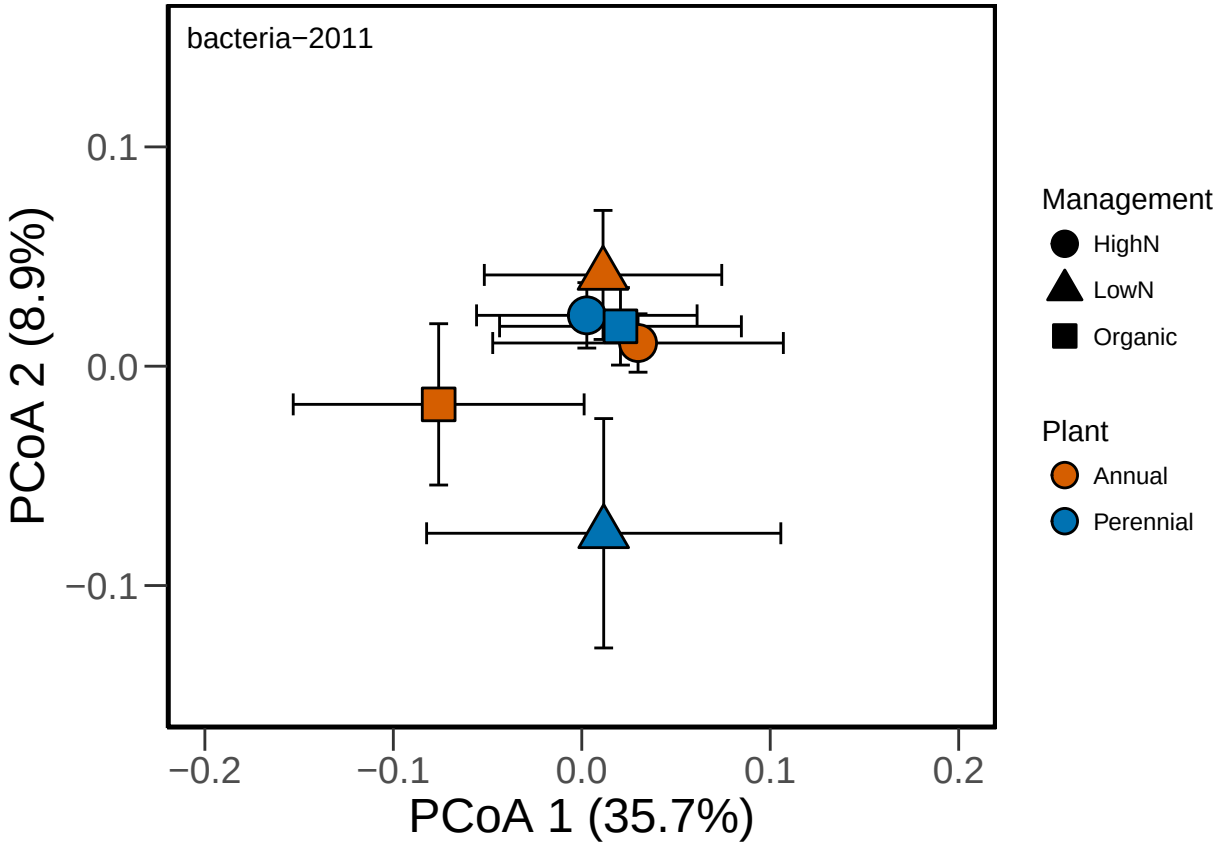
```
dataREL.dist <- vegdist(new.data.2011[,-c(1:9)], method="bray")

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #35.7
```

```
## [1] 35.7
```

```
explainvar2b #8.9
```

```
## [1] 8.9
# Added by Mario ######
#soil2011 <- soil[soil$Year == 2011, 17:22]
#fit <- envfit(pcoa,soil2011,perm=1000)
#fit.coords <- as.data.frame(fit$vectors$arrows) * 0.1
############

# Principal Coordinates Analysis (Mgmt*Plant) - 2011 only
pcoa.groups <- paste(new.data.2011$Management, new.data.2011$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

#Plot
df8a <- as.data.frame(pcoa.cent.dataframe)
plot8a <- ggplot(df8a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
bact.2011<- plot8a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +

  # Added By Mario ####
  #geom_segment(data=fit.coords,aes(x=0,xend=Dim1,y=0,yend=Dim2),
               #arrow = arrow(length = unit(0.25, "cm")),
               #colour="grey20", linetype=4, inherit.aes = FALSE) +
  #geom_segment(data=fit.coords,aes(x=Dim1 * 0.98,xend=Dim1,y=Dim2 * 0.98,yend=Dim2),
               #arrow = arrow(length = unit(0.25, "cm")),
               #colour="grey20", inherit.aes = FALSE) +
  #geom_text(data=fit.coords,aes(x=Dim1 * 1.125,y=Dim2 * 1.125, label=rownames(fit.coords)),
            #size=3, inherit.aes = FALSE) +
  ####################


  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
```

```
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.2), ylim = c(-0.15, 0.15)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (35.7%)") + ylab("PCoA 2 (8.9%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.16, y = 0.15, label = "bacteria-2011")

bact.2011
```




```
ggsave("../figures/bact.2011_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/bact.2011_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
# PERMANOVA - 2012 only
new.data.2012 <- subset(new.data, Year == 2012)
adonis.2012 = adonis(new.data.2012[,-c(1:9)] ~Block+Management*Plant, method = "bray", data = new.data.2
adonis.2012
```

```
##
```

```
## Call:
## adonis(formula = new.data.2012[, -c(1:9)] ~ Block + Management *      Plant, data = new.data.2012, p
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##                  Df SumsOfSqs  MeanSqs F.Model      R2  Pr(>F)
## Block             1   0.04580 0.045801 0.81911 0.03347 0.60739
## Management        2   0.19234 0.096172 1.71997 0.14055 0.02298 *
## Plant             1   0.07910 0.079096 1.41459 0.05780 0.12987
## Management:Plant  2   0.10070 0.050351 0.90050 0.07359 0.55045
## Residuals        17   0.95055 0.055915         0.69460
## Total            23   1.36850                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
dataREL.dist <- vegdist(new.data.2012[,-c(1:9)], method="bray")

# Added by Mario ######
#soil2012 <- soil[soil$Year == 2012, 17:22]
#fit <- envfit(pcoa,soil2012,perm=1000)
#fit
#fit.coords <- as.data.frame(fit$vectors$arrows) * 0.1
#############

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #28.9
```

