

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

*Christine D. Sprunger, Ariane L. Peralta, Steven Culman, Jay T. Lennon, Sieglinde S. Snapp*

*Last updated on 13 February, 2018*

Project Description: Fill out

## Initial Setup

## Import Files

### Environmental Data

```
# Import Environmental Data
design <- read.csv("../data/PWES_KBS_design.csv", header=TRUE)
```

### Nematode Data

```
# Import Environmental Data
nema <- read.csv("../data/PWES_nema.csv", header=TRUE)
nema <- na.omit(nema)
dim(nema)

## [1] 66 61

nema.design <- nema[,c(1:9)]
nema.comm <- nema[,-c(1:9)]
dim(nema.comm)

## [1] 66 52

# Remove taxa with less than one occurrences across all sites
nema.comm.a <- nema.comm[, which(colSums(nema.comm) >= 1)]
dim(nema.comm.a)

## [1] 66 37

# Make Relative Abundance Matrices
dataRELnema <- nema.comm.a
for(i in 1:dim(nema.comm.a)[1]){
  dataRELnema[i,] <- nema.comm.a[i,]/sum(nema.comm.a[i,])
}
dim(dataRELnema)

## [1] 66 37

nemaREL <- cbind(nema.design,dataRELnema)
dim(nemaREL)
```

```
## [1] 66 46
```

## 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)
```

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

```
##
```

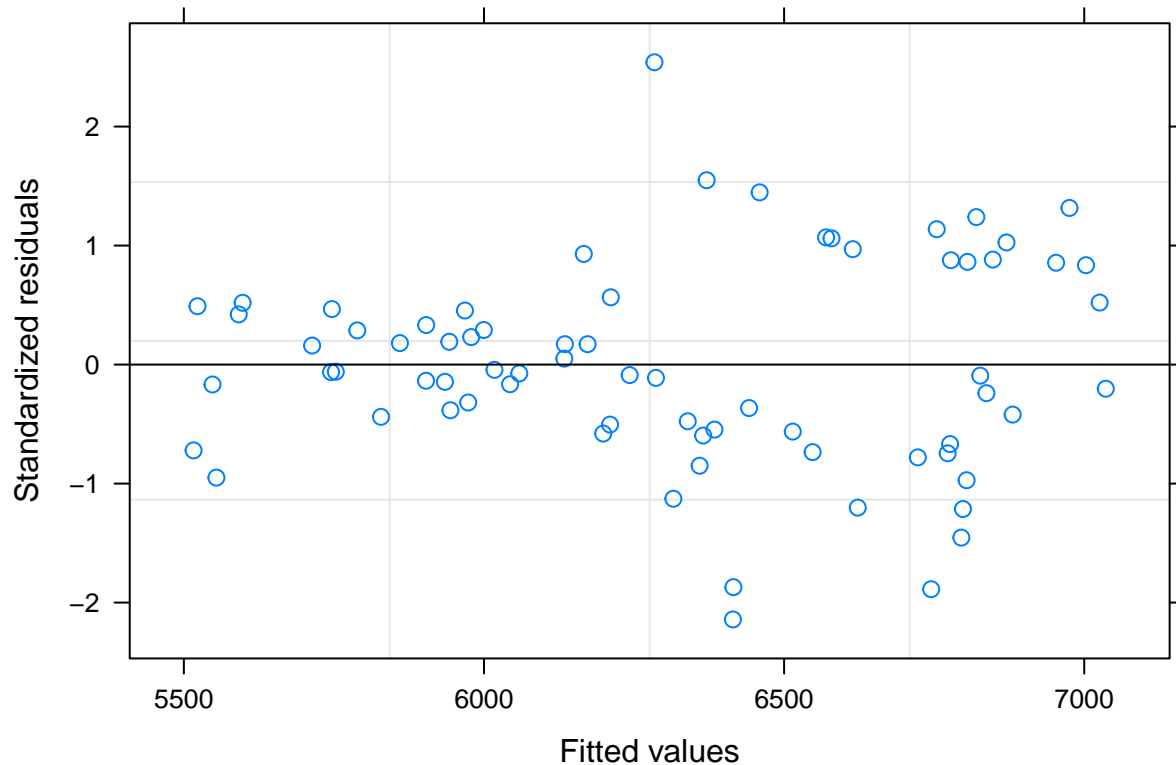
```
## Attaching package: 'Matrix'
```

