Field Old and Young Leaves Secondary Metabolites Analysis

Kenia E. Segura Aba

4/30/2020

This file was run in R version 3.5.3. The packages used are `tidyverse` version 1.3.0, `readr` version 1.3.1, `RRPP` version 0.4.2.9000, `mixOmics` version 6.6.2, and `labdsv` version 2.0-1. The following analysis of secondary metabolites was conducted using a split-plot analysis of variance (ANOVA) of Young and Old *P. virgatum* leaves using residual randomization permutation procedure (`RRPP`). Patterns in metabolite classification were visualized using `mixOmics` for principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Dufrene-Legendre indicator analysis was performed to identify specific metabolites indicative of plant response to water treatment and fungal treatment (`labdsv`).

1. Load necessary packages

```
library(tidyverse)
library(readr)
library(RRPP)
library(mixOmics)
library(labdsv)
```

Secondary Metabolites (Neg)

RRPP

2. Define dependent variable matrix and class matrix.

```
path <- "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/"

O_SM_neg <- read_tsv(paste(path, "XCMS Online Results/O_SM_Neg/XCMS.annotated.Report_1394387.tsv",
    sep = ""))

Y_SM_neg <- read_tsv(paste(path, "XCMS Online Results/Y_SM_Neg/XCMS.annotated.Report_1394397.tsv",
    sep = ""))

# dependent variable: metabolite intensities
Y_old <- O_SM_neg[, c(2, 12:54)] %>% data.frame(row.names = 1) %>%
    t %>% data.frame()
scaled_Y_old <- scale(Y_old)

Y_young <- Y_SM_neg[, c(2, 12:54)] %>% data.frame(row.names = 1) %>%
    t %>% data.frame()
scaled_Y_young <- scale(Y_young)

# class: sample factors
class <- read.csv(paste(path, "XCMS Online Results/class.csv",
    sep = ""), header = T, row.names = 1)
```

3. Define and run multivariate regression models, then print out the results.

```
O_LMneg <- lm.rrpp(scaled_Y_old ~ Block * Water * Fungus, data = class,
    SS.type = "III", print.progress = F)
summary(O_LMneg)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 3734
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Full Model Analysis of Variance
##
##                         Df Residual Df       SS Residual SS       Rsq        F
## Block * Water * Fungus  7          35 46010.13    110817.9 0.2933796 2.075935
##                         Z (from F) Pr(>F)
## Block * Water * Fungus   4.423368  0.001
##
##
## Redundancy Analysis (PCA on fitted values and residuals)
##
##              Trace Proportion Rank
## Fitted    1095.479  0.2933795    7
## Residuals 2638.521  0.7066205   35
## Total     3734.000  1.0000000   42
##
## Eigenvalues
##
##                 PC1      PC2      PC3      PC4      PC5      PC6      PC7
## Fitted     564.1858 153.9587 125.7033  96.0683  72.5213  48.1336  34.9085
## Residuals  603.9575 244.3193 216.0016 156.1993 129.7743 107.1920 106.3001
## Total     1006.1235 296.2684 272.7497 229.4345 201.7779 159.0190 137.0564
##                 PC8      PC9     PC10     PC11     PC12     PC13     PC14
## Fitted
## Residuals   84.3344  83.2693  73.9679  64.4639  60.8570  54.7399  50.4119
## Total      117.3648  99.5651  83.3967  82.1249  73.8888  70.7485  62.0878
##                PC15     PC16     PC17     PC18     PC19     PC20     PC21
## Fitted
## Residuals   48.0455  45.1743  44.2448  38.6085  36.2209  33.6145  32.5220
## Total       56.5008  54.4177  49.8007  46.9897  42.8943  41.9063  38.2559
##                PC22     PC23     PC24     PC25     PC26     PC27     PC28
## Fitted
## Residuals   31.0776  29.3703  28.4226  26.8550  25.5566  24.1440  23.7667
## Total       37.3648  34.9422  32.5619  31.2924  30.5917  28.3578  27.7198
##                PC29     PC30     PC31     PC32     PC33     PC34     PC35
## Fitted
## Residuals   21.6762  21.4874  20.8524  20.3145  18.5993  17.4643  14.7149
## Total       26.3828  26.1749  24.7238  23.5483  22.9331  22.1958  21.1991
##                PC36     PC37     PC38     PC39     PC40     PC41     PC42
## Fitted
## Residuals
```

```
## Total       19.8343   19.0164   18.6900   17.8323   17.2688   15.2883   13.7105
```

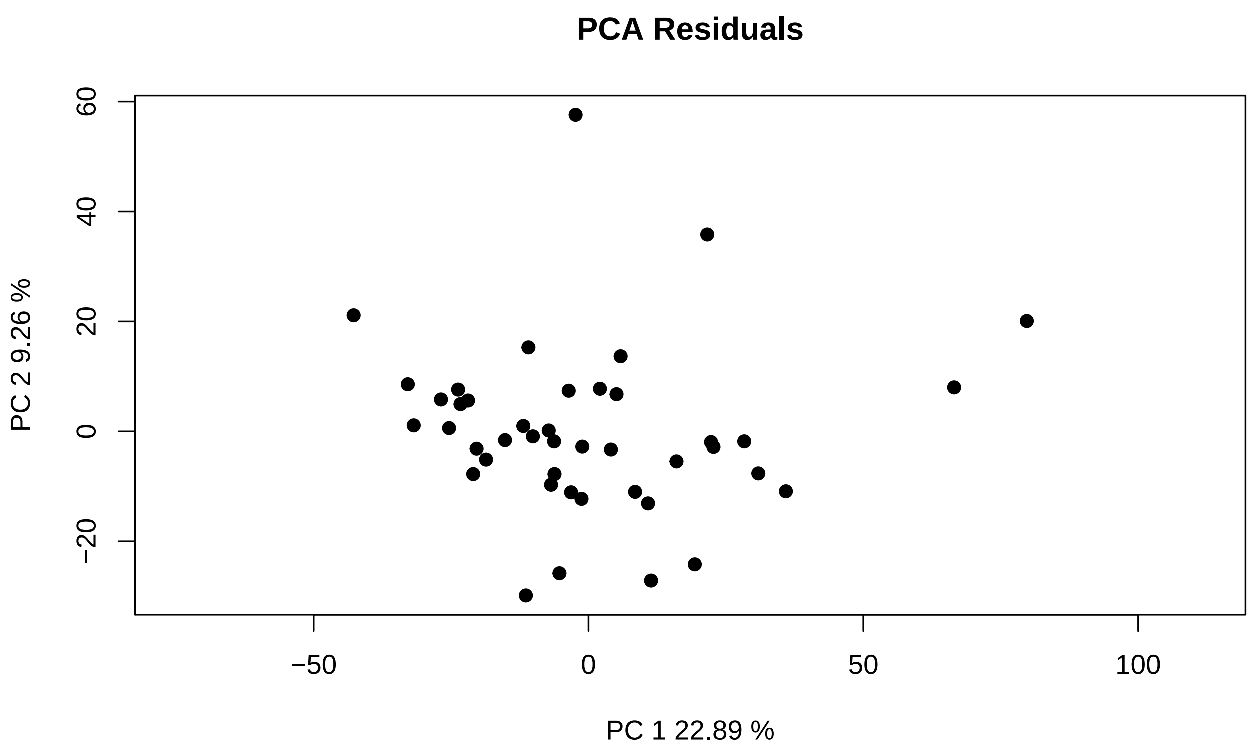
```r
Y_LMneg <- lm.rrpp(scaled_Y_young ~ Block * Water * Fungus, data = class,
    SS.type = "III", print.progress = F)
summary(Y_LMneg)
```

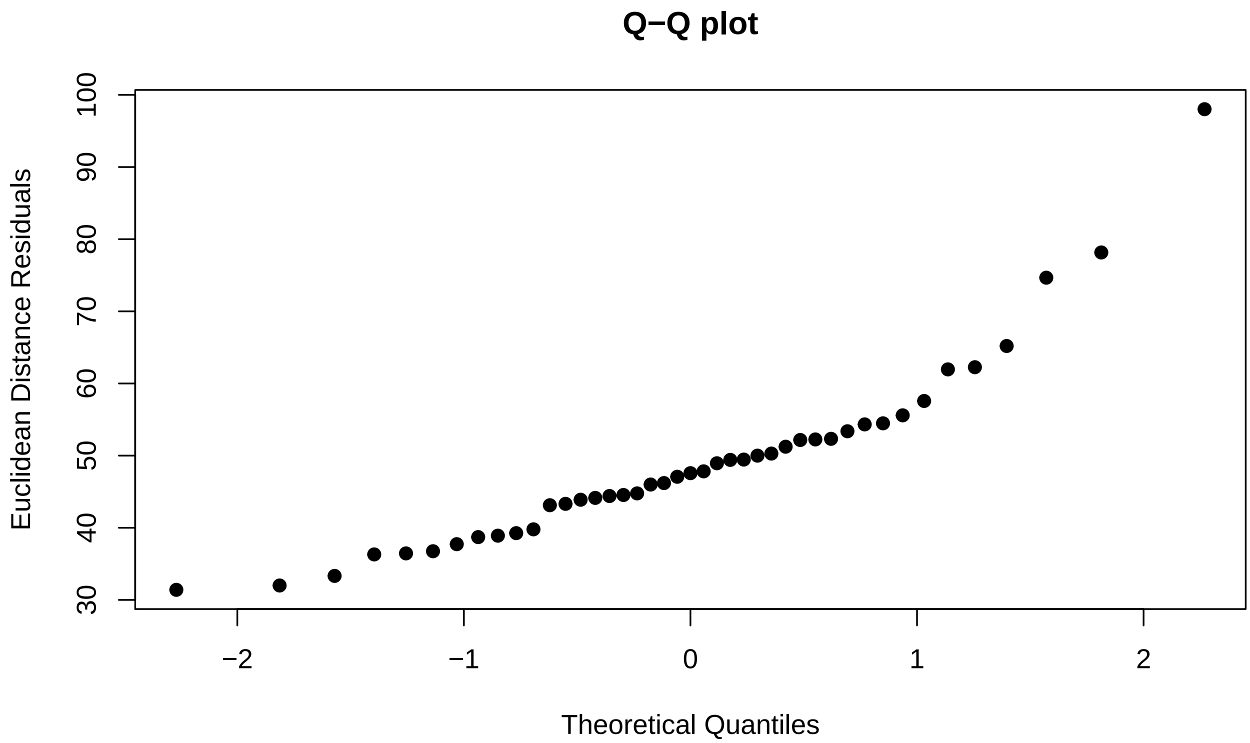
```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 2564
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Full Model Analysis of Variance
##
##                        Df Residual Df       SS Residual SS       Rsq        F
## Block * Water * Fungus  7          35 31681.28    76006.72 0.2941951 2.084111
##                        Z (from F) Pr(>F)
## Block * Water * Fungus   3.555953  0.001
##
##
## Redundancy Analysis (PCA on fitted values and residuals)
##
##                 Trace Proportion Rank
## Fitted     754.3162  0.2941951    7
## Residuals 1809.6838  0.7058049   35
## Total     2564.0000  1.0000000   42
##
## Eigenvalues
##
##                 PC1      PC2      PC3      PC4      PC5      PC6      PC7
## Fitted    479.1294  91.1236  60.3231  48.3523  34.7519  22.3644  18.2714
## Residuals 453.7519 244.4531 180.4838 108.3626  99.0782  86.2482  70.5464
## Total     741.2963 412.4162 243.1708 131.7368 117.9416  99.9943  90.3451
##                 PC8      PC9     PC10     PC11     PC12     PC13     PC14
## Fitted
## Residuals  64.5324  43.4411  42.9112  32.4887  29.6020  27.9724  26.3722
## Total      80.2716  46.9199  45.5122  42.9432  35.3433  34.7238  32.1156
##                PC15     PC16     PC17     PC18     PC19     PC20     PC21
## Fitted
## Residuals  24.6029  23.8238  21.4968  20.4037  18.8184  17.5014  16.1502
## Total      28.6321  27.6384  26.6384  22.9269  21.5609  20.5311  19.2592
##                PC22     PC23     PC24     PC25     PC26     PC27     PC28
## Fitted
## Residuals  15.6273  14.9691  13.2887  13.0737  12.8391  11.8027  11.5692
## Total      18.1625  17.3365  16.2670  15.2323  14.2357  13.7407  13.6344
##                PC29     PC30     PC31     PC32     PC33     PC34     PC35
## Fitted
## Residuals  10.4926  10.3096   9.4148   9.1406   8.4518   7.8754   7.7879
## Total      12.3507  11.8622  11.3577  10.9042  10.5546  10.1456   9.5823
##                PC36     PC37     PC38     PC39     PC40     PC41     PC42
## Fitted
## Residuals
```

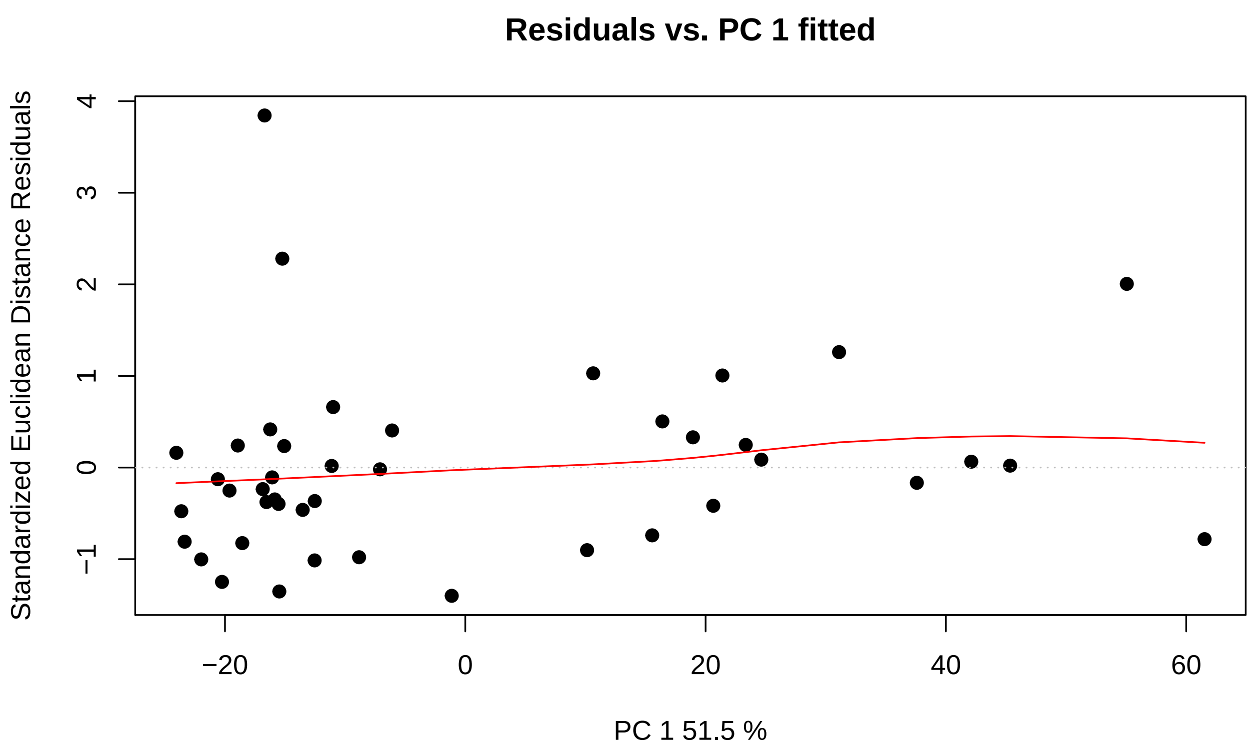
```
## Total        9.2737   8.8000   8.6340   8.0915   7.7601   7.6078   6.5488
```

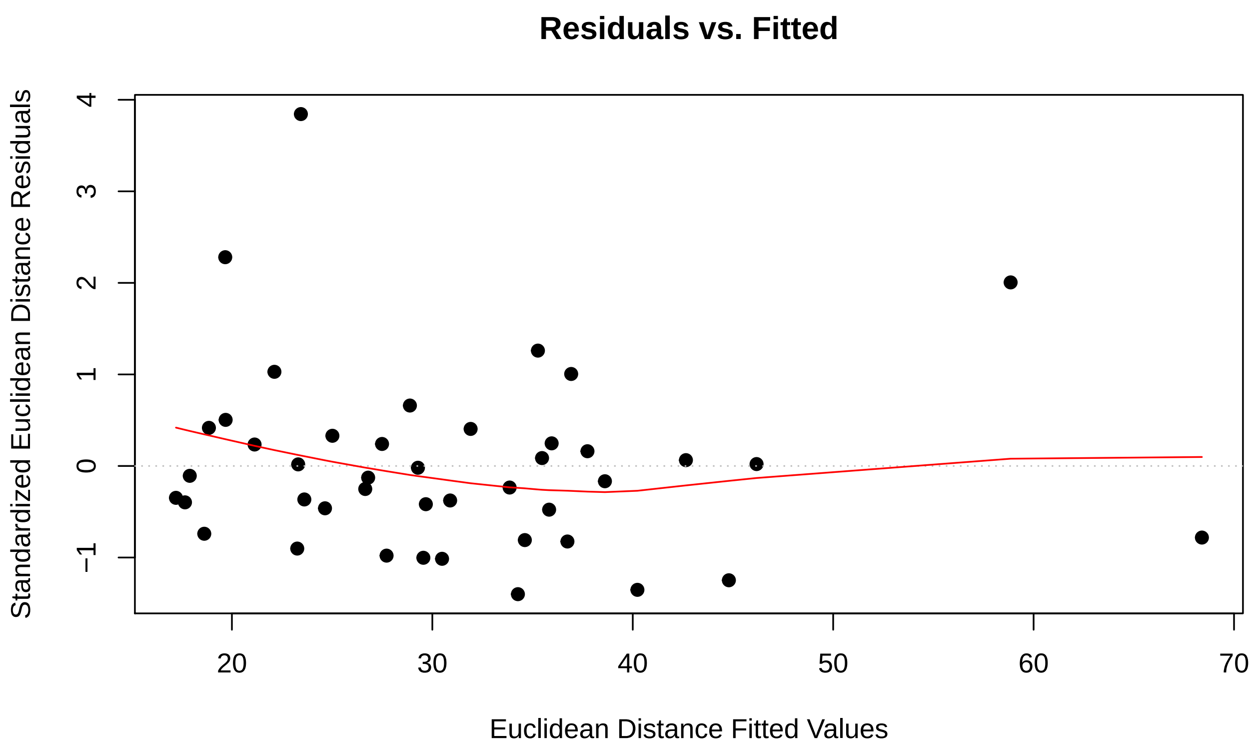
4. Examine RRPP plots to check for assumptions.

```
## Old Leaves residuals vs fitted values (homoscedasticity
## check)
Odiagnostics <- plot(O_LMneg, type = "diagnostics")
```

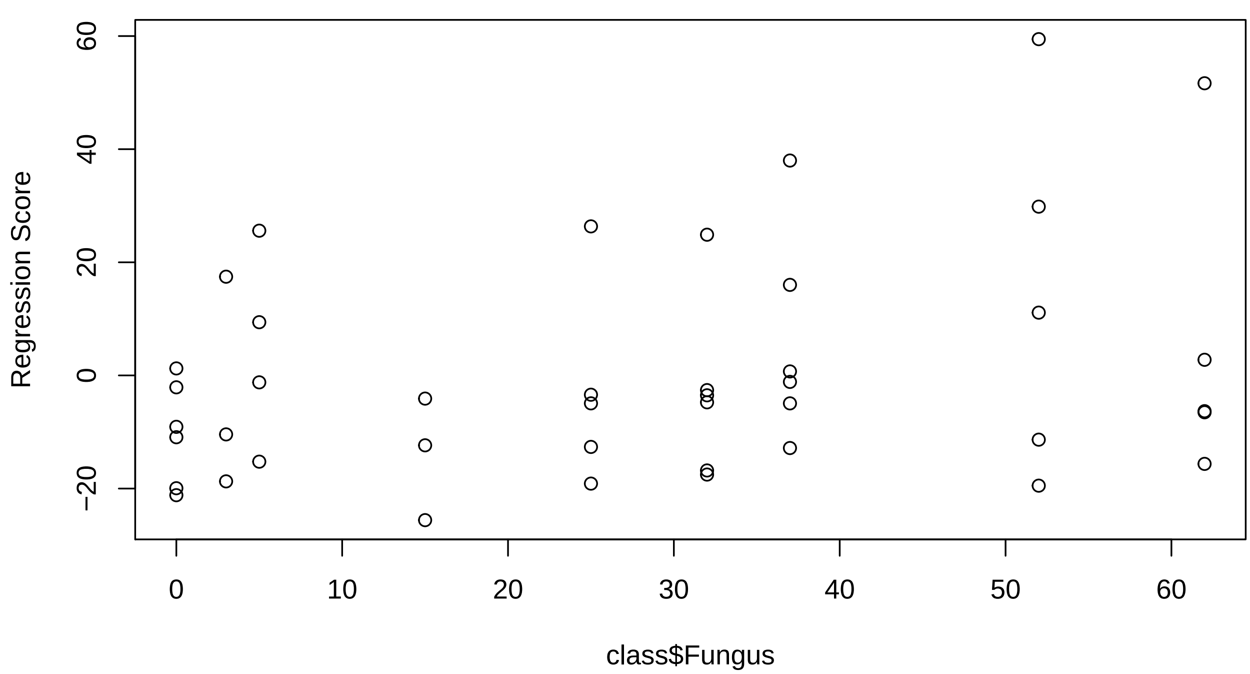



Q−Q plot
Euclidean Distance Residuals
30
40
50
60
70
80
90
100
−2
−1
0
1
2
Theoretical Quantiles

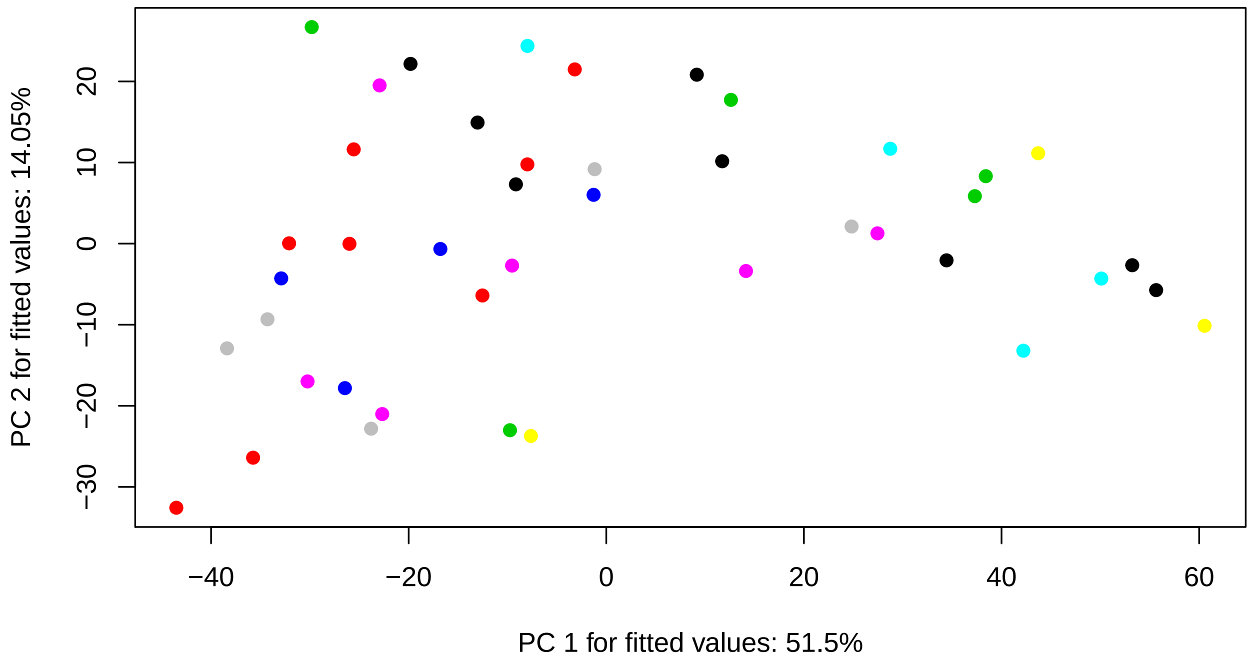

Residuals vs. PC 1 fitted
Standardized Euclidean Distance Residuals
−1
0
1
2
3
4
−20
0
20
40
60
PC 1 51.5 %



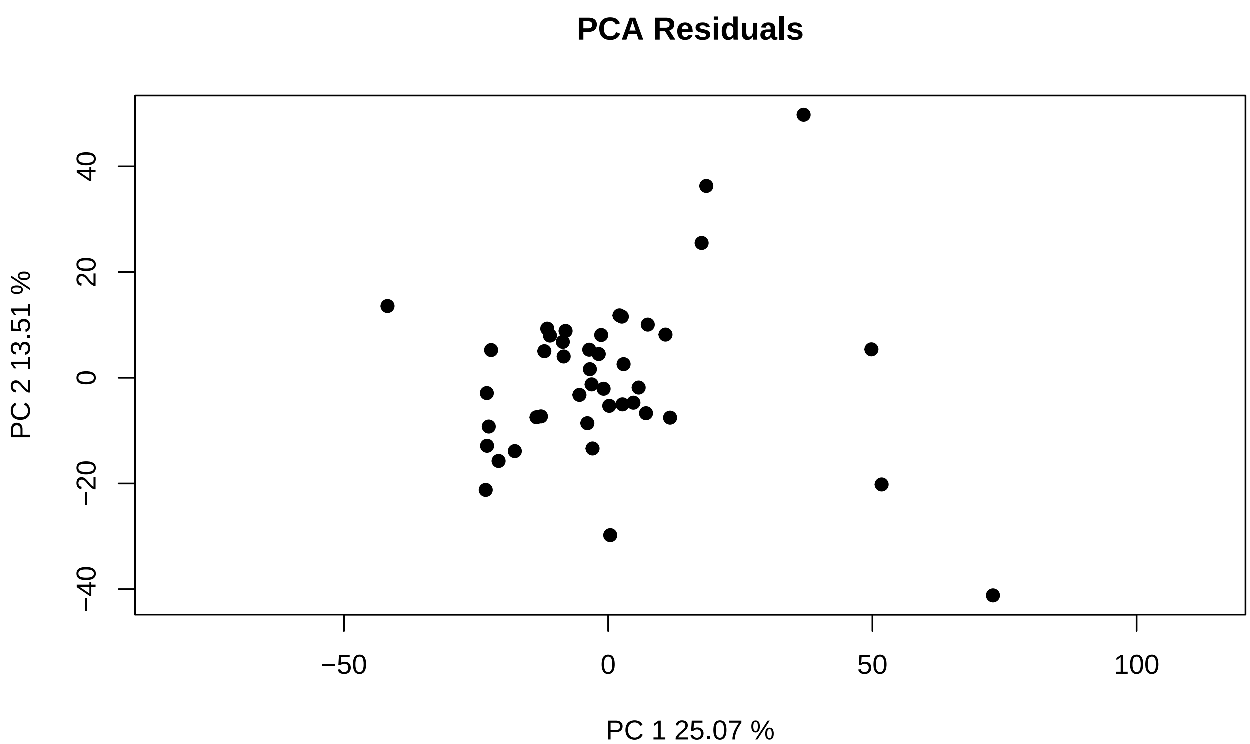

```r
# linear regression plot
Oregression <- plot(O_LMneg, type = "regression", predictor = class$Fungus,
    reg.type = "RegScore")
```

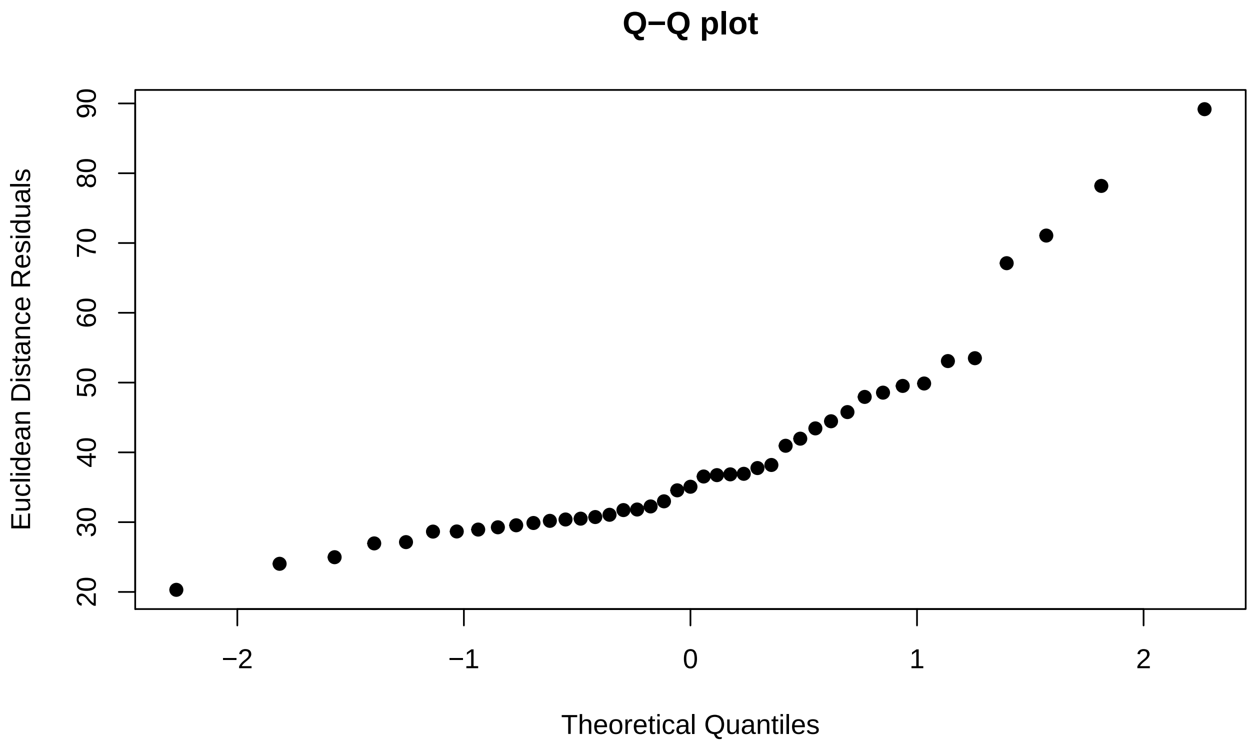


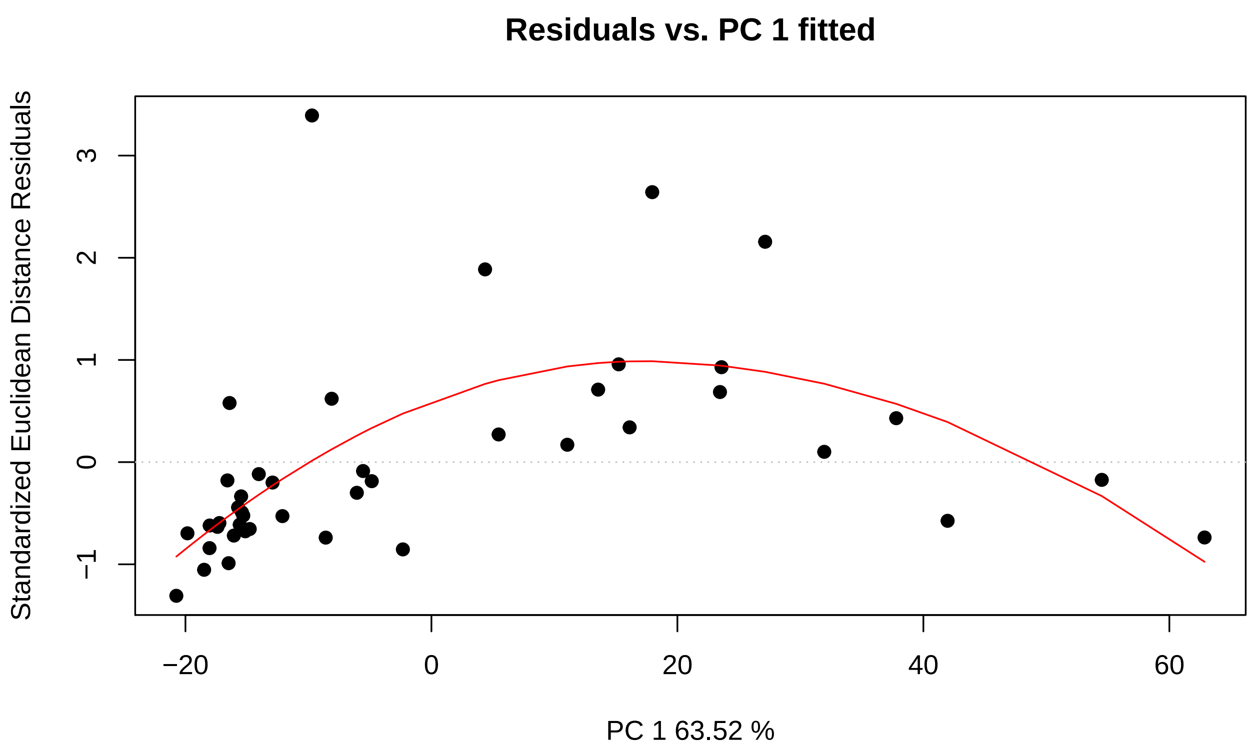

```r
# pca plot
Opcplot <- plot(O_LMneg, type = "PC", pch = 19, col = interaction(class$Water,
    class$Fungus))
```

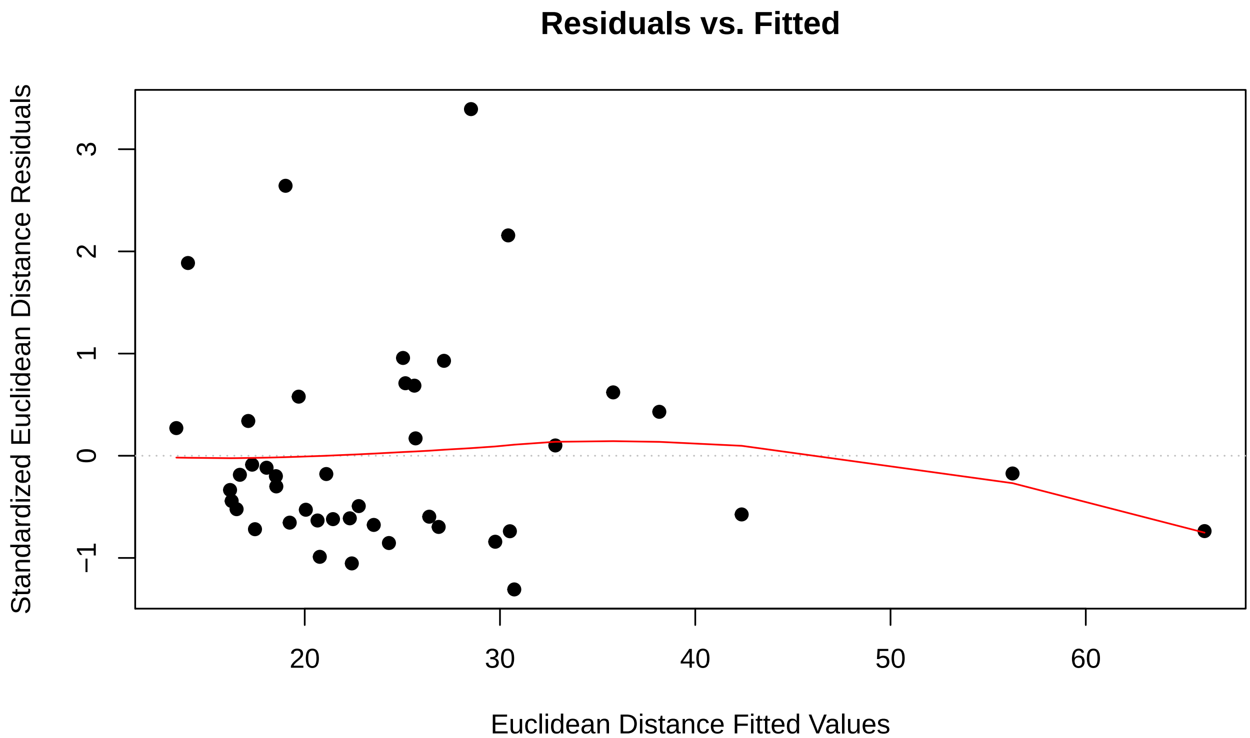



```r
## Young Leaves residuals vs fitted values (homoscedasticity
## check)
Ydiagnostics <- plot(Y_LMneg, type = "diagnostics")
```