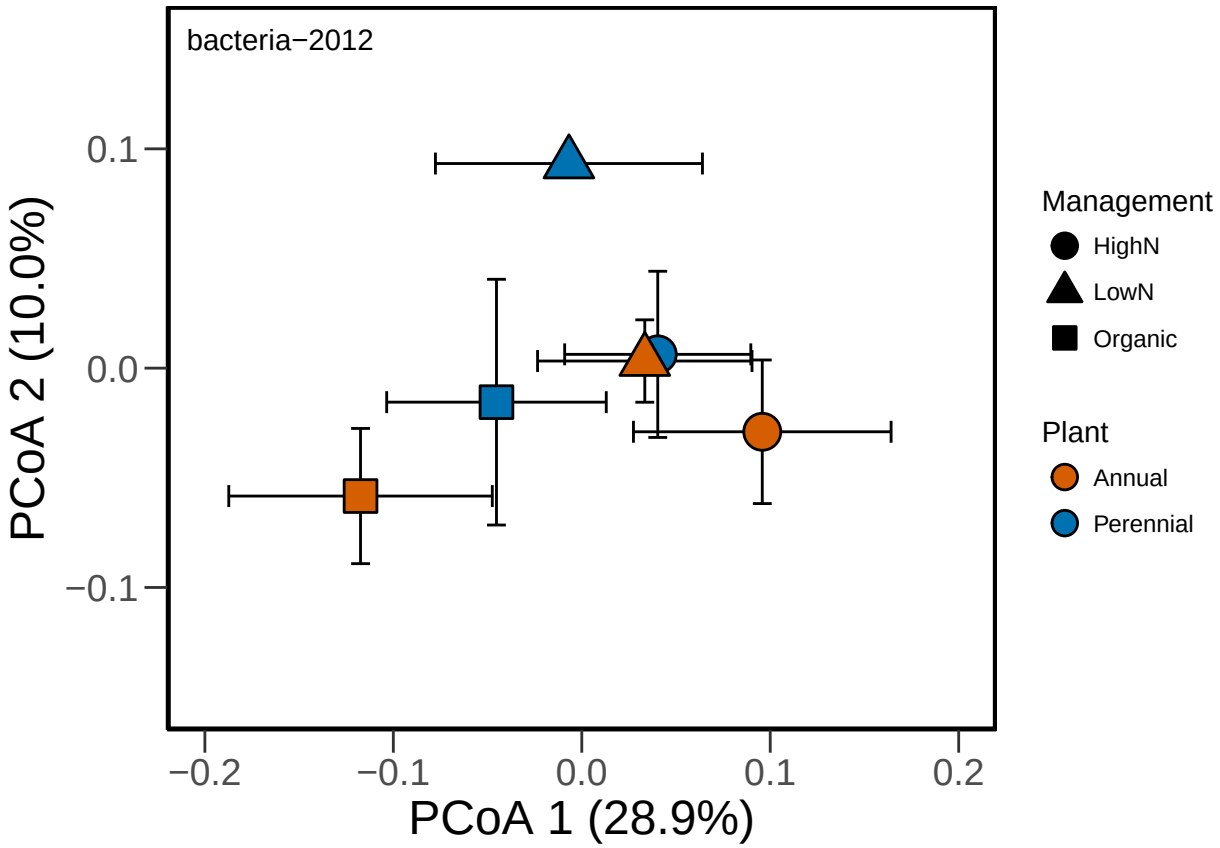
```
## [1] 28.9
```

```
explainvar2b #10.0
```

```
## [1] 10
```

```
# Principal Coordinates Analysis (Mgmt*Plant) - 2012 only
pcoa.groups <- paste(new.data.2012$Management, new.data.2012$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
```

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

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

#Plot
df9a <- as.data.frame(pcoa.cent.dataframe)
plot9a <- ggplot(df9a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
bact.2012<- plot9a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +

   # Added By Mario ####
  #geom_segment(data=fit.coords,aes(x=0,xend=Dim1,y=0,yend=Dim2),
               #arrow = arrow(length = unit(0.25, "cm")),
               #colour="grey20", linetype=c(4,4,4,4,1), inherit.aes = FALSE) +
  #geom_segment(data=fit.coords,aes(x=Dim1 * 0.98,xend=Dim1,y=Dim2 * 0.98,yend=Dim2),
               #arrow = arrow(length = unit(0.25, "cm")),
               #colour="grey20", inherit.aes = FALSE) +
  #geom_text(data=fit.coords,aes(x=Dim1 * 1.125,y=Dim2 * 1.125, label=rownames(fit.coords)),
            #size=3, inherit.aes = FALSE) +
  ######################