```
## 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

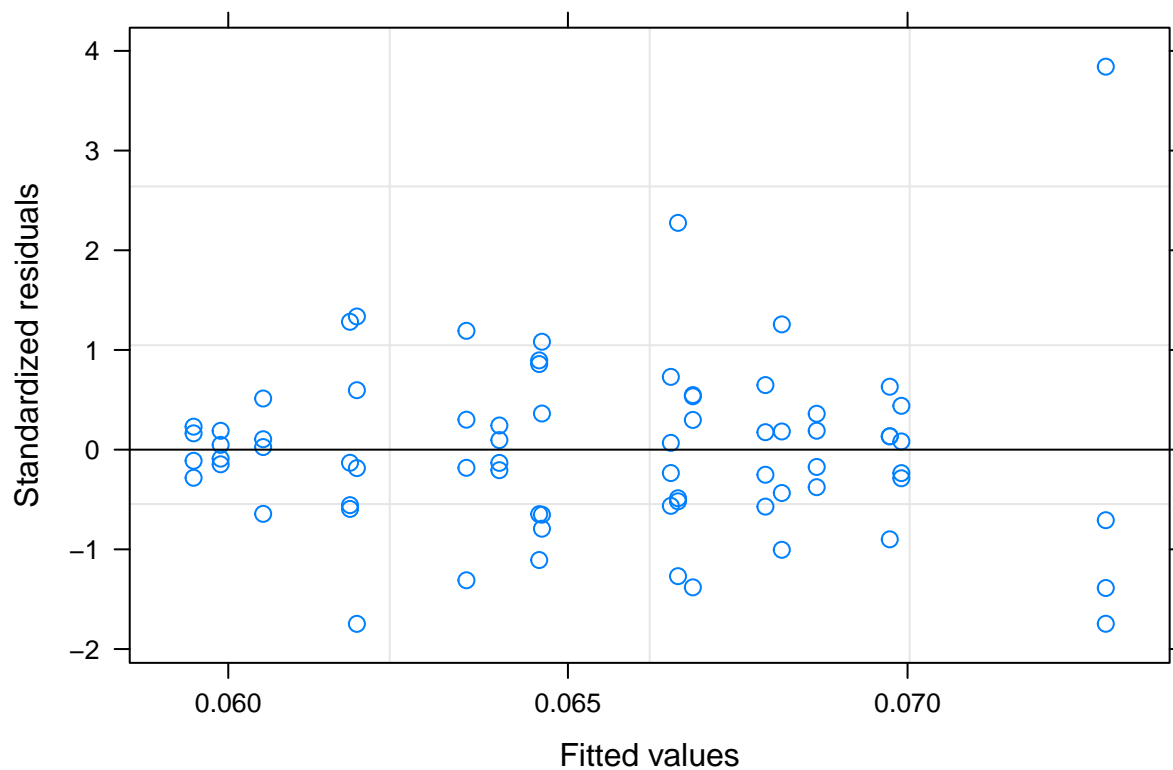
## Linear mixed-effects model fit by REML
## Data: diversity
## Log-restricted-likelihood: -437.3691
## Fixed: richness ~ Date * Management * Plant
##              (Intercept)
##              5600.50
##              DateJun-12
##              426.00
##              DateJun-13
##              823.25
##              ManagementLowN
##              6.50
##              ManagementOrganic
##              452.00
##              PlantIntWheatgrass
##              387.25
##              DateJun-12:ManagementLowN
##              335.25
##              DateJun-13:ManagementLowN
```

```
##                                449.25
##      DateJun-12:ManagementOrganic
##                                -183.00
##      DateJun-13:ManagementOrganic
##                                -19.00
##      DateJun-12:PlantIntWheatgrass
##                                218.00
##      DateJun-13:PlantIntWheatgrass
##                                -592.00
##      ManagementLowN:PlantIntWheatgrass
##                                -196.25
##      ManagementOrganic:PlantIntWheatgrass
##                                -527.00
##      DateJun-12:ManagementLowN:PlantIntWheatgrass
##                                -309.00
##      DateJun-13:ManagementLowN:PlantIntWheatgrass
##                                411.25
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                                455.50
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                                682.00
##
## Random effects:
## Formula: ~1 | Block
##      (Intercept) Residual
## StdDev:      145.0771 620.4259
##
## Number of Observations: 72
## Number of Groups: 4
```

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

```
##      numDF denDF  F-value p-value
## (Intercept)      1    51 3758.256 <.0001
## Date          2    51  12.040 0.0001
## Management    2    51   1.526 0.2272
## Plant         1    51   1.186 0.2813
## Date:Management 4    51   0.599 0.6653
## Date:Plant      2    51   0.955 0.3917
## Management:Plant 2    51   0.126 0.8822
## Date:Management:Plant 4    51   0.348 0.8444
```

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



```
evenness.lm
```

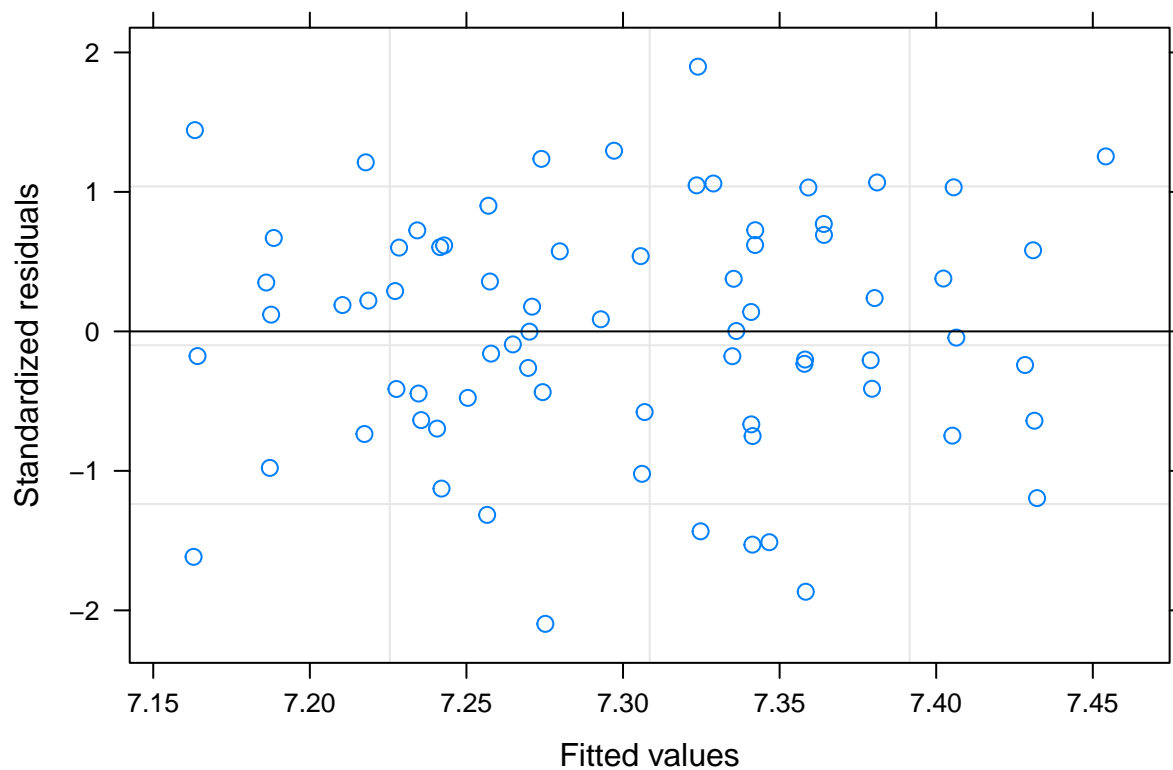
```
## Linear mixed-effects model fit by REML
## Data: diversity
## Log-restricted-likelihood: 173.6959
## Fixed: simp.even ~ Date * Management * Plant
##              (Intercept)
##              0.0681490363
##              DateJun-12
##              -0.0046430325
##              DateJun-13
##              -0.0082615881
##              ManagementLowN
##              -0.0002414019
##              ManagementOrganic
##              -0.0076356449
##              PlantIntWheatgrass
##              0.0017596198
##              DateJun-12:ManagementLowN
##              -0.0013713740
##              DateJun-13:ManagementLowN
##              -0.0001570066
##              DateJun-12:ManagementOrganic
##              0.0106444857
##              DateJun-13:ManagementOrganic
##              0.0123659814
##              DateJun-12:PlantIntWheatgrass
##              -0.0034737315
##              DateJun-13:PlantIntWheatgrass
##              0.0112710764
```