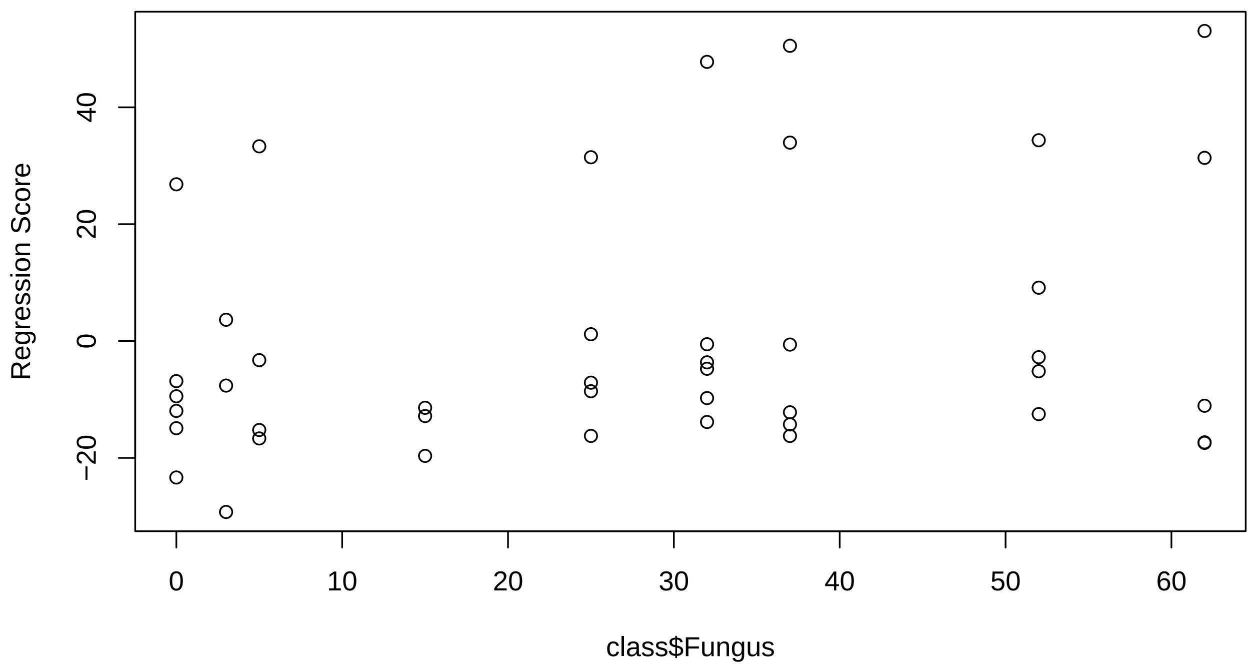



Q−Q plot
Euclidean Distance Residuals
20
30
40
50
60
70
80
90
−2
−1
0
1
2
Theoretical Quantiles

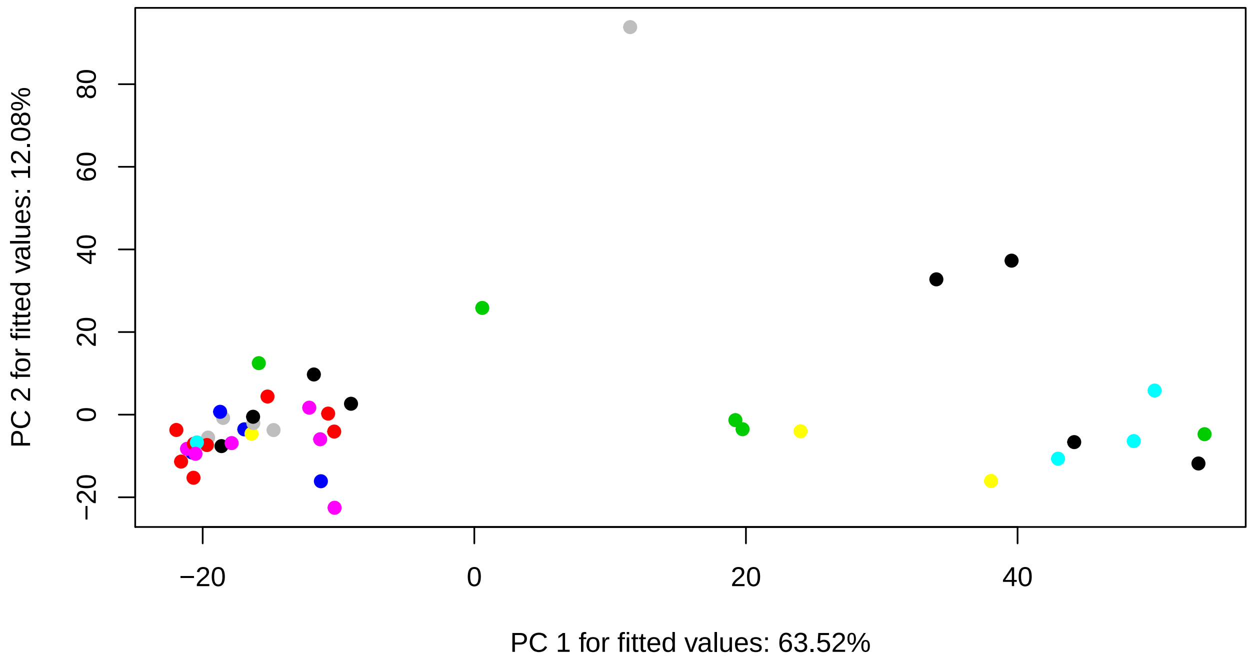

Residuals vs. PC 1 fitted
Standardized Euclidean Distance Residuals
−1
0
1
2
3
−20
0
20
40
60
PC 1 63.52 %




```
# linear regression plot
Yregression <- plot(Y_LMneg, type = "regression", predictor = class$Fungus,
    reg.type = "RegScore")
```




```
# pca plot
Ypcplot <- plot(Y_LMneg, type = "PC", pch = 19, col = interaction(class$Water,
    class$Fungus))
```



5. Perform an RRPP ANOVA and print results.

```
## Old Leaves
OnegANOVA <- anova(O_LMneg, effect.type = "F", error = c("Residuals",
    "Block:Water", "Block:Water:Fungus", "Residuals", "Block:Water:Fungus",
    "Block:Water:Fungus", "Residuals"))
summary(OnegANOVA, formula = T)
```

```
##
## Analysis of Variance, using Residual Randomization
## Permutation procedure: Randomization of null model residuals
## Number of permutations: 1000
## Estimation method: Ordinary Least Squares
## Sums of Squares and Cross-products: Type III
## Effect sizes (Z) based on F distributions
##
##                    Df     SS     MS     Rsq      F       Z Pr(>F)
## Block               1   3202 3201.5 0.02041 1.0112 0.16985  0.380
## Water               1   5610 5610.4 0.03577 1.5380 1.96387  0.023 *
## Fungus              1   5175 5174.6 0.03300 1.4954 1.21500  0.101
## Block:Water         1   3648 3647.8 0.02326 1.1521 0.53559  0.264
## Block:Fungus        1   3692 3691.6 0.02354 1.0669 0.24281  0.413
## Water:Fungus        1   3935 3935.5 0.02509 1.1373 0.61054  0.268
## Block:Water:Fungus  1   3460 3460.3 0.02206 1.0929 0.36419  0.318
## Residuals          35 110818 3166.2 0.70662
## Total              42 156828
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call: lm.rrpp(f1 = scaled_Y_old ~ Block * Water * Fungus, SS.type = "III",
##     data = class, print.progress = F)
```

```
## Young Leaves
YnegANOVA <- anova(Y_LMneg, effect.type = "F", error = c("Residuals",
```

```r
    "Block:Water", "Block:Water:Fungus", "Residuals", "Block:Water:Fungus",
    "Block:Water:Fungus", "Residuals"))
summary(YnegANOVA, formula = T)
```

```
##
## Analysis of Variance, using Residual Randomization
## Permutation procedure: Randomization of null model residuals
## Number of permutations: 1000
## Estimation method: Ordinary Least Squares
## Sums of Squares and Cross-products: Type III
## Effect sizes (Z) based on F distributions
##
##                    Df     SS     MS     Rsq      F        Z Pr(>F)
## Block               1   1885 1885.0 0.01750 0.8680 -0.09773  0.492
## Water               1   2056 2056.1 0.01909 1.5851  1.77758  0.029 *
## Fungus              1   4496 4496.5 0.04175 1.8526  1.52838  0.062 .
## Block:Water         1   1297 1297.1 0.01205 0.5973 -1.16378  0.885
## Block:Fungus        1   2939 2939.1 0.02729 1.2109  0.58033  0.308
## Water:Fungus        1   2993 2992.7 0.02779 1.2330  0.80289  0.212
## Block:Water:Fungus  1   2427 2427.1 0.02254 1.1177  0.43661  0.301
## Residuals          35  76007 2171.6 0.70580
## Total              42 107688
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call: lm.rrpp(f1 = scaled_Y_young ~ Block * Water * Fungus, SS.type = "III",
##     data = class, print.progress = F)
```

6. Test lm.rrpp model coefficients. "d" is the amount of change in a variable for the coefficient indicated.

```r
## Old Leaves test model coefficients
Onegcoef <- coef(O_LMneg, test = T)
summary(Onegcoef)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 3734
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Statistics (distances) of coefficients with 95 percent confidence intervals,
## effect sizes, and probabilities of exceeding observed values based on
## 1000 random permutations using RRPP
##
##                          d.obs  UCL (95%)         Zd Pr(>d)
## (Intercept)         60.3518088 94.8615309 -1.6121305  0.971
## Block               26.9816761 32.9077884  0.5512534  0.248
## WaterLow            92.4671749 84.3645613  2.5422317  0.022
## Fungus               1.8892146  1.8085027  2.1748588  0.035
## Block:WaterLow      38.4181644 43.1363024  0.9567060  0.158
## Block:Fungus         0.8183189  0.9491407  0.9231061  0.156
## WaterLow:Fungus      2.2856809  2.4777582  1.2147799  0.104
```

```
## Block:WaterLow:Fungus  1.0601248  1.2422484  0.7793295  0.190
```

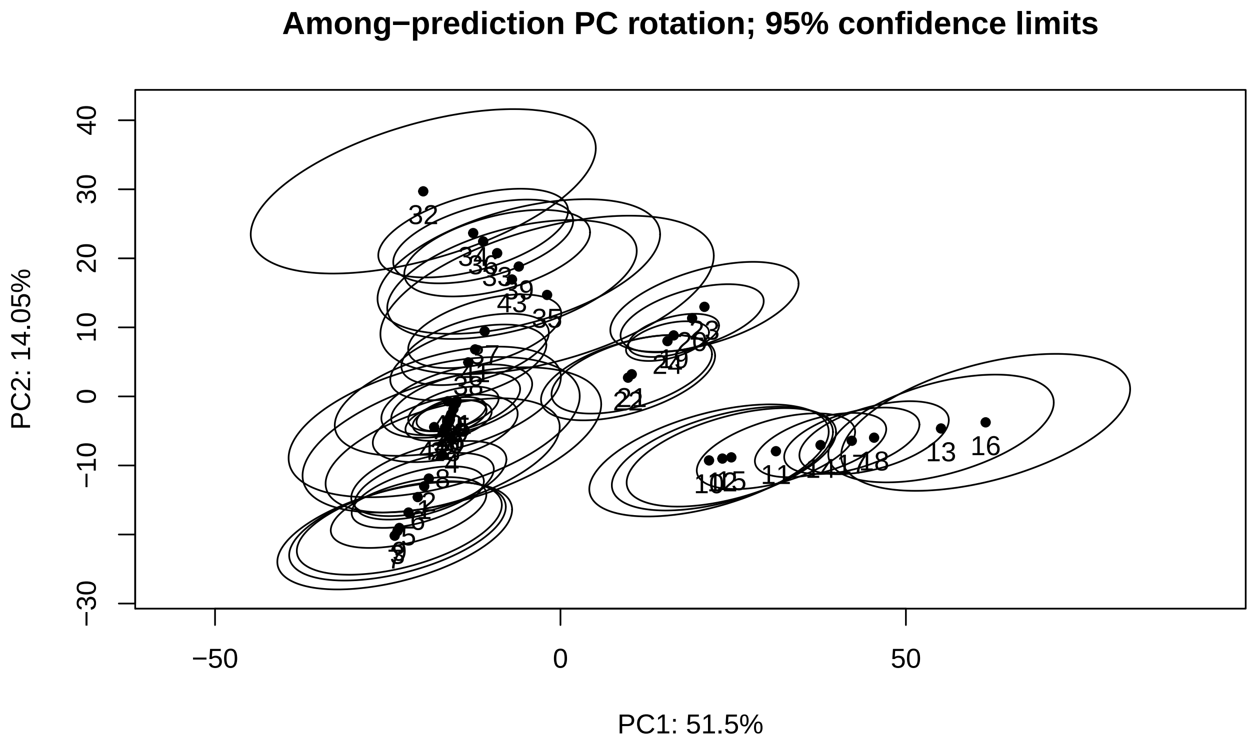
```r
## Young Leaves test model coefficients
Ynegcoef <- coef(Y_LMneg, test = T)
summary(Ynegcoef)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 2564
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Statistics (distances) of coefficients with 95 percent confidence intervals,
## effect sizes, and probabilities of exceeding observed values based on
## 1000 random permutations using RRPP
##
##                            d.obs  UCL (95%)         Zd Pr(>d)
## (Intercept)           44.4893714 78.2131969 -1.6657168  0.981
## Block                 20.7034191 28.5283175  0.1593677  0.369
## WaterLow              55.9771863 70.4398099  0.3803943  0.312
## Fungus                 1.7610770  1.5766022  2.6584165  0.017
## Block:WaterLow        22.9093040 36.4303968 -0.7532173  0.764
## Block:Fungus           0.7301684  0.8048526  1.2662781  0.111
## WaterLow:Fungus        1.9932055  2.1471403  1.3875655  0.093
## Block:WaterLow:Fungus  0.8878702  1.0670980  0.7758973  0.181
```

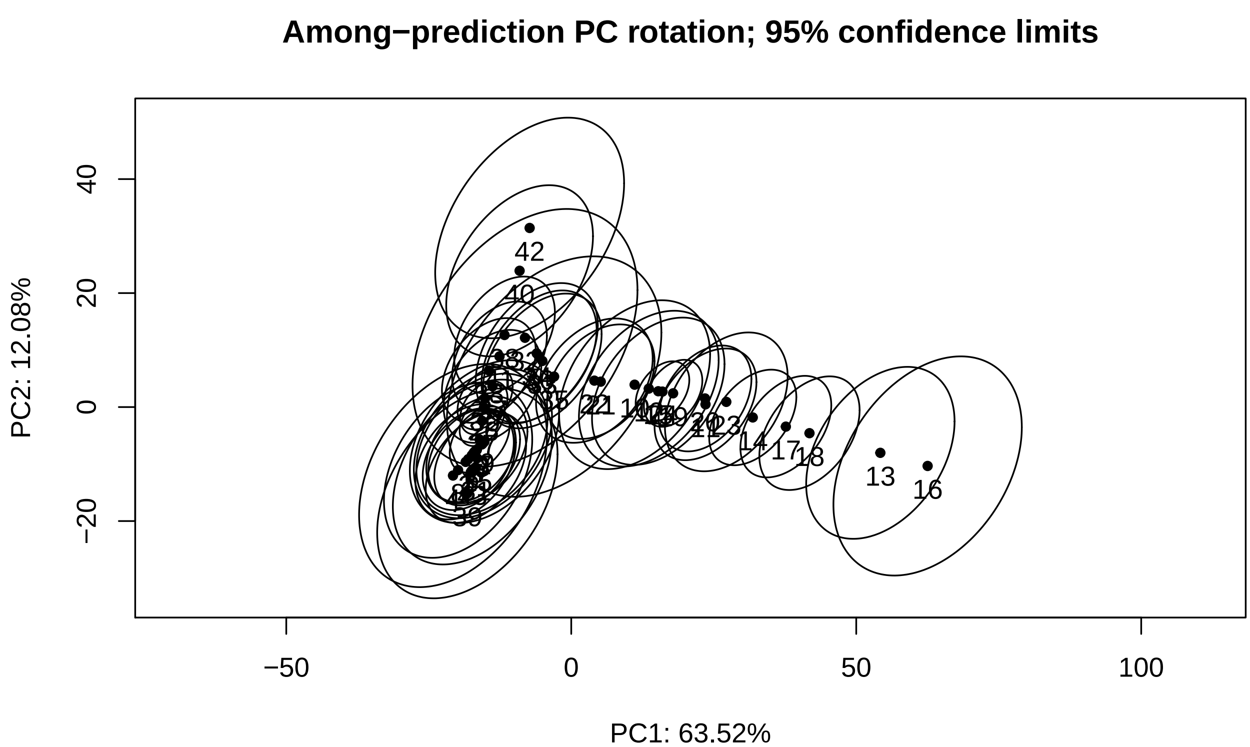
WaterLow has the largest effect on the model. The standard is the mean for High water treatment.

7. Compute predicted values from the lm.rrpp model fit using bootstrapped residuals to generate confidence intervals (precision of group mean estimates).

```r
O_pred <- predict(O_LMneg)
plot(O_pred, PC = T, ellipse = T)
```




```r
Y_pred <- predict(Y_LMneg)
plot(Y_pred, PC = T, ellipse = T)
```



8. Test pairwise differences between least squares means. Similar to tukeyHSD function in the r stats package. The pairwise function will generate tables with confidence intervals and p-values for the pairwise statistic, Euclidean distance between least-squares means.

```r
## Old Leaves pairwise differences of water
Onegpw <- pairwise(O_LMneg, groups = class$Water)
summary(Onegpw, confidence = 0.95, stat.table = T)
```

```
##
## Pairwise comparisons
##
## Groups: High Low
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##                 d UCL (95%)          Z Pr > d
## High:Low 38.34694  50.24041 -0.5513654  0.703
```

```r
## Young Leaves pairwise differences of water
Ynegpw <- pairwise(Y_LMneg, groups = class$Water)
summary(Ynegpw, confidence = 0.95, stat.table = T)
```

```
##
## Pairwise comparisons
##
## Groups: High Low
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##                 d UCL (95%)          Z Pr > d
## High:Low 34.62897  44.30378 -0.5379515  0.691
```

```r
Ynegpw2 <- pairwise(Y_LMneg, groups = class$Fungus)
summary(Ynegpw2, confidence = 0.95, stat.table = T)
```

```
##
## Pairwise comparisons
##
## Groups: 0 3 5 15 25 32 37 52 62
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##               d UCL (95%)          Z Pr > d
## 0:3   11.967287 18.555949 -1.4978925  0.959
## 0:5    7.073755 13.428834 -1.6020527  0.992
## 0:15   8.463239 16.172610 -2.5549409  1.000
## 0:25   7.249913 15.315498 -1.8878668  1.000
```

```
## 0:32  10.797658 20.956386 -1.4256607  0.968
## 0:37  12.484792 24.230822 -1.4256607  0.968
## 0:52  19.500474 34.375969 -1.1517552  0.902
## 0:62  23.410947 43.486293 -1.2916990  0.930
## 3:5   14.149716 20.429021 -0.8980665  0.816
## 3:15   8.923562 14.505974 -1.4600123  0.953
## 3:25  12.647085 19.868156 -1.9614455  0.990
## 3:32  12.397093 21.117445 -2.0916543  0.996
## 3:37  13.281103 23.284780 -2.0439032  0.994
## 3:52  18.290922 32.392207 -1.5941758  0.984
## 3:62  20.897656 38.852087 -1.6092736  0.993
## 5:15   7.559166 10.988748 -0.8778605  0.825
## 5:25  10.045844 16.181730 -0.7737974  0.772
## 5:32  13.913808 21.424391 -0.5543023  0.662
## 5:37  15.397669 24.193320 -0.6177830  0.698
## 5:52  22.064981 33.778284 -0.4747036  0.640
## 5:62  25.678135 41.667127 -0.7354210  0.757
## 15:25  7.934798 12.790631 -1.8608519  0.991
## 15:32  9.834148 16.366952 -1.2818876  0.918
## 15:37 11.031471 18.597077 -1.2207255  0.907
## 15:52 16.772019 26.718885 -0.7896441  0.764
## 15:62 20.435333 35.226049 -1.0375491  0.858
## 25:32  4.796356  7.089027 -0.6425309  0.745
## 25:37  6.280954 10.039236 -0.8332043  0.794
## 25:52 13.445915 20.526435 -0.5790940  0.689
## 25:62 17.520881 29.512969 -1.0224704  0.853
## 32:37  1.687134  3.274435 -1.4256607  0.968
## 32:52  9.437433 14.770629 -0.7956615  0.770
## 32:62 12.949955 22.831397 -1.1768566  0.893
## 37:52  8.039163 12.185987 -0.6900703  0.732
## 37:62 11.372417 19.728841 -1.1405778  0.882
## 52:62  7.696395 15.191814 -1.4596150  0.968
```

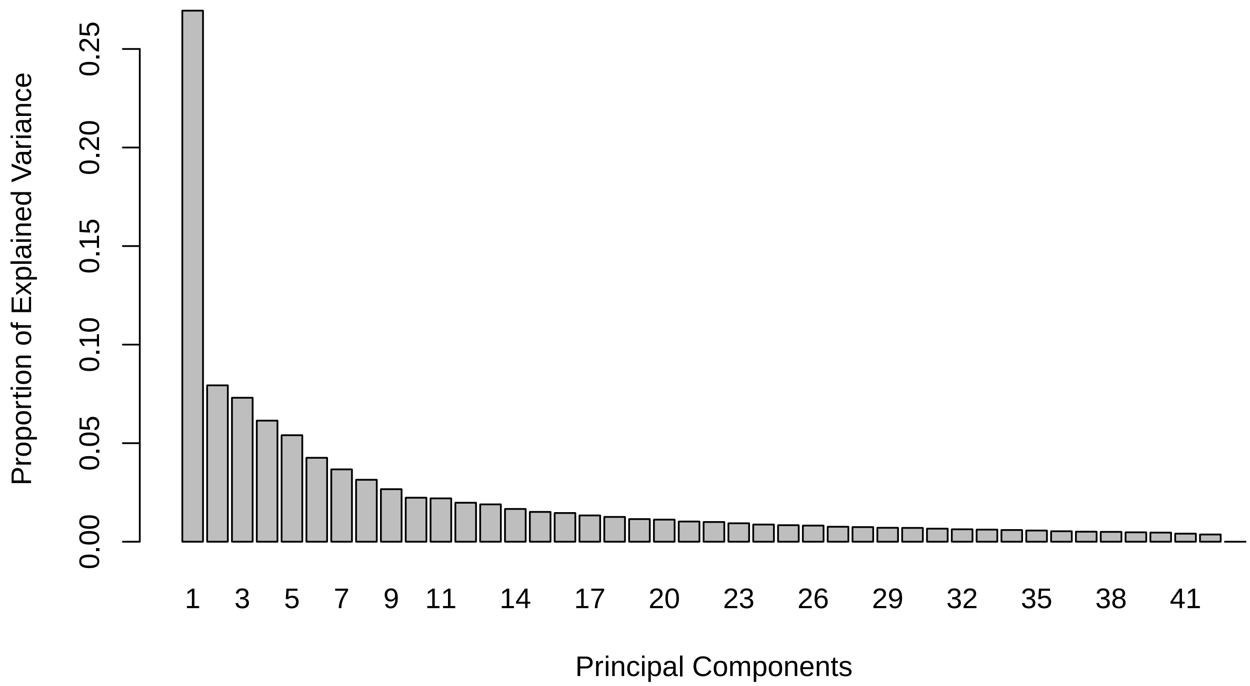
PCA

8. Identify the major source of variation in data and determine if the variation is sourced from experimental bias or biological conditions.

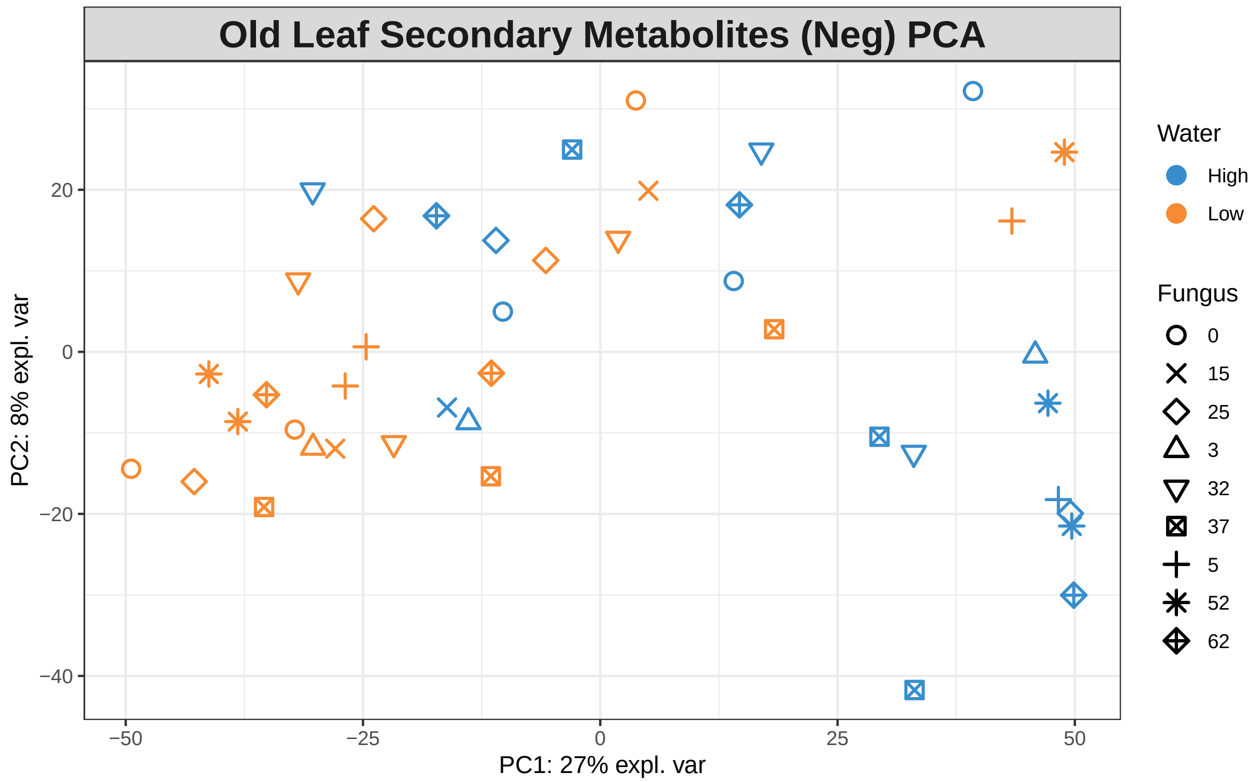
```r
# Old Leaf Secondary Metabolites (Neg) tune how many
# components to use
tune.pca(scaled_Y_old)
```

```
## Eigenvalues for the first 10 principal components, see object$sdev^2:
##        PC1        PC2        PC3        PC4        PC5        PC6        PC7
## 1006.12346  296.26837  272.74967  229.43449  201.77791  159.01902  137.05643
##        PC8        PC9       PC10
##  117.36477   99.56505   83.39669
##
## Proportion of explained variance for the first 10 principal components, see object$explained_varianc
##        PC1        PC2        PC3        PC4        PC5        PC6        PC7
## 0.26944924 0.07934343 0.07304490 0.06144470 0.05403801 0.04258678 0.03670499
##        PC8        PC9       PC10
## 0.03143138 0.02666445 0.02233441
##
## Cumulative proportion explained variance for the first 10 principal components, see object$cum.var:
```

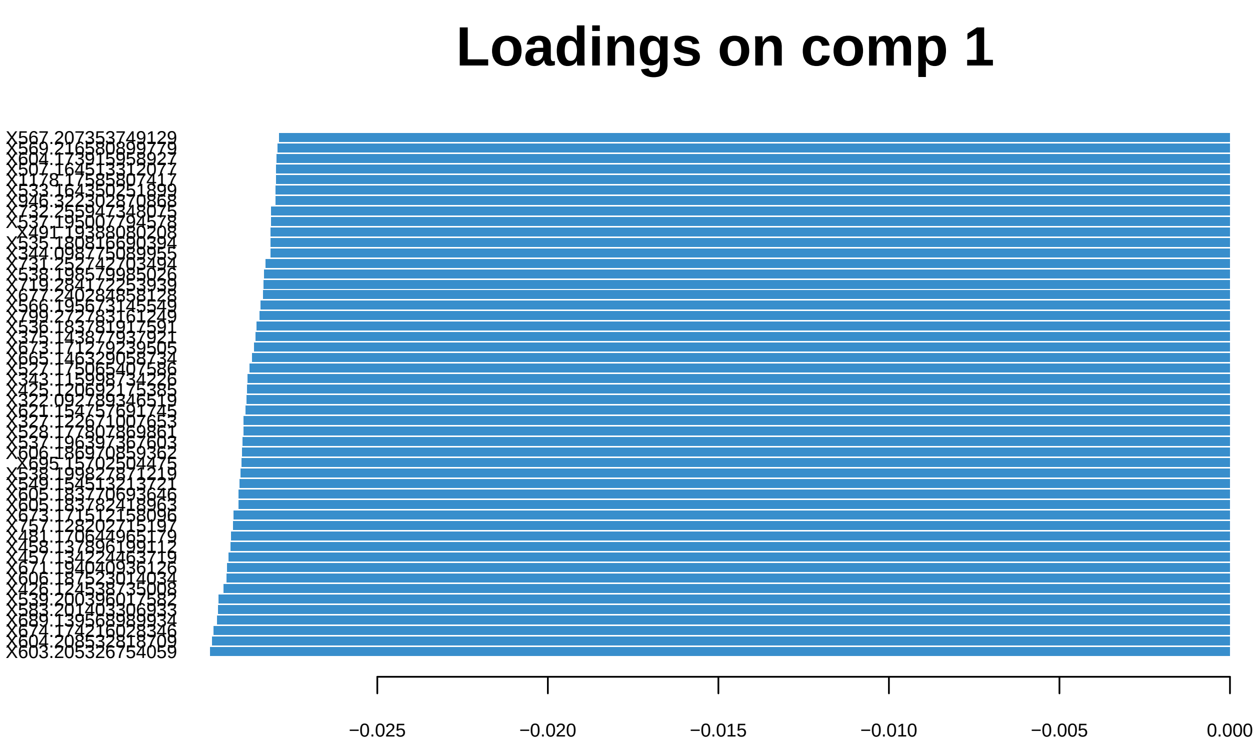
```
##        PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 0.2694492 0.3487927 0.4218376 0.4832823 0.5373203 0.5799070 0.6166120 0.6480434
##        PC9      PC10
## 0.6747079 0.6970423
##
##  Other available components:
##  --------------------
##  loading vectors: see object$rotation
```




```
pca.res <- mixOmics::pca(scaled_Y_old, ncomp = 4, scale = F)

# plot pca
plotIndiv(pca.res, group = class$Water, ind.names = F, pch = as.factor(class$Fungus),
    legend = T, legend.title = "Water", legend.title.pch = "Fungus",
    title = "Old Leaf Secondary Metabolites (Neg) PCA")
```

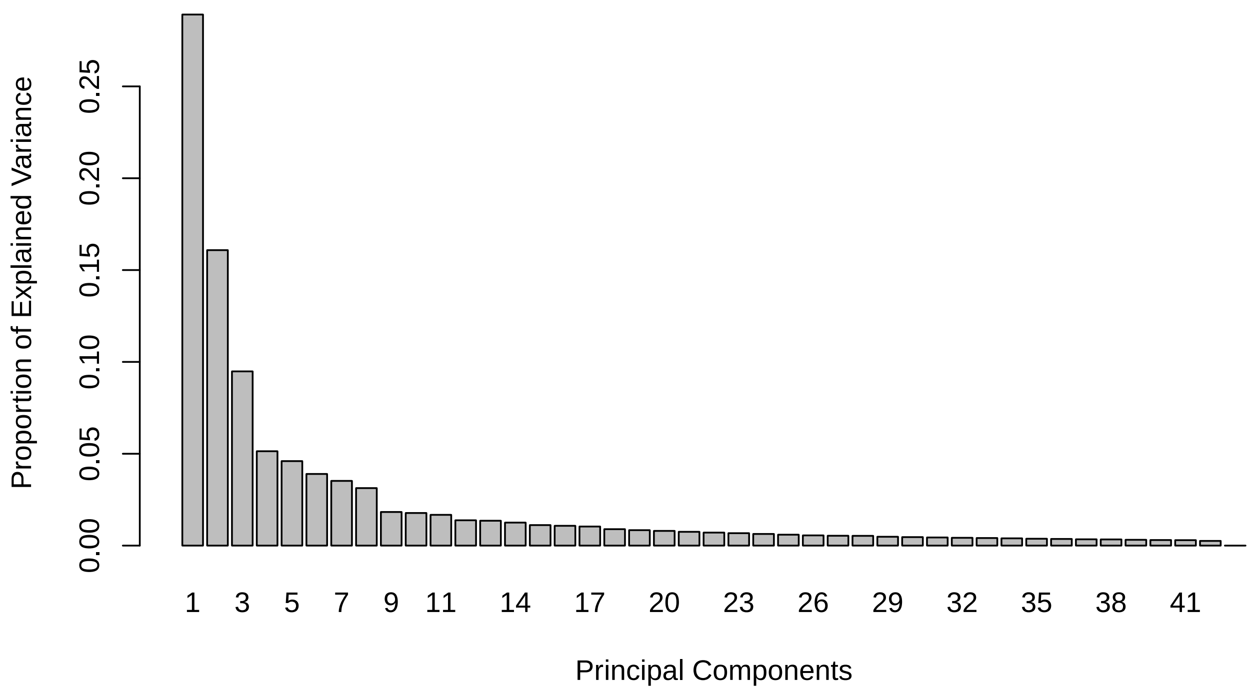



```r
# Look at variable coefficients in each component with the
# loading vectors The absolute value of loading vectors
# represent the importance of each variable to define each PC
plotLoadings(pca.res, ndisplay = 50)
```



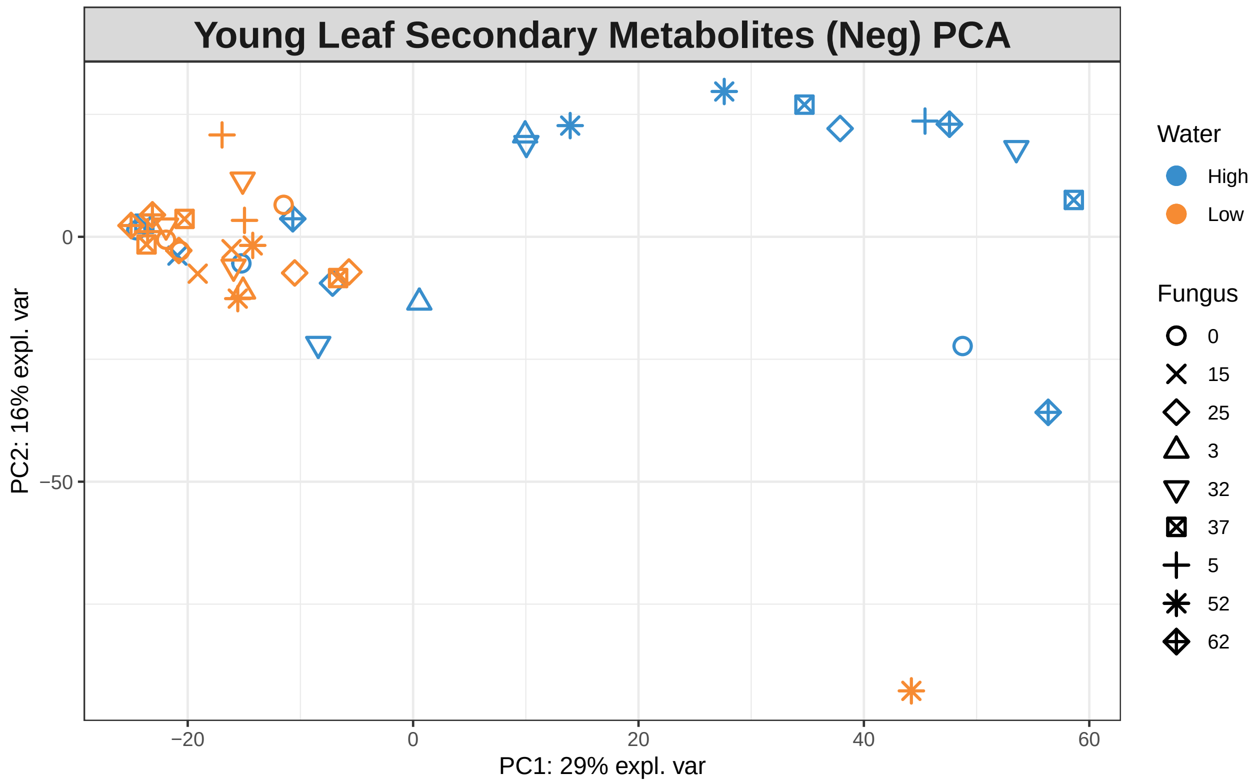
```r
# Young Leaf Secondary Metabolites (Neg) tune how many
# components to use
tune.pca(scaled_Y_young)
```

```
## Eigenvalues for the first 10 principal components, see object$sdev^2:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 741.29628 412.41616 243.17081 131.73675 117.94156  99.99428  90.34507  80.27160
##       PC9      PC10
##  46.91985  45.51224
##
## Proportion of explained variance for the first 10 principal components, see object$explained_varianc
##        PC1        PC2        PC3        PC4        PC5        PC6        PC7
## 0.28911711 0.16084874 0.09484041 0.05137939 0.04599905 0.03899933 0.03523599
##        PC8        PC9       PC10
## 0.03130718 0.01829947 0.01775048
##
## Cumulative proportion explained variance for the first 10 principal components, see object$cum.var:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 0.2891171 0.4499658 0.5448063 0.5961856 0.6421847 0.6811840 0.7164200 0.7477272
##       PC9      PC10
## 0.7660267 0.7837771
##
##  Other available components:
##  --------------------
##  loading vectors: see object$rotation
```