  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.2), ylim = c(-0.15, 0.15)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (28.9%)") + ylab("PCoA 2 (10.0%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.16, y = 0.15, label = "bacteria-2012")
bact.2012
```




```
ggsave("../figures/bact.2012_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/bact.2012_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
# PERMANOVA - 2013 only
new.data.2013 <- subset(new.data, Year == 2013)
adonis.2013 = adonis(new.data.2013[,-c(1:9)] ~Block+Management*Plant, method = "bray", data = new.data.1
adonis.2013
```

```
##
## Call:
## adonis(formula = new.data.2013[, -c(1:9)] ~ Block + Management *      Plant, data = new.data.2013, pe
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##                  Df SumsOfSqs  MeanSqs F.Model      R2   Pr(>F)
## Block             1   0.08052 0.080516 1.44926 0.05650 0.084915 .
## Management        2   0.18090 0.090448 1.62803 0.12694 0.016983 *
## Plant             1   0.11567 0.115672 2.08206 0.08117 0.006993 **
## Management:Plant  2   0.10355 0.051775 0.93193 0.07266 0.578422
## Residuals        17   0.94446 0.055557         0.66274
## Total            23   1.42510                  1.00000
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
dataREL.dist <- vegdist(new.data.2013[,-c(1:9)], method="bray")

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #26.2
```

```
## [1] 26.2
```

```
explainvar2b #13.8
```

```
## [1] 13.8
```

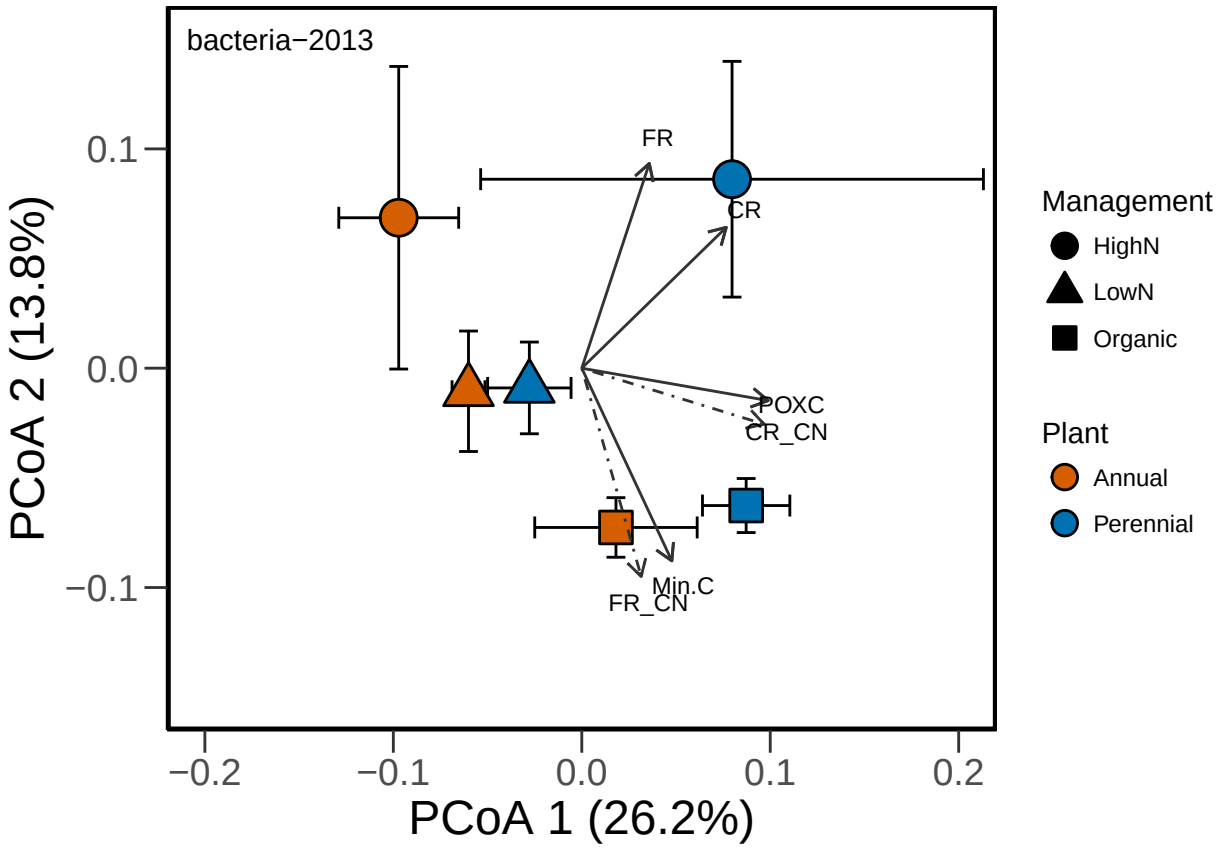
```
# Principal Coordinates Analysis (Mgmt*Plant) - 2013 only
pcoa.groups <- paste(new.data.2013$Management, new.data.2013$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

# Added by Mario ######
#soil2013 <- soil[soil$Year == 2013, 17:22]
#fit <- envfit(pcoa,soil2013,perm=1000)
#fit
#fit.coords <- as.data.frame(fit$vectors$arrows) * 0.1

# Updated by AP with env2013 data
env2013 <- read.csv("../data/EnvironronmentalFactors2013.csv", header=TRUE)
soil2013 <- env2013[,(9:14)]
fit <- envfit(pcoa,soil2013,perm=1000, na.rm=TRUE)
fit
```