```
##           ManagementLowN:PlantIntWheatgrass
##                                     -0.0028281857
##           ManagementOrganic:PlantIntWheatgrass
##                                     0.0063893845
##   DateJun-12:ManagementLowN:PlantIntWheatgrass
##                                     0.0092697098
##   DateJun-13:ManagementLowN:PlantIntWheatgrass
##                                     -0.0057010057
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                                     -0.0066130065
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                                     -0.0142998730
##
## Random effects:
## Formula: ~1 | Block
##           (Intercept)      Residual
## StdDev: 2.736817e-07 0.007699659
##
## Number of Observations: 72
## Number of Groups: 4
```

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

	numDF	denDF	F-value	p-value
## (Intercept)	1	51	5197.382	<.0001
## Date	2	51	0.849	0.4337
## Management	2	51	0.287	0.7515
## Plant	1	51	3.976	0.0515
## Date:Management	4	51	0.922	0.4584
## Date:Plant	2	51	1.343	0.2701
## Management:Plant	2	51	0.070	0.9326
## Date:Management:Plant	4	51	0.964	0.4351

```
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: 41.29656
## Fixed: shannon ~ Date * Management * Plant
##              (Intercept)
##              7.193441699
##              DateJun-12
##              0.047080651
##              DateJun-13
##              0.086712706
##              ManagementLowN
##              -0.024342192
##              ManagementOrganic
##              0.030212517
##              PlantIntWheatgrass
##              0.082486769
##              DateJun-12:ManagementLowN
##              0.046647116
##              DateJun-13:ManagementLowN
##              0.091311181
##              DateJun-12:ManagementOrganic
##              0.070399949
##              DateJun-13:ManagementOrganic
##              0.074932962
##              DateJun-12:PlantIntWheatgrass
##              -0.011148069
##              DateJun-13:PlantIntWheatgrass
##              -0.032914641
```

```
##           ManagementLowN:PlantIntWheatgrass
##                               -0.018131711
##           ManagementOrganic:PlantIntWheatgrass
##                               -0.058299982
##   DateJun-12:ManagementLowN:PlantIntWheatgrass
##                               0.031140590
##   DateJun-13:ManagementLowN:PlantIntWheatgrass
##                               0.032820028
## DateJun-12:ManagementOrganic:PlantIntWheatgrass
##                               0.009969838
## DateJun-13:ManagementOrganic:PlantIntWheatgrass
##                               0.060592005
##
## Random effects:
## Formula: ~1 | Block
##      (Intercept)   Residual
## StdDev:  0.01755267 0.08806107
##
## Number of Observations: 72
## Number of Groups: 4
```

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

	numDF	denDF	F-value	p-value
## (Intercept)	1	51	288485.24	<.0001
## Date	2	51	15.72	<.0001
## Management	2	51	2.96	0.0607
## Plant	1	51	7.61	0.0080
## Date:Management	4	51	1.02	0.4056
## Date:Plant	2	51	0.00	0.9963
## Management:Plant	2	51	0.34	0.7111
## Date:Management:Plant	4	51	0.09	0.9864