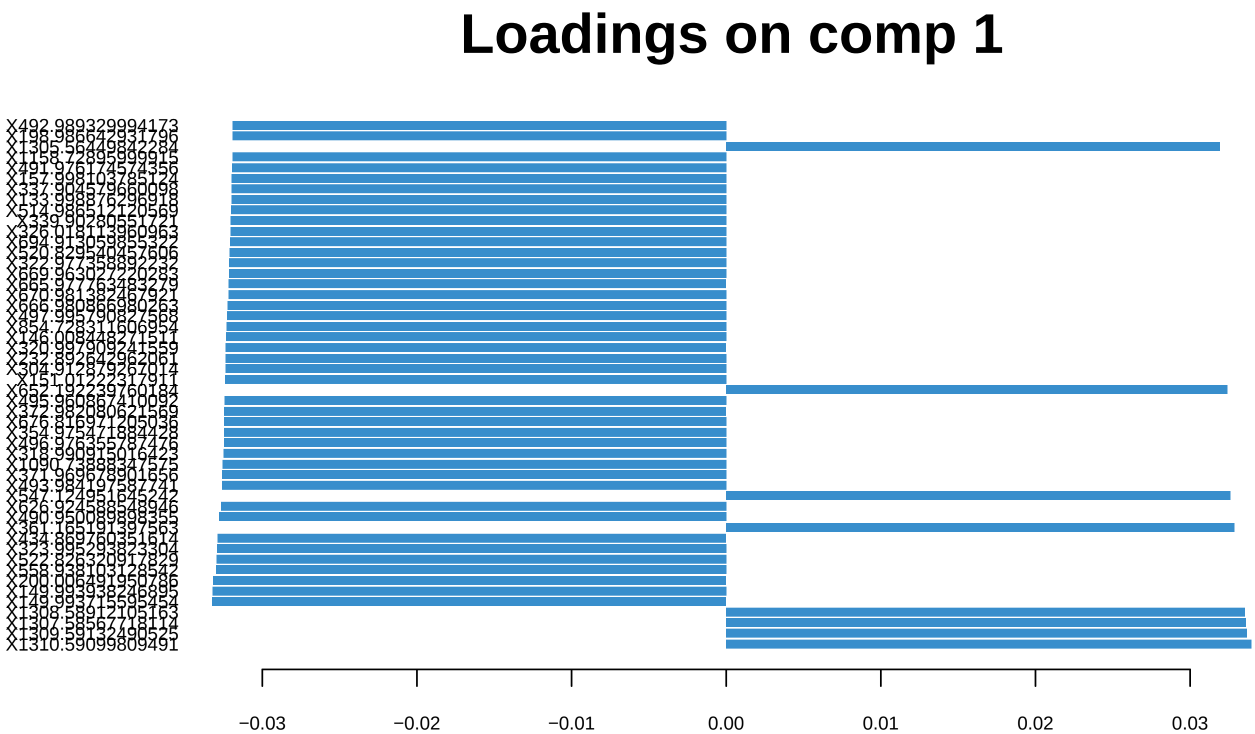



```r
pca.res <- mixOmics::pca(scaled_Y_young, ncomp = 3, scale = F)

# plot pca
plotIndiv(pca.res, group = class$Water, ind.names = F, pch = as.factor(class$Fungus),
    legend = T, legend.title = "Water", legend.title.pch = "Fungus",
```

```
    title = "Young Leaf Secondary Metabolites (Neg) PCA")
```

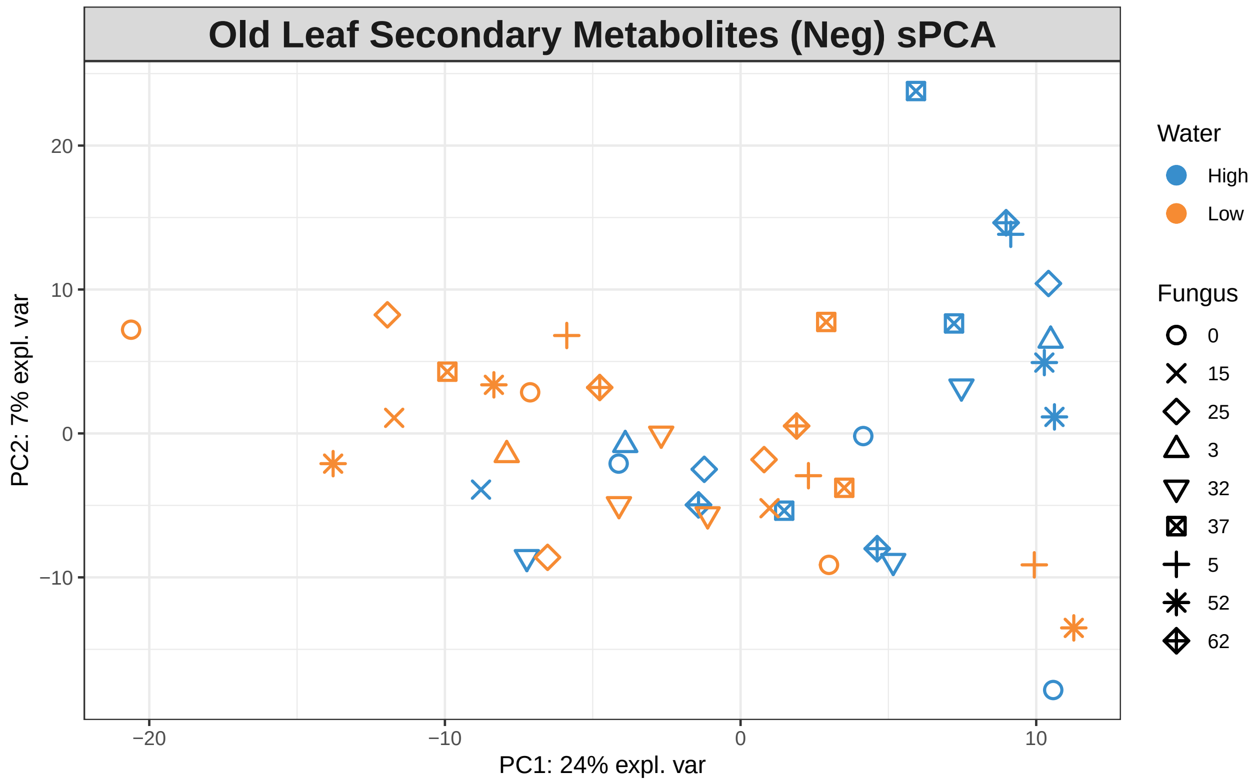



```
# Look at variable coefficients in each component with the
# loading vectors The absolute value of loading vectors
# represent the importance of each variable to define each PC
plotLoadings(pca.res, ndisplay = 50)
```

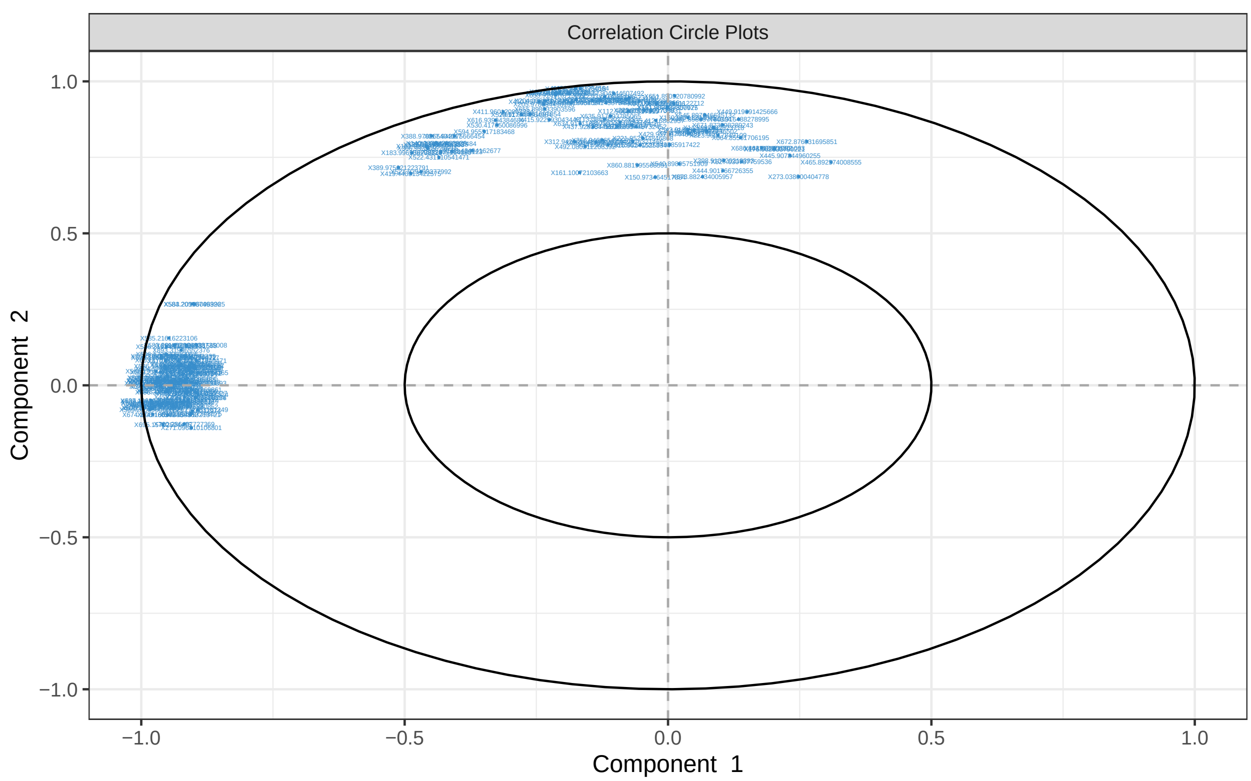


9. Identify the variables that contribute to the explained variance.

```
# Old Leaf
spca.res <- mixOmics::spca(scaled_Y_old, ncomp = 4, keepX = c(100,
    100, 10, 10))

# plot spca
plotIndiv(spca.res, group = class$Water, ind.names = F, pch = as.factor(class$Fungus),
    legend = T, legend.title = "Water", legend.title.pch = "Fungus",
    title = "Old Leaf Secondary Metabolites (Neg) sPCA")
```




```r
# variables contributing to each component
plotVar(spca.res, cex = 1)
```



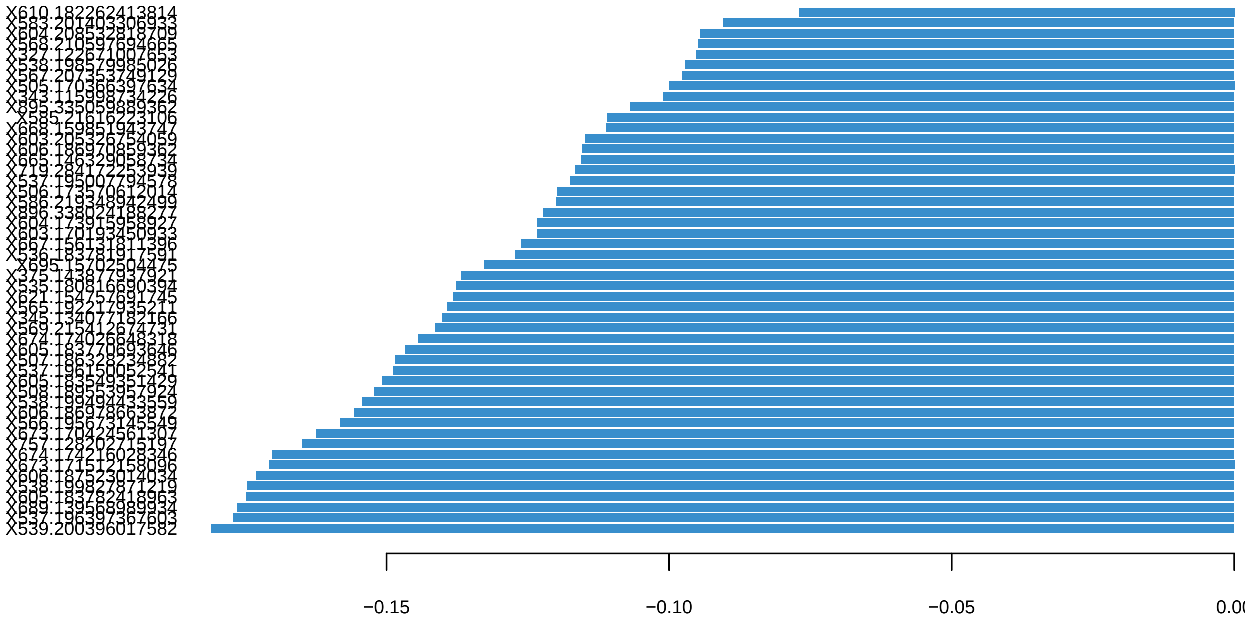
```r
selectVar(spca.res, comp = 1)$value  # view loading value of each metabolite
```

```
##                        value.var
## X539.200396017582 -0.181082951
## X537.196397367603 -0.177118557
## X689.139568989934 -0.176379501
## X605.183782418963 -0.174908432
## X538.199827871219 -0.174730140
## X606.187523014034 -0.173086641
## X673.171512158096 -0.170842206
## X674.174216028346 -0.170227465
## X757.128202715197 -0.164840111
## X673.170424561307 -0.162356814
## X566.195673145549 -0.158134925
## X606.186978663872 -0.155743982
## X538.199494433559 -0.154379050
## X508.189553957924 -0.152111850
## X605.183549351429 -0.150829867
## X537.196150052541 -0.148851518
## X507.186328234882 -0.148529026
## X605.183770693646 -0.146771927
## X674.174026648318 -0.144312231
## X569.215412674731 -0.141371483
## X345.134077182166 -0.140059056
## X565.192217935211 -0.139170771
## X621.154757691745 -0.138209140
## X535.180816690394 -0.137719228
## X375.143877937921 -0.136720111
## X695.15702504475  -0.132672803
## X536.183781917591 -0.127150509
## X667.156131811396 -0.126185948
## X603.170193450933 -0.123359380
## X604.173915958927 -0.123282579
## X896.338024188277 -0.122276357
## X586.219348942499 -0.120032704
## X506.173570612014 -0.119838045
## X537.195007794578 -0.117466739
## X719.284172253939 -0.116608140
## X665.146329058734 -0.115605747
## X606.186970859362 -0.115319414
## X603.205326754059 -0.114899908
## X668.159851943747 -0.111049129
## X585.21616223106  -0.110936526
## X895.335059889362 -0.106853883
## X343.115998734226 -0.101118943
## X505.170366397634 -0.100061246
## X567.207353749129 -0.097753184
## X538.198579985026 -0.097218515
## X327.122671007653 -0.095158658
## X568.210597694665 -0.094806479
## X604.208532818709 -0.094428877
## X583.201403306933 -0.090439718
## X610.182262413814 -0.076973370
## X346.140373954959 -0.069376435
```

```
## X570.219543443364 -0.067921138
## X149.059073075148 -0.065194016
## X893.31996552376  -0.065091823
## X833.181144939018 -0.062452460
## X727.178420224907 -0.058258959
## X671.194040936126 -0.054279193
## X720.286487727369 -0.053767576
## X457.134224463719 -0.052892192
## X878.329634092335 -0.049771260
## X731.252742703494 -0.048911141
## X735.146845005385 -0.048670427
## X540.215185799472 -0.047788147
## X803.137206404634 -0.046133144
## X613.210520581568 -0.045375543
## X653.178879325056 -0.042429292
## X507.164513312077 -0.042115852
## X508.174573318256 -0.042074589
## X817.214095360852 -0.040853774
## X458.137896199112 -0.040246045
## X481.170644965179 -0.039170292
## X877.32639228893  -0.037250992
## X677.166894727587 -0.036928503
## X716.259282733113 -0.032230304
## X549.154513213721 -0.031683394
## X879.340062720795 -0.030671329
## X376.132023520547 -0.030608230
## X894.32300346217  -0.030321790
## X527.175065407586 -0.028133102
## X732.255947348075 -0.027703876
## X271.096810106801 -0.027347261
## X528.177807869861 -0.027319604
## X521.201790047362 -0.026797953
## X589.18940461419  -0.025996732
## X516.148124468326 -0.025001523
## X584.20513749326  -0.023886833
## X717.21276012974  -0.023767090
## X698.250989243722 -0.018573314
## X515.14524575838  -0.016156106
## X583.201760969925 -0.015767460
## X880.34365929126  -0.014726899
## X796.233580680884 -0.012552486
## X439.157924608228 -0.010533966
## X489.162112886893 -0.010227638
## X359.148741949471 -0.009070205
## X426.124538735008 -0.007881174
## X795.230947111927 -0.007269924
## X799.272783161249 -0.003948915
## X330.141938124165 -0.003220751
## X993.322387841374 -0.002683019
```

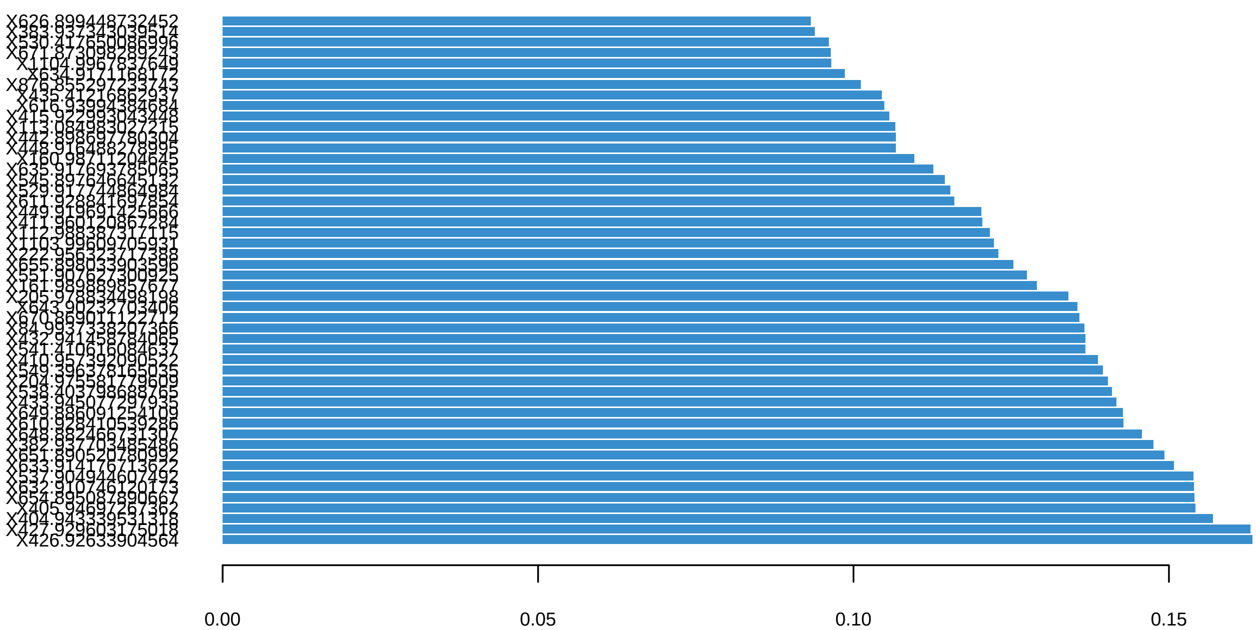
```
# plot loadings for comp 1
plotLoadings(spca.res, ndisplay = 50)
```

Loadings on comp 1



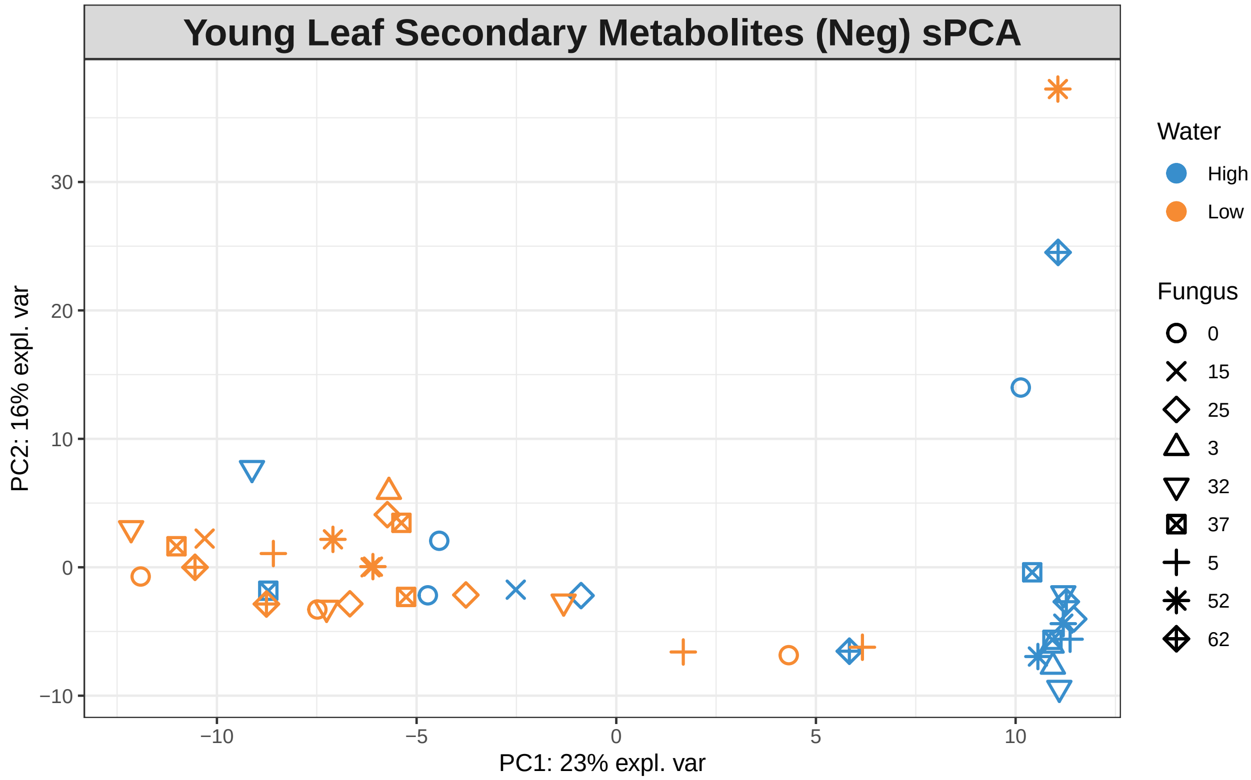

```r
# plot loadings for comp 2
plotLoadings(spca.res, comp = 2, ndisplay = 50)
```

Loadings on comp 2

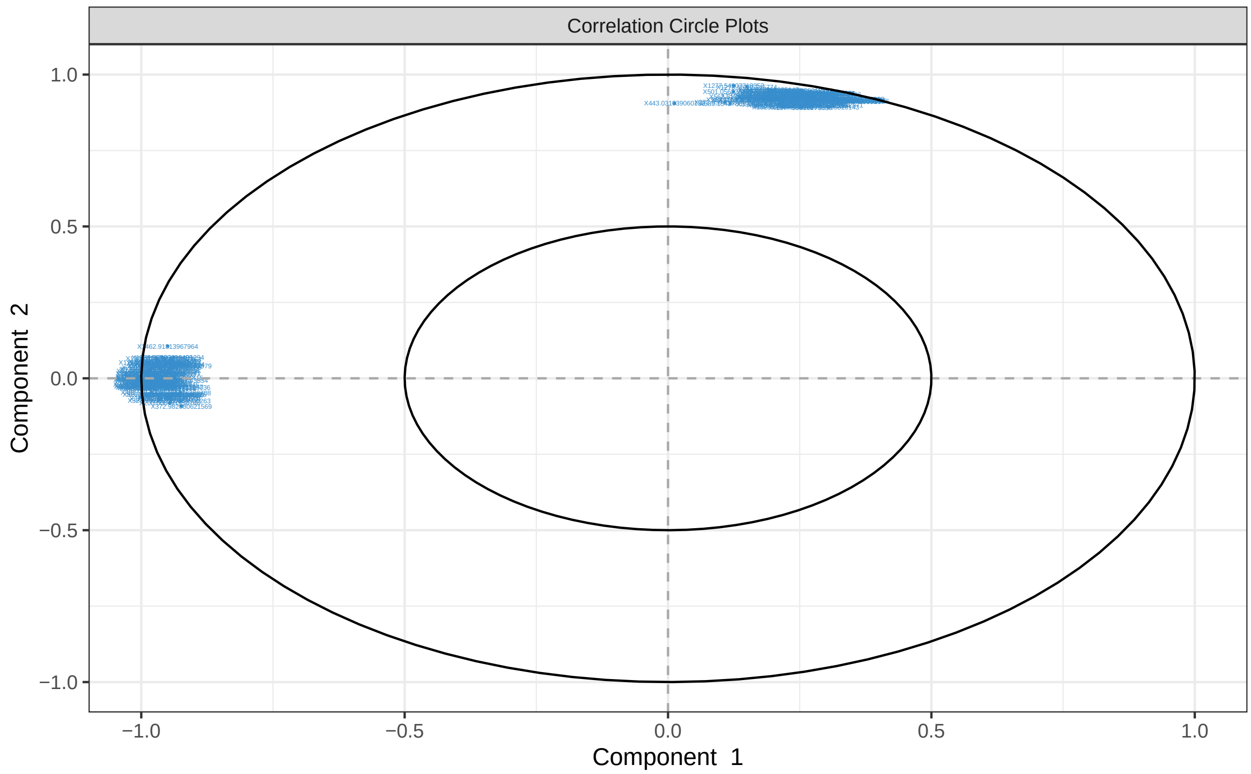



```r
# Young Leaf
spca.res <- mixOmics::spca(scaled_Y_young, ncomp = 3, keepX = c(100,
    100, 10))
```

```r
# plot spca
plotIndiv(spca.res, group = class$Water, ind.names = F, pch = as.factor(class$Fungus),
    legend = T, legend.title = "Water", legend.title.pch = "Fungus",
    title = "Young Leaf Secondary Metabolites (Neg) sPCA")
```




```r
# variables contributing to each component
plotVar(spca.res, cex = 1)
```



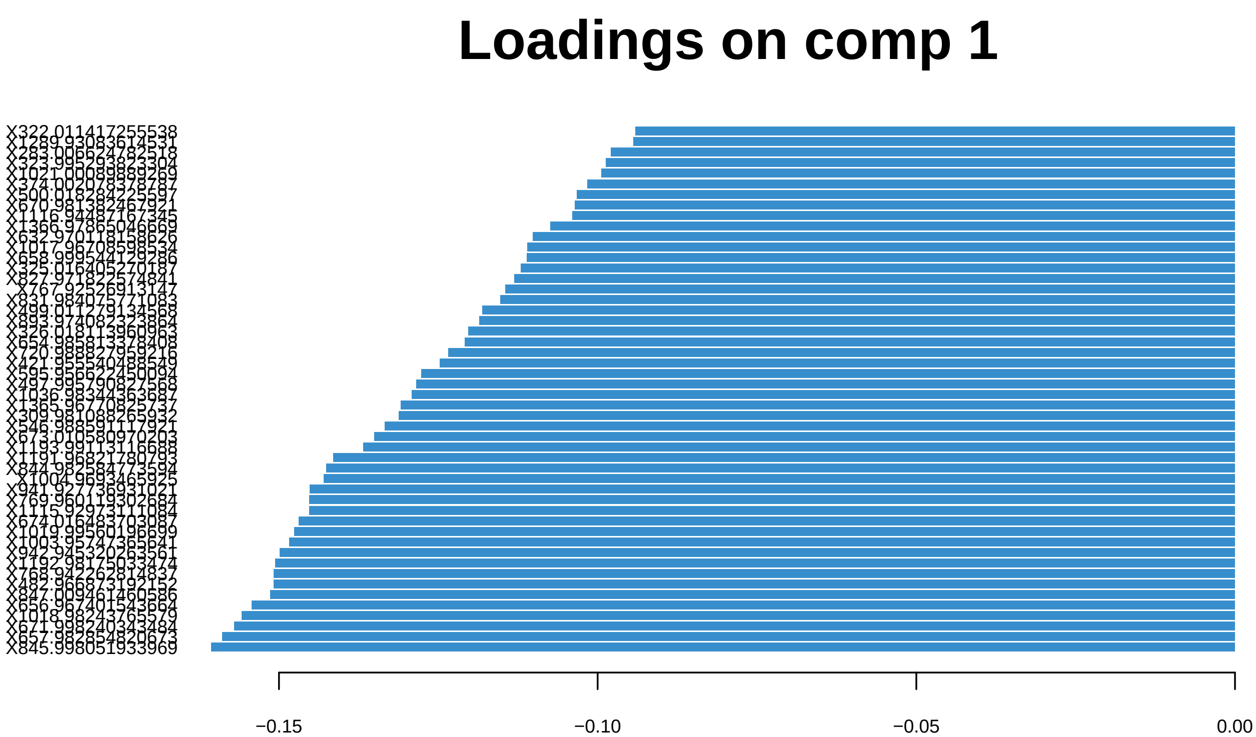

```r
selectVar(spca.res, comp = 1)$value  # view loading value of each metabolite
```

```
##                       value.var
## X845.998051933969 -0.1606330732
## X657.982854820673 -0.1588913910
## X671.998240343484 -0.1570044653
## X1018.98243765579 -0.1558509302
## X656.967401543664 -0.1542198021
## X847.009461460586 -0.1513489387
## X482.966873192152 -0.1507840595
## X768.942262814837 -0.1507794924
## X1192.98175033474 -0.1505850124
## X942.945320263561 -0.1498940009
## X1003.95747365641 -0.1483768457
## X1019.99560196699 -0.1475759336
## X674.016483703087 -0.1468869753
## X1115.92973111084 -0.1452322948
## X769.960119302684 -0.1452289240
## X941.927736931021 -0.1451711041
## X1004.9693465925  -0.1429543394
## X844.982584773594 -0.1425938797
## X1191.96821780793 -0.1414755075
## X1193.99113116688 -0.1367830091
## X673.010580970203 -0.1350161100
## X546.988591117921 -0.1333992604
## X309.981088265932 -0.1311783631
## X1365.96770825737 -0.1308576944
## X1036.98344363687 -0.1291475627
## X497.995790827568 -0.1284198692
```

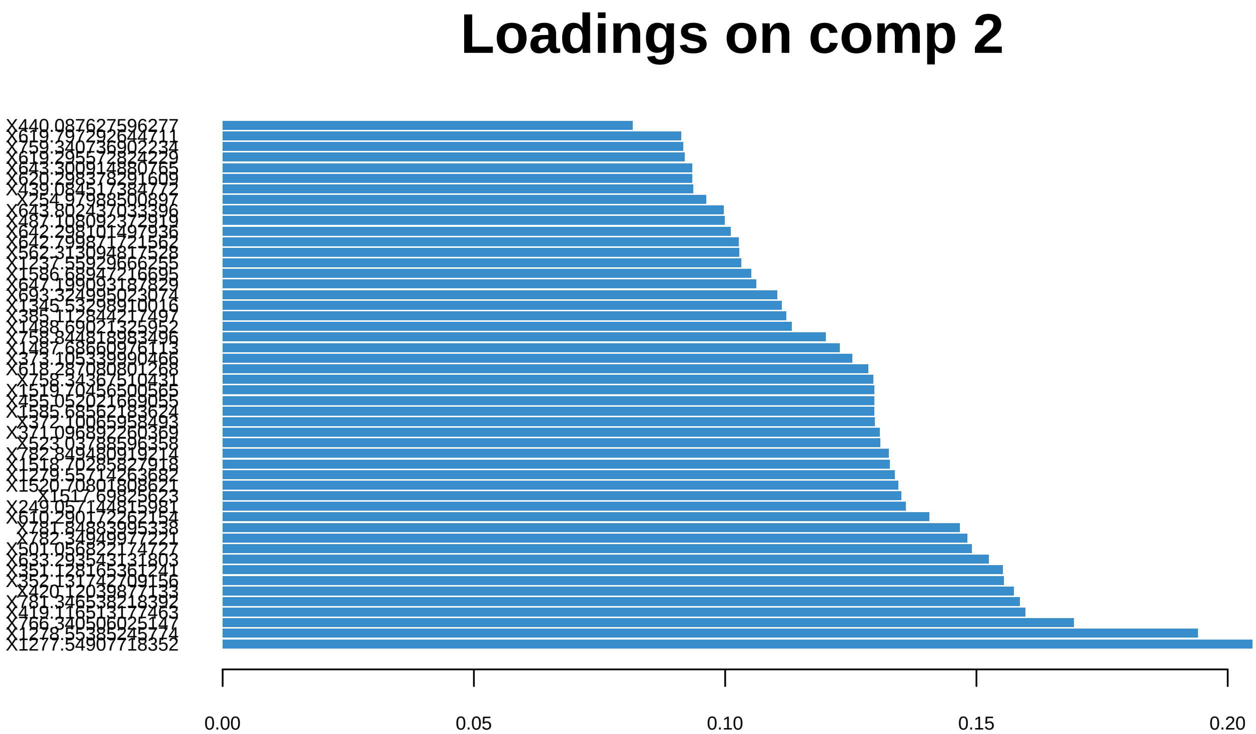
```
## X595.956622450094 -0.1276729024
## X421.955540488549 -0.1247260506
## X720.988827959216 -0.1233755750
## X654.985813378408 -0.1208239906
## X326.018113960963 -0.1202548234
## X893.974082323864 -0.1185839446
## X499.011279134568 -0.1180404580
## X831.984075771083 -0.1152274812
## X767.92526913147  -0.1144326983
## X827.971822574841 -0.1130674947
## X325.016405270187 -0.1120384107
## X658.999544129286 -0.1111185579
## X1017.96708598534 -0.1110301332
## X632.970118158626 -0.1101401694
## X1366.97865046669 -0.1073823625
## X1116.94487167345 -0.1039494146
## X670.981382467921 -0.1035217899
## X500.018284225597 -0.1032308499
## X374.002078378787 -0.1015634926
## X1021.00089889269 -0.0993725768
## X323.995293823304 -0.0987291486
## X283.006624782518 -0.0979302194
## X1289.93083614531 -0.0944013782
## X322.011417255538 -0.0940995915
## X1002.95537959577 -0.0924342460
## X943.961229494259 -0.0915650916
## X320.997909241559 -0.0907305440
## X849.977486523324 -0.0900179868
## X548.002503827387 -0.0864849042
## X1364.95342014471 -0.0848014939
## X667.985410425584 -0.0825819686
## X1539.96588681403 -0.0818807443
## X439.973315198955 -0.0747832594
## X1177.95950779409 -0.0705672962
## X1537.94035488602 -0.0702813560
## X1176.95174154844 -0.0693124850
## X311.000291548275 -0.0673917656
## X1190.95179773088 -0.0660990078
## X297.965942084671 -0.0659084293
## X151.01222317911  -0.0641287500
## X828.975599111753 -0.0627652369
## X1462.91813967964 -0.0582347094
## X492.989329994173 -0.0568171097
## X655.964796055963 -0.0556936893
## X1209.9715082933  -0.0554816541
## X1001.96773728958 -0.0511680114
## X496.976355787476 -0.0504382102
## X843.965940610961 -0.0496897756
## X200.006491950786 -0.0493434127
## X485.000453042681 -0.0492835816
## X1005.98287863099 -0.0484741503
## X894.987582775265 -0.0484167703
## X1288.91771445373 -0.0466471937
## X892.958412895177 -0.0465485025
```

```
## X840.974050128923 -0.0462979834
## X675.97602596745  -0.0462678146
## X493.984197587741 -0.0442490380
## X832.995567847355 -0.0441367913
## X1066.96015657453 -0.0439418244
## X483.981165431415 -0.0373671386
## X862.985775931247 -0.0364052212
## X653.970909319968 -0.0362206773
## X839.970909295556 -0.0334228170
## X1067.97416491294 -0.0331244928
## X494.99544329672  -0.0317928369
## X1538.95350383304 -0.0308769093
## X1114.9157769402  -0.0284955747
## X1016.95098645776 -0.0219369799
## X506.971749443834 -0.0166512968
## X676.816971205036 -0.0063743103
## X666.980866980263 -0.0054246355
## X308.001278383708 -0.0041169721
## X1187.95480089979 -0.0008532640
## X372.982080621569 -0.0001506316
```

```r
# plot loadings for comp 1
plotLoadings(spca.res, ndisplay = 50)
```