```
##
## ***VECTORS
##
##           Dim1     Dim2     r2   Pr(>r)
## CR     0.76663  0.64209 0.2708 0.047952 *
```

```
## FR     0.35827  0.93362 0.4993 0.000999 ***
## CR_CN  0.96693 -0.25504 0.1669 0.143856
## FR_CN  0.31501 -0.94909 0.0654 0.487512
## POXC   0.98904 -0.14768 0.3089 0.019980 *
## Min.C  0.47785 -0.87844 0.4036 0.007992 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 1000
##
## 1 observation deleted due to missingness
```

```
fit.coords <- as.data.frame(fit$vectors$arrows) * 0.1

############
#Plot
df10a <- as.data.frame(pcoa.cent.dataframe)
plot10a <- ggplot(df10a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
bact.2013<- plot10a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +

     # Added By Mario ####
  geom_segment(data=fit.coords,aes(x=0,xend=Dim1,y=0,yend=Dim2),
               arrow = arrow(length = unit(0.25, "cm")),
               colour="grey20", linetype=c(1,1,4,4,1,1), inherit.aes = FALSE) +
  geom_segment(data=fit.coords,aes(x=Dim1 * 0.98,xend=Dim1,y=Dim2 * 0.98,yend=Dim2),
               arrow = arrow(length = unit(0.25, "cm")),
               colour="grey20", inherit.aes = FALSE) +
  geom_text(data=fit.coords,aes(x=Dim1 * 1.125,y=Dim2 * 1.125, label=rownames(fit.coords)),
            size=3, inherit.aes = FALSE) +
  ######################

  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.2), ylim = c(-0.15, 0.15)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (26.2%)") + ylab("PCoA 2 (13.8%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.16, y = 0.15, label = "bacteria-2013")
```

```
bact.2013
```




```
ggsave("../figures/bact.2013_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/bact.2013_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

Simple Hypothesis Testing - Nematodes

```
#PERMANOVA Date|Plant|Management
str(nemaREL)
```

```
## 'data.frame':    65 obs. of  61 variables:
##  $ Year             : int  2011 2011 2011 2011 2011 2011 2011 2011 2011 2011 ...
##  $ Micro_ID         : Factor w/ 72 levels "micro-1","micro-10",..: 1 12 23 34 45 56 67 71 5 6 ...
##  $ Field.ID         : int  1 16 31 46 61 76 91 106 11 26 ...
##  $ Date             : Factor w/ 3 levels "11-Jun","12-Jun",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ Sample           : Factor w/ 24 levels "1-HA1","1-HA2",..: 17 9 1 18 10 2 19 11 21 13 ...
##  $ Depth            : Factor w/ 1 level "0-10cm": 1 1 1 1 1 1 1 1 1 1 ...
##  $ Management       : Factor w/ 3 levels "HighN","LowN",..: 3 2 1 3 2 1 3 2 3 2 ...
##  $ Plant            : Factor w/ 2 levels "Annual","IntWheatgrass": 1 1 1 1 1 1 1 1 2 2 ...
##  $ Block            : int  1 1 1 2 2 2 3 3 1 1 ...
##  $ Monhysteridae    : num  0.0233 0 0.0201 0.0228 0.0881 ...
```

```
##  $ Monhysteridae..  : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Panagrolaimus    : num  0.00465 0 0.00503 0.02283 0.05031 ...
##  $ Rhabditidae      : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Rhabditis        : num  0 0 0.0101 0.0137 0.0881 ...
##  $ Dauerlarvae      : num  0.0558 0 0 0.0685 0.0252 ...
##  $ Mesorhabditis    : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Diploscapter     : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Diplogasteridae  : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Acrobeles        : num  0 0 0 0.06849 0.00629 ...
##  $ Acrobeloides     : num  0.1721 0.1393 0.1357 0.1279 0.0881 ...
##  $ Chiloplacus      : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Cephalobidae     : num  0.00465 0.01639 0.00503 0.03196 0.01887 ...
##  $ Metacrolobus     : num  0 0 0 0.00457 0 ...
##  $ Plectus          : num  0 0.0082 0.0201 0 0 ...
##  $ AnaPlectus       : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Wilsonema        : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Prismatolaimus   : num  0 0 0.00503 0.04566 0 ...
##  $ Alaimus          : num  0.00465 0 0.00503 0.0137 0.01887 ...
##  $ Aphelenchus      : num  0.1023 0.0738 0.0955 0.0228 0.044 ...
##  $ Aphelenchoides   : num  0 0 0 0.0457 0.0314 ...
##  $ Aprutides        : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Tylenchidae      : num  0.335 0.082 0.397 0.1 0.27 ...
##  $ Diphterophora    : num  0.00465 0.03279 0.0201 0 0.00629 ...
##  $ Tylencholaimus   : num  0.01395 0 0 0.00913 0 ...
##  $ Tylencholaimellus: num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Gracilacus       : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Paratylenchus    : num  0 0.0082 0 0.0183 0 ...
##  $ Pratylenchus     : num  0.1163 0.4672 0.1407 0.3196 0.0629 ...
##  $ Meloigogyne      : num  0 0 0 0 0.00629 ...
##  $ Mesocriconema    : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Hopolaimus       : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Tylenchorhynchus : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Helicotylenchus  : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Paratrichodorus  : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Xiphinema        : num  0 0.01639 0.00503 0 0 ...
##  $ Chromadoridae    : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Achromadora      : num  0 0 0 0 0 ...
##  $ Dorylaimidae     : num  0.1163 0.1148 0.0804 0.0502 0.1761 ...
##  $ Qudsianematidae  : num  0 0 0.0101 0 0 ...
##  $ Thornematidae    : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Aporcelaimidae   : num  0.0186 0.03279 0.04523 0.00457 0.01258 ...
##  $ Mesodorylaimus   : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Tripyla          : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Tripylina        : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Mylonchulus      : num  0.00465 0 0 0 0 ...
##  $ Mononchus        : num  0.02326 0.0082 0 0.00913 0.00629 ...
##  $ Clarkus          : num  0 0 0 0 0 ...
##  $ Mononchidae      : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Granonchulus     : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Anatonchus       : num  0 0 0 0 0 0 0 0 0 0 ...
##  $ Discolaimus      : num  0 0 0 0 0 ...
```

```
adonis2 = adonis(nemaREL[,-c(1:9)] ~Block+Date*Management*Plant, method = "bray", data = nemaREL, perm=
adonis2
```

```
##
## Call:
## adonis(formula = nemaREL[, -c(1:9)] ~ Block + Date * Management *      Plant, data = nemaREL, permuta
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##                       Df SumsOfSqs MeanSqs F.Model      R2   Pr(>F)
## Block                  1    0.1843 0.18426  2.1896 0.02731 0.022977 *
## Date                   2    0.9286 0.46428  5.5170 0.13761 0.000999 ***
## Management             2    0.4542 0.22708  2.6983 0.06731 0.001998 **
## Plant                  1    0.4169 0.41694  4.9544 0.06179 0.000999 ***
## Date:Management        4    0.1756 0.04391  0.5218 0.02603 0.993007
## Date:Plant             2    0.2199 0.10997  1.3068 0.03260 0.168831
## Management:Plant       2    0.2650 0.13251  1.5746 0.03928 0.062937 .
## Date:Management:Plant  4    0.2319 0.05798  0.6889 0.03437 0.911089
## Residuals             46    3.8711 0.08416         0.57371
## Total                 64    6.7476                 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Nematode Ordinations

Principal Coordinates Ordination

Principal Coordinates Ordination - NEMATODES seaparate years