```
#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)
```



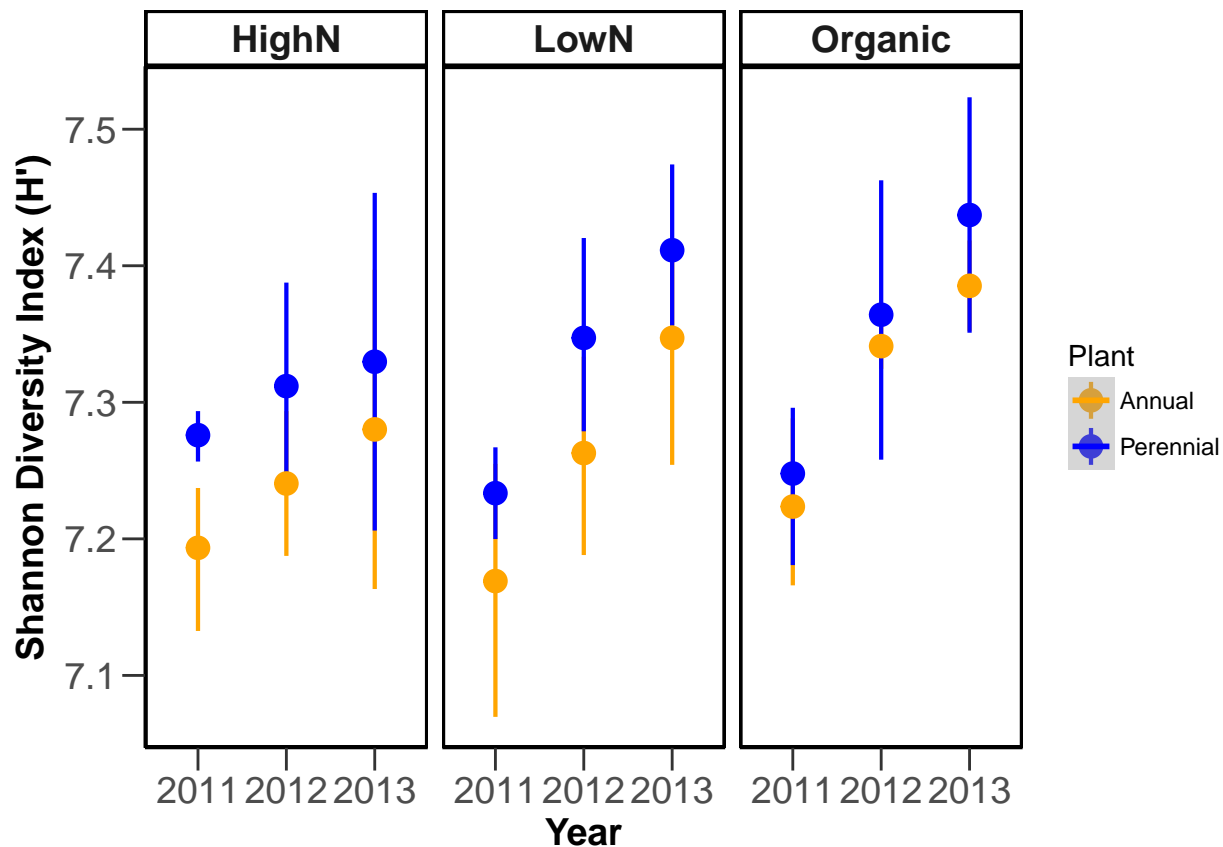
```
## [1] 24 13
```

```
summary <- diversity.1 %>% group_by(Management, Plant) %>% summarise(mean.richness=mean(richness), se.richness=sd(richness)/sqrt(n()),  
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          6424          533          7.28      0.0739  
## 2 HighN      IntWheatgr~    6219          189          7.33      0.0731  
## 3 LowN       Annual          6880          365          7.35      0.0495  
## 4 LowN      IntWheatgr~    6890          163          7.41      0.0323  
## 5 Organic   Annual          6857          349          7.39      0.0207  
## 6 Organic   IntWheatgr~    6807          386          7.44      0.0535
```

## 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", values=c("HighN"="red", "LowN"="blue", "Organic"="green"))  
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)] ~ Date*Management*Plant+Block, method = "bray", data = new.data, perm=999)
adonis
```

```
##
## Call:
## adonis(formula = new.data[, -c(1:9)] ~ Date * Management * Plant + Block, data = new.data, perm=999)
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##              Df SumsOfSqs  MeanSqs F.Model    R2  Pr(>F)
## Date           2    0.4027  0.201328   3.6942 0.09233 0.000999 ***
## Management     2    0.2698  0.134918   2.4756 0.06188 0.002997 **
## Plant          1    0.1084  0.108392   1.9889 0.02486 0.018981 *
## Block          1    0.0783  0.078300   1.4367 0.01795 0.091908 .
## Date:Management 4    0.1796  0.044909   0.8241 0.04119 0.866134
```

```
## Date:Plant      2      0.1342 0.067076  1.2308 0.03076 0.137862
## Management:Plant 2      0.1323 0.066139  1.2136 0.03033 0.167832
## Date:Management:Plant 4      0.1673 0.041820  0.7674 0.03836 0.958042
## 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
```

```
new.data.2011 <- subset(new.data, Year == 2011)
```

```
adonis.2011 = adonis(new.data.2011[, -c(1:9)] ~ Management*Plant+Block, method = "bray", data = new.data.2011)
adonis.2011
```

```
##
```

```
## Call:
```

```
## adonis(formula = new.data.2011[, -c(1:9)] ~ Management * Plant +      Block, 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)
## Management	2	0.07623	0.038117	0.72092	0.06545	0.8102
## Plant	1	0.04778	0.047775	0.90359	0.04102	0.4366
## Block	1	0.04654	0.046544	0.88030	0.03996	0.4486
## Management:Plant	2	0.09531	0.047653	0.90127	0.08183	0.4865
## Residuals	17	0.89884	0.052873		0.77174	
## Total	23	1.16469			1.00000	

```
new.data.2012 <- subset(new.data, Year == 2012)
```

```
adonis.2012 = adonis(new.data.2012[, -c(1:9)] ~ Management*Plant+Block, method = "bray", data = new.data.2012)
adonis.2012
```