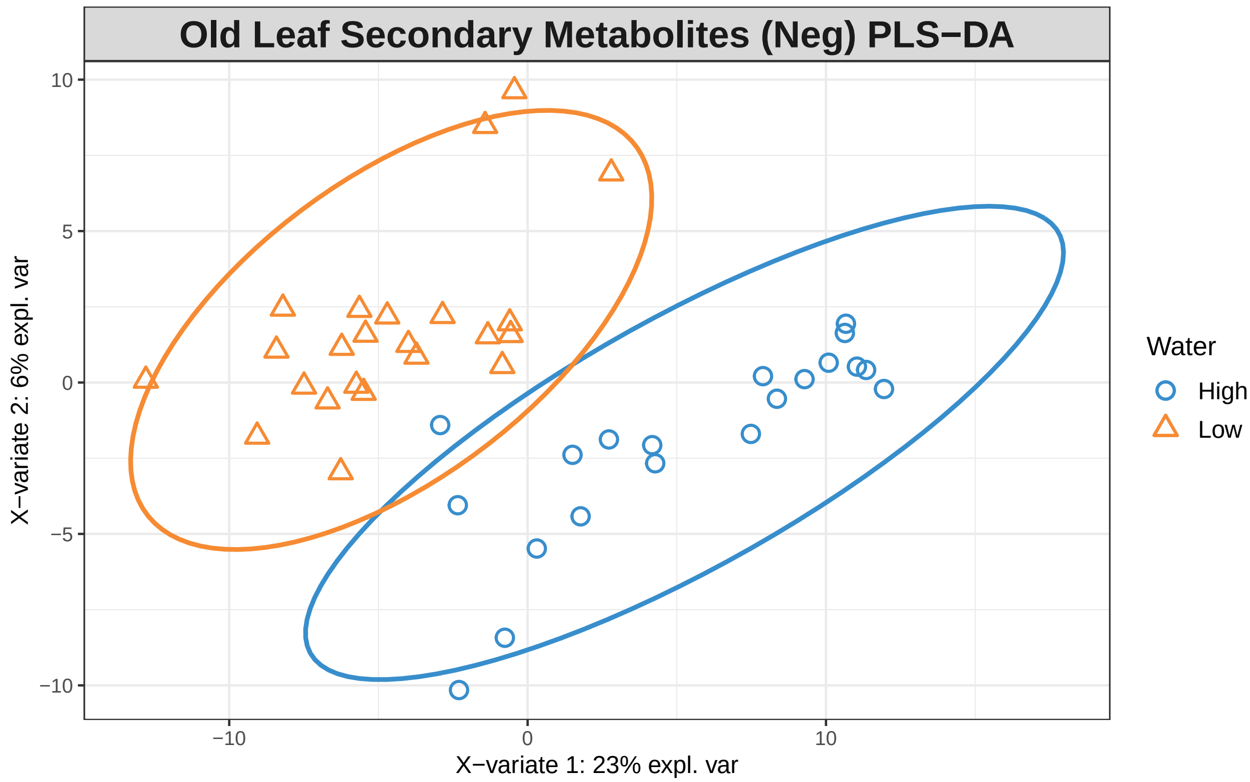

```r
# plot loadings for comp 2
plotLoadings(spca.res, comp = 2, ndisplay = 50)
```



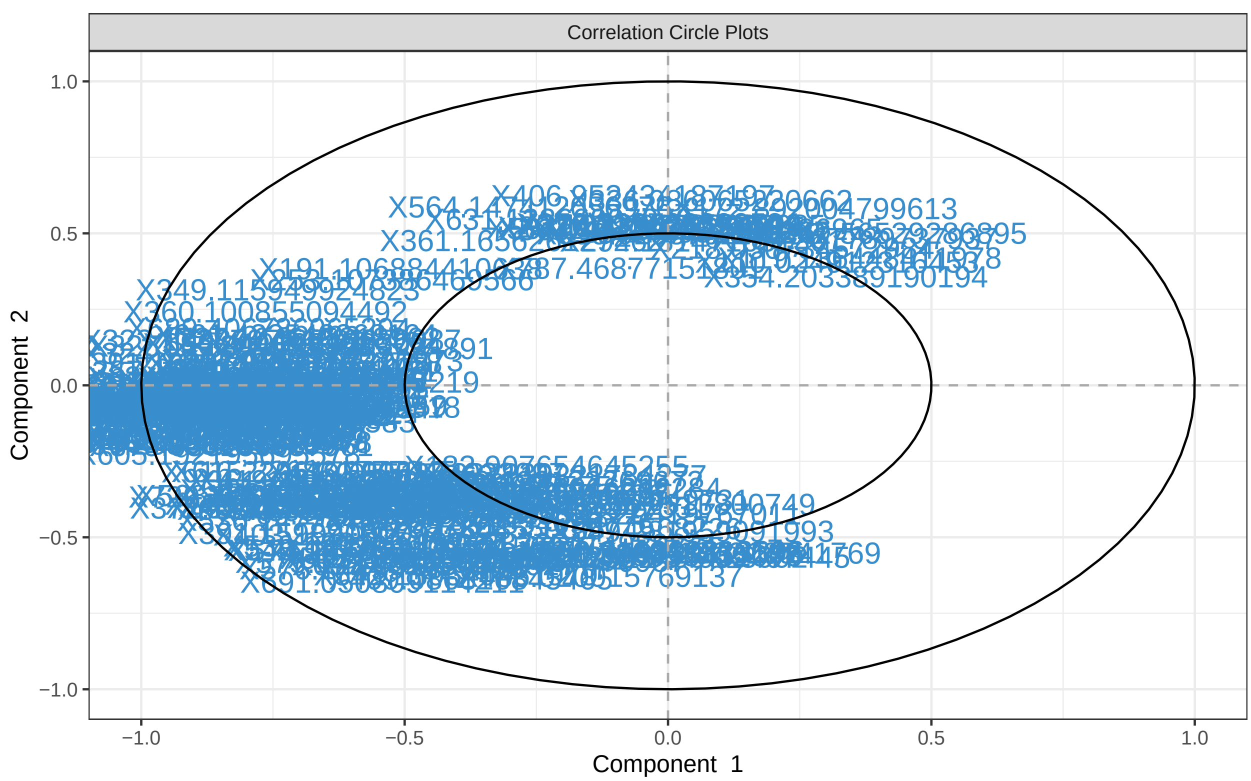
PLS-DA

10. Classify samples into known groups and predict the class of new samples.

```
# Old Leaf
old.splsda <- mixOmics::splsda(scaled_Y_old, class$Water, keepX = c(100,
    100))

# plot pls-da
plotIndiv(old.splsda, ind.names = F, legend = T, title = "Old Leaf Secondary Metabolites (Neg) PLS-DA",
    legend.title = "Water", ellipse = T)
```




```
# plot and select the variables
plotVar(old.splsda)
```



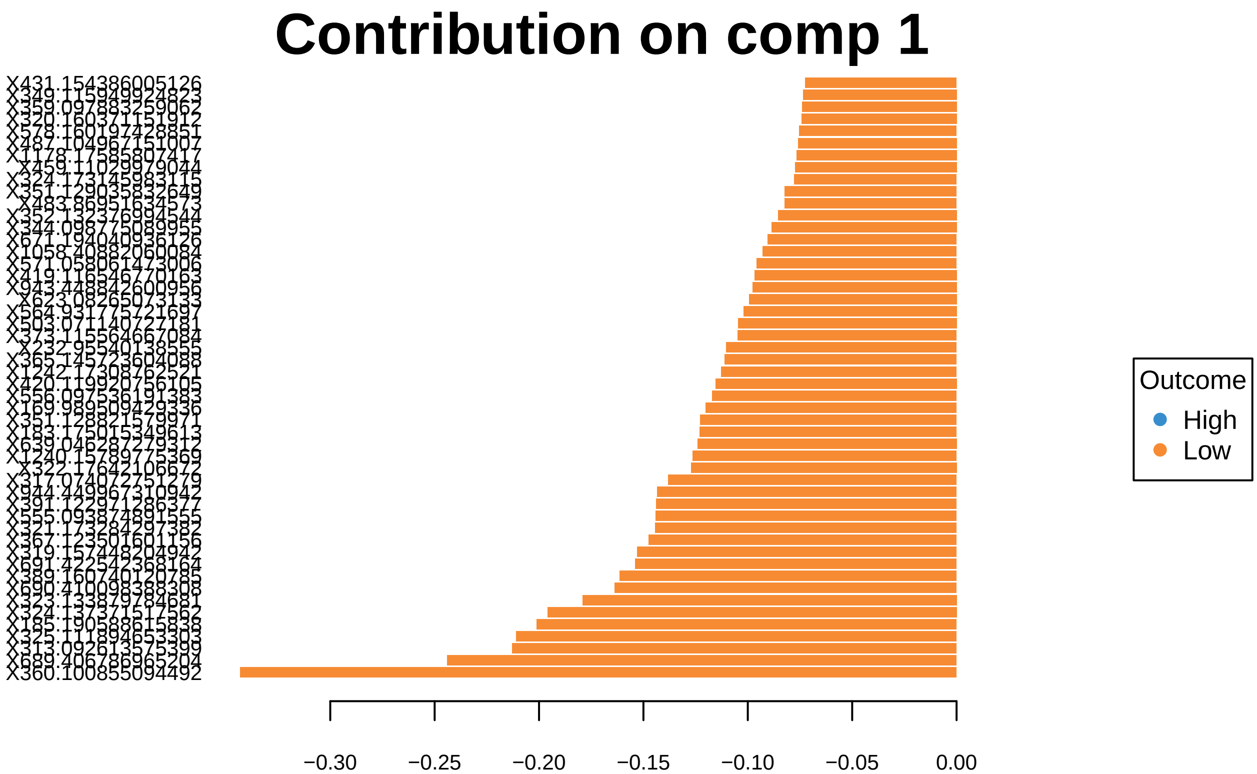
```r
selectVar(old.splsda, comp = 1)
```

```
## $name
##   [1] "X360.100855094492" "X689.406786965204" "X313.092613575399"
##   [4] "X325.111894653303" "X185.190588615838" "X324.137371517562"
##   [7] "X323.133879784681" "X690.410098388308" "X389.160740120785"
##  [10] "X691.422542368164" "X319.157448204942" "X367.123501601156"
##  [13] "X321.173284297382" "X555.093874891555" "X391.12297128637"
##  [16] "X944.449967310942" "X317.074072751279" "X322.17642106672"
##  [19] "X1240.15789775369" "X639.046287279312" "X183.175015349613"
##  [22] "X351.128821579971" "X169.989509429336" "X556.097536191383"
##  [25] "X420.119920756105" "X1242.17308762521" "X365.145723604088"
##  [28] "X232.95540138555"  "X373.115564667084" "X503.071140727181"
##  [31] "X564.931775721697" "X623.08265073133"  "X943.448842600956"
##  [34] "X419.116546770163" "X571.058061473006" "X1058.40882060084"
##  [37] "X671.194040936126" "X344.098775089955" "X352.132376994544"
##  [40] "X483.86951634573"  "X351.129035832649" "X324.173145983115"
##  [43] "X459.11029979044"  "X1178.17585807417" "X487.104967151007"
##  [46] "X578.160197428851" "X320.160371151912" "X359.097883259062"
##  [49] "X349.115949924823" "X431.154386005126" "X604.208532818709"
##  [52] "X340.075145186925" "X779.244559972528" "X537.082677183086"
##  [55] "X481.088866272944" "X435.08241905927"  "X1243.17676615566"
##  [58] "X707.029978476026" "X488.109293490248" "X461.06951805573"
##  [61] "X380.158184370181" "X561.03604963324"  "X737.232626061852"
##  [64] "X403.160178776661" "X648.205609857975" "X402.11215295727"
##  [67] "X483.119144506346" "X341.965577057768" "X531.066415927912"
##  [70] "X727.164615818962" "X379.328828228171" "X253.107386469566"
##  [73] "X582.225274749511" "X538.198579985026" "X509.112370096629"
##  [76] "X797.114458482459" "X170.985886728112" "X339.118846056087"
##  [79] "X605.192195695578" "X581.222187754695" "X317.075176414668"
##  [82] "X480.872661584228" "X191.106884410638" "X337.077569276891"
##  [85] "X813.080943994947" "X274.831245724891" "X1244.17196607701"
##  [88] "X125.083041728111" "X403.124191936883" "X745.249207080709"
##  [91] "X492.892293308974" "X173.831930748219" "X420.157767082518"
##  [94] "X1411.56721539583" "X173.117683745721" "X326.186522086171"
##  [97] "X177.054713421273" "X363.089853013778" "X338.08099104949"
## [100] "X379.056256037961"
##
## $value
##                    value.var
## X360.100855094492 -0.343087113
## X689.406786965204 -0.243980813
## X313.092613575399 -0.212848057
## X325.111894653303 -0.210978045
## X185.190588615838 -0.201115999
## X324.137371517562 -0.195958410
## X323.133879784681 -0.179196447
## X690.410098388308 -0.163741506
## X389.160740120785 -0.161347090
## X691.422542368164 -0.153900693
## X319.157448204942 -0.152947273
## X367.123501601156 -0.147500421
## X321.173284297382 -0.144279341
## X555.093874891555 -0.144164122
```

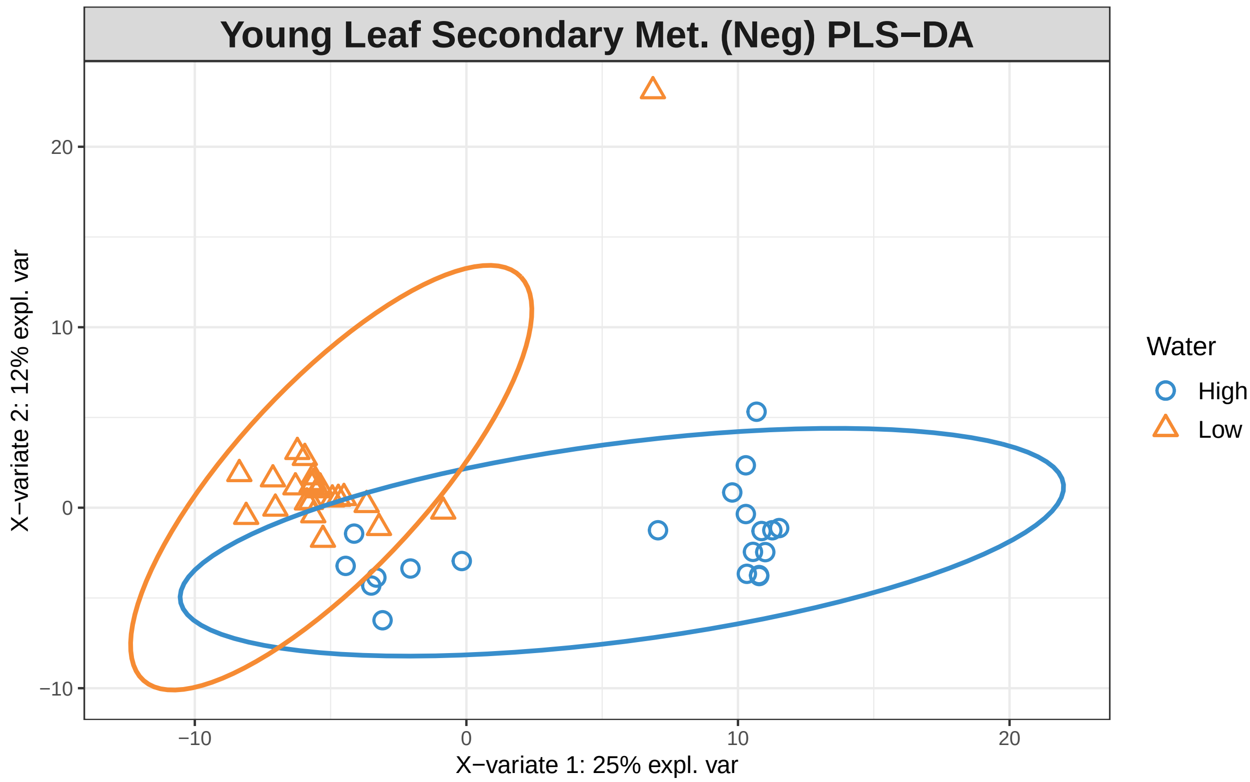
```
## X391.122971286377 -0.143791645
## X944.449967310942 -0.143389701
## X317.074072751279 -0.138157675
## X322.17642106672  -0.127191868
## X1240.15789775369 -0.126435373
## X639.046287279312 -0.124081960
## X183.175015349613 -0.122999679
## X351.128821579971 -0.122763557
## X169.989509429336 -0.120186465
## X556.097536191383 -0.117034237
## X420.119920756105 -0.115473266
## X1242.17308762521 -0.112731694
## X365.145723604088 -0.111058298
## X232.95540138555  -0.110330724
## X373.115564667084 -0.104760137
## X503.071140727181 -0.104718524
## X564.931775721697 -0.102057012
## X623.08265073133  -0.099408492
## X943.448842600956 -0.097763217
## X419.116546770163 -0.096815661
## X571.058061473006 -0.095732735
## X1058.40882060084 -0.092767379
## X671.194040936126 -0.090464559
## X344.098775089955 -0.088669610
## X352.132376994544 -0.085510281
## X483.86951634573  -0.082308021
## X351.129035832649 -0.082287182
## X324.173145983115 -0.077698371
## X459.11029979044  -0.077395460
## X1178.17585807417 -0.076698722
## X487.104967151007 -0.075986692
## X578.160197428851 -0.075288966
## X320.160371151912 -0.074244898
## X359.097883259062 -0.074035136
## X349.115949924823 -0.073591353
## X431.154386005126 -0.072490068
## X604.208532818709 -0.072236378
## X340.075145186925 -0.071596818
## X779.244559972528 -0.071183880
## X537.082677183086 -0.069883004
## X481.088866272944 -0.068707232
## X435.08241905927  -0.068085888
## X1243.17676615566 -0.056126462
## X707.029978476026 -0.055657940
## X488.109293490248 -0.055151086
## X461.06951805573  -0.053574443
## X380.158184370181 -0.051874729
## X561.03604963324  -0.050211614
## X737.232626061852 -0.049763044
## X403.160178776661 -0.049653036
## X648.205609857975 -0.048848009
## X402.11215295727  -0.045746915
## X483.119144506346 -0.043102012
## X341.965577057768 -0.042460376
```

```
## X531.066415927912 -0.040675698
## X727.164615818962 -0.040650908
## X379.328828228171 -0.033741877
## X253.107386469566 -0.033311519
## X582.225274749511 -0.029564631
## X538.198579985026 -0.028853103
## X509.112370096629 -0.028625407
## X797.114458482459 -0.028095612
## X170.985886728112 -0.027017507
## X339.118846056087 -0.026546405
## X605.192195695578 -0.026545106
## X581.222187754695 -0.026514681
## X317.075176414668 -0.025846378
## X480.872661584228 -0.024447803
## X191.106884410638 -0.019600042
## X337.077569276891 -0.017707061
## X813.080943994947 -0.015917969
## X274.831245724891 -0.013837994
## X1244.17196607701 -0.012832722
## X125.083041728111 -0.011628397
## X403.124191936883 -0.011363122
## X745.249207080709 -0.010654157
## X492.892293308974 -0.008953790
## X173.831930748219 -0.008696817
## X420.157767082518 -0.007923736
## X1411.56721539583 -0.006699450
## X173.117683745721 -0.005112417
## X326.186522086171 -0.002636670
## X177.054713421273 -0.002103826
## X363.089853013778 -0.002103020
## X338.08099104949  -0.001091795
## X379.056256037961 -0.001047459
##
## $comp
## [1] 1
```

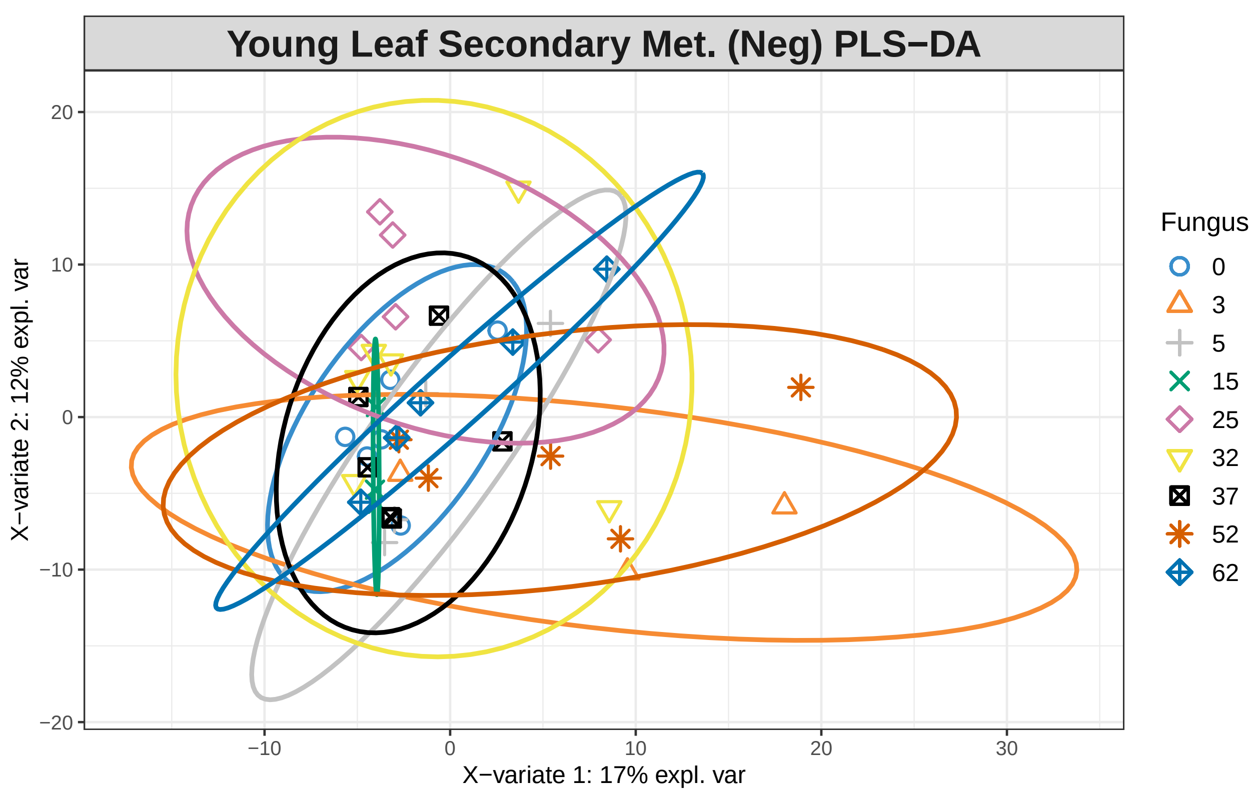
```r
plotLoadings(old.splsda, contrib = "max", method = "mean", ndisplay = 50)
```



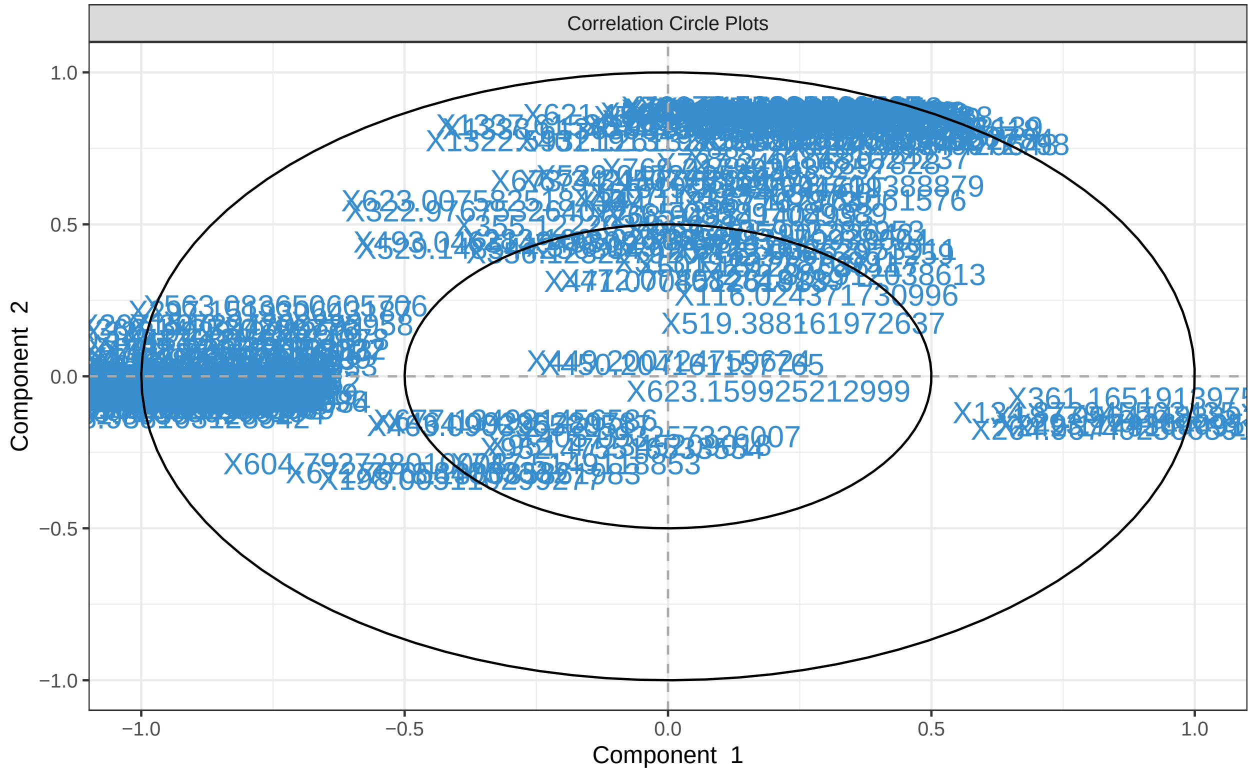

```r
# Young Leaf
young.splsda <- mixOmics::splsda(scaled_Y_young, class$Water,
    keepX = c(100, 100))
young.splsda2 <- mixOmics::splsda(scaled_Y_young, class$Fungus,
    keepX = c(100, 100))

# plot pls-da
plotIndiv(young.splsda, ind.names = F, legend = T, title = "Young Leaf Secondary Met. (Neg) PLS-DA",
    legend.title = "Water", ellipse = T)
```




```r
plotIndiv(young.splsda2, ind.names = F, legend = T, title = "Young Leaf Secondary Met. (Neg) PLS-DA",
    legend.title = "Fungus", ellipse = T)
```



```r
# plot and select the variables
plotVar(young.splsda)
```




```r
selectVar(young.splsda, comp = 1)
```

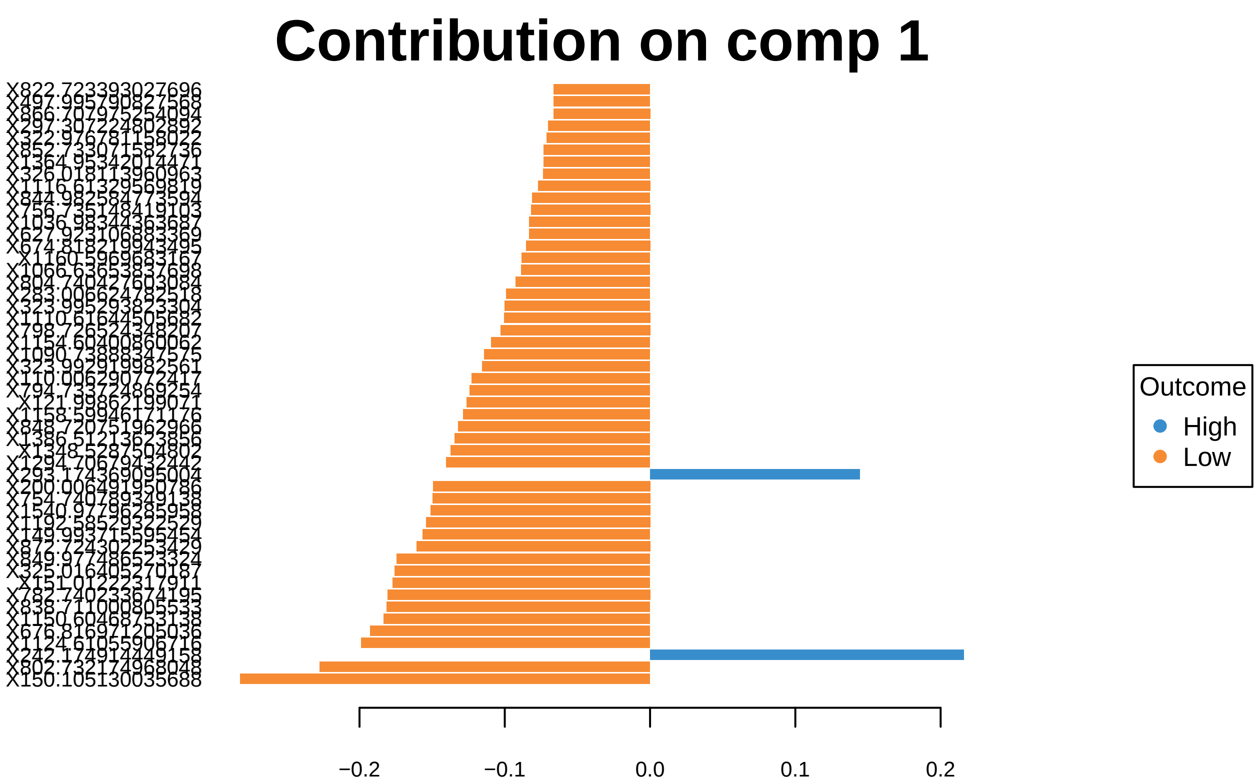
```
## $name
##   [1] "X150.105130035688" "X802.732174968048" "X242.174914449158"
##   [4] "X1124.61055906716" "X676.816971205036" "X1150.60468753138"
##   [7] "X838.711000805533" "X782.740233674195" "X151.01222317911"
##  [10] "X325.016405270187" "X849.977486523324" "X872.724302253429"
##  [13] "X149.993715595454" "X1192.58529322529" "X1540.97796285958"
##  [16] "X754.740789349138" "X200.006491950786" "X293.174369095004"
##  [19] "X1294.70679432442" "X1348.5287504802"  "X1386.51213623856"
##  [22] "X848.720751962966" "X1158.59946171176" "X121.99862199071"
##  [25] "X794.733724869254" "X110.006290772417" "X323.992919982561"
##  [28] "X1090.73888347575" "X1154.60400860062" "X798.726524348207"
##  [31] "X1110.61644505682" "X323.995293823304" "X283.006624782518"
##  [34] "X804.740427603084" "X1066.63653837698" "X1160.5969683167"
##  [37] "X674.818219943495" "X627.923106883369" "X1036.98344363687"
##  [40] "X756.735148419103" "X844.982584773594" "X1116.61329569819"
##  [43] "X326.018113960963" "X1364.95342014471" "X852.733071582736"
##  [46] "X322.976781158022" "X297.307224802892" "X866.707975254094"
##  [49] "X497.995790827568" "X822.723393027696" "X830.737149656595"
##  [52] "X133.998876296918" "X482.966873192152" "X874.707571794695"
##  [55] "X424.843687850262" "X1158.72895999915" "X671.998240343484"
##  [58] "X369.877177619296" "X1350.52689216505" "X1539.96588681403"
##  [61] "X297.151930643187" "X850.821164746369" "X484.12840584078"
##  [64] "X1078.62469612196" "X134.872945563435" "X845.998051933969"
```

```
##  [67] "X840.71024774808"  "X514.986512120569" "X421.955540488549"
##  [70] "X751.803644269174" "X828.732413615017" "X563.083650605706"
##  [73] "X1019.99560196699" "X1191.96821780793" "X152.014000365393"
##  [76] "X1018.98243765579" "X1138.61401223754" "X892.958412895177"
##  [79] "X1192.98175033474" "X632.970118158626" "X361.165191397563"
##  [82] "X558.938103128542" "X854.728311606954" "X896.710609558084"
##  [85] "X843.965940610961" "X670.981382467921" "X657.982854820673"
##  [88] "X366.142530296776" "X538.815638527027" "X264.967492566591"
##  [91] "X187.096414888931" "X1128.61230614359" "X824.820684339506"
##  [94] "X826.731366769468" "X1177.95950779409" "X337.904579660098"
##  [97] "X1711.93957530353" "X916.69988248716"  "X1103.61628997104"
## [100] "X339.90280551721"
##
## $value
##                      value.var
## X150.105130035688 -0.282039719
## X802.732174968048 -0.227414352
## X242.174914449158  0.215910257
## X1124.61055906716 -0.198782419
## X676.816971205036 -0.192655075
## X1150.60468753138 -0.183273749
## X838.711000805533 -0.181332267
## X782.740233674195 -0.180686657
## X151.01222317911  -0.176989099
## X325.016405270187 -0.175849728
## X849.977486523324 -0.174259645
## X872.724302253429 -0.160769967
## X149.993715595454 -0.156421706
## X1192.58529322529 -0.154190387
## X1540.97796285958 -0.151174229
## X754.740789349138 -0.149717546
## X200.006491950786 -0.149376121
## X293.174369095004  0.144319007
## X1294.70679432442 -0.140253834
## X1348.5287504802  -0.137292240
## X1386.51213623856 -0.134315079
## X848.720751962966 -0.131984710
## X1158.59946171176 -0.128594472
## X121.99862199071  -0.126133016
## X794.733724869254 -0.124014822
## X110.006290772417 -0.122819961
## X323.992919982561 -0.115547980
## X1090.73888347575 -0.114224424
## X1154.60400860062 -0.109269030
## X798.726524348207 -0.102943924
## X1110.61644505682 -0.100529543
## X323.995293823304 -0.100100667
## X283.006624782518 -0.098992349
## X804.740427603084 -0.092445129
## X1066.63653837698 -0.088592213
## X1160.5969683167  -0.088291119
## X674.818219943495 -0.085416018
## X627.923106883369 -0.083266055
## X1036.98344363687 -0.083040165
```

```
## X756.735148419103 -0.081939466
## X844.982584773594 -0.081009163
## X1116.61329569819 -0.077158656
## X326.018113960963 -0.073578175
## X1364.95342014471 -0.073276382
## X852.733071582736 -0.073097286
## X322.976781158022 -0.071190101
## X297.307224802892 -0.070158011
## X866.707975254094 -0.066433552
## X497.995790827568 -0.066224702
## X822.723393027696 -0.066184178
## X830.737149656595 -0.063541450
## X133.998876296918 -0.063294993
## X482.966873192152 -0.063122686
## X874.707571794695 -0.059919434
## X424.843687850262 -0.059134367
## X1158.72895999915 -0.058494755
## X671.998240343484 -0.058106054
## X369.877177619296 -0.053828920
## X1350.52689216505 -0.052171922
## X1539.96588681403 -0.049501193
## X297.151930643187 -0.048493255
## X850.821164746369 -0.047328889
## X484.12840584078  -0.046426405
## X1078.62469612196 -0.043632423
## X134.872945563435  0.042506082
## X845.998051933969 -0.041902578
## X840.71024774808  -0.041651192
## X514.986512120569 -0.041251783
## X421.955540488549 -0.040089622
## X751.803644269174 -0.039688088
## X828.732413615017 -0.038866785
## X563.083650605706 -0.038776647
## X1019.99560196699 -0.038232314
## X1191.96821780793 -0.037011527
## X152.014000365393 -0.036591298
## X1018.98243765579 -0.034731819
## X1138.61401223754 -0.033671300
## X892.958412895177 -0.033540932
## X1192.98175033474 -0.033489661
## X632.970118158626 -0.028243242
## X361.165191397563  0.026629148
## X558.938103128542 -0.026275125
## X854.728311606954 -0.025659016
## X896.710609558084 -0.023886971
## X843.965940610961 -0.023711050
## X670.981382467921 -0.021465443
## X657.982854820673 -0.020212723
## X366.142530296776 -0.019123888
## X538.815638527027 -0.017573798
## X264.967492566591  0.015608624
## X187.096414888931  0.015526290
## X1128.61230614359 -0.014892070
## X824.820684339506 -0.013553869
```

```
## X826.731366769468 -0.012032830
## X1177.95950779409 -0.011616170
## X337.904579660098 -0.011374628
## X1711.93957530353 -0.009008417
## X916.69988248716  -0.008695822
## X1103.61628997104 -0.006663306
## X339.90280551721  -0.006540303
##
## $comp
## [1] 1
```

```r
plotLoadings(young.splsda, contrib = "max", method = "mean",
    ndisplay = 50)
```



Heatmaps of Averaged Data

11. Create averaged metabolite matrices and rerun PLS-DA to create a heatmap.

```r
## Old Leaves
av_Y_old <- aggregate(Y_old, by = list(class$Water, class$Fungus),
    FUN = "mean", simplify = T, data = class)
av.old.plsda <- mixOmics::plsda(av_Y_old[, 3:3735], av_Y_old$Group.1)  # water

# heatmap
oldcim <- cim(av.old.plsda, title = "Old Leaf Secondary Met. (neg) Averaged Over Water",
    col.names = F, xlab = "Secondary Metabolites", save = "png",
    name.save = "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/

## Young Leaves
```

```r
av_Y_young <- aggregate(Y_young, by = list(class$Water, class$Fungus),
    FUN = "mean", simplify = T, data = class)
av.young.plsda <- mixOmics::plsda(av_Y_young[, 3:2566], av_Y_young$Group.1)  # water
av.young.plsda2 <- mixOmics::plsda(av_Y_young[, 3:2566], av_Y_young$Group.2)  # fungus

# heatmap
youngcim <- cim(av.young.plsda, title = "Young Leaf Secondary Met. (neg) Averaged Over Water",
    col.names = F, xlab = "Secondary Metabolites", save = "png",
    name.save = "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/S

# heatmap
youngcim2 <- cim(av.young.plsda2, title = "Young Leaf Secondary Met. (neg) Averaged Over Fungi",
    col.names = F, xlab = "Secondary Metabolites", save = "png",
    name.save = "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/S
```

Indicator Analysis

12. Identify indicator metabolites characteristic of each treatment using Dufrene-Legendre Indicator Analysis.

```r
# Old Leaf
indicator_Water <- indval(Y_young, clustering = class$Water,
    numitr = 999, type = "long")

# Young Leaf
indicator_Water <- indval(Y_young, clustering = class$Water,
    numitr = 999, type = "long")
```

13. Disect indval object.

```r
Orelfrq <- indicator_Water$relfrq  # relative frequency of species in classes
Orelabu <- indicator_Water$relabu  # relative abundance of species in classes
Oindval <- indicator_Water$indval  # the indicator value for each species
Omaxcls <- data.frame(indicator_Water$maxcls)  # the class each species has max indicator value for
Oindcls <- data.frame(indicator_Water$indcls)  # the indicator value for each species to its max class
Opval <- data.frame(indicator_Water$pval)  # the probability of obtaining as high an indicator value as

Yrelfrq <- indicator_Water$relfrq  # relative frequency of species in classes
Yrelabu <- indicator_Water$relabu  # relative abundance of species in classes
Yindval <- indicator_Water$indval  # the indicator value for each species
Ymaxcls <- data.frame(indicator_Water$maxcls)  # the class each species has max indicator value for
Yindcls <- data.frame(indicator_Water$indcls)  # the indicator value for each species to its max class
Ypval <- data.frame(indicator_Water$pval)  # the probability of obtaining as high an indicator value as
```