```
nemaREL.2011 <- subset(nemaREL, Year == 2011)
dim(nemaREL)
```

```
## [1] 65 61
```

```
dim(nemaREL.2011)
```

```
## [1] 17 61
```

```
# PERMANOVA - 2011 only
adonis(nemaREL.2011[,-c(1:9)]~Block+Management*Plant, data=nemaREL.2011)
```

```
##
## Call:
## adonis(formula = nemaREL.2011[, -c(1:9)] ~ Block + Management *      Plant, data = nemaREL.2011)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Block             1   0.14772 0.147722 1.64279 0.09953  0.114
## Management        2   0.16951 0.084756 0.94256 0.11421  0.524
```

```
## Plant             1   0.16219 0.162185 1.80362 0.10928  0.076 .
## Management:Plant  2   0.10552 0.052761 0.58675 0.07110  0.884
## Residuals        10   0.89922 0.089922         0.60588
## Total            16   1.48416                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
dataREL.dist <- vegdist(nemaREL.2011[,-c(1:9)], method="bray")

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #28.6
```

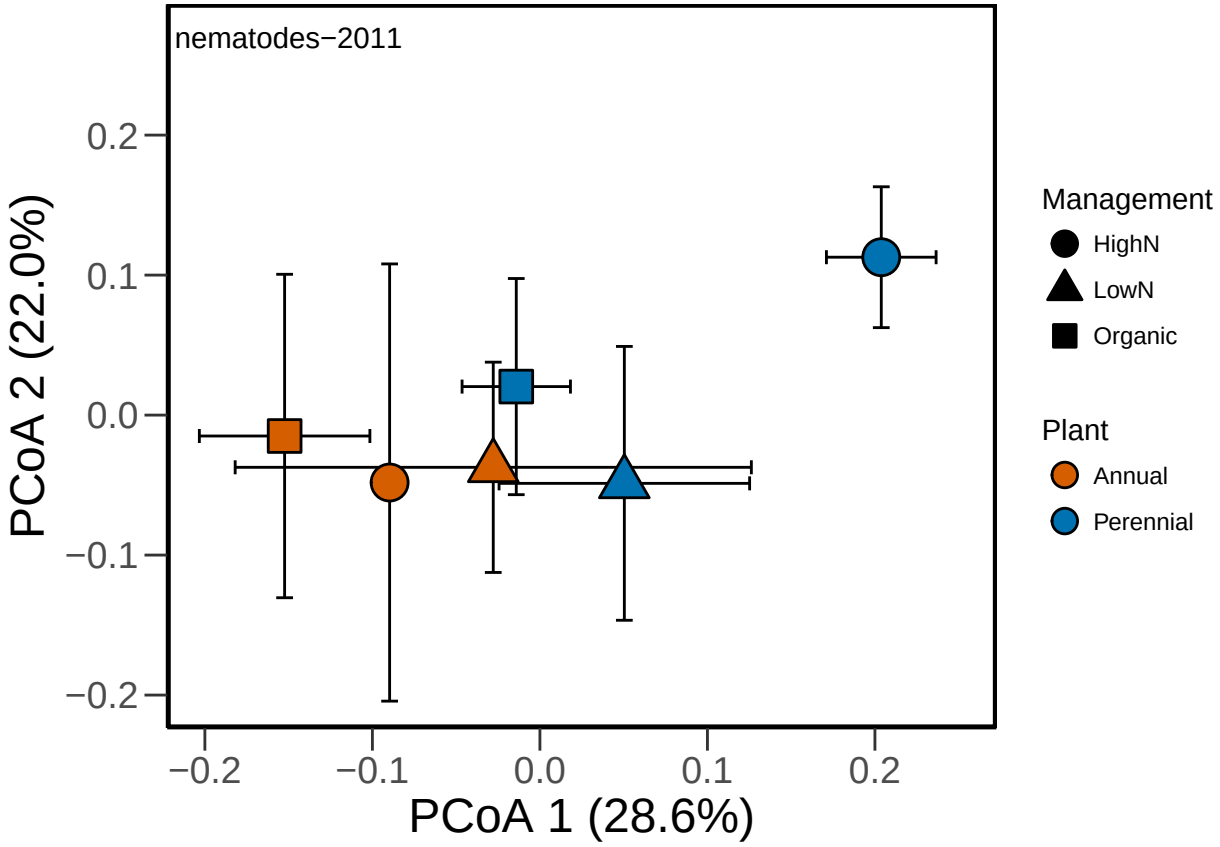
```
## [1] 28.6
```