```
##
```

```
## Call:
```

```
## adonis(formula = new.data.2012[, -c(1:9)] ~ Management * Plant +      Block, 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)
## Management	2	0.19234	0.096172	1.71997	0.14055	0.03497 *
## Plant	1	0.07910	0.079096	1.41459	0.05780	0.13487
## Block	1	0.04580	0.045801	0.81911	0.03347	0.60539
## Management:Plant	2	0.10070	0.050351	0.90050	0.07359	0.57542
## 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
```

```
new.data.2013 <- subset(new.data, Year == 2013)
```

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

```
##
```

```
## Call:
## adonis(formula = new.data.2013[, -c(1:9)] ~ Management * Plant + Block, data = new.data.2013, p
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##              Df SumsOfSqs  MeanSqs F.Model    R2  Pr(>F)
## Management    2   0.18090  0.090448  1.62803  0.12694  0.01499 *
## Plant          1   0.11567  0.115672  2.08206  0.08117  0.01199 *
## Block          1   0.08052  0.080516  1.44926  0.05650  0.09790 .
## Management:Plant 2   0.10355  0.051775  0.93193  0.07266  0.58541
## 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
```

## Microbial Ordinations

### Principal Coordinates Ordination

```
# Principal Coordinates Analysis
dataREL.dist <- vegdist(dataREL, 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 #23.1

## [1] 23.1
explainvar2b #9.1

## [1] 9.1

# Principal Coordinates Analysis Mgmt*Date
all.equal(rownames(design), rownames(dataREL))

## [1] TRUE

pcoa.groups <- paste(new.data$Management, new.data$Date, 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)
```

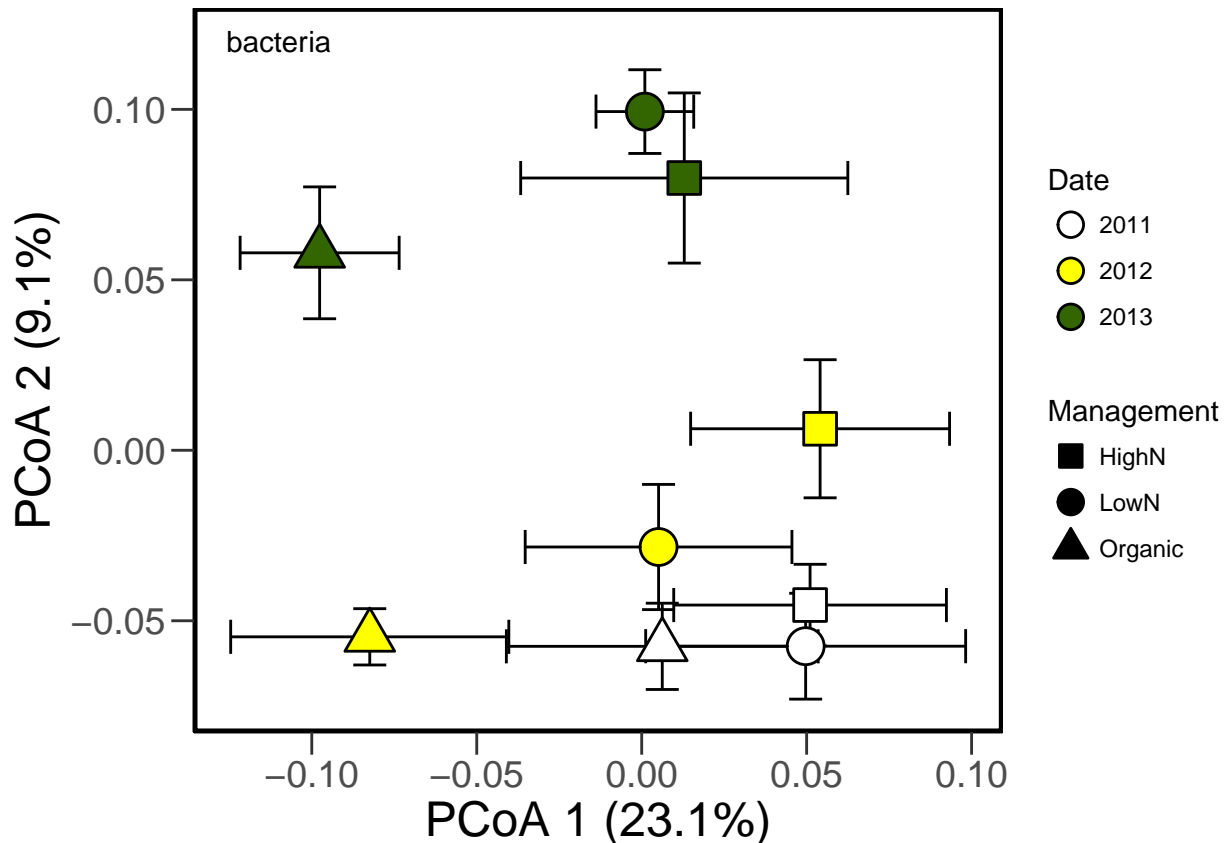
```

# 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)) # Date

# Principal Coordinates Analysis (Mgmt*Date)
df1a <- as.data.frame(pcoa.cent.dataframe)
plot1a <- ggplot(df1a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                          group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
plot1a + 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("2011", "2012", "2013"),
                      values = c("#FFFFFF", "#FFFF00", "#336600")) +
  scale_fill_manual(labels = c("2011", "2012", "2013"),
                    values = c("#FFFFFF", "#FFFF00", "#336600")) +
  scale_shape_manual(labels = c("HighN", "LowN", "Organic"),
                     values = c(22, 21, 24)) +
#coord_cartesian(xlim = c(-0.25, 0.5), ylim = c(-0.35, 0.3)) +
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 (23.1%)") + ylab("PCoA 2 (9.1%)") +
labs(fill = "Date", 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 = -0.11, y = 0.12, label = "bacteria")

```