14. Export results to a csv file.

```r
write.csv(cbind(Orelfrq, Orelabu, Oindval, Omaxcls, Oindcls,
    Opval), "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/Seco
write.csv(cbind(Yrelfrq, Yrelabu, Yindval, Ymaxcls, Yindcls,
    Ypval), "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/Seco
```

Secondary Metabolites (Pos)

RRPP

2. Define dependent variable matrix and class matrix.

```r
path <- "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/"

O_SM_pos <- read_tsv(paste(path, "XCMS Online Results/O_SM_Pos/XCMS.annotated.Report_1394418.tsv",
    sep = ""))

Y_SM_pos <- read_tsv(paste(path, "XCMS Online Results/Y_SM_Pos/XCMS.annotated.Report_1394440.tsv",
    sep = ""))

# dependent variable: metabolite intensities
Y_old <- O_SM_pos[, c(2, 12:54)] %>% data.frame(row.names = 1) %>%
    t %>% data.frame()
scaled_Y_old <- scale(Y_old)

Y_young <- Y_SM_pos[, c(2, 12:54)] %>% data.frame(row.names = 1) %>%
    t %>% data.frame()
scaled_Y_young <- scale(Y_young)

# class: sample factors
class <- read.csv(paste(path, "XCMS Online Results/class.csv",
    sep = ""), header = T, row.names = 1)
```

3. Define and run multivariate regression models, then print out the results.

```r
O_LMpos <- lm.rrpp(scaled_Y_old ~ Block * Water * Fungus, data = class,
    SS.type = "III", print.progress = F)
summary(O_LMpos)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 5800
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Full Model Analysis of Variance
##
##                        Df Residual Df       SS Residual SS       Rsq        F
## Block * Water * Fungus  7          35 66102.35    177497.7 0.2713561 1.862063
##                        Z (from F) Pr(>F)
## Block * Water * Fungus    3.72936  0.002
##
##
## Redundancy Analysis (PCA on fitted values and residuals)
##
##              Trace Proportion Rank
## Fitted    1573.865   0.271356    7
## Residuals 4226.135   0.728644   35
## Total     5800.000   1.000000   42
```

```
##
## Eigenvalues
##
##                  PC1       PC2       PC3       PC4       PC5       PC6       PC7
## Fitted     613.0058  377.3605  245.4204  108.9341   90.8147   75.5811   62.7487
## Residuals 1125.9274  456.5069  301.3978  228.3176  221.2095  152.5997  143.8765
## Total     1563.9592  668.0796  447.6527  365.5013  244.1181  236.9154  180.9496
##                  PC8       PC9      PC10      PC11      PC12      PC13      PC14
## Fitted
## Residuals  138.6110  120.4927  105.6991  100.1973   86.5102   79.7117   76.6642
## Total      164.0427  148.5100  136.1229  116.6738  109.9659   96.2741   89.0995
##                 PC15      PC16      PC17      PC18      PC19      PC20      PC21
## Fitted
## Residuals   71.8936   67.2005   61.6455   60.0418   56.5602   54.0174   51.0623
## Total       84.0573   78.2452   75.8352   68.1756   62.8428   61.6040   59.1024
##                 PC22      PC23      PC24      PC25      PC26      PC27      PC28
## Fitted
## Residuals   49.5758   45.6077   44.4495   42.9826   38.1437   35.0037   32.8700
## Total       56.0853   53.7059   49.4072   48.9840   46.9481   46.5683   43.3817
##                 PC29      PC30      PC31      PC32      PC33      PC34      PC35
## Fitted
## Residuals   32.0639   28.5369   27.4785   24.8828   23.9346   21.1897   19.2722
## Total       41.0579   37.8943   34.1106   32.7965   31.8990   29.7583   28.4563
##                 PC36      PC37      PC38      PC39      PC40      PC41      PC42
## Fitted
## Residuals
## Total       26.1381   25.2956   24.1190   24.0956   22.7724   20.3350   18.4638
```

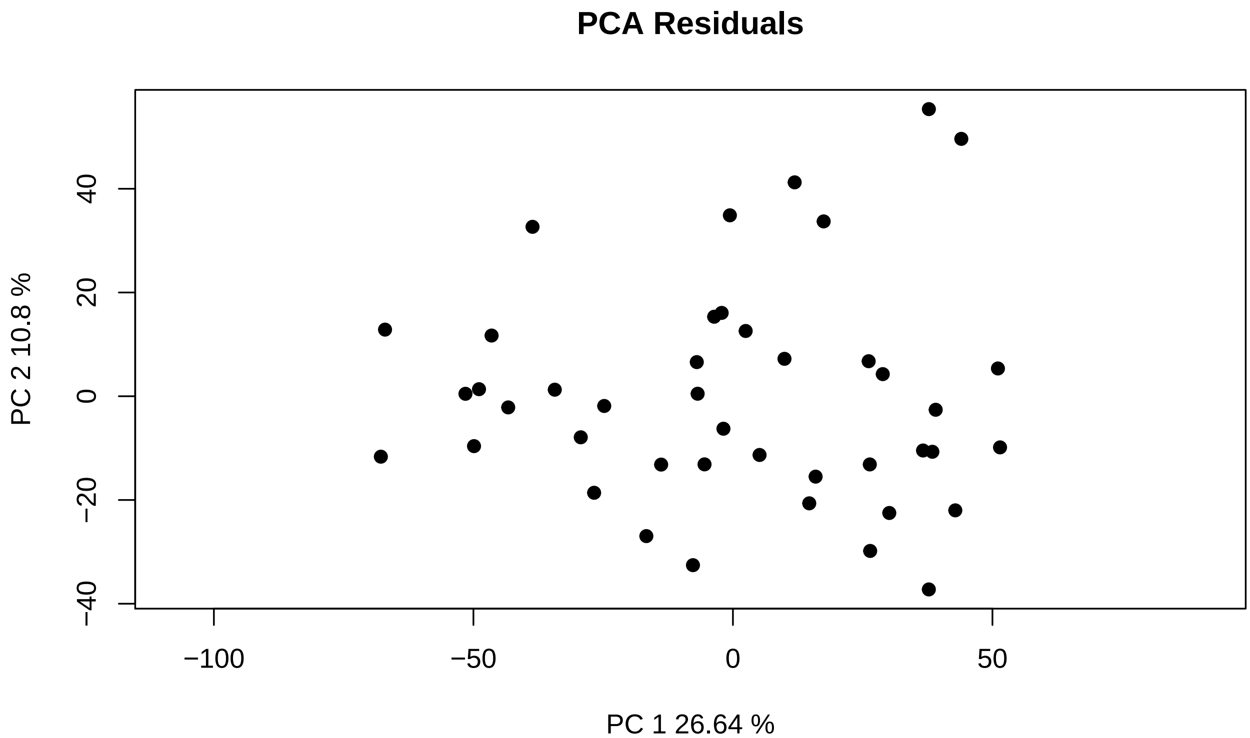
```r
Y_LMpos <- lm.rrpp(scaled_Y_young ~ Block * Water * Fungus, data = class,
    SS.type = "III", print.progress = F)
summary(Y_LMpos)
```

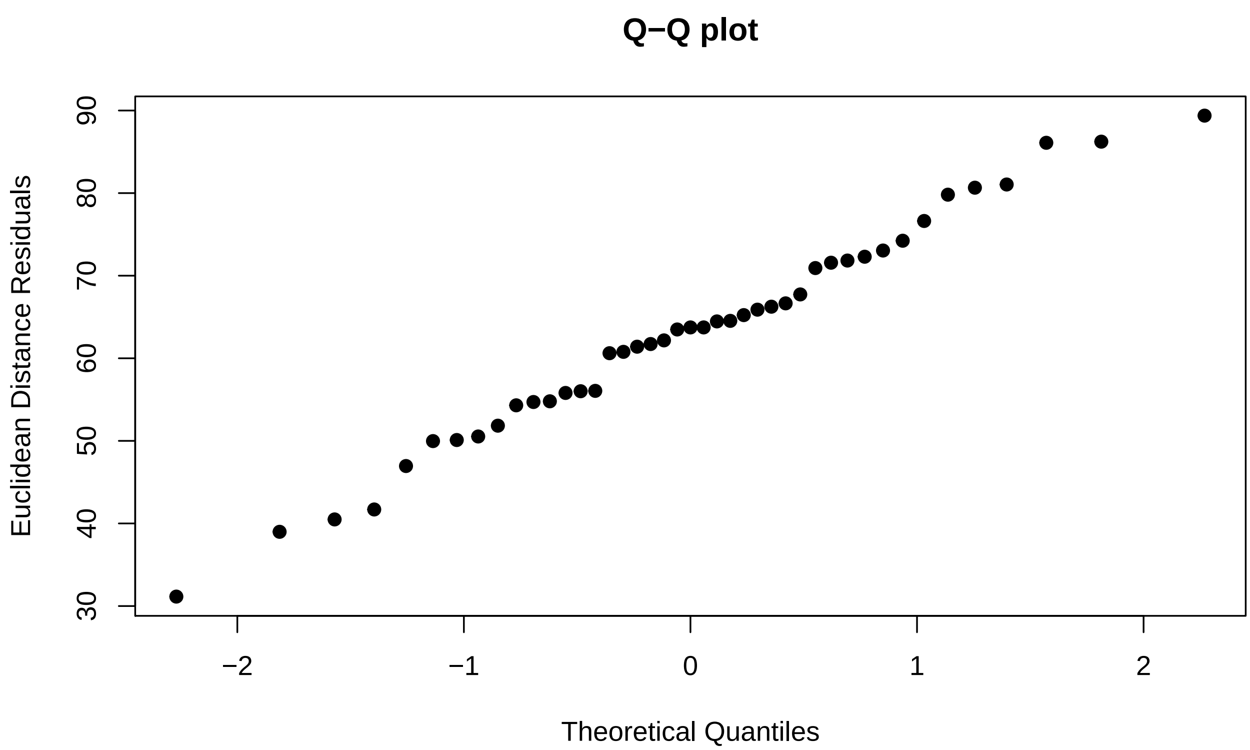
```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 3559
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Full Model Analysis of Variance
##
##                       Df Residual Df       SS Residual SS       Rsq        F
## Block * Water * Fungus  7          35 38642.68    110835.3 0.2585175 1.743248
##                       Z (from F) Pr(>F)
## Block * Water * Fungus    3.36302  0.001
##
##
## Redundancy Analysis (PCA on fitted values and residuals)
##
##              Trace Proportion Rank
## Fitted     920.064  0.2585176    7
## Residuals 2638.936  0.7414824   35
## Total     3559.000  1.0000000   42
```

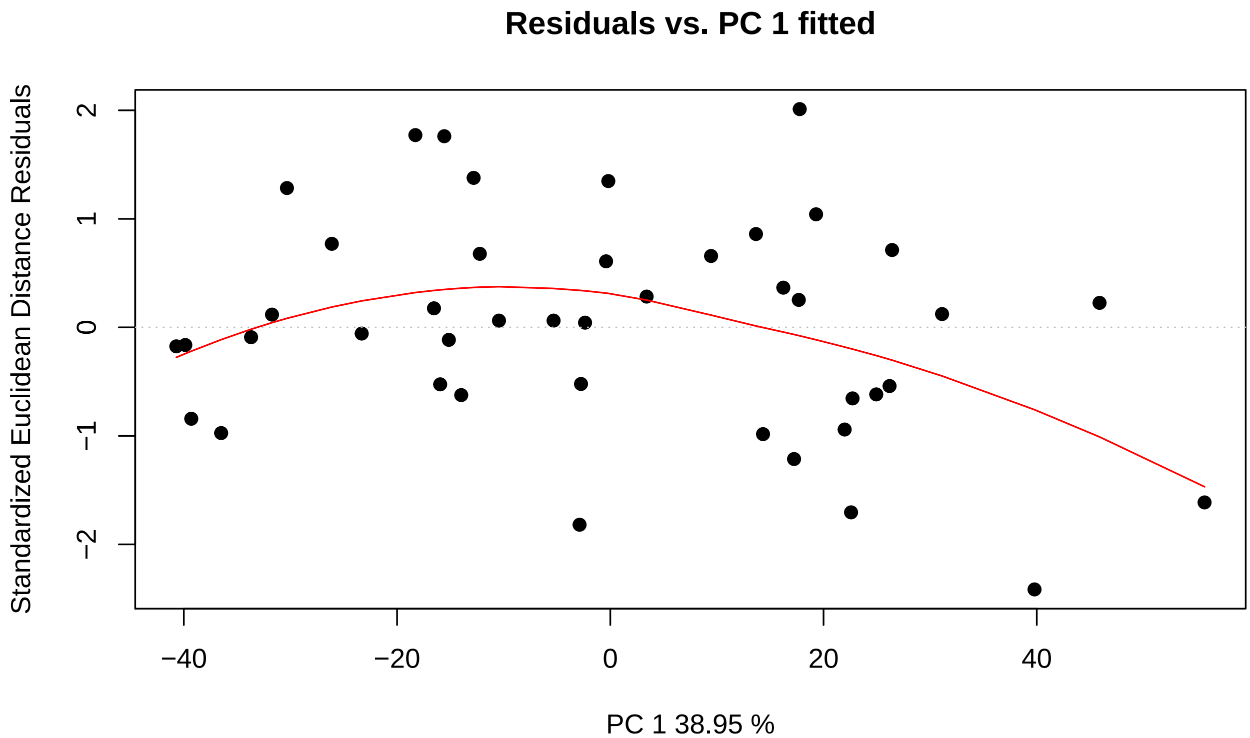
```
##
## Eigenvalues
##
##                PC1      PC2      PC3      PC4      PC5      PC6     PC7
## Fitted    397.7443 240.1652  87.8892  66.1666  47.2456  44.1174 36.7354
## Residuals 633.3110 249.1598 226.5129 162.1190 123.8237  98.7414 86.2987
## Total     812.6211 580.6484 274.7611 195.5419 135.8900 111.6667 91.3427
##                PC8      PC9     PC10     PC11     PC12     PC13    PC14
## Fitted
## Residuals  74.0692  62.7979  62.2331  54.0214  50.0254  49.2183 47.9739
## Total      81.8073  74.4560  68.9230  65.8313  58.9405  52.9731 51.5130
##               PC15     PC16     PC17     PC18     PC19     PC20    PC21
## Fitted
## Residuals  46.9945  42.0306  40.1348  39.0245  37.6032  36.6949 35.0499
## Total      49.3036  48.1786  45.4179  43.1774  42.9165  40.3182 39.0791
##               PC22     PC23     PC24     PC25     PC26     PC27    PC28
## Fitted
## Residuals  33.5945  32.0128  31.2296  30.3538  29.6785  28.2886 27.7751
## Total      37.6835  35.9477  35.1338  33.9837  33.6972  32.1289 31.3560
##               PC29     PC30     PC31     PC32     PC33     PC34    PC35
## Fitted
## Residuals  27.0825  26.2620  25.4541  24.0211  23.4611  22.1253 19.7593
## Total      30.5374  29.9458  28.8928  28.0758  27.1667  26.5044 26.1782
##               PC36     PC37     PC38     PC39     PC40     PC41    PC42
## Fitted
## Residuals
## Total      25.4687  25.1620  23.5154  22.1223  21.2005  19.9071 19.0846
```

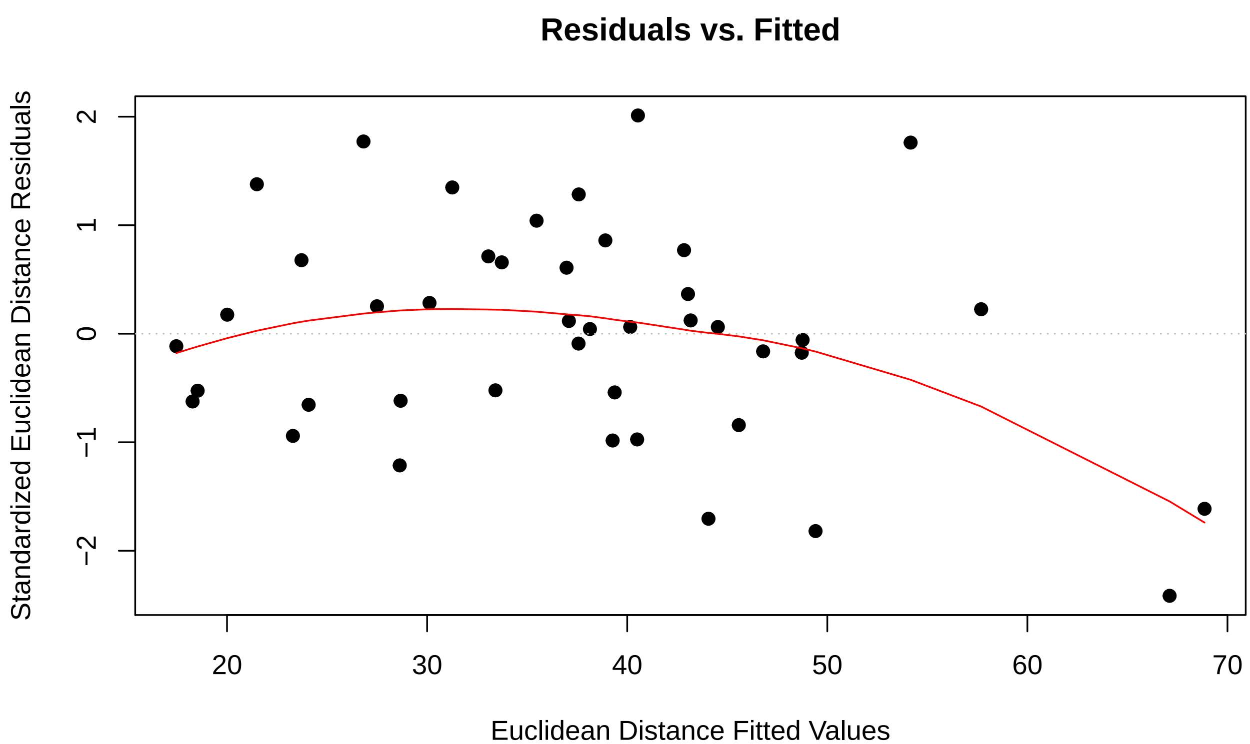
4. Examine RRPP plots to check for assumptions.

```
## Old Leaves residuals vs fitted values (homoscedasticity
## check)
Odiagnostics <- plot(O_LMpos, type = "diagnostics")
```

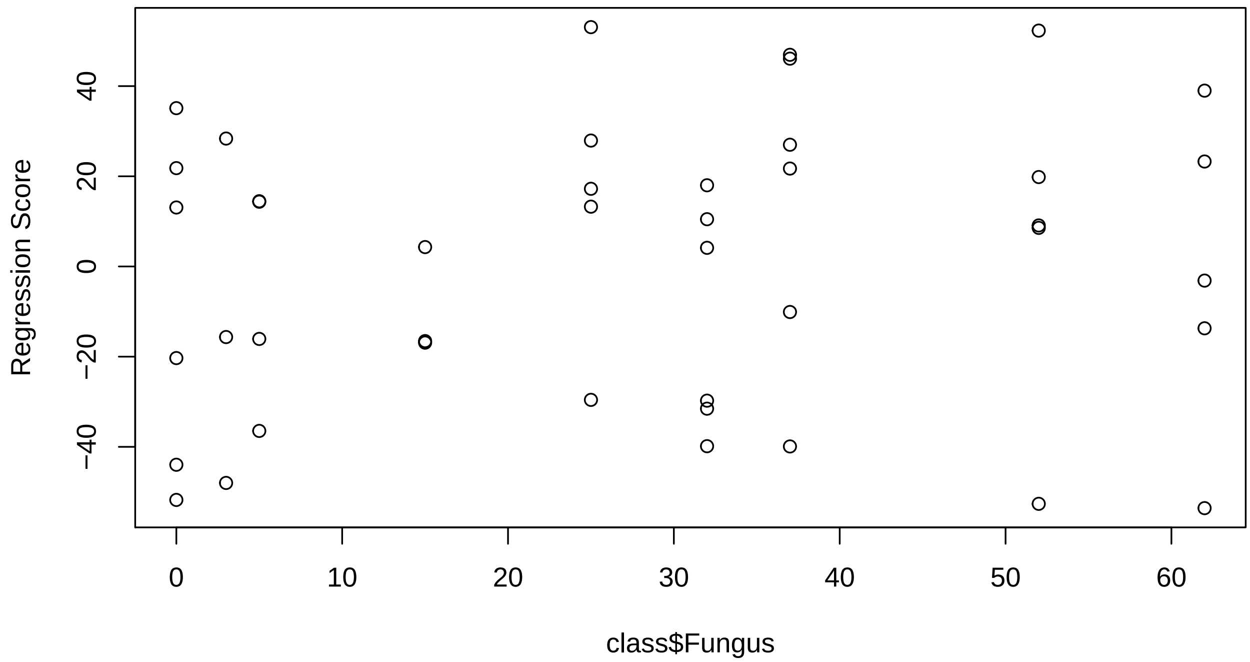

PCA Residuals
PC 2 10.8 %
PC 1 26.64 %
−40
−20
0
20
40
−100
−50
0
50


Q−Q plot
Euclidean Distance Residuals
Theoretical Quantiles
30
40
50
60
70
80
90
−2
−1
0
1
2

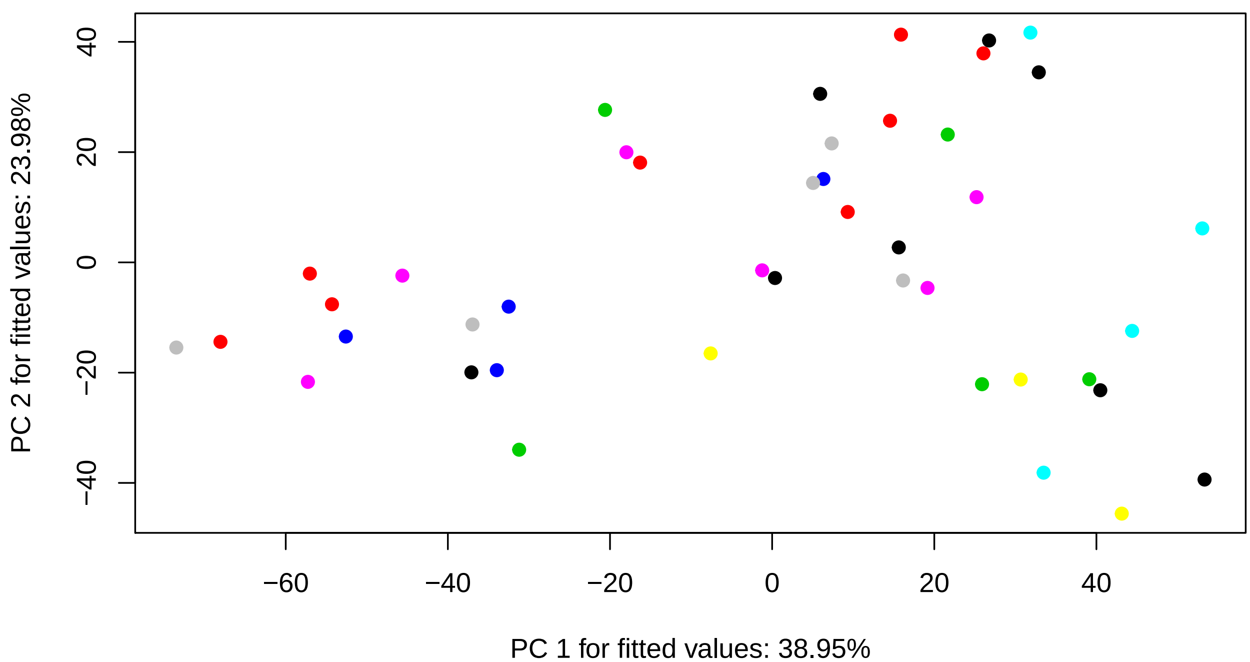




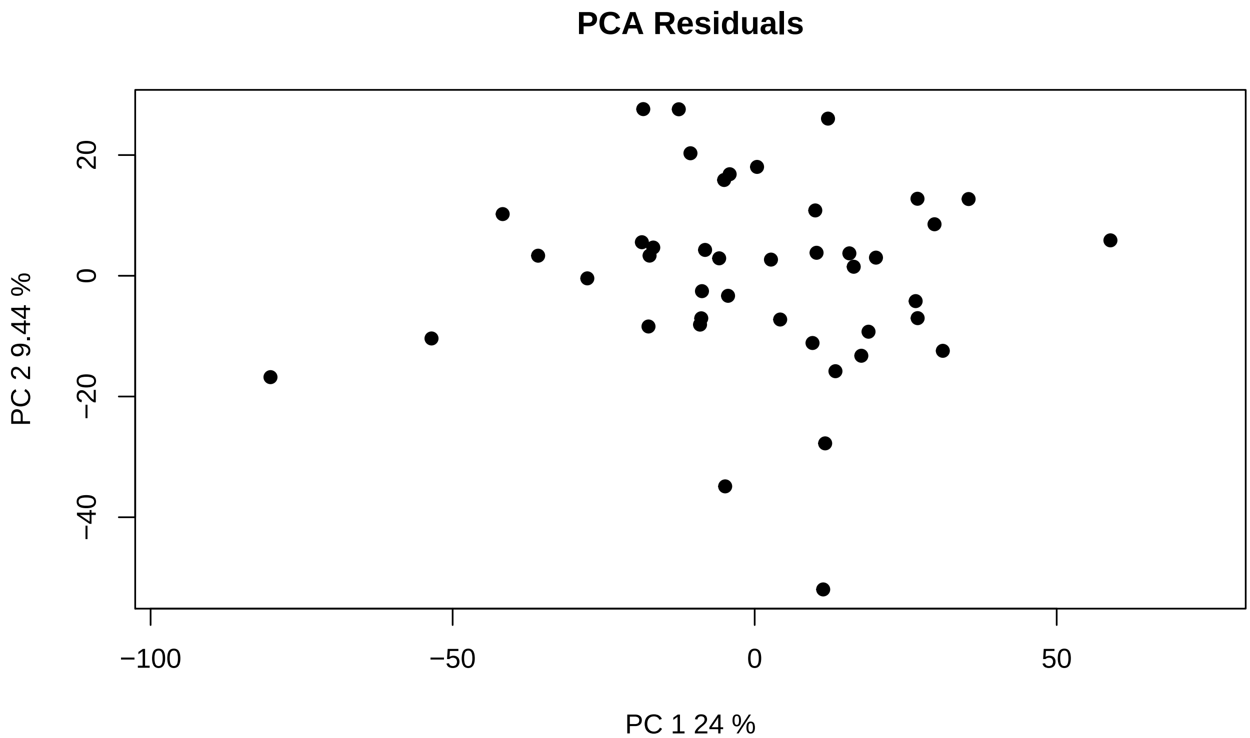

```
# linear regression plot
Oregression <- plot(O_LMpos, type = "regression", predictor = class$Fungus,
    reg.type = "RegScore")
```

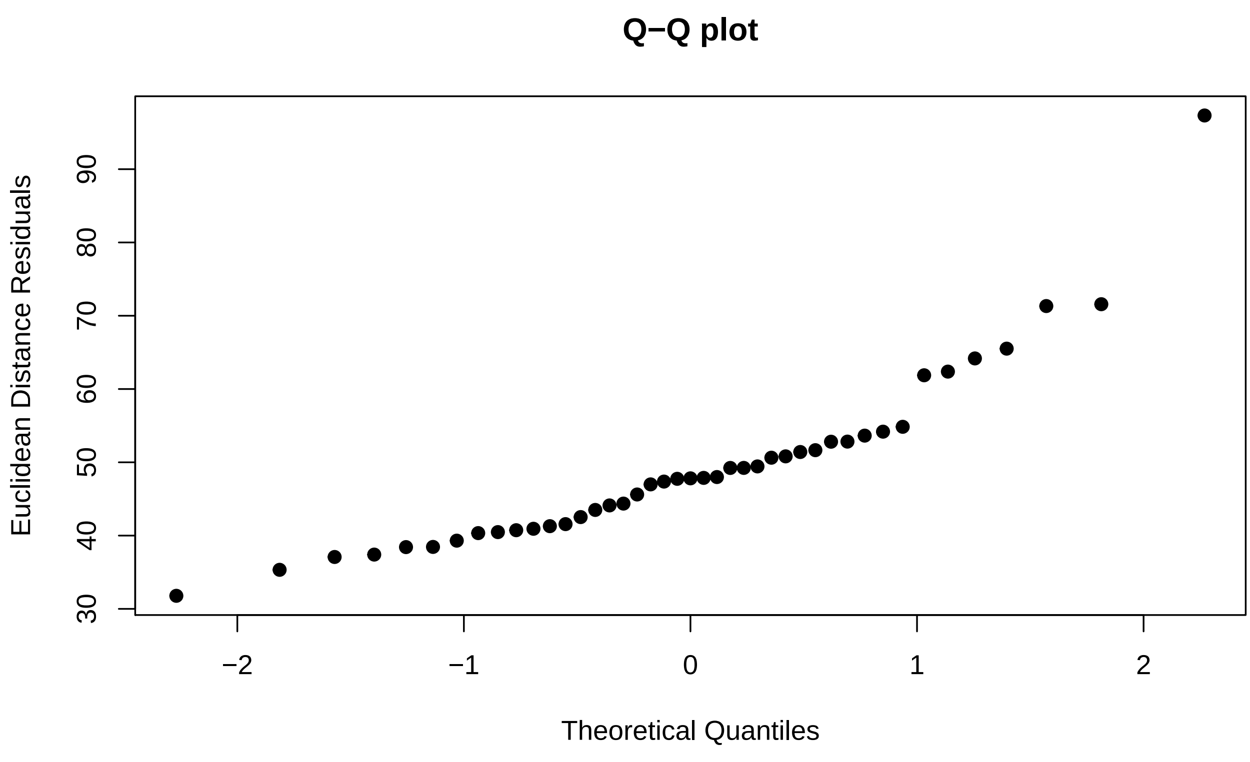


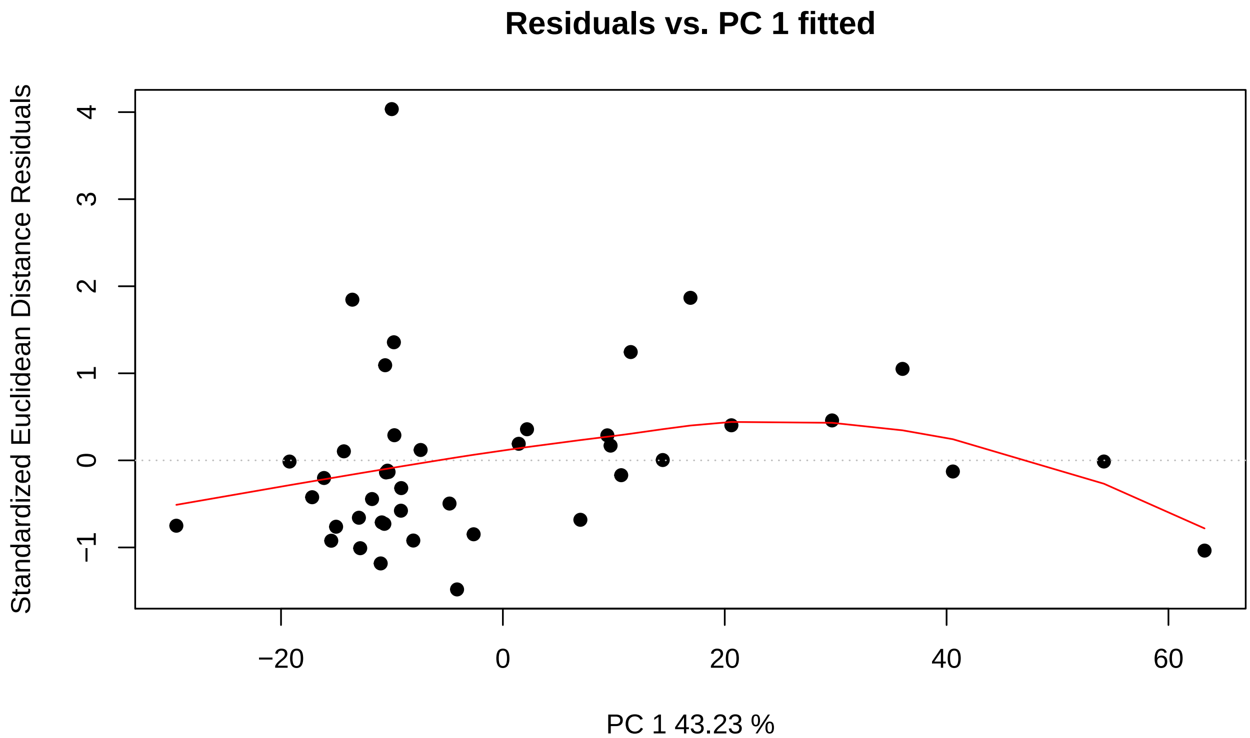

```
# pca plot
Opcplot <- plot(O_LMpos, type = "PC", pch = 19, col = interaction(class$Water,
    class$Fungus))
```

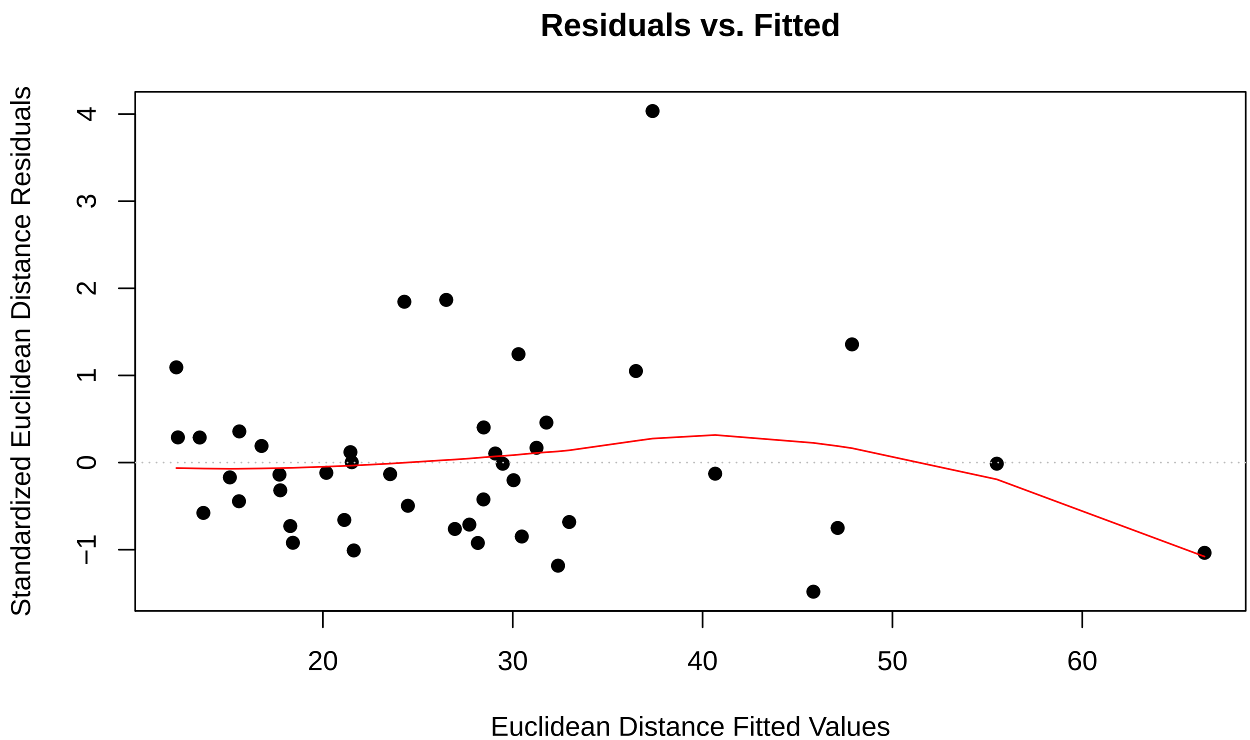



```
## Young Leaves residuals vs fitted values (homoscedasticity
## check)
Ydiagnostics <- plot(Y_LMpos, type = "diagnostics")
```