```
explainvar2b #22.0
```

```
## [1] 22
```

```
# Principal Coordinates Analysis (Mgmt*Plant) - 2011 only
pcoa.groups <- paste(nemaREL.2011$Management, nemaREL.2011$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

#Plot
df7a <- as.data.frame(pcoa.cent.dataframe)
plot7a <- ggplot(df7a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
nema.2011<- plot7a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +
  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
```

```
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.25), ylim = c(-0.2, 0.27)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (28.6%)") + ylab("PCoA 2 (22.0%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.15, y = 0.27, label = "nematodes-2011")
nema.2011
```




```
ggsave("../figures/nema.2011_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/nema.2011_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
nemaREL.2012 <- subset(nemaREL, Year == 2012)
dim(nemaREL)
```

```
## [1] 65 61
```

```r
dim(nemaREL.2012)
```

```
## [1] 24 61
```

```r
# PERMANOVA  - 2012 only
adonis(nemaREL.2012[,-c(1:9)]~Block+Management*Plant, data=nemaREL.2012)
```

```
##
## Call:
## adonis(formula = nemaREL.2012[, -c(1:9)] ~ Block + Management *      Plant, data = nemaREL.2012)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                  Df SumsOfSqs  MeanSqs F.Model      R2 Pr(>F)
## Block             1   0.18669 0.186692 2.07835 0.08561  0.031 *
## Management        2   0.15376 0.076880 0.85587 0.07050  0.639
## Plant             1   0.15116 0.151159 1.68277 0.06931  0.096 .
## Management:Plant  2   0.16217 0.081086 0.90269 0.07436  0.580
## Residuals        17   1.52706 0.089827         0.70022
## Total            23   2.18084                  1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
dataREL.dist <- vegdist(nemaREL.2012[,-c(1:9)], method="bray")

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #25.8
```

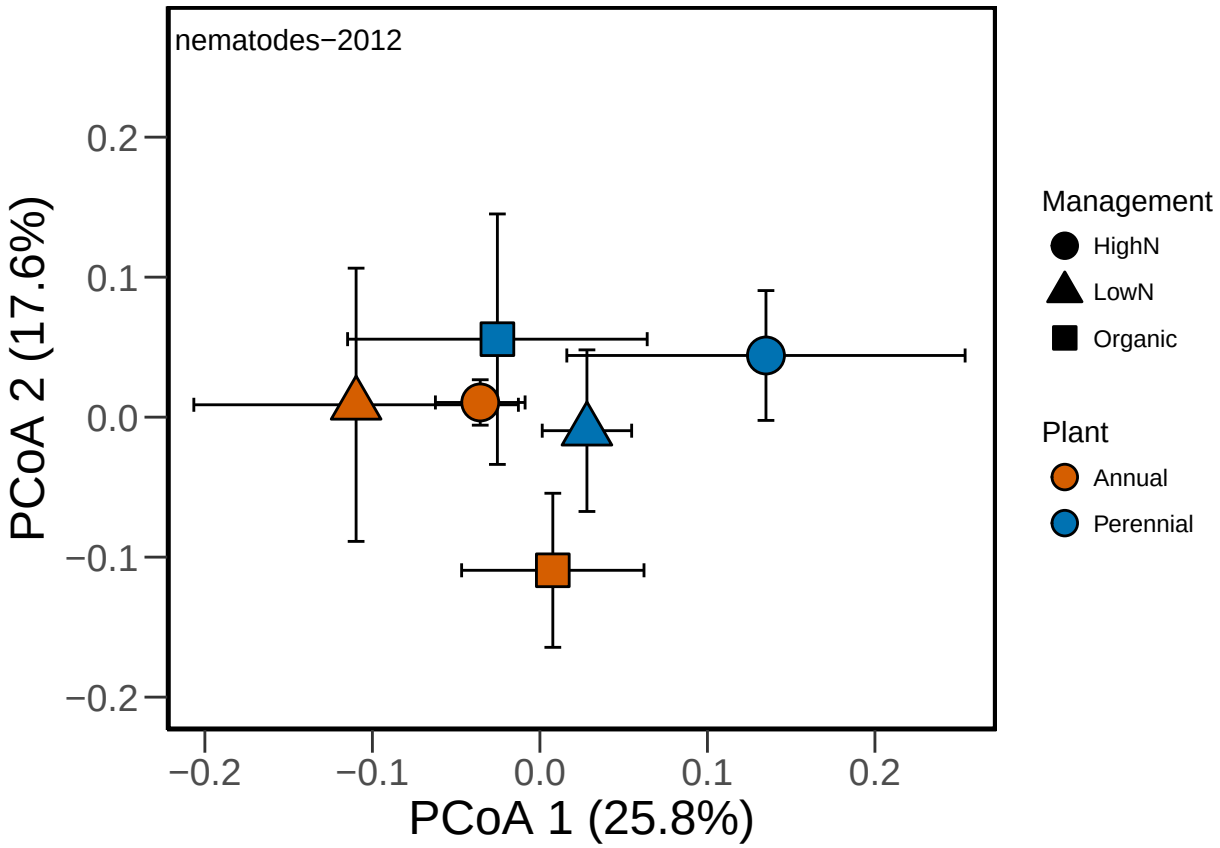
```
## [1] 25.8
```