```
ggsave("../figures/16SrRNA_PWES_YrMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=N
```

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

```
# Principal Coordinates Analysis (Mgmt*Plant)
all.equal(rownames(design), rownames(dataREL))
```

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

```
pcoa.groups <- paste(new.data$Management, new.data$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
```

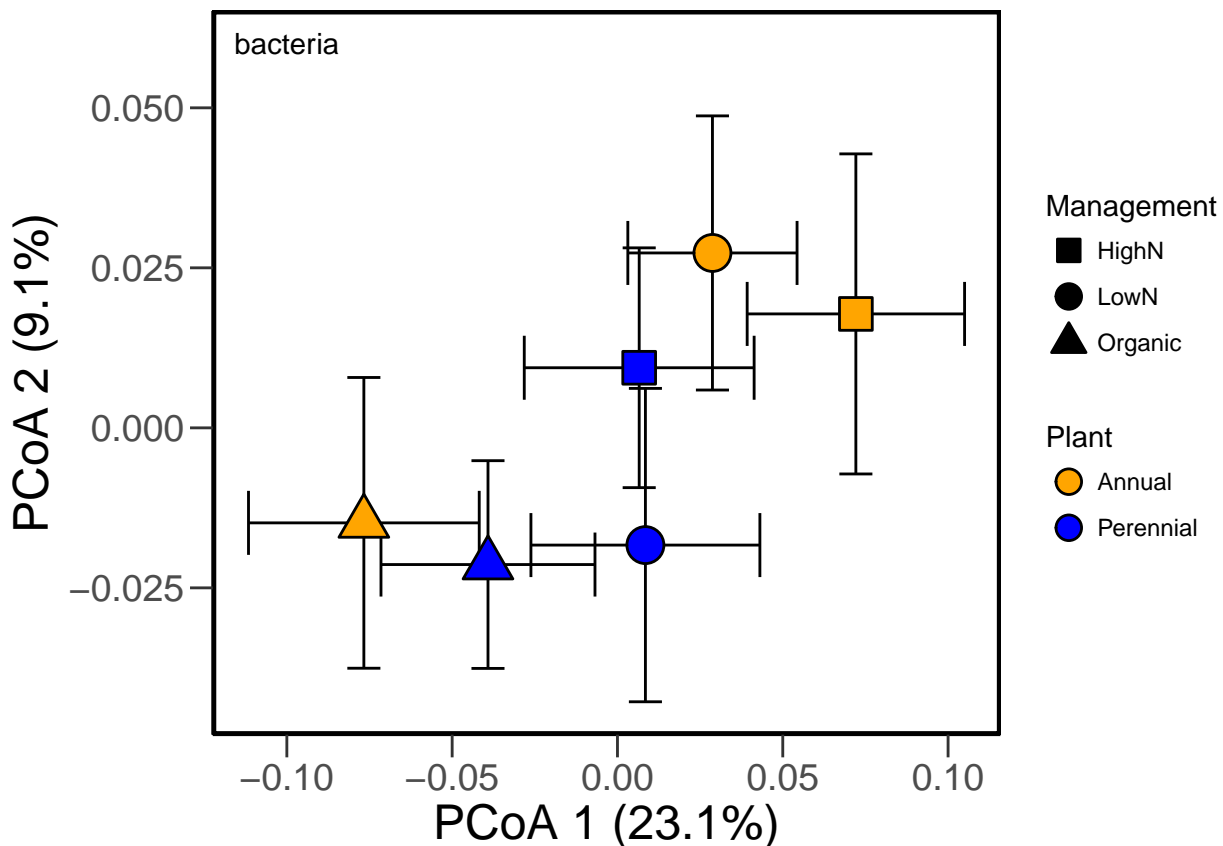
```
#Plot
```

```
df2a <- as.data.frame(pcoa.cent.dataframe)
```

```

plot2a <- ggplot(df2a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
  group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
plot2a + 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("orange", "blue")) +
  scale_fill_manual(labels = c("Annual", "Perennial"),
    values = c("orange", "blue")) +
  scale_shape_manual(labels = c("HighN", "LowN", "Organic"),
    values = c(22, 21, 24)) +
  #coord_cartesian(xlim = c(-0.25, 0.5), ylim = c(-0.35, 0.3)) +
  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 (23.1%)") + ylab("PCoA 2 (9.1%)") +
  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 = -0.10, y = 0.06, label = "bacteria")

```



```
ggsave("../figures/16SrRNA_PWES_PlantMgmt.pdf", plot=last_plot(), device=NULL, path=NULL, scale=1, width=
```

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

## Simple Hypothesis Testing - Nematodes

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

```
## 'data.frame': 66 obs. of 46 variables:  
## $ Micro_ID : Factor w/ 72 levels "micro-1","micro-10",...: 1 12 23 34 45 56 67 71 72 5 ...  
## $ Field.ID : int 1 16 31 46 61 76 91 106 121 11 ...  
## $ 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 3 21 ...  
## $ 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 1 3 ...  
## $ Plant : Factor w/ 2 levels "Annual","Perennial": 1 1 1 1 1 1 1 1 1 2 ...  
## $ Year : int 2011 2011 2011 2011 2011 2011 2011 2011 2011 2011 ...  
## $ Block : Factor w/ 4 levels "I","II","III",...: 1 1 1 2 2 2 3 3 3 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 ...  
## $ Acrobeles : num 0 0 0 0.06849 0.00629 ...  
## $ Acrobeloides : num 0.1721 0.1393 0.1357 0.1279 0.0881 ...  
## $ 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 ...  
## $ 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 ...  
## $ 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 ...  
## $ Tylenchorhynchus : num 0 0 0 0 0 0 0 0 0 0 ...  
## $ Xiphinema : num 0 0.01639 0.00503 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 ...  
## $ Aporcelaimidae : num 0.0186 0.03279 0.04523 0.00457 0.01258 ...  
## $ Tripyla : 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 ...
## $ Discolaimus     : num  0 0 0 0 0 ...