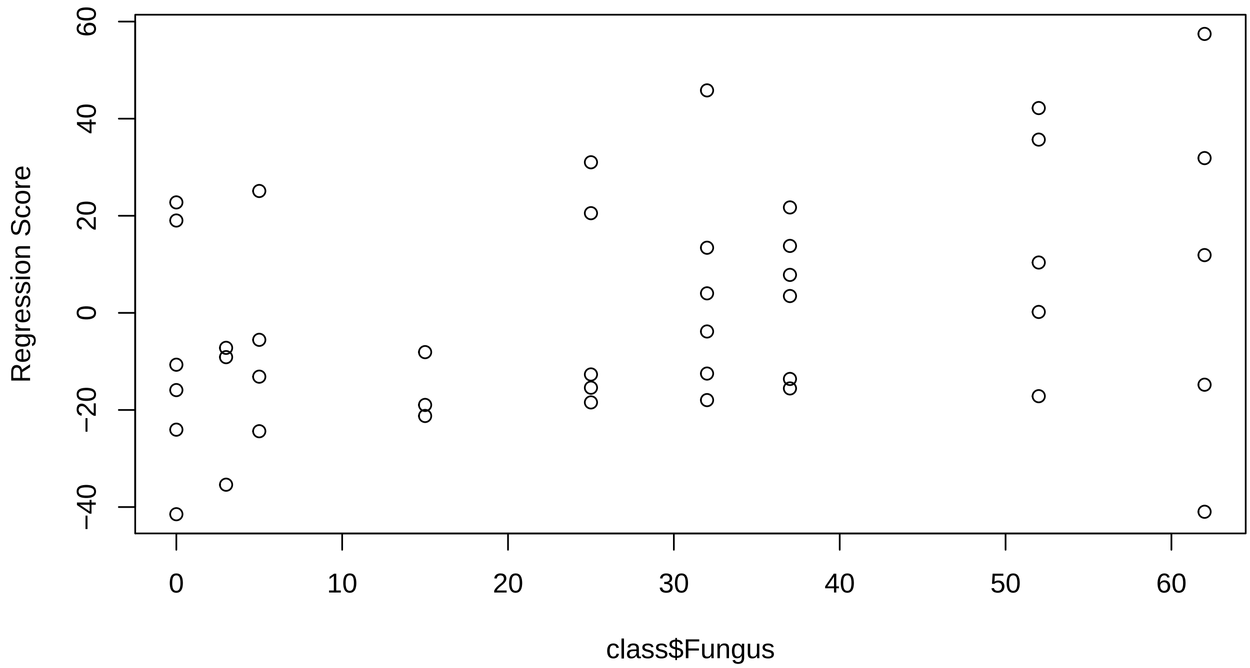

PCA Residuals
PC 2 9.44 %
PC 1 24 %
20
0
−20
−40
−100
−50
0
50


Q−Q plot
Euclidean Distance Residuals
Theoretical Quantiles
30
40
50
60
70
80
90
−2
−1
0
1
2

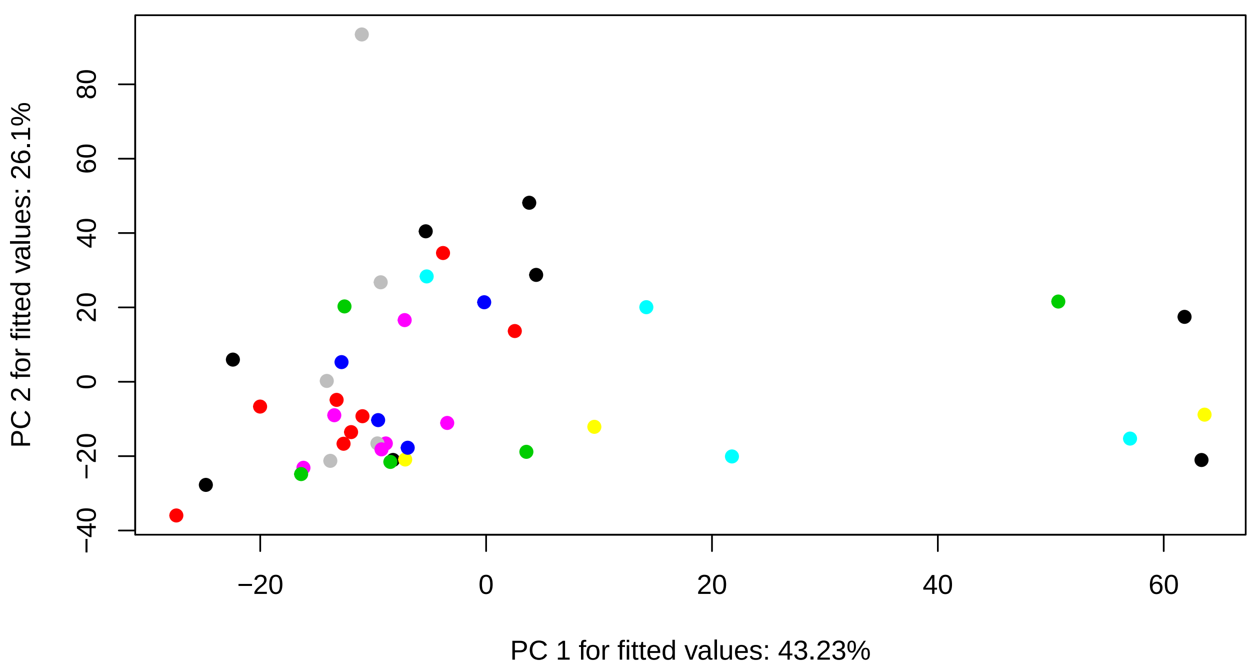





```
# linear regression plot
Yregression <- plot(Y_LMpos, type = "regression", predictor = class$Fungus,
    reg.type = "RegScore")
```




```r
# pca plot
Ypcplot <- plot(Y_LMpos, type = "PC", pch = 19, col = interaction(class$Water,
    class$Fungus))
```



5. Perform an RRPP ANOVA and print results.

```r
## Old Leaves
OposANOVA <- anova(O_LMpos, effect.type = "F", error = c("Residuals",
    "Block:Water", "Block:Water:Fungus", "Residuals", "Block:Water:Fungus",
    "Block:Water:Fungus", "Residuals"))
summary(OposANOVA, formula = T)
```

```
##
## Analysis of Variance, using Residual Randomization
## Permutation procedure: Randomization of null model residuals
## Number of permutations: 1000
## Estimation method: Ordinary Least Squares
## Sums of Squares and Cross-products: Type III
## Effect sizes (Z) based on F distributions
##
##                    Df     SS     MS     Rsq      F        Z Pr(>F)
## Block               1   9842 9842.3 0.04040 1.9408  1.92490  0.038 *
## Water               1   6015 6014.6 0.02469 1.2215  0.95268  0.170
## Fungus              1   8415 8414.6 0.03454 1.9912  1.81123  0.037 *
## Block:Water         1   4924 4923.9 0.02021 0.9709  0.06657  0.444
## Block:Fungus        1   7570 7569.9 0.03108 1.7913  1.60229  0.048 *
## Water:Fungus        1   4625 4625.0 0.01899 1.0944  0.42231  0.321
## Block:Water:Fungus  1   4226 4225.9 0.01735 0.8333 -0.34680  0.605
## Residuals          35 177498 5071.4 0.72864
## Total              42 243600
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call: lm.rrpp(f1 = scaled_Y_old ~ Block * Water * Fungus, SS.type = "III",
##     data = class, print.progress = F)
```

```
## Young Leaves
YposANOVA <- anova(Y_LMpos, effect.type = "F", error = c("Residuals",
    "Block:Water", "Block:Water:Fungus", "Residuals", "Block:Water:Fungus",
    "Block:Water:Fungus", "Residuals"))
summary(YposANOVA, formula = T)
```

```
##
## Analysis of Variance, using Residual Randomization
## Permutation procedure: Randomization of null model residuals
## Number of permutations: 1000
## Estimation method: Ordinary Least Squares
## Sums of Squares and Cross-products: Type III
## Effect sizes (Z) based on F distributions
##
##                    Df     SS     MS     Rsq      F        Z Pr(>F)
## Block               1   5774 5773.8 0.03863 1.8233  1.93604  0.047 *
## Water               1   3326 3325.5 0.02225 0.8231 -0.93297  0.825
## Fungus              1   7286 7285.7 0.04874 1.4310  1.01284  0.143
## Block:Water         1   4040 4040.2 0.02703 1.2758  0.92712  0.181
## Block:Fungus        1   6299 6299.0 0.04214 1.2372  0.67087  0.254
## Water:Fungus        1   3839 3839.1 0.02568 0.7540 -1.30998  0.899
## Block:Water:Fungus  1   5091 5091.4 0.03406 1.6078  1.51617  0.078 .
## Residuals          35 110835 3166.7 0.74148
## Total              42 149478
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call: lm.rrpp(f1 = scaled_Y_young ~ Block * Water * Fungus, SS.type = "III",
##     data = class, print.progress = F)
```

6. Test lm.rrpp model coefficients. "d" is the amount of change in a variable for the coefficient indicated.

```r
## Old Leaves test model coefficients
Oposcoef <- coef(O_LMpos, test = T)
summary(Oposcoef)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 5800
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Statistics (distances) of coefficients with 95 percent confidence intervals,
## effect sizes, and probabilities of exceeding observed values based on
## 1000 random permutations using RRPP
##
##                           d.obs  UCL (95%)          Zd Pr(>d)
## (Intercept)           82.950278 123.646305 -0.98387899  0.845
## Block                 47.308227  42.733029  2.65864993  0.016
## WaterLow              95.740528 108.018897  1.04798116  0.129
## Fungus                 2.409128   2.341365  2.12191890  0.035
## Block:WaterLow        44.635517  55.938812  0.41818423  0.286
## Block:Fungus           1.171821   1.192261  1.73997035  0.062
## WaterLow:Fungus        2.477853   3.156799  0.26381705  0.333
## Block:WaterLow:Fungus  1.171555   1.583269  0.01146787  0.427
```

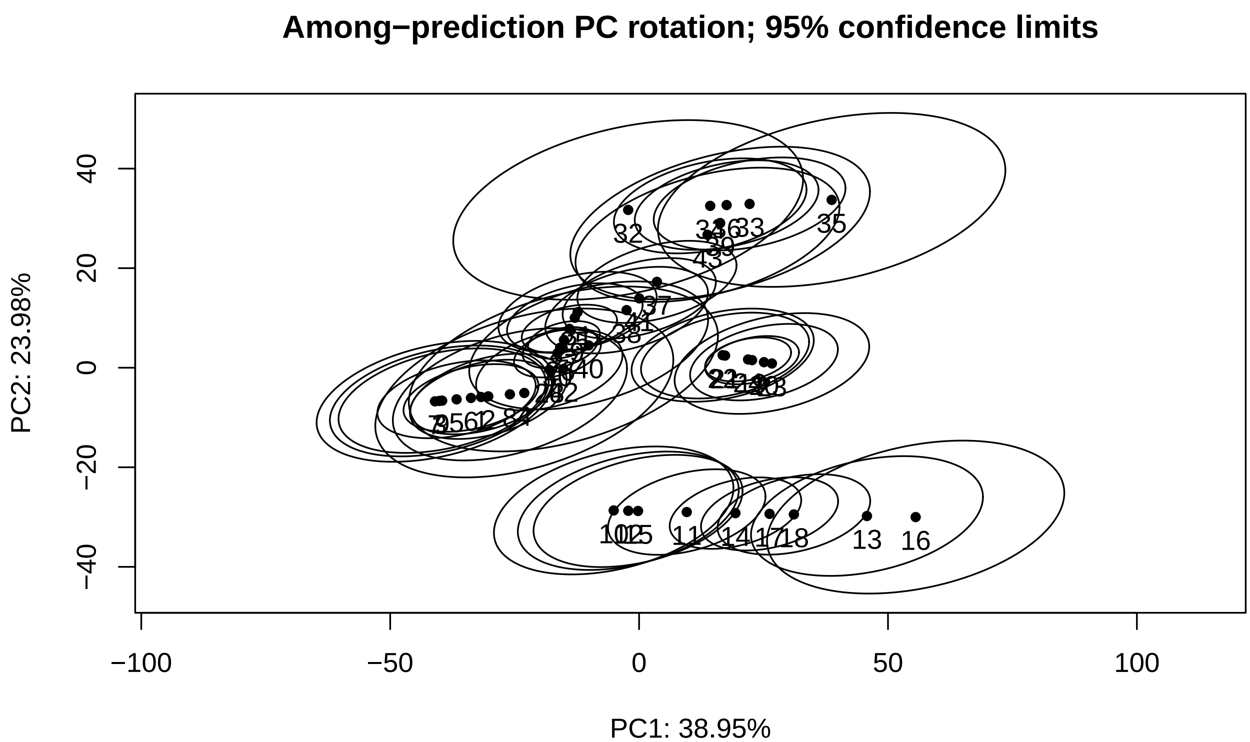
```r
## Young Leaves test model coefficients
Yposcoef <- coef(Y_LMpos, test = T)
summary(Yposcoef)
```

```
##
## Linear Model fit with lm.rrpp
##
## Number of observations: 43
## Number of dependent variables: 3559
## Data space dimensions: 42
## Sums of Squares and Cross-products: Type III
## Number of permutations: 1000
##
## Statistics (distances) of coefficients with 95 percent confidence intervals,
## effect sizes, and probabilities of exceeding observed values based on
## 1000 random permutations using RRPP
##
##                           d.obs  UCL (95%)         Zd Pr(>d)
## (Intercept)           67.224931 85.2935706 -0.3209761  0.579
## Block                 36.234157 33.0151212  2.6598470  0.025
## WaterLow              71.190457 83.2740846  0.7403659  0.221
## Fungus                 2.241702  1.8123137  3.7077004  0.001
## Block:WaterLow        40.431917 42.7689207  1.4380824  0.099
## Block:Fungus           1.068939  0.9294049  2.9339124  0.012
## WaterLow:Fungus        2.257521  2.4701231  1.1719248  0.120
## Block:WaterLow:Fungus  1.285945  1.2527744  2.1256950  0.040
```

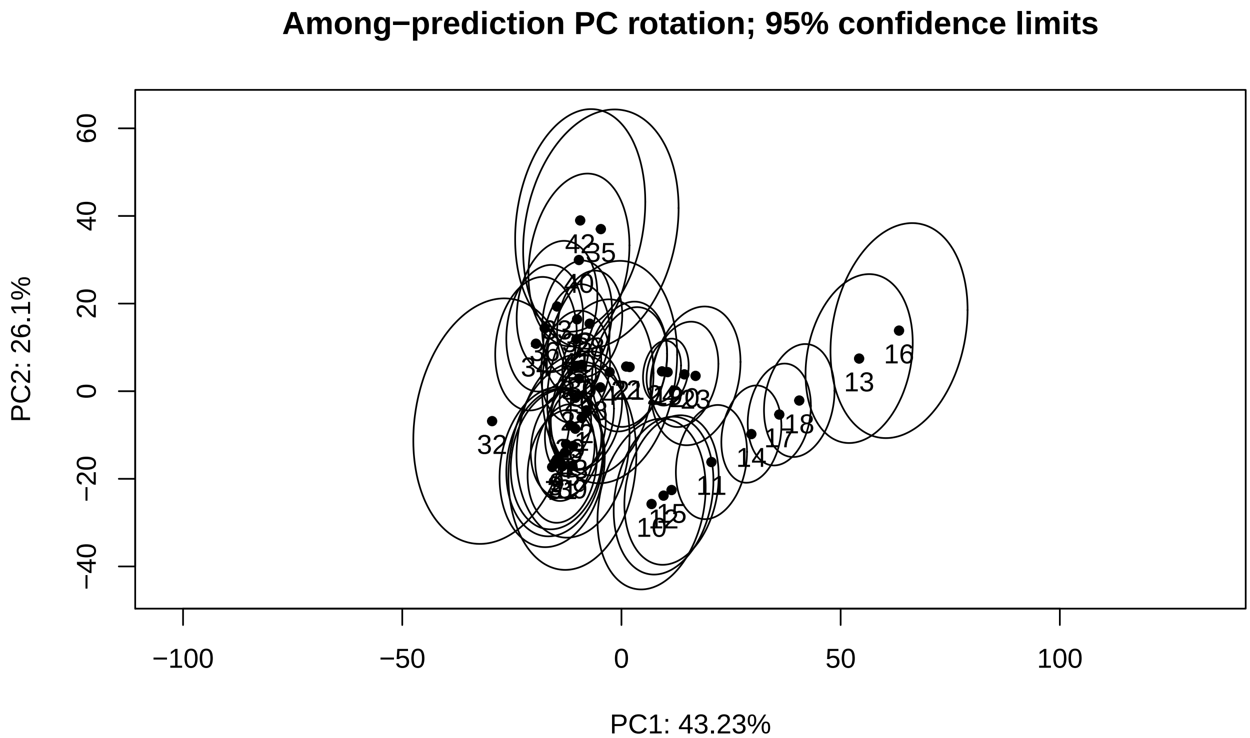
Block and Fungus have the largest effect on the model for old leaves, but not their interaction. The standard is the mean for High water treatment. For young leaves, Block, Fungus, Block:Fungus, and Block:WaterLow:Fungus have the largest effect on the model.

7. Compute predicted values from the lm.rrpp model fit using bootstrapped residuals to generate confidence intervals (precision of group mean estimates).

```
O_pred <- predict(O_LMpos)
plot(O_pred, PC = T, ellipse = T)
```




```
Y_pred <- predict(Y_LMpos)
plot(Y_pred, PC = T, ellipse = T)
```



8. Test pairwise differences between least squares means. Similar to tukeyHSD function in the r stats package. The pairwise function will generate tables with confidence intervals and p-values for the pairwise statistic, Euclidean distance between least-squares means.

```r
## Old Leaves pairwise differences of fungus
Opospw <- pairwise(O_LMpos, groups = class$Fungus)
summary(Opospw, confidence = 0.95, stat.table = T)
```

```
##
## Pairwise comparisons
##
## Groups: 0 3 5 15 25 32 37 52 62
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##                 d UCL (95%)          Z Pr > d
## 0:3   22.190429 32.492258 -1.0112629  0.871
## 0:5   17.798195 27.718783 -0.6148004  0.675
## 0:15  24.519599 36.089254 -0.7116512  0.713
## 0:25  13.615879 23.913372 -1.6164696  0.992
## 0:32  16.139136 27.978037 -2.0455341  1.000
## 0:37  18.660876 32.349605 -2.0455341  1.000
## 0:52  29.207175 48.868672 -1.7103422  0.988
## 0:62  31.133050 53.704651 -2.2106751  0.999
## 3:5   17.786466 28.050413 -2.3037793  1.000
## 3:15  12.322880 20.691802 -1.8704914  0.998
## 3:25  22.264469 32.814810 -1.8676372  0.992
```

```
## 3:32  24.176171 35.969951 -1.7329771  0.988
## 3:37  25.415180 38.317480 -1.8993074  0.993
## 3:52  30.040035 46.627327 -2.2954309  0.998
## 3:62  35.208499 55.206965 -2.1168574  0.995
## 5:15  12.706131 19.539228 -1.5781163  0.986
## 5:25  15.112813 24.619394 -1.5400619  0.987
## 5:32  19.099319 30.945100 -1.4950982  0.980
## 5:37  20.477870 33.363854 -1.7278335  0.996
## 5:52  27.654743 43.340933 -1.9945684  0.995
## 5:62  31.206660 51.072831 -2.0517931  0.999
## 15:25 19.500368 27.260373 -0.8707305  0.802
## 15:32 22.187833 31.553845 -0.8906080  0.806
## 15:37 22.854770 33.147243 -1.0502674  0.860
## 15:52 25.865764 38.617452 -1.6053809  0.969
## 15:62 31.688647 48.705388 -1.5662315  0.970
## 25:32  5.003006  7.943417 -1.5254417  0.959
## 25:37  6.857942 11.218972 -2.0548343  0.995
## 25:52 17.391686 27.046073 -1.5571375  0.961
## 25:62 19.775068 33.378674 -2.1970900  0.998
## 32:37  2.521740  4.371568 -2.0455341  1.000
## 32:52 14.379824 22.957411 -1.2948364  0.923
## 32:62 15.526671 26.608905 -2.2598326  0.999
## 37:52 12.363495 19.284901 -1.1660058  0.892
## 37:62 13.204385 22.606916 -2.2420805  0.998
## 52:62 11.324188 19.324377 -1.7097974  0.991
```

```r
## Young Leaves pairwise differences of fungus
Ypospw <- pairwise(Y_LMpos, groups = class$Fungus)
summary(Ypospw, confidence = 0.95, stat.table = T)
```

```
##
## Pairwise comparisons
##
## Groups: 0 3 5 15 25 32 37 52 62
##
## RRPP: 1000 permutations
##
## LS means:
## Vectors hidden (use show.vectors = TRUE to view)
##
## Pairwise distances between means, plus statistics
##               d UCL (95%)           Z Pr > d
## 0:3   12.197518 19.533049 -2.18210327  0.999
## 0:5   12.541925 17.640986 -0.45336070  0.626
## 0:15  12.091486 20.090658 -2.20630400  1.000
## 0:25  10.411956 20.531180 -1.62296440  0.999
## 0:32  14.320542 26.976690 -1.42750418  0.966
## 0:37  16.558127 31.191798 -1.42750418  0.966
## 0:52  27.084454 44.686627 -1.04659723  0.858
## 0:62  28.135960 52.171108 -1.47302204  0.977
## 3:5   13.070454 20.501668 -1.88308937  0.994
## 3:15   7.883536 14.837528 -2.99055259  1.000
## 3:25  16.269685 26.813298 -1.53362088  0.980
## 3:32  18.616016 31.045268 -1.33954226  0.942
## 3:37  20.359490 34.322890 -1.31471920  0.937
```

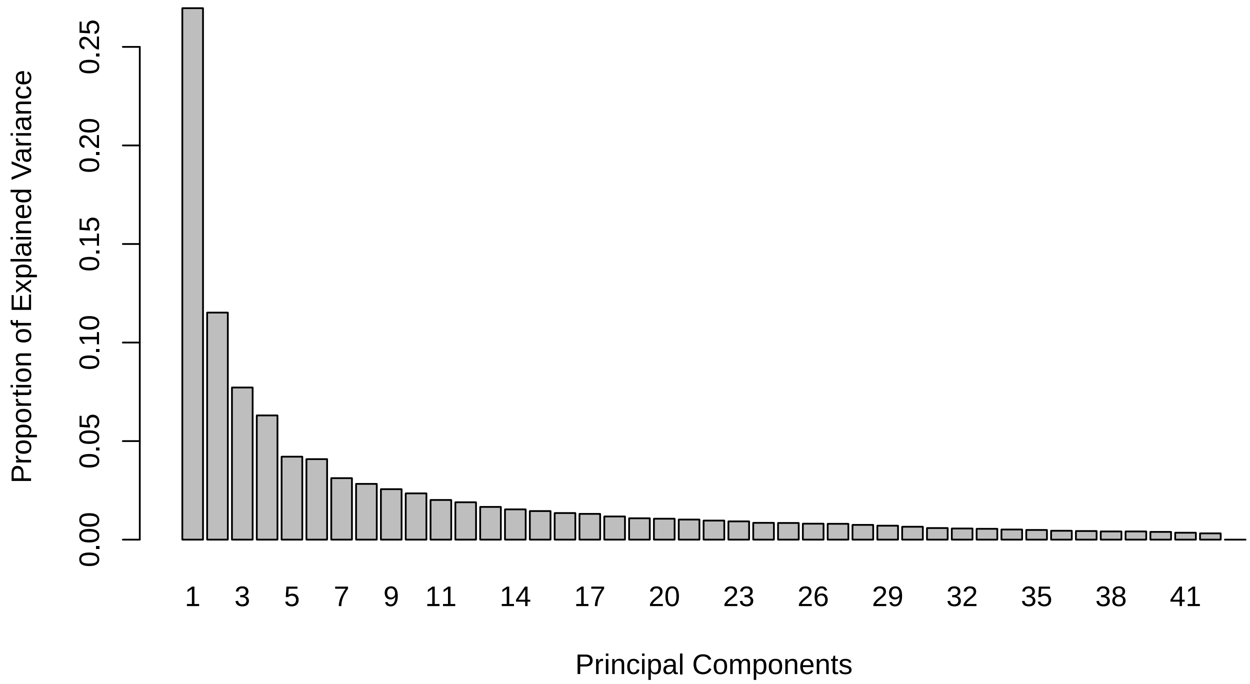
```
## 3:52  28.906708 45.815463 -0.93800711  0.807
## 3:62  30.000546 53.206821 -1.35456031  0.950
## 5:15   9.303381 12.951065 -0.82847173  0.785
## 5:25  18.191487 23.841544  0.02334391  0.466
## 5:32  21.913465 29.682924 -0.14472777  0.529
## 5:37  23.826525 33.112910 -0.26316380  0.576
## 5:52  33.680353 45.964895 -0.11233241  0.509
## 5:62  33.745873 52.272289 -0.71572121  0.730
## 15:25 14.077529 20.070660 -0.77476608  0.744
## 15:32 16.806159 24.208033 -0.71075530  0.739
## 15:37 18.460367 27.189715 -0.74491770  0.757
## 15:52 26.902869 37.988611 -0.38296203  0.626
## 15:62 27.791185 45.557752 -1.00799975  0.830
## 25:32  4.657594  7.293248 -1.01624336  0.843
## 25:37  6.722654 11.101488 -1.13391459  0.878
## 25:52 17.852807 25.600287 -0.52038638  0.664
## 25:62 18.564809 32.707464 -1.38240218  0.955
## 32:37  2.237585  4.215108 -1.42750418  0.966
## 32:52 13.890120 19.541887 -0.48604206  0.650
## 32:62 14.160482 25.893366 -1.51208894  0.982
## 37:52 12.062830 16.119982 -0.27986236  0.566
## 37:62 12.050231 21.807837 -1.52461705  0.985
## 52:62  9.354537 16.144669 -1.47222656  0.994
```

PCA

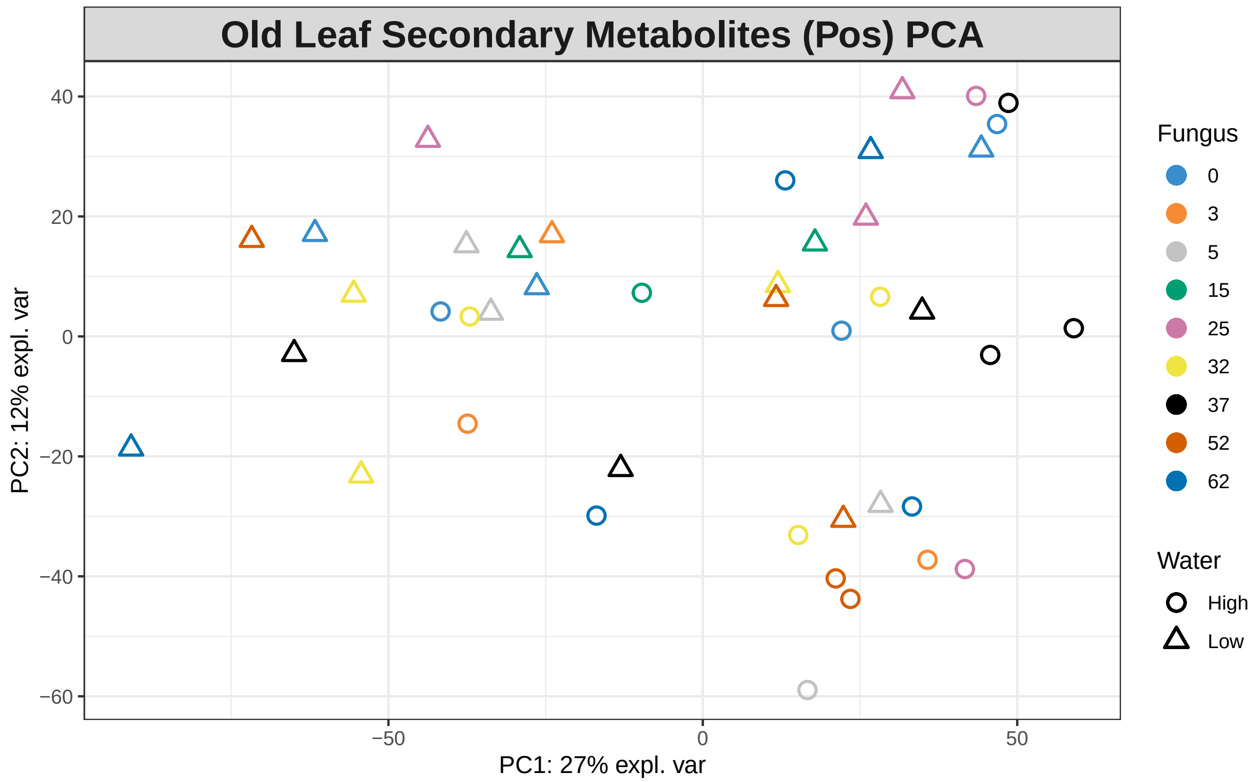
8. Identify the major source of variation in data and determine if the variation is sourced from experimental bias or biological conditions.

```
# Old Leaf Secondary Metabolites (Pos) tune how many
# components to use
tune.pca(scaled_Y_old)
```

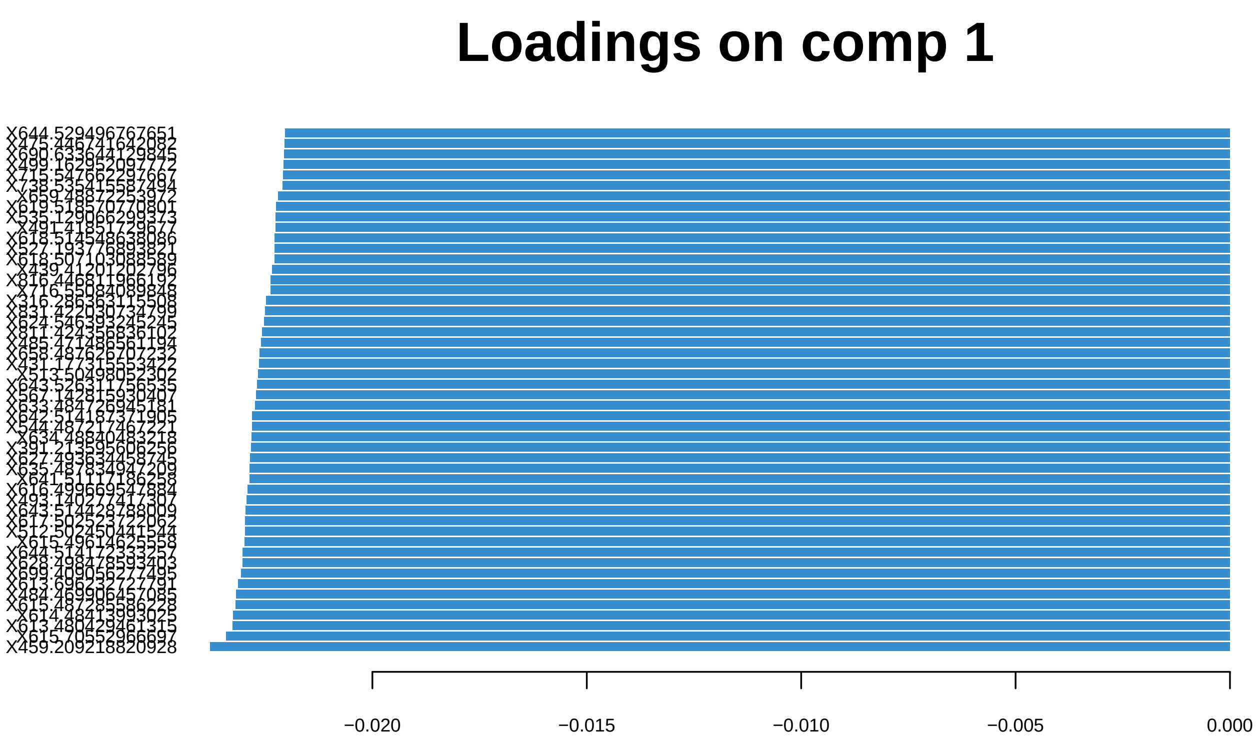
```
## Eigenvalues for the first 10 principal components, see object$sdev^2:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 1563.9592  668.0796  447.6527  365.5013  244.1181  236.9154  180.9496  164.0427
##       PC9      PC10
##  148.5100  136.1229
##
## Proportion of explained variance for the first 10 principal components, see object$explained_varianc
##        PC1        PC2        PC3        PC4        PC5        PC6        PC7
## 0.26964813 0.11518614 0.07718151 0.06301746 0.04208933 0.04084749 0.03119821
##        PC8        PC9       PC10
## 0.02828322 0.02560518 0.02346946
##
## Cumulative proportion explained variance for the first 10 principal components, see object$cum.var:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 0.2696481 0.3848343 0.4620158 0.5250332 0.5671226 0.6079701 0.6391683 0.6674515
##       PC9      PC10
## 0.6930567 0.7165261
##
##  Other available components:
##  --------------------
##  loading vectors: see object$rotation
```




```
pca.res <- mixOmics::pca(scaled_Y_old, ncomp = 3, scale = F)

# plot pca
plotIndiv(pca.res, group = class$Fungus, ind.names = F, pch = as.factor(class$Water),
    legend = T, legend.title = "Fungus", legend.title.pch = "Water",
    title = "Old Leaf Secondary Metabolites (Pos) PCA")
```

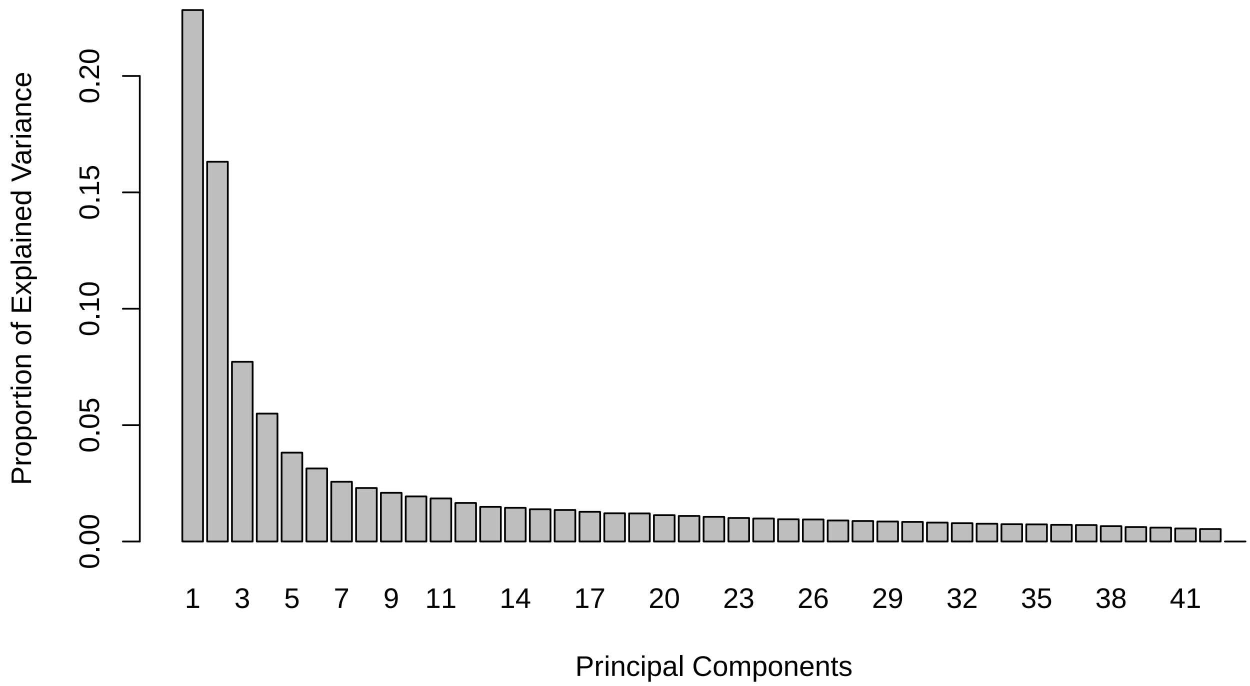



```r
# Look at variable coefficients in each component with the
# loading vectors The absolute value of loading vectors
# represent the importance of each variable to define each PC
plotLoadings(pca.res, ndisplay = 50)
```



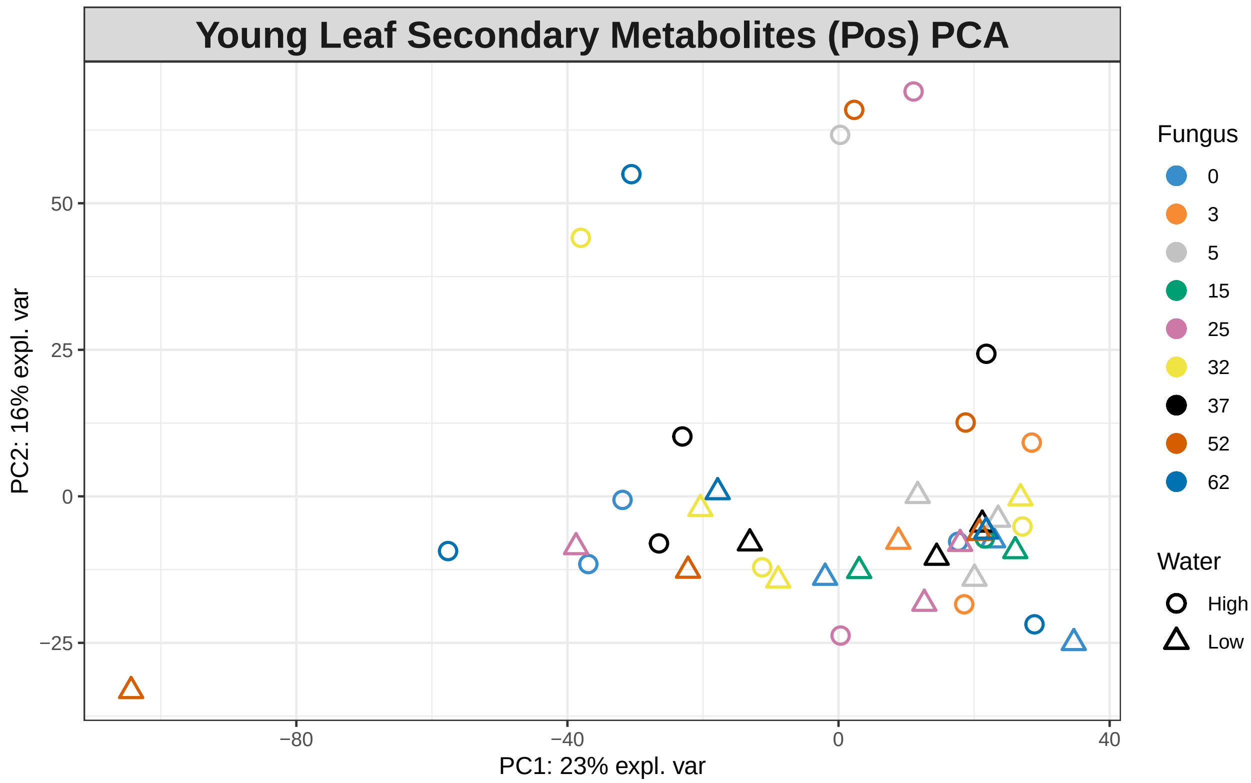
```r
# Young Leaf Secondary Metabolites (Pos) tune how many
# components to use
tune.pca(scaled_Y_young)
```

```
## Eigenvalues for the first 10 principal components, see object$sdev^2:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 812.62108 580.64841 274.76111 195.54194 135.89003 111.66667  91.34267  81.80728
##       PC9      PC10
##  74.45598  68.92299
##
## Proportion of explained variance for the first 10 principal components, see object$explained_varianc
##        PC1        PC2        PC3        PC4        PC5        PC6        PC7
## 0.22832849 0.16314931 0.07720177 0.05494294 0.03818208 0.03137586 0.02566526
##        PC8        PC9       PC10
## 0.02298603 0.02092048 0.01936583
##
## Cumulative proportion explained variance for the first 10 principal components, see object$cum.var:
##       PC1       PC2       PC3       PC4       PC5       PC6       PC7       PC8
## 0.2283285 0.3914778 0.4686796 0.5236225 0.5618046 0.5931805 0.6188457 0.6418317
##       PC9      PC10
## 0.6627522 0.6821181
##
##  Other available components:
##  --------------------
##  loading vectors: see object$rotation
```