```r
explainvar2b #17.6
```

```
## [1] 17.6
```

```r
# Principal Coordinates Analysis (Mgmt*Plant) - 2012 only
pcoa.groups <- paste(nemaREL.2012$Management, nemaREL.2012$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
```

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

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

#Plot
df6a <- as.data.frame(pcoa.cent.dataframe)
plot6a <- ggplot(df6a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
nema.2012<- plot6a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +
  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.25), ylim = c(-0.2, 0.27)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (25.8%)") + ylab("PCoA 2 (17.6%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.15, y = 0.27, label = "nematodes-2012")
nema.2012
```




```
ggsave("../figures/nema.2012_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/nema.2012_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
nemaREL.2013 <- subset(nemaREL, Year == 2013)

# PERMANOVA  - 2013 only
adonis(nemaREL.2013[,-c(1:9)]~Block+Management*Plant, data=nemaREL.2013)
```

```
##
## Call:
## adonis(formula = nemaREL.2013[, -c(1:9)] ~ Block + Management *      Plant, data = nemaREL.2013)
##
## Permutation: free
## Number of permutations: 999
##
## Terms added sequentially (first to last)
##
##                  Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
## Block             1   0.14070 0.14070  2.0469 0.06525  0.058 .
## Management        2   0.31947 0.15973  2.3238 0.14815  0.009 **
## Plant             1   0.30920 0.30920  4.4981 0.14338  0.001 ***
## Management:Plant  2   0.21851 0.10925  1.5894 0.10133  0.088 .
## Residuals        17   1.16858 0.06874         0.54190
## Total            23   2.15646                 1.00000
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
dataREL.dist <- vegdist(nemaREL.2013[,-c(1:9)], method="bray")

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

explainvar1b <- round(pcoa$eig[1] / sum(pcoa$eig), 3) * 100
explainvar2b <- round(pcoa$eig[2] / sum(pcoa$eig), 3) * 100
sum.eigb <- sum(explainvar1b, explainvar2b)

explainvar1b #27.0
```

```
## [1] 27
```

```
explainvar2b #24.2
```