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

##
## Call:
## adonis(formula = nemaREL[, -c(1:9)] ~ Date * Management * Plant +      Block, data = nemaREL, permut
##
## Permutation: free
## Number of permutations: 1000
##
## Terms added sequentially (first to last)
##
##              Df SumsOfSqs MeanSqs F.Model      R2    Pr(>F)
## Date          2    0.9454 0.47269  5.1880 0.12393 0.000999 ***
## Management    2    0.4685 0.23423  2.5708 0.06141 0.000999 ***
## Plant         1    0.4133 0.41330  4.5362 0.05418 0.000999 ***
## Block         3    0.6045 0.20150  2.2115 0.07924 0.000999 ***
## Date:Management 4    0.2490 0.06225  0.6832 0.03264 0.950050
## Date:Plant      2    0.2794 0.13969  1.5331 0.03662 0.062937 .
## Management:Plant 2    0.2884 0.14418  1.5825 0.03780 0.038961 *
## Date:Management:Plant 4    0.2799 0.06998  0.7680 0.03669 0.852148
## Residuals      45    4.1000 0.09111      0.53748
## Total          65    7.6283      1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Nematode Ordinations

### Principal Coordinates Ordination

```
# Principal Coordinates Analysis
# nema.comm.a is relativized nematode community
dataREL.dist <- vegdist(nema.comm.a, 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 #17.3

## [1] 17.3
explainvar2b #13.6

## [1] 13.6
```

```

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

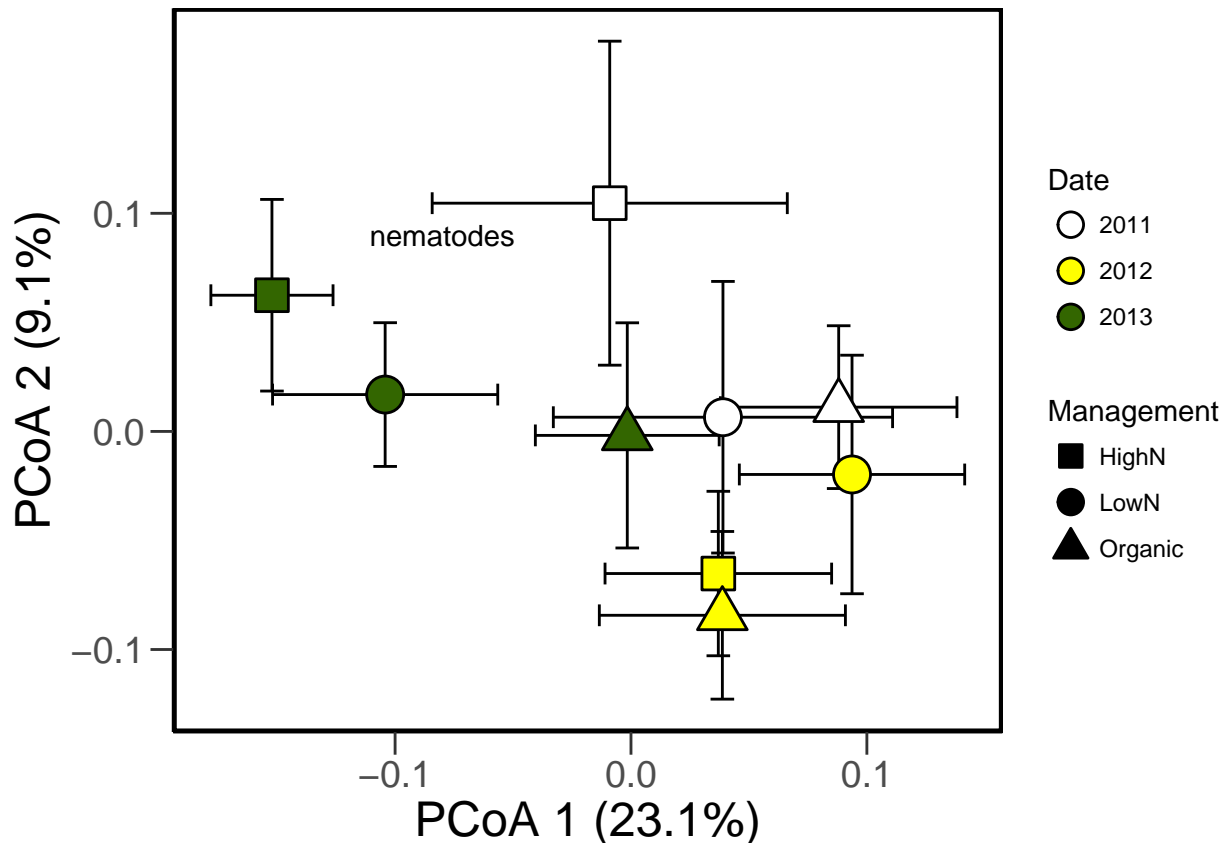
# Calculate Centroids (mean and SE)
pcoa.L.centroids <- melt(pcoa.points, id="group", measure.vars = c("X1", "X2"))
pcoa.centroids <- acast(pcoa.L.centroids, variable ~ group, mean)
pcoa.centroids.se <- acast(pcoa.L.centroids, variable ~ group, se)
pcoa.centroids.sd <- acast(pcoa.L.centroids, variable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

# Principal Coordinates Analysis (Mgmt*Date)
df3a <- as.data.frame(pcoa.cent.dataframe)
plot3a <- ggplot(df3a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
                          group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
plot3a + 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("2011", "2012", "2013"),
                      values = c("#FFFFFF", "#FFFF00", "#336600")) +
  scale_fill_manual(labels = c("2011", "2012", "2013"),
                    values = c("#FFFFFF", "#FFFF00", "#336600")) +
  scale_shape_manual(labels = c("HighN", "LowN", "Organic"),
                     values = c(22, 21, 24)) +
#coord_cartesian(xlim = c(-0.25, 0.5), ylim = c(-0.35, 0.3)) +
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 (23.1%)") + ylab("PCoA 2 (9.1%)") +
labs(fill = "Date", 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 = -0.08, y = 0.09, label = "nematodes")