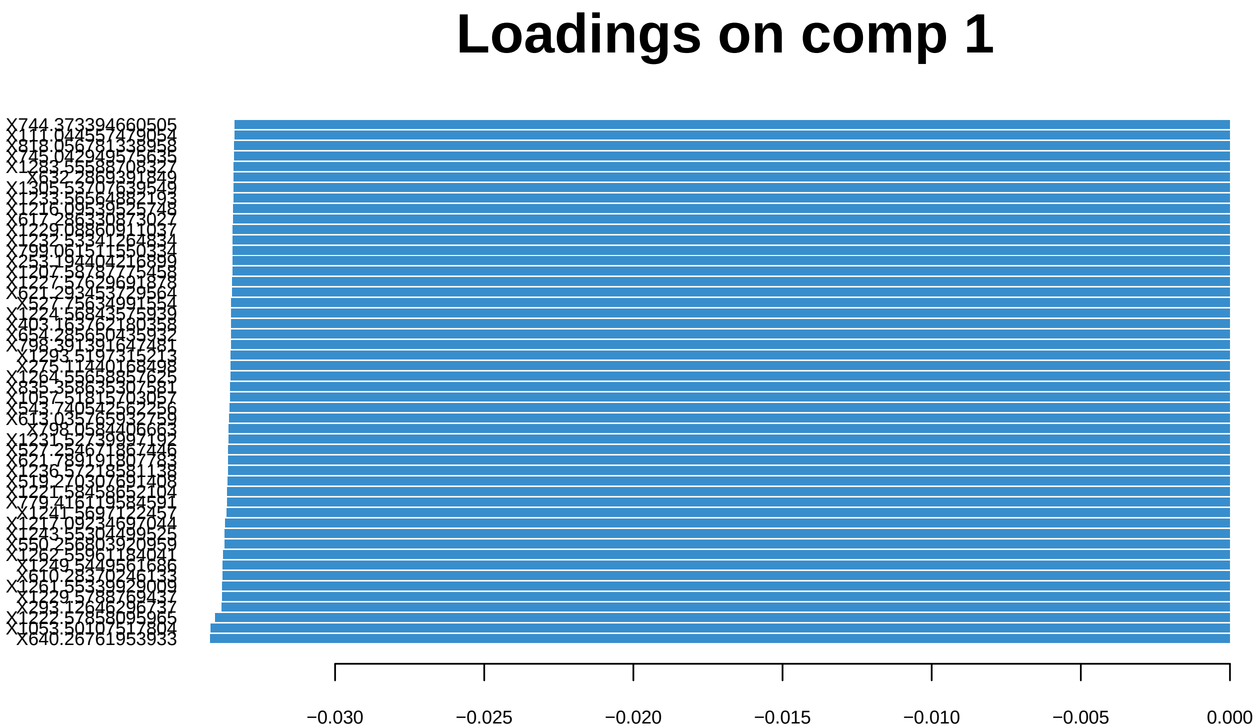



```r
pca.res <- mixOmics::pca(scaled_Y_young, ncomp = 3, scale = F)

# plot pca
plotIndiv(pca.res, group = class$Fungus, ind.names = F, pch = as.factor(class$Water),
    legend = T, legend.title = "Fungus", legend.title.pch = "Water",
```

```r
    title = "Young Leaf Secondary Metabolites (Pos) PCA")
```

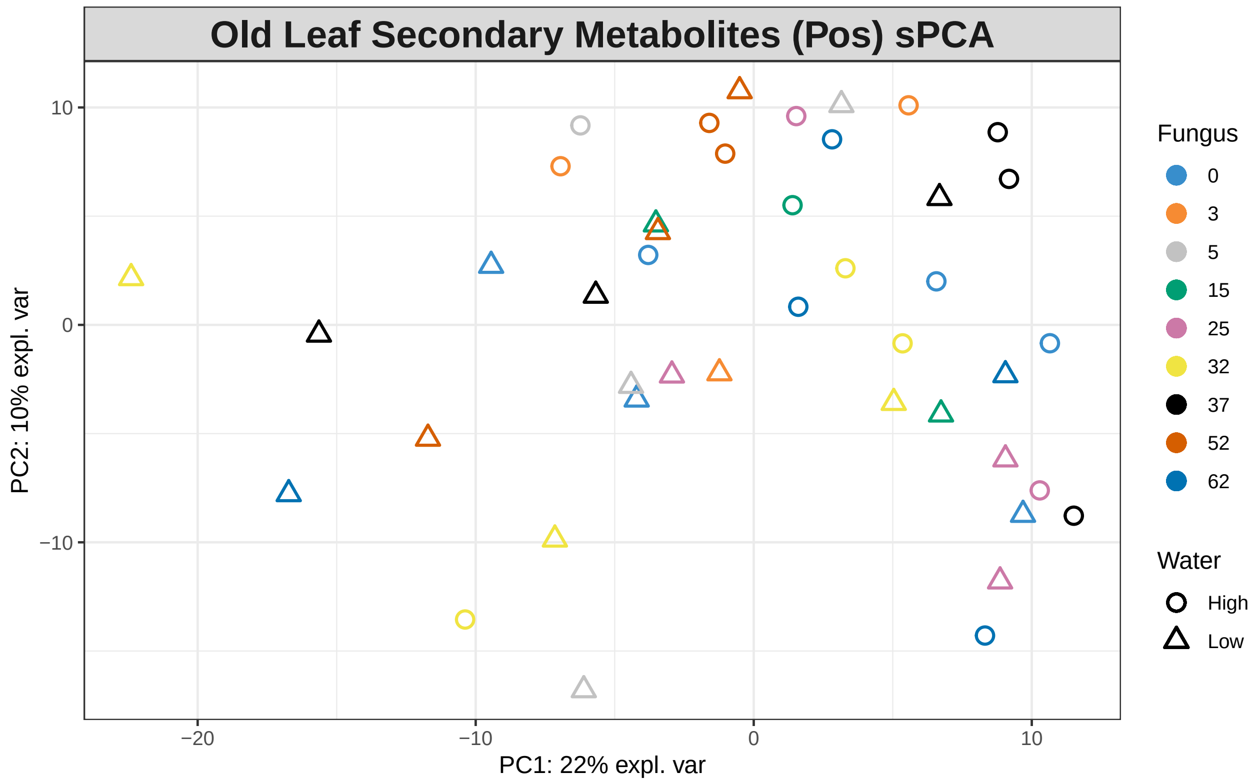



```r
# Look at variable coefficients in each component with the
# loading vectors The absolute value of loading vectors
# represent the importance of each variable to define each PC
plotLoadings(pca.res, ndisplay = 50)
```

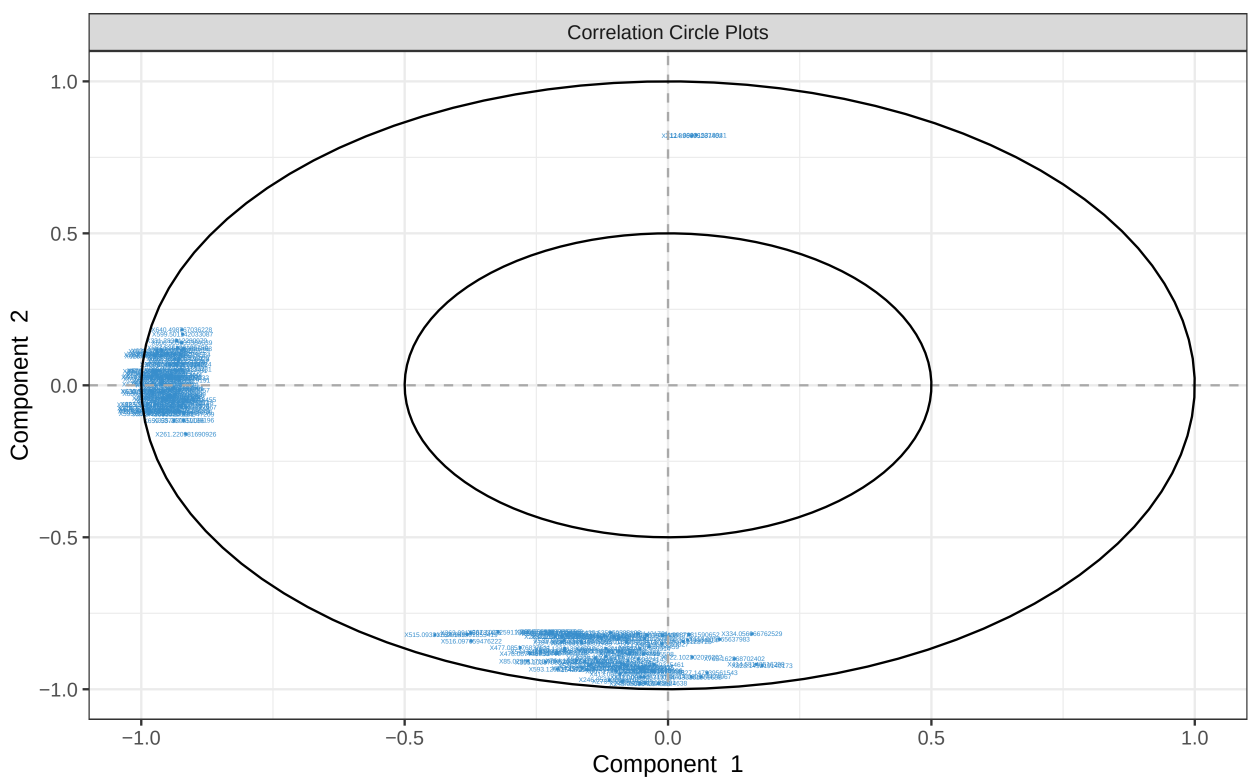


9. Identify the variables that contribute to the explained variance.

```r
# Old Leaf
spca.res <- mixOmics::spca(scaled_Y_old, ncomp = 3, keepX = c(100,
    100, 10))

# plot spca
plotIndiv(spca.res, group = class$Fungus, ind.names = F, pch = as.factor(class$Water),
    legend = T, legend.title = "Fungus", legend.title.pch = "Water",
    title = "Old Leaf Secondary Metabolites (Pos) sPCA")
```




```r
# variables contributing to each component
plotVar(spca.res, cex = 1)
```

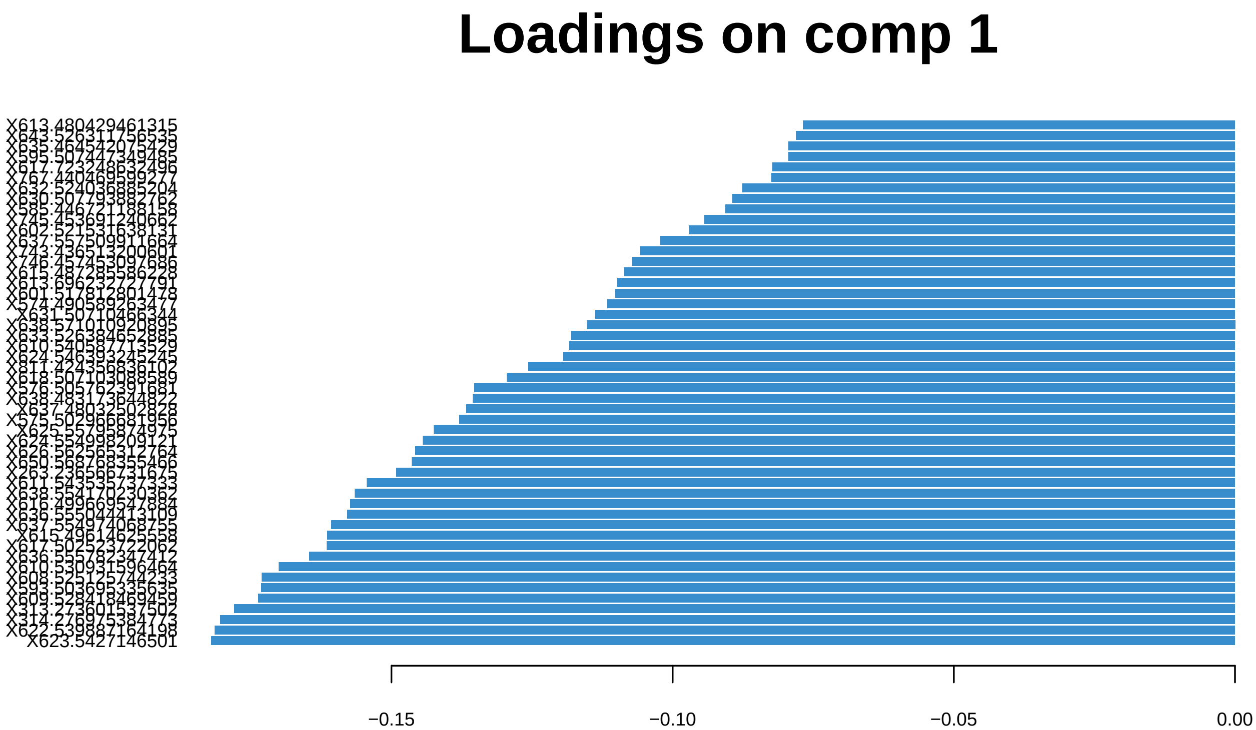


```r
selectVar(spca.res, comp = 1)$value  # view loading value of each metabolite
```

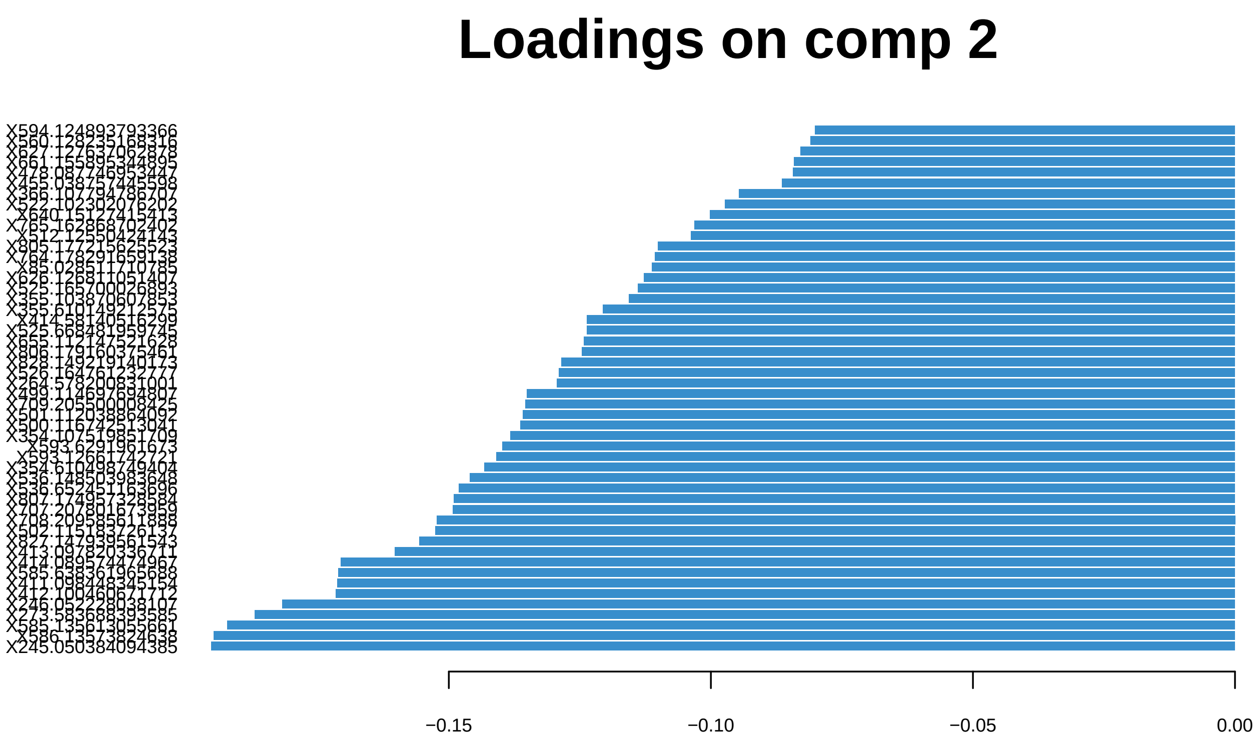
```
##                      value.var
## X623.5427146501   -0.182055114
## X622.539887164198 -0.181404088
## X314.276975384773 -0.180391891
## X313.273601537502 -0.177913462
## X609.528418469459 -0.173659127
## X593.503695335635 -0.173111385
## X608.525125744233 -0.173085253
## X610.530931596464 -0.170019964
## X636.555782347412 -0.164596649
## X617.502523722062 -0.161488471
## X615.49614625558  -0.161372201
## X637.554974068755 -0.160689106
## X636.555044413109 -0.157801079
## X616.499669547884 -0.157311441
## X638.554170230362 -0.156500296
## X611.543535737333 -0.154368634
## X263.236566731675 -0.149120932
## X650.568768355466 -0.146359898
## X626.562565312764 -0.145730463
## X624.554998209121 -0.144372113
## X625.55795874975  -0.142472966
## X575.502966681956 -0.137889376
## X637.48032502828  -0.136679304
## X638.483173644822 -0.135515131
## X576.505762391681 -0.135267793
## X618.507103088589 -0.129459479
## X811.424356836102 -0.125604358
## X624.546393245245 -0.119384220
## X610.540587713529 -0.118351586
## X633.526384652885 -0.117956330
## X638.571010920895 -0.115276197
## X631.50710466344  -0.113748627
## X574.490589263477 -0.111560071
## X601.517812801478 -0.110208018
## X613.696232727791 -0.109824109
## X615.487285586228 -0.108647615
## X746.457453097686 -0.107262835
## X743.436513200601 -0.105806758
## X637.557509911664 -0.102124150
## X602.521531638131 -0.097096716
## X745.453691240662 -0.094310581
## X585.446721188158 -0.090565970
## X630.507793882762 -0.089309333
## X632.524036885204 -0.087580472
## X767.440469599277 -0.082429634
## X617.723248632496 -0.082259964
## X595.507447349485 -0.079387805
## X635.464542075429 -0.079367125
## X643.526311756535 -0.078010992
## X613.480429461315 -0.076824176
## X659.48872253972  -0.076643589
```

```
## X577.506622179924 -0.075085714
## X614.48413993025  -0.071693614
## X645.515984064549 -0.071351379
## X651.564511662028 -0.069707813
## X636.467534222444 -0.069163816
## X626.570188705153 -0.065764285
## X573.487260848141 -0.065535248
## X829.406046163401 -0.062453335
## X658.538868443561 -0.060418449
## X580.492441620954 -0.059736350
## X641.51117186258  -0.058555426
## X642.514187371905 -0.058042934
## X659.537339659066 -0.058033262
## X768.443158772313 -0.055913361
## X598.456409633744 -0.055656048
## X664.585958751482 -0.053832451
## X652.58279371971  -0.053087021
## X643.514428788009 -0.052008040
## X673.509123378608 -0.051285990
## X331.283812280979 -0.045554142
## X644.529496767651 -0.045034037
## X657.484442714608 -0.043683074
## X667.525186185759 -0.042049347
## X660.503817215267 -0.038786678
## X633.484726945181 -0.037949634
## X658.487626707232 -0.037612021
## X744.439887639683 -0.036573902
## X663.551426151608 -0.035986321
## X665.510410370928 -0.035924141
## X665.586601431923 -0.034656733
## X644.514172333257 -0.033719869
## X666.546022519191 -0.032138035
## X662.551874509279 -0.031995479
## X639.565404224584 -0.029863137
## X620.52328522026  -0.025732640
## X668.53044299629  -0.025132634
## X645.530094137731 -0.024834541
## X640.498767036228 -0.022619778
## X600.503635384639 -0.021720501
## X599.501142033087 -0.018378543
## X638.57096397224  -0.017188350
## X617.511254268748 -0.016288781
## X634.48840483218  -0.015300095
## X603.53233885458  -0.014696303
## X335.257931139196 -0.013030594
## X635.487834947209 -0.012811617
## X336.261554038455 -0.004335658
## X261.220981690926 -0.003217427
## X262.224903017467 -0.001379185
```

```r
# plot loadings for comp 1
plotLoadings(spca.res, ndisplay = 50)
```

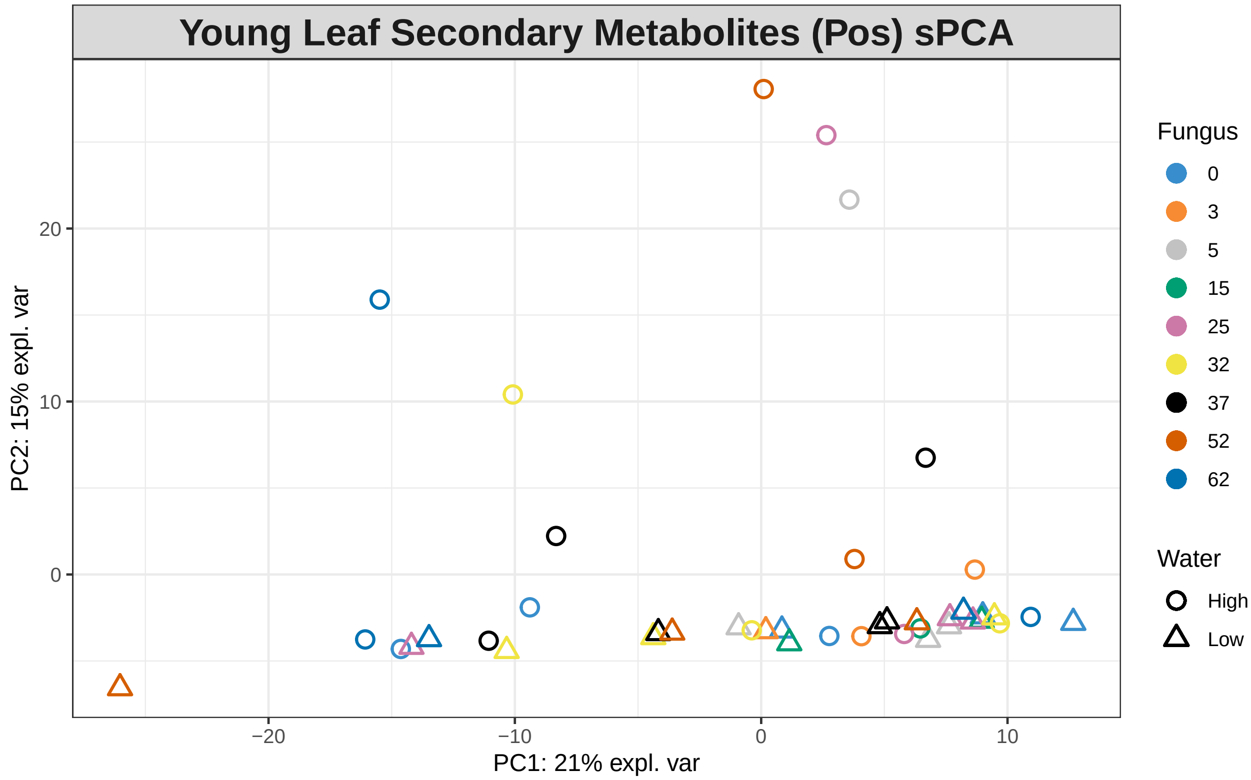



```r
# plot loadings for comp 2
plotLoadings(spca.res, comp = 2, ndisplay = 50)
```

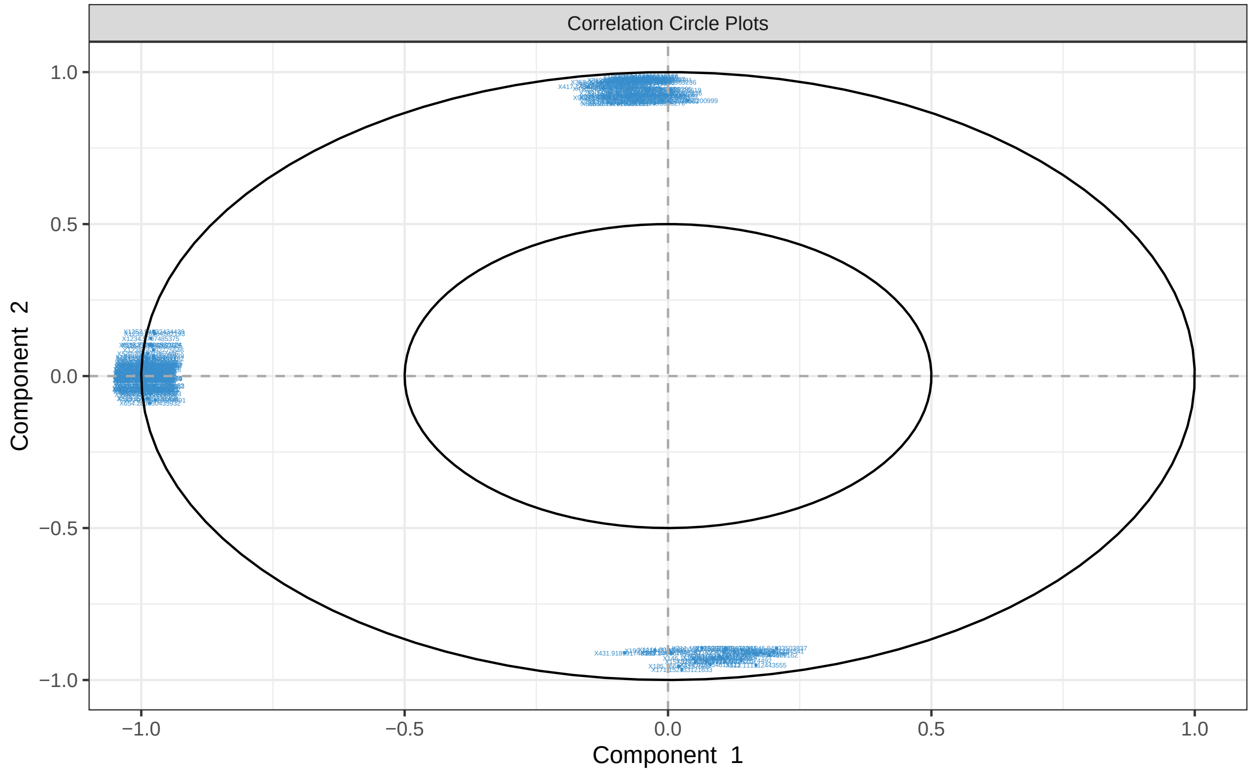



```r
# Young Leaf
spca.res <- mixOmics::spca(scaled_Y_young, ncomp = 3, keepX = c(100,
    100, 10))
```

```r
# plot spca
plotIndiv(spca.res, group = class$Fungus, ind.names = F, pch = as.factor(class$Water),
    legend = T, legend.title = "Fungus", legend.title.pch = "Water",
    title = "Young Leaf Secondary Metabolites (Pos) sPCA")
```




```r
# variables contributing to each component
plotVar(spca.res, cex = 1)
```



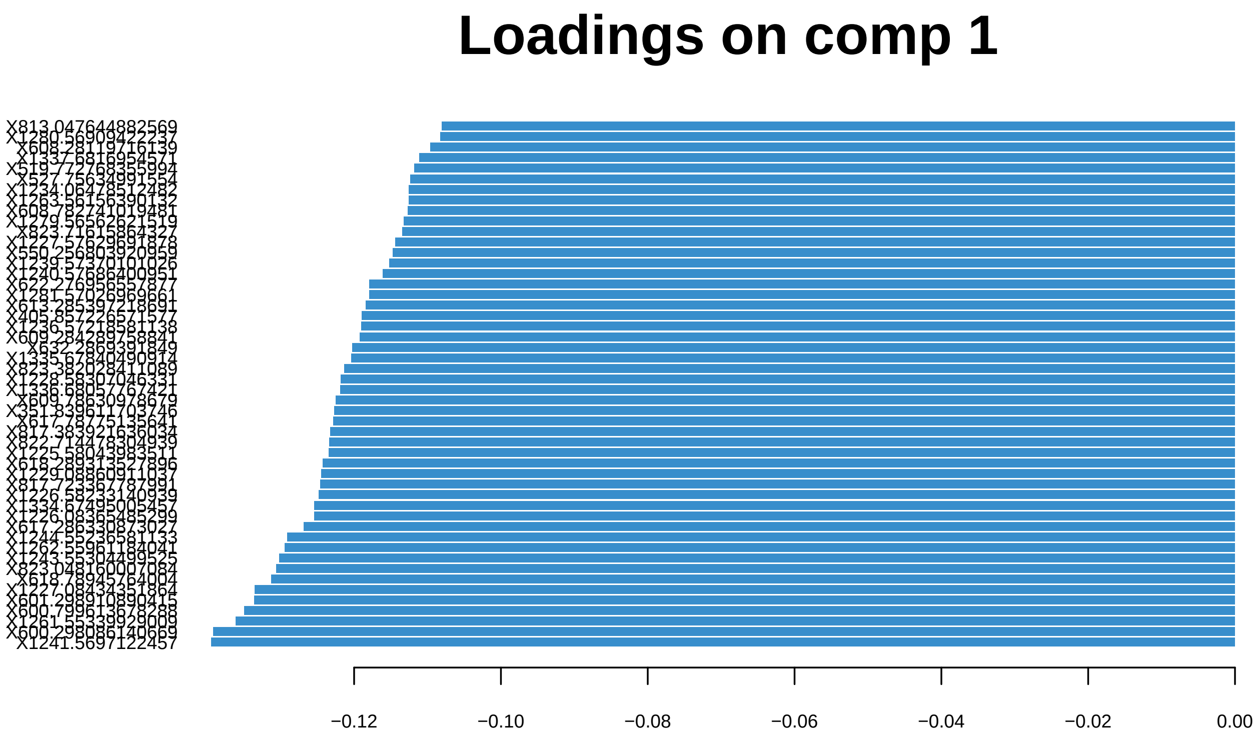

```r
selectVar(spca.res, comp = 1)$value  # view loading value of each metabolite
```

```
##                        value.var
## X1241.5697122457  -0.139470661
## X600.298086140669 -0.139175646
## X1261.55339929009 -0.136132665
## X600.799613678288 -0.134941320
## X601.298910890415 -0.133563771
## X1227.08434351864 -0.133521416
## X618.78945764004  -0.131283246
## X823.048160007084 -0.130587035
## X1243.55304499525 -0.130195212
## X1262.55961184041 -0.129412563
## X1244.55236581133 -0.129116682
## X617.286330873027 -0.126845950
## X1226.08365485299 -0.125392084
## X1334.67495005457 -0.125382708
## X1226.58233140939 -0.124832612
## X817.723367787991 -0.124618167
## X1229.08860911037 -0.124494169
## X618.289313527896 -0.124274416
## X1225.58043983511 -0.123439455
## X822.714478304939 -0.123348570
## X817.383921636034 -0.123223780
## X617.78775135641  -0.122810308
## X351.839611703746 -0.122681247
## X609.78630978679  -0.122479407
## X1336.68057767421 -0.121862820
## X1228.58307046331 -0.121824091
```

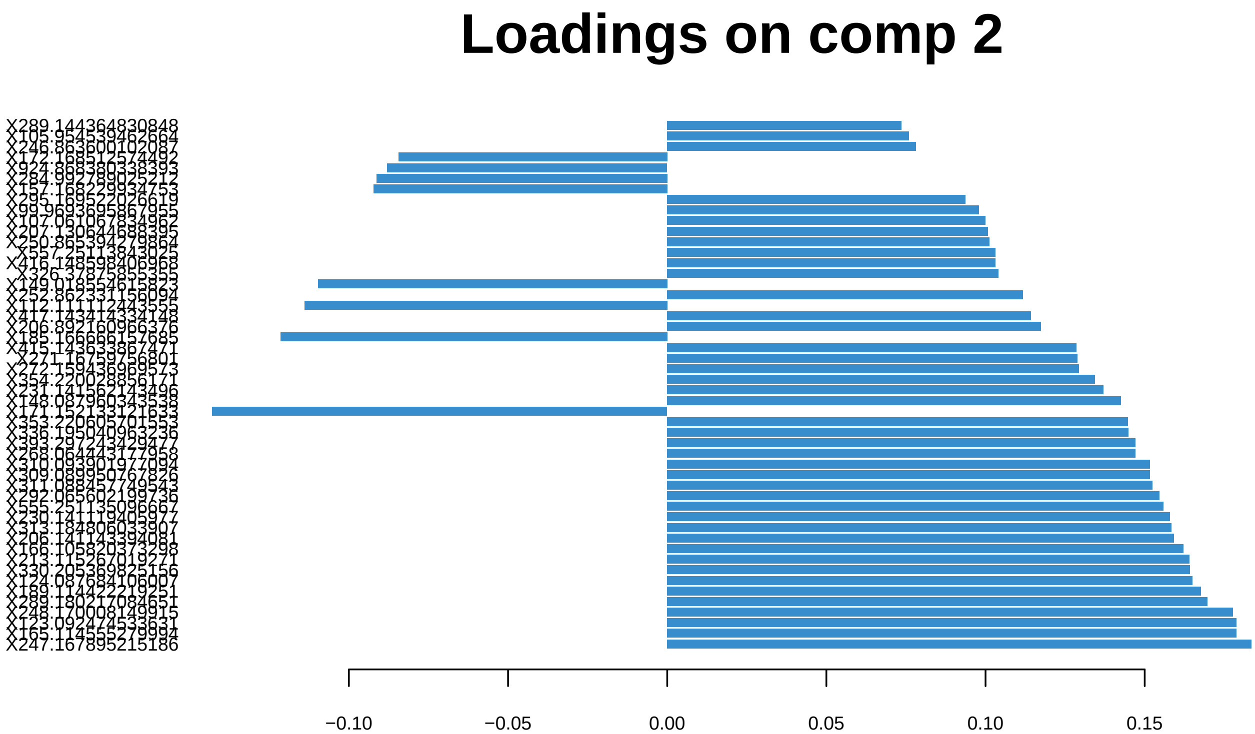
```
## X823.382028411089 -0.121349640
## X1335.67840490914 -0.120383444
## X632.2869391849   -0.120231642
## X609.284289758841 -0.119187887
## X1236.57218581138 -0.119006491
## X405.857226571577 -0.118923159
## X613.285397218691 -0.118398301
## X1281.57026969661 -0.117923790
## X622.276956557877 -0.117908484
## X1240.57686400951 -0.116112366
## X1239.57370101026 -0.115179222
## X550.256803920959 -0.114748723
## X1227.57629691878 -0.114347942
## X823.71615864327  -0.113434602
## X1279.56562621519 -0.113192877
## X608.782741019481 -0.112651258
## X1263.56156390132 -0.112569764
## X1234.06478512482 -0.112525354
## X527.75634991554  -0.112363717
## X519.772768355994 -0.111812009
## X1337.6816954571  -0.111093133
## X608.28119716139  -0.109601512
## X1280.56909422237 -0.108275587
## X813.047644882569 -0.108059038
## X406.192592684012 -0.107786086
## X631.785508659045 -0.106529676
## X817.043002326106 -0.105118952
## X1235.06677264887 -0.104034681
## X611.287897439172 -0.103000870
## X824.049066021535 -0.102789556
## X631.284156543571 -0.102303243
## X352.174656446785 -0.101512752
## X1054.50423500233 -0.099237844
## X601.799037963433 -0.098831516
## X1242.56522633851 -0.096006929
## X1228.07642334574 -0.094508987
## X613.035765932759 -0.093906496
## X818.056781338958 -0.091141400
## X612.785606728103 -0.087550336
## X651.781625860394 -0.085040210
## X527.254671867446 -0.084059004
## X1237.57084462266 -0.081084178
## X1230.08774541731 -0.079219075
## X519.270307691408 -0.078746834
## X816.707450462089 -0.078302171
## X651.28036531701  -0.076416993
## X632.786594301567 -0.074710660
## X813.376589649202 -0.073640219
## X520.264679977611 -0.073469371
## X550.759359335189 -0.069502467
## X1229.5788769437  -0.068918200
## X528.257744262133 -0.064983429
## X1283.55588708327 -0.060901976
## X654.285650435932 -0.060037126
```

```
## X613.536347716772 -0.057915722
## X1234.5707485375  -0.051899728
## X838.372738715693 -0.049536575
## X613.772591530124 -0.045920191
## X535.247917849353 -0.044255968
## X1037.52045054275 -0.040974710
## X1305.53707639549 -0.039625742
## X1053.50107517804 -0.037969267
## X1264.55658857625 -0.037667817
## X1222.57858095965 -0.036063657
## X1237.0724873078  -0.034444605
## X610.28370246133  -0.027216591
## X1238.56292775646 -0.022181468
## X1224.56843575939 -0.021649724
## X812.714817179162 -0.020333869
## X1231.52739997192 -0.020296654
## X1252.54032434439 -0.015880601
## X621.789191807783 -0.015879632
## X1233.56564882193 -0.009690989
## X779.416119584591 -0.001035685
```

```
# plot loadings for comp 1
plotLoadings(spca.res, ndisplay = 50)
```