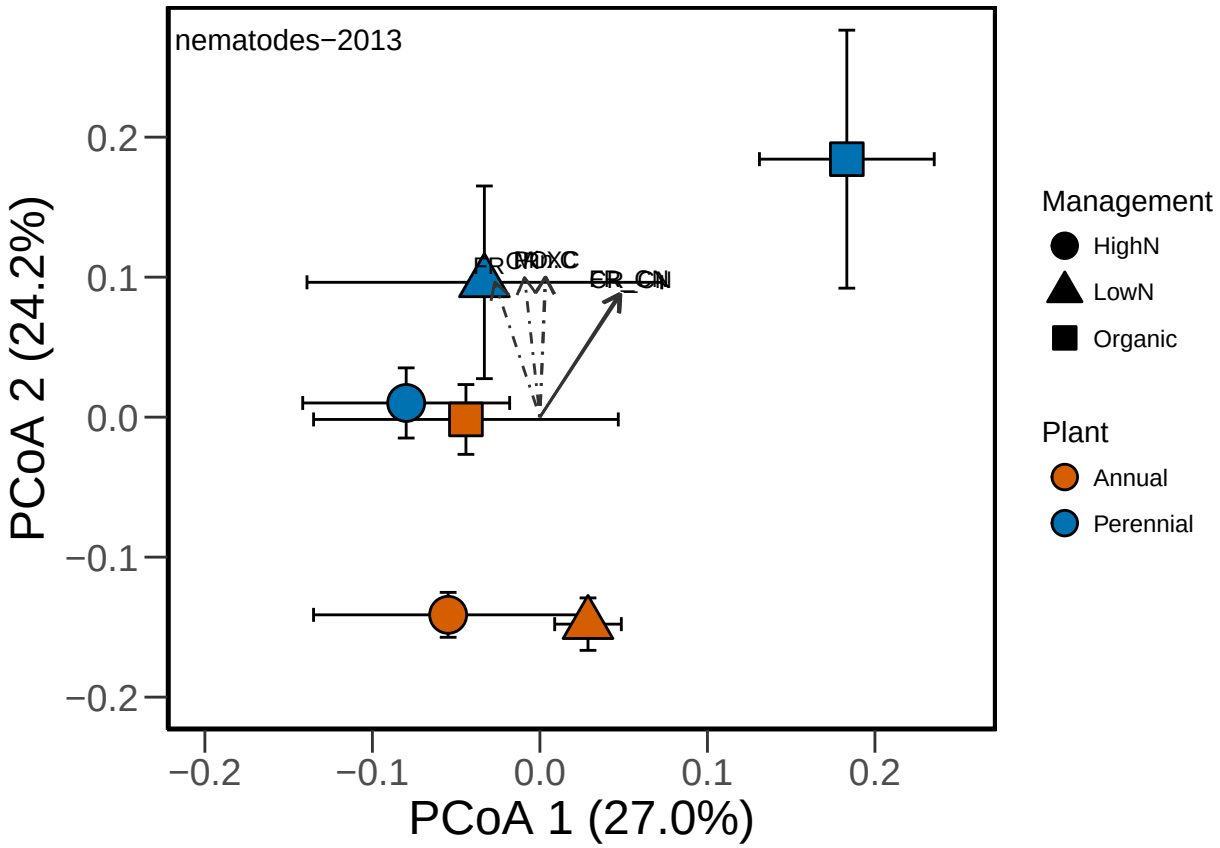
```
## [1] 24.2
```

```
# Principal Coordinates Analysis (Mgmt*Plant) - 2013 only
pcoa.groups <- paste(nemaREL.2013$Management, nemaREL.2013$Plant, sep = "_")
pcoa.points <- data.frame(pcoa$points, group = pcoa.groups)

# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable ~ group, sd)

# Combine
pcoa.cent.dataframe <- cbind(t(pcoa.centroids), t(pcoa.centroids.se))
colnames(pcoa.cent.dataframe) <- c("V1", "V2", "V1e", "V2e")
pcoa.cent.treats <- rownames(pcoa.cent.dataframe)

pcoa.col <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 2)) # Management
pcoa.shape <- as.factor(sapply(strsplit(pcoa.cent.treats, "_"), `[`, 1))  # Plant

# Updated by AP with env2013 data
env2013 <- read.csv("../data/EnvironronmentalFactors2013.csv", header=TRUE)
soil2013 <- env2013[,(9:14)]
fit <- envfit(pcoa,soil2013,perm=1000, na.rm=TRUE)
fit
```

```
##
## ***VECTORS
##
##           Dim1     Dim2     r2   Pr(>r)
## CR    -0.09149  0.99581 0.1871 0.124875
## FR    -0.26941  0.96303 0.0698 0.497502
## CR_CN  0.48298  0.87563 0.5643 0.000999 ***
## FR_CN  0.47323  0.88094 0.4497 0.003996 **
## POXC   0.03552  0.99937 0.0771 0.448551
## Min.C  0.03088  0.99952 0.1660 0.157842
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 1000
##
## 1 observation deleted due to missingness
```

```
fit.coords <- as.data.frame(fit$vectors$arrows) * 0.1

#Plot
df5a <- as.data.frame(pcoa.cent.dataframe)
plot5a <- ggplot(df5a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                 group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
nema.2013<- plot5a + theme(panel.grid.major = element_blank(),
               panel.grid.minor = element_blank(),
               axis.line = element_line(colour = "black")) +
theme(panel.background = element_blank()) +
  geom_errorbarh(aes(xmax=V1+V1e, xmin=V1-V1e, height=0.01), colour="black") +
  geom_errorbar(aes(ymax=V2+V2e, ymin=V2-V2e, width=0.01), colour="black") +
  geom_point(aes(fill=pcoa.col), colour = "black", size=6, stroke = 0.75) +

       # Added By Mario ####
  geom_segment(data=fit.coords,aes(x=0,xend=Dim1,y=0,yend=Dim2),
               arrow = arrow(length = unit(0.25, "cm")),
               colour="grey20", linetype=c(4,4,1,1,4,4), inherit.aes = FALSE) +
  geom_segment(data=fit.coords,aes(x=Dim1 * 0.98,xend=Dim1,y=Dim2 * 0.98,yend=Dim2),
               arrow = arrow(length = unit(0.25, "cm")),
               colour="grey20", inherit.aes = FALSE) +
  geom_text(data=fit.coords,aes(x=Dim1 * 1.125,y=Dim2 * 1.125, label=rownames(fit.coords)),
            size=3, inherit.aes = FALSE) +
  ####################

  scale_colour_manual(labels = c("Annual","Perennial"),
                      values = c("#D55E00", "#0072B2")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
                    values = c("#D55E00", "#0072B2")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
                     values = c(21, 24, 22)) +
  coord_cartesian(xlim = c(-0.2, 0.25), ylim = c(-0.2, 0.27)) +
  theme(axis.title = element_text(size=18), axis.text=element_text(size=14),
          axis.text.x = element_text(size=14),
          panel.border = element_rect(colour = "black", size=1.25)) +
  theme(axis.ticks.length=unit(0.3,"cm")) +
  xlab("PCoA 1 (27.0%)") + ylab("PCoA 2 (24.2%)") +
  labs(fill = "Plant", shape = "Management") +
  guides(fill = guide_legend(override.aes = list(pch=21, size = 4, colour="black")),
         shape = guide_legend(override.aes = list(size = 4, fill="black"))) +
         annotate("text", x = -0.15, y = 0.27, label = "nematodes-2013")
nema.2013
```




```
ggsave("../figures/nema.2013_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

```
ggsave("../figures/nema.2013_PlantMgmt.png", plot=last_plot(), device=NULL, path=NULL, scale=1, width=NA
```

```
## Saving 6.5 x 4.5 in image
```

Bacterial community indicator species analysis 2013 only - plant

Nematode community indicator species analysis 2013 only - plant and mgmt