```



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

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

```
# Principal Coordinates Analysis (Mgmt*Plant)
```

```
pcoa.groups <- paste(nemaREL$Management, nemaREL$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
```

```
df4a <- as.data.frame(pcoa.cent.dataframe)
```

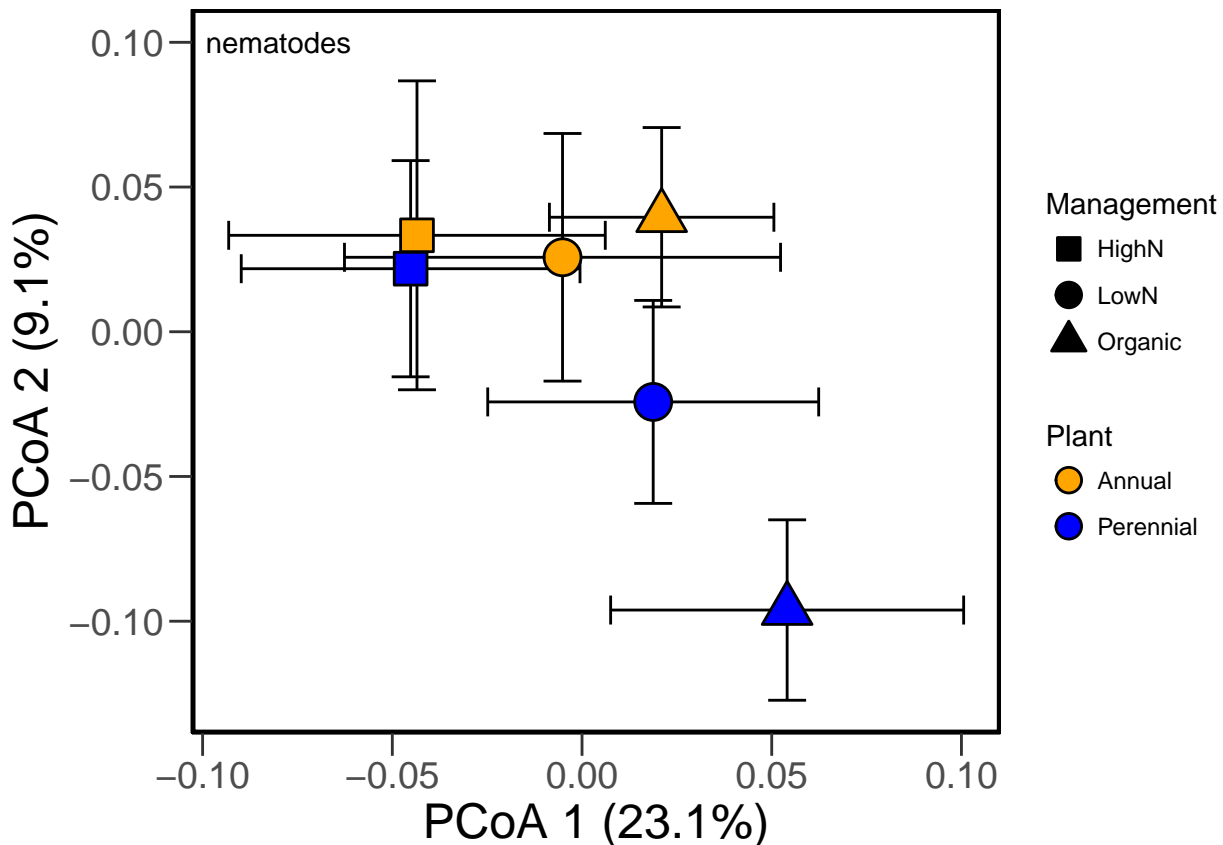
```
plot4a <- ggplot(df4a, aes(x=V1, y=V2, colour=pcoa.col, shape = pcoa.shape,
  group = interaction(pcoa.col, pcoa.shape))) + theme_bw()
```

```
plot4a + 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("orange", "blue")) +
  scale_fill_manual(labels = c("Annual","Perennial"),
    values = c("orange", "blue")) +
  scale_shape_manual(labels = c("HighN","LowN","Organic"),
    values = c(22, 21, 24)) +
  #coord_cartesian(xlim = c(-0.25, 0.5), ylim = c(-0.35, 0.3)) +
  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 (23.1%)") + ylab("PCoA 2 (9.1%)") +
  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 = -0.08, y = 0.1, label = "nematodes")

```



```

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

```

```

## Saving 6.5 x 4.5 in image

```