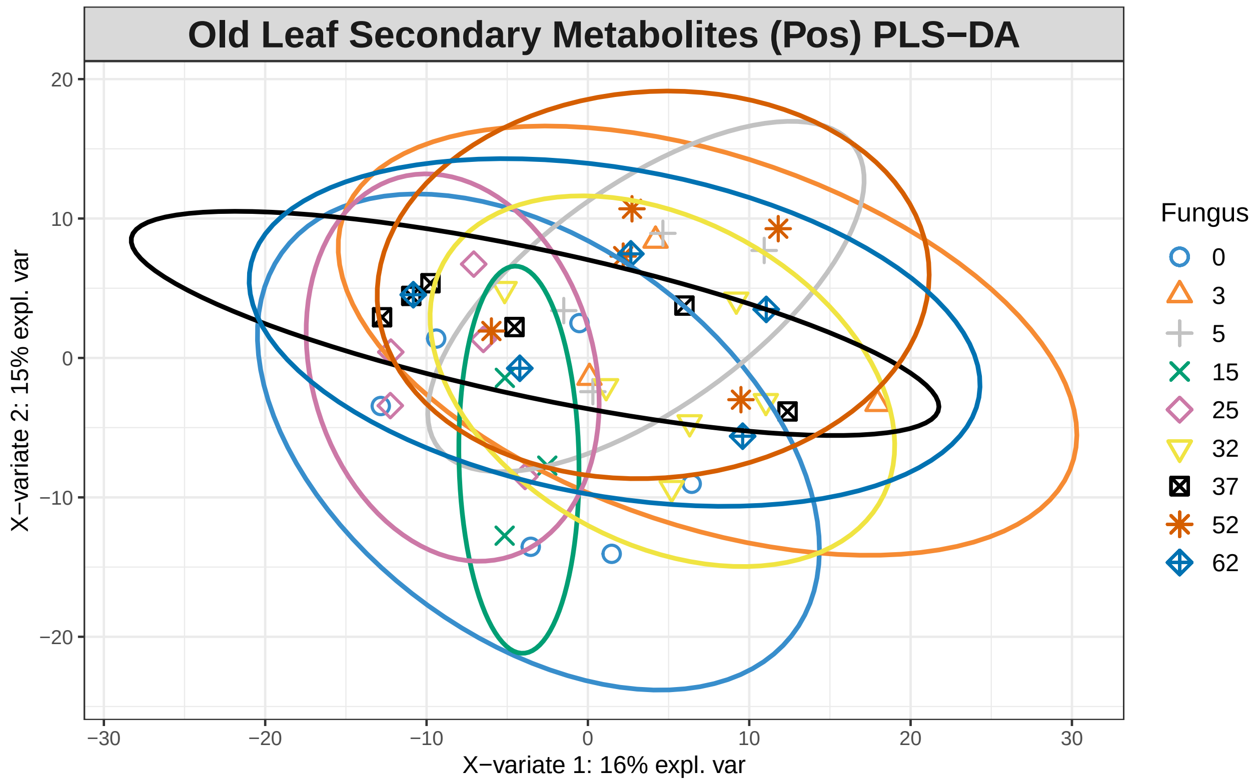

```
# plot loadings for comp 2
plotLoadings(spca.res, comp = 2, ndisplay = 50)
```



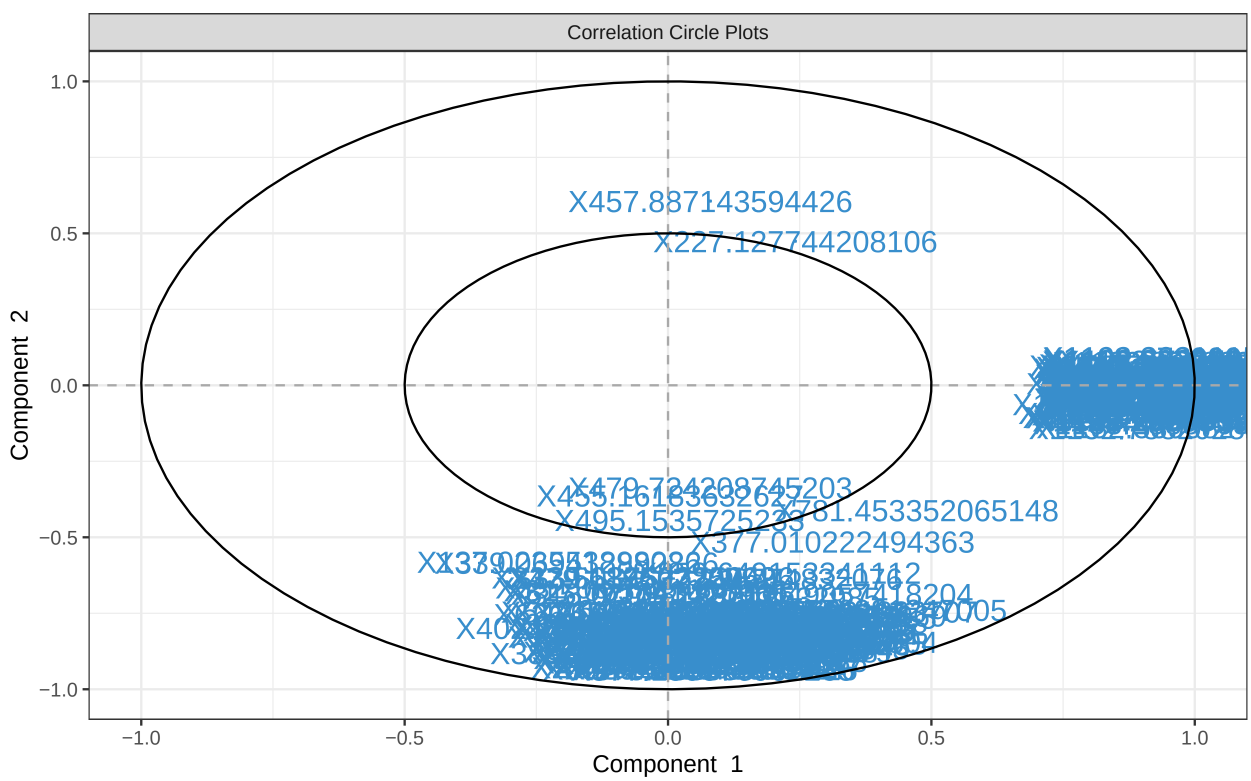
PLS-DA

10. Classify samples into known groups and predict the class of new samples.

```
# Old Leaf
old.splsda <- mixOmics::splsda(scaled_Y_old, class$Fungus, keepX = c(100,
    100))

# plot pls-da
plotIndiv(old.splsda, ind.names = F, legend = T, title = "Old Leaf Secondary Metabolites (Pos) PLS-DA",
    legend.title = "Fungus", ellipse = T)
```




```r
# plot and select the variables
plotVar(old.splsda)
```



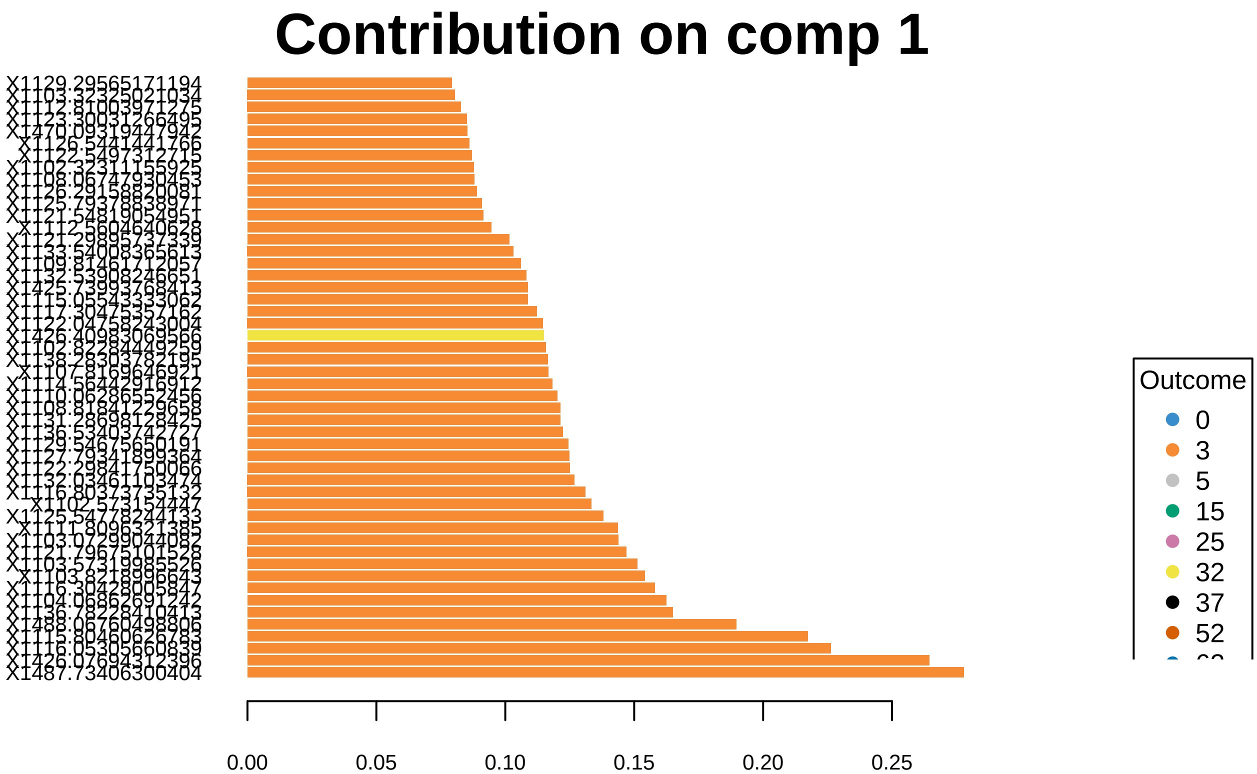
```r
selectVar(old.splsda, comp = 1)
```

```
## $name
##   [1] "X1487.73406300404" "X1426.07694312396" "X1116.05305660839"
##   [4] "X1115.80460626783" "X1488.06760498806" "X1136.78228410413"
##   [7] "X1104.06862691242" "X1116.30428005847" "X1103.8218996643"
##  [10] "X1103.57319985526" "X1121.79675101528" "X1103.07299044082"
##  [13] "X1111.8096321385"  "X1125.54778244133" "X1102.573154447"
##  [16] "X1116.80373735132" "X1132.03461103474" "X1122.29841750066"
##  [19] "X1127.79341899364" "X1129.54675650191" "X1136.53403742727"
##  [22] "X1131.28698128425" "X1108.81841229658" "X1110.06286552456"
##  [25] "X1114.56442916912" "X1107.8169646921"  "X1138.28303782195"
##  [28] "X1102.82284449259" "X1426.40983069566" "X1122.04758243004"
##  [31] "X1117.30475357162" "X1115.05543333062" "X1425.73993768413"
##  [34] "X1132.53908246651" "X1109.81461712057" "X1133.54008365613"
##  [37] "X1121.29895737339" "X1112.5604640628"  "X1121.54819054951"
##  [40] "X1125.79378838971" "X1126.29158820081" "X1108.06747930453"
##  [43] "X1102.32311155925" "X1122.5497312715"  "X1126.5441441766"
##  [46] "X1470.09319447942" "X1123.30031266495" "X1112.81003971275"
##  [49] "X1103.32325021034" "X1129.29565171194" "X1112.30949170708"
##  [52] "X1108.31811054051" "X1488.40051943368" "X1471.42767786981"
##  [55] "X1117.04932871798" "X1469.75976780245" "X1470.42704875471"
##  [58] "X1127.04008421696" "X1131.78505133898" "X1470.76081907912"
##  [61] "X1113.56329850353" "X1098.56831395271" "X1469.42570146433"
##  [64] "X1135.53727745968" "X1112.05929178221" "X1113.31183863808"
##  [67] "X1108.56822391305" "X1131.03795724898" "X1471.09532086136"
##  [70] "X1127.29255917483" "X1114.06358094469" "X1129.79457145018"
##  [73] "X1109.06854325948" "X1125.30053856127" "X1485.74884256861"
##  [76] "X1135.28675162713" "X1489.399551935"   "X1478.08739359233"
##  [79] "X1133.28887059062" "X1132.78820284397" "X1476.75330076931"
##  [82] "X1113.06007098305" "X1131.53755678079" "X1109.56623050873"
##  [85] "X1136.28695855805" "X1478.42215498284" "X1132.28893553294"
##  [88] "X1124.55354253246" "X1117.80491567323" "X1118.30495999252"
##  [91] "X1109.31799268981" "X1114.31354083018" "X1118.05504416426"
##  [94] "X1477.4205825873"  "X1113.81293085655" "X1483.41064725665"
##  [97] "X1119.80974545454" "X1126.79208167921" "X1490.40376922173"
## [100] "X1119.30886967612"
##
## $value
##                    value.var
## X1487.73406300404 0.2778109819
## X1426.07694312396 0.2644217191
## X1116.05305660839 0.2261700396
## X1115.80460626783 0.2173284797
## X1488.06760498806 0.1896116468
## X1136.78228410413 0.1650091785
## X1104.06862691242 0.1623645986
## X1116.30428005847 0.1580176449
## X1103.8218996643  0.1540609727
## X1103.57319985526 0.1512505345
## X1121.79675101528 0.1470020181
## X1103.07299044082 0.1437882774
## X1111.8096321385  0.1436423860
## X1125.54778244133 0.1379262355
```

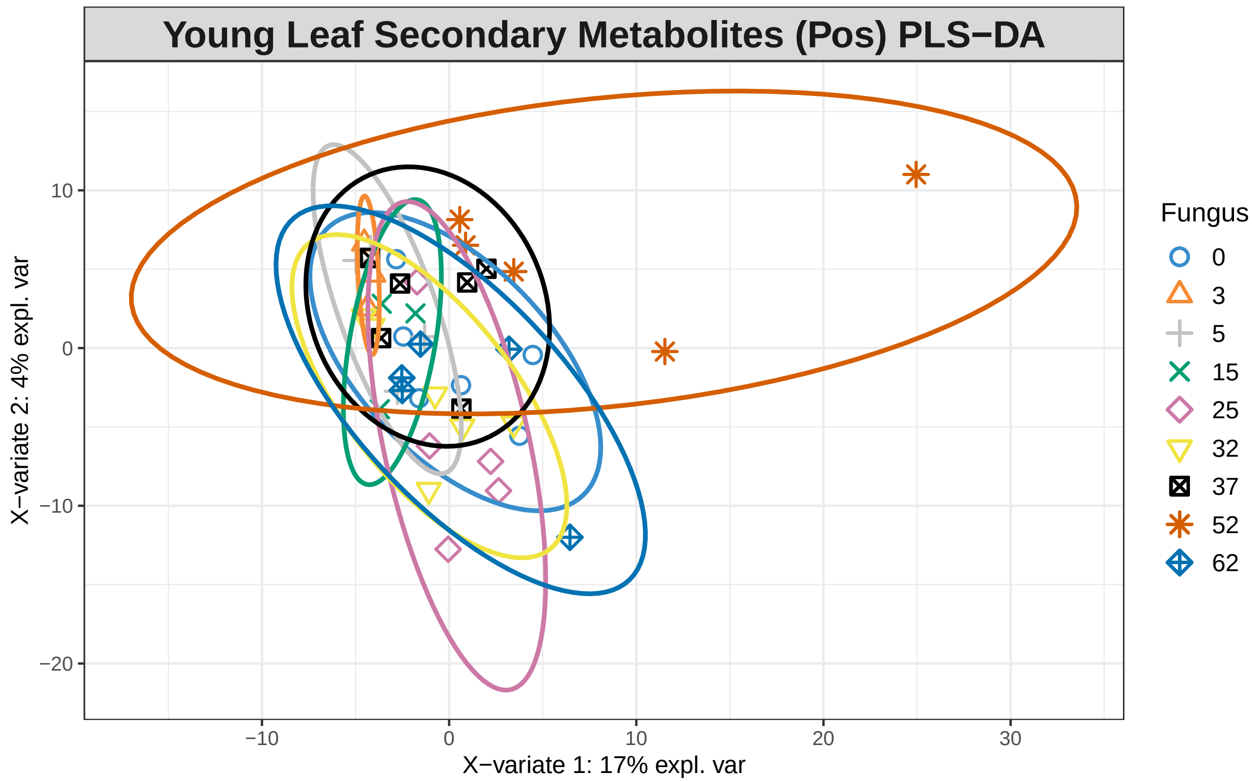
```
## X1102.573154447   0.1333348419
## X1116.80373735132 0.1311062999
## X1132.03461103474 0.1268407388
## X1122.29841750066 0.1249396606
## X1127.79341899364 0.1247654543
## X1129.54675650191 0.1244549039
## X1136.53403742727 0.1223155601
## X1131.28698128425 0.1212771304
## X1108.81841229658 0.1212334363
## X1110.06286552456 0.1201315088
## X1114.56442916912 0.1181915572
## X1107.8169646921  0.1167675478
## X1138.28303782195 0.1165180686
## X1102.82284449259 0.1156617829
## X1426.40983069566 0.1149926752
## X1122.04758243004 0.1146371796
## X1117.30475357162 0.1121319305
## X1115.05543333062 0.1087284570
## X1425.73993768413 0.1086871253
## X1132.53908246651 0.1080661707
## X1109.81461712057 0.1059478384
## X1133.54008365613 0.1031746286
## X1121.29895737339 0.1015076025
## X1112.5604640628  0.0945782327
## X1121.54819054951 0.0914253886
## X1125.79378838971 0.0909061512
## X1126.29158820081 0.0889916026
## X1108.06747930453 0.0879526826
## X1102.32311155925 0.0878277012
## X1122.5497312715  0.0870585837
## X1126.5441441766  0.0860669559
## X1470.09319447942 0.0851941163
## X1123.30031266495 0.0849895564
## X1112.81003971275 0.0828129627
## X1103.32325021034 0.0804834482
## X1129.29565171194 0.0792222380
## X1112.30949170708 0.0755750879
## X1108.31811054051 0.0741827212
## X1488.40051943368 0.0738000496
## X1471.42767786981 0.0705795509
## X1117.04932871798 0.0680631925
## X1469.75976780245 0.0677773708
## X1470.42704875471 0.0670220233
## X1127.04008421696 0.0617318264
## X1131.78505133898 0.0614552161
## X1470.76081907912 0.0607021936
## X1113.56329850353 0.0575110057
## X1098.56831395271 0.0552270918
## X1469.42570146433 0.0547297316
## X1135.53727745968 0.0525023044
## X1112.05929178221 0.0499647663
## X1113.31183863808 0.0499299039
## X1108.56822391305 0.0484990599
## X1131.03795724898 0.0467007621
```

```
## X1471.09532086136 0.0465308757
## X1127.29255917483 0.0465304054
## X1114.06358094469 0.0430392118
## X1129.79457145018 0.0426737863
## X1109.06854325948 0.0425454117
## X1125.30053856127 0.0384018869
## X1485.74884256861 0.0361639550
## X1135.28675162713 0.0355602328
## X1489.399551935   0.0355471964
## X1478.08739359233 0.0338542992
## X1133.28887059062 0.0332983021
## X1132.78820284397 0.0314452831
## X1476.75330076931 0.0312544730
## X1113.06007098305 0.0307244558
## X1131.53755678079 0.0306259262
## X1109.56623050873 0.0297866618
## X1136.28695855805 0.0275403531
## X1478.42215498284 0.0271748784
## X1132.28893553294 0.0270411862
## X1124.55354253246 0.0221139805
## X1117.80491567323 0.0220497801
## X1118.30495999252 0.0208404964
## X1109.31799268981 0.0198891505
## X1114.31354083018 0.0182593046
## X1118.05504416426 0.0178664769
## X1477.4205825873  0.0169645660
## X1113.81293085655 0.0158796336
## X1483.41064725665 0.0132492924
## X1119.80974545454 0.0098193383
## X1126.79208167921 0.0071816142
## X1490.40376922173 0.0020508425
## X1119.30886967612 0.0006404074
##
## $comp
## [1] 1
```

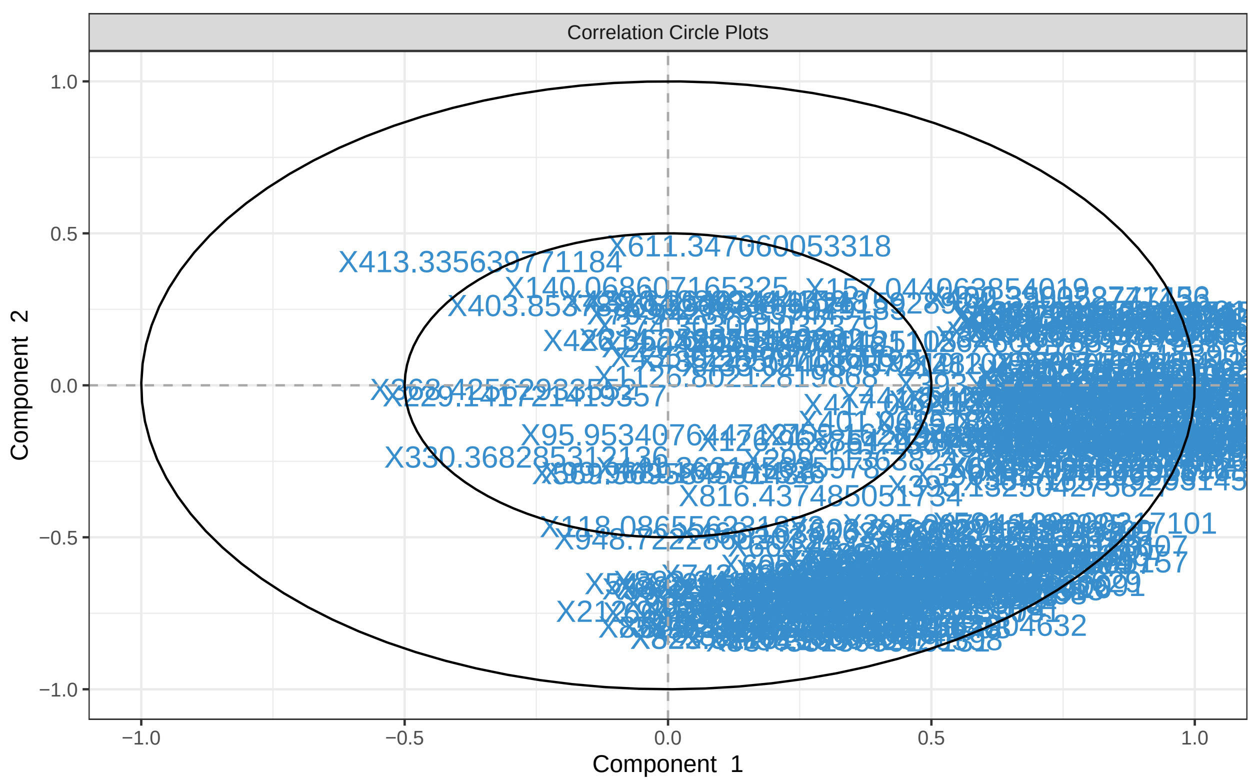
```
plotLoadings(old.splsda, contrib = "max", method = "mean", ndisplay = 50)
```




```r
# Young Leaf
young.splsda <- mixOmics::splsda(scaled_Y_young, class$Fungus,
    keepX = c(100, 100))

# plot pls-da
plotIndiv(young.splsda, ind.names = F, legend = T, title = "Young Leaf Secondary Metabolites (Pos) PLS-D
    legend.title = "Fungus", ellipse = T)
```




```r
# plot and select the variables
plotVar(young.splsda)
```



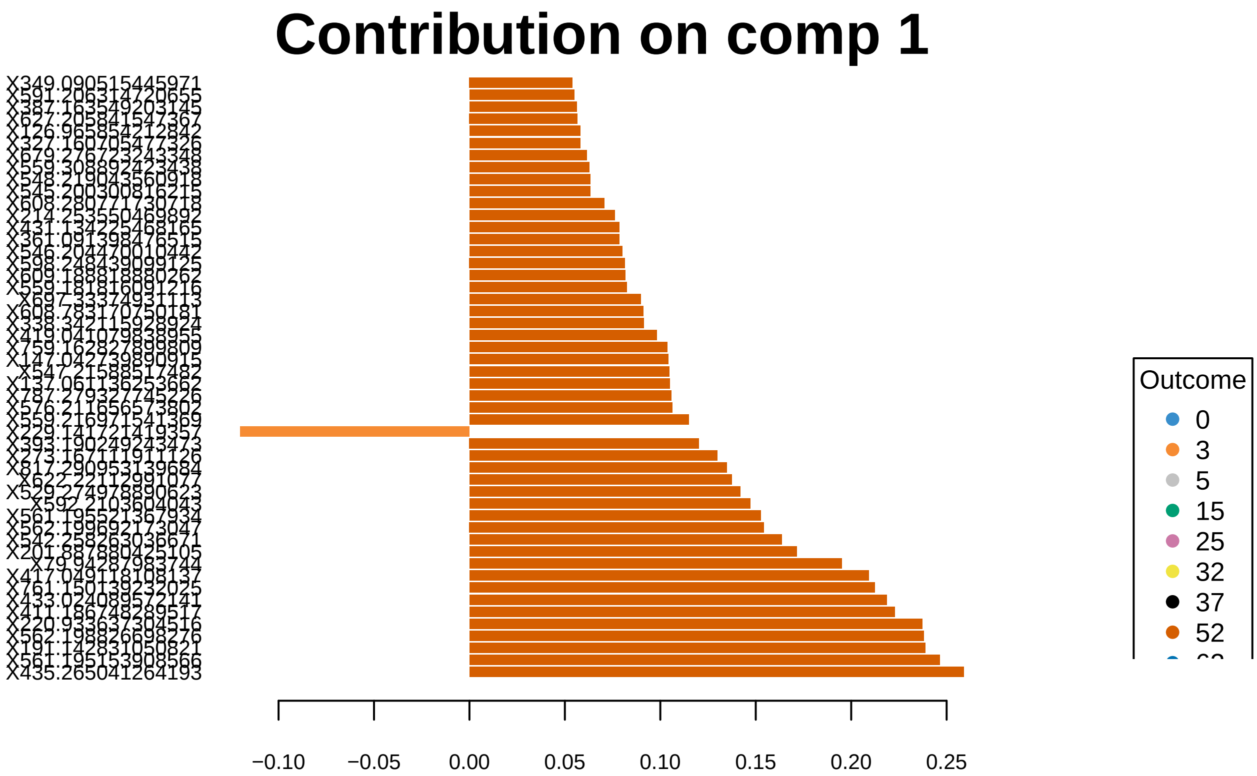
```r
selectVar(young.splsda, comp = 1)
```

```
## $name
##   [1] "X435.265041264193" "X561.195153908566" "X191.142831050821"
##   [4] "X562.198826698276" "X220.933637304516" "X411.186748289517"
##   [7] "X433.024089572141" "X761.150139232025" "X417.049118108137"
##  [10] "X79.94287983744"   "X201.887880425105" "X542.258263036671"
##  [13] "X562.199692173047" "X561.195521367934" "X592.2103604043"
##  [16] "X529.274978890623" "X622.22112991077"  "X817.290953139684"
##  [19] "X273.167111911126" "X393.190249243473" "X229.141721419357"
##  [22] "X559.216971541369" "X576.211656573802" "X787.279327745226"
##  [25] "X137.061136253662" "X547.21588517482"  "X147.042739890915"
##  [28] "X759.162827899809" "X419.041079838955" "X338.342115928924"
##  [31] "X608.783170750181" "X697.33374931113"  "X559.181816091216"
##  [34] "X609.188818880262" "X598.248439099125" "X546.204470010442"
##  [37] "X361.091398476515" "X431.134225468165" "X214.253550469892"
##  [40] "X608.280771730718" "X545.200300816215" "X548.219043560918"
##  [43] "X559.308892423438" "X679.276723243348" "X327.160705477326"
##  [46] "X126.965854212842" "X627.205841547367" "X387.163549203145"
##  [49] "X591.206314720655" "X349.090515445971" "X678.27479236805"
##  [52] "X149.095689289718" "X219.102286597348" "X579.103775337199"
##  [55] "X295.087943490825" "X381.116290473037" "X396.136174296936"
##  [58] "X769.269913578792" "X368.42562933552"  "X281.589541788573"
##  [61] "X771.283948428587" "X611.208506435124" "X607.209814001622"
##  [64] "X575.208493952074" "X459.153301042731" "X549.219252720572"
##  [67] "X282.095747416245" "X628.210632485529" "X547.216368593675"
##  [70] "X159.101695076237" "X655.189885840188" "X395.132504275827"
##  [73] "X330.368285312136" "X603.206683298622" "X402.358358777433"
##  [76] "X430.390022741156" "X595.222226186161" "X548.220348742993"
##  [79] "X157.044063854019" "X608.21522833073"  "X179.070702928982"
##  [82] "X723.248094849444" "X375.14623697476"  "X707.430538895667"
##  [85] "X540.245574294723" "X604.209992086111" "X593.221770649434"
##  [88] "X346.111503639577" "X374.303001032379" "X851.311747586913"
##  [91] "X498.217452455795" "X621.216851121727" "X500.210549877182"
##  [94] "X594.2254897838"   "X435.022999713606" "X501.138397567176"
##  [97] "X1379.64356208646" "X441.244799903401" "X435.128173248124"
## [100] "X401.068518225837"
##
## $value
##                     value.var
## X435.265041264193  0.258963679
## X561.195153908566  0.246358522
## X191.142831050821  0.238693289
## X562.198826698276  0.237963492
## X220.933637304516  0.237248512
## X411.186748289517  0.222867792
## X433.024089572141  0.218606675
## X761.150139232025  0.212332555
## X417.049118108137  0.209195273
## X79.94287983744    0.195117208
## X201.887880425105  0.171412375
## X542.258263036671  0.163553226
## X562.199692173047  0.154299165
## X561.195521367934  0.152634222
```

```
## X592.2103604043    0.147199177
## X529.274978890623  0.141830874
## X622.22112991077   0.137377777
## X817.290953139684  0.134882524
## X273.167111911126  0.129870163
## X393.190249243473  0.120262756
## X229.141721419357 -0.120047587
## X559.216971541369  0.114792705
## X576.211656573802  0.106310189
## X787.279327745226  0.105770084
## X137.061136253662  0.104912721
## X547.21588517482   0.104665651
## X147.042739890915  0.104086004
## X759.162827899809  0.103561657
## X419.041079838955  0.098130782
## X338.342115928924  0.091404048
## X608.783170750181  0.090990343
## X697.33374931113   0.089838998
## X559.181816091216  0.082511640
## X609.188818880262  0.081541900
## X598.248439099125  0.081463692
## X546.204470010442  0.080079798
## X361.091398476515  0.078486608
## X431.134225468165  0.078440925
## X214.253550469892  0.076089624
## X608.280771730718  0.070627804
## X545.200300816215  0.063329224
## X548.219043560918  0.063225972
## X559.308892423438  0.062664063
## X679.276723243348  0.061463371
## X327.160705477326  0.058134774
## X126.965854212842  0.058067992
## X627.205841547367  0.056609462
## X387.163549203145  0.056225981
## X591.206314720655  0.055010844
## X349.090515445971  0.053976768
## X678.27479236805   0.053139991
## X149.095689289718  0.052029242
## X219.102286597348  0.051889353
## X579.103775337199  0.051834367
## X295.087943490825  0.050970519
## X381.116290473037  0.049560180
## X396.136174296936  0.048273374
## X769.269913578792  0.047746198
## X368.42562933552  -0.046740608
## X281.589541788573  0.045612957
## X771.283948428587  0.042215253
## X611.208506435124  0.041706940
## X607.209814001622  0.040487259
## X575.208493952074  0.040183163
## X459.153301042731  0.039493252
## X549.219252720572  0.038285218
## X282.095747416245  0.036745489
## X628.210632485529  0.036450168
```

```
## X547.216368593675  0.036022686
## X159.101695076237  0.033758763
## X655.189885840188  0.033010829
## X395.132504275827  0.032253564
## X330.368285312136 -0.031975536
## X603.206683298622  0.031005958
## X402.358358777433  0.030979833
## X430.390022741156  0.030323872
## X595.222226186161  0.029635867
## X548.220348742993  0.029628293
## X157.044063854019  0.028907790
## X608.21522833073   0.024580253
## X179.070702928982  0.024301928
## X723.248094849444  0.023929859
## X375.14623697476   0.020281866
## X707.430538895667  0.017609269
## X540.245574294723  0.016430810
## X604.209992086111  0.015782657
## X593.221770649434  0.014237139
## X346.111503639577  0.013165190
## X374.303001032379  0.011635550
## X851.311747586913  0.009264566
## X498.217452455795  0.008973775
## X621.216851121727  0.008646167
## X500.210549877182  0.007840429
## X594.2254897838    0.006764113
## X435.022999713606  0.005054371
## X501.138397567176  0.004307501
## X1379.64356208646  0.001845171
## X441.244799903401  0.001827014
## X435.128173248124  0.001329296
## X401.068518225837  0.001178817
##
## $comp
## [1] 1
```

```r
plotLoadings(young.splsda, contrib = "max", method = "mean",
    ndisplay = 50)
```



Heatmaps of Averaged Data

11. Create averaged metabolite matrices and rerun PLS-DA to create a heatmap.

```
## Old Leaves
av_Y_old <- aggregate(Y_old, by = list(class$Water, class$Fungus),
    FUN = "mean", simplify = T, data = class)
av.old.plsda <- mixOmics::plsda(av_Y_old[, 3:5802], av_Y_old$Group.2)  # fungus

# heatmap
oldcim <- cim(av.old.plsda, title = "Old Leaf Secondary Met. (pos) Averaged Over Fungi",
    col.names = F, xlab = "Secondary Metabolites", save = "png",
    name.save = "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/S
```

Indicator Analysis

12. Identify indicator metabolites characteristic of each treatment using Dufrene-Legendre Indicator Analysis.

```
# Old Leaf
indicator_Fungus <- indval(Y_young, clustering = class$Fungus,
    numitr = 999, type = "long")

# Young Leaf
indicator_Fungus <- indval(Y_young, clustering = class$Fungus,
    numitr = 999, type = "long")
```

13. Disect indval object.

```
Orelfrq <- indicator_Fungus$relfrq  # relative frequency of species in classes
Orelabu <- indicator_Fungus$relabu  # relative abundance of species in classes
```

```r
Oindval <- indicator_Fungus$indval  # the indicator value for each species
Omaxcls <- data.frame(indicator_Fungus$maxcls)  # the class each species has max indicator value for
Oindcls <- data.frame(indicator_Fungus$indcls)  # the indicator value for each species to its max class
Opval <- data.frame(indicator_Fungus$pval)  # the probability of obtaining as high an indicator value a

Yrelfrq <- indicator_Fungus$relfrq  # relative frequency of species in classes
Yrelabu <- indicator_Fungus$relabu  # relative abundance of species in classes
Yindval <- indicator_Fungus$indval  # the indicator value for each species
Ymaxcls <- data.frame(indicator_Fungus$maxcls)  # the class each species has max indicator value for
Yindcls <- data.frame(indicator_Fungus$indcls)  # the indicator value for each species to its max class
Ypval <- data.frame(indicator_Fungus$pval)  # the probability of obtaining as high an indicator value a
```

14. Export results to a csv file.

```r
write.csv(cbind(Orelfrq, Orelabu, Oindval, Omaxcls, Oindcls,
    Opval), "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/Secon
write.csv(cbind(Yrelfrq, Yrelabu, Yindval, Ymaxcls, Yindcls,
    Ypval), "~/Box/Summer 2018 TX Endo Field Samples and Analysis/Statistics/Kenia_Thesis_Analysis/Secon
```

References

Collyer, M.L., Adams, D.C. 2018. RRPP: An r package for fitting linear models to high-dimensional data using residual randomization. Methods in Ecology and Evolution. 9(7):1772-1779.

Dufrene, M. and Legendre, P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67(3):345-366.

Rohart, F., Gautier, B., Singh, A., & Lê Cao, K. A. 2017. mixOmics: An R package for ‘omics feature selection and multiple data integration. PLoS computational biology, 13(11):e1005752.