## pcwOrd: (partial) (constrained) (weighted) ordination

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## ${\bf Contents}$

1	Mo	tivation	2
2	Bas	sic Functions	3
	2.1	Ordination	3
		2.1.1 Eigenvalues	4
		2.1.2 Axis Variances	4
		2.1.3 Scree plots	5
		2.1.4 Calculate scores	6
		2.1.5 Identify top features	7
3	Mo	ore ordinations	8
	3.1	Log-ratio analysis	8
	3.2	Weighted ordinations	9
	3.3	Constrained ordination	10
		3.3.1 Significance testing	11
	3.4	Partial ordination	11
	3.5	Partialed, constrained, weighted ordination	13
4	Cor	mparison to vegan and easyCODA	13
	4.1	Eigenvalues	14
	4.2	Scores	15
	4.3	Permutation Tests	16
5	Additional plotting options		
	5.1	Added categories	17
	5.2	Different axes	18
	5.3	Limit column labels	18
	5.4	colors and shapes	19
	5.5	Screeplots as grobs	21

#### 1 Motivation

Many ordination packages exist for ecologists using R - vegan is excellent, for example. But none readily perform (to my knowledge) weighted, partial constrained ordinations.

The motivation for this is to analyze compositional ecological data - especially high throughput sequencing data - with methods that are robust, reproducible, and transparent. Compositional data contains only relative data (think relative abundance), and so requires a bit of extra care to analyze. The dominant approach is to perform a log-ratio transformation, then use principle components analysis - combined, this is called log-ratio analysis. For an enterance into this literature, see Greenacre and Aitchison (2002), Greenacre and Lewi (2009), and Quinn et al (2018).

In particular, Greenacre and Lewi (2009) suggest using weighted log-ratio analysis, as unweighted log-ratio analysis is susceptible to noise in low-abundance features. Raw values of low-abundance features have higher relative variance than raw values of high-abundance features; therefore, the log-ratios of low abundance features are also of lower certainty. When low-abundance features dominate a solution the distances and inferences made about samples is less robust to error and noise. Note that correspondence analysis also is suceptible to dominance by low-abundance features.

Weighted log-ratio analysis down-weights these uncertain, low-abundance features. As a result, the observed distances among samples should be more reproducible across experiments.

vegan can perform constrained and partialed log-ratio analysis if the community matrix is log-transformed beforehand. However, it does not provide a straightforward way to weight columns and working with ordination objects is not intuitive. easyCODA allows weighted and constrained log-ratio analysis, but does not allow partialing of nuisance effects and does not provide hypothesis testing. pcwOrd allows partialed, constrained, and weighted ordinations, and also provides utility functions for investigating ordination objects, visualization, and hypothesis testing. The code is written to be self-documenting. Maybe you'll agree.

This isn't an R package yet, so load the functions with source():

This vignette will demonstrate the features of pcwOrd: ordination, hypothesis-testing, and visualization. I'll use the spiders dataset from mvabund. This dataset has two tables:

- 1. A data.frame of environmental variables, to which I'll add a categorical version of soil.dry\*.
- 2. A matrix of community data.

Certain pcwOrd functions use the rownames of the environmental and community data to cross-reference, so we want to ensure both tables have rownames. Usually these are something meaningful, like sample IDs.

<sup>\*</sup>technically this should be ordinal, but we'll treat it as categorical.

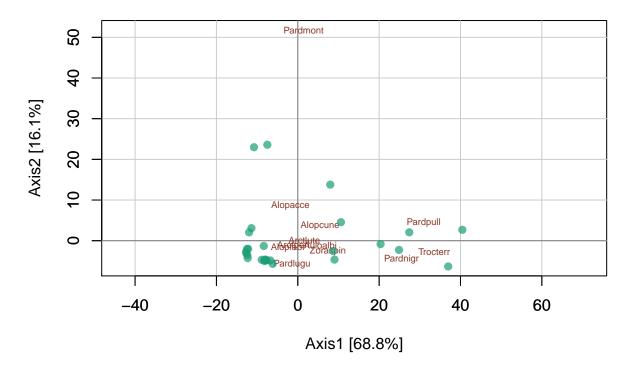
```
# environmental data
envi = as.data.frame(spider$x)
# categorical soil wetness
cats = c('dry', 'damp', 'wet', 'soaking')
cc = ceiling(envi$soil.dry)
envi$soil.cat = factor(cats[cc],
                      levels=cats)
# community data
comm = as.matrix(spider$abund)
# rownames
nobs = nrow(comm)
row_names = c(letters[], LETTERS[])[1:nobs]
rownames(envi) = row_names
rownames(comm) = row_names
str(envi)
                 28 obs. of 7 variables:
## 'data.frame':
## $ soil.dry
                 : num 2.33 3.05 2.56 2.67 3.02 ...
## $ bare.sand
                : num 00000...
## $ fallen.leaves: num 0 1.79 0 0 0 ...
## $ moss
                 : num 3.04 1.1 2.4 2.4 0 ...
## $ herb.layer : num 4.45 4.56 4.61 4.62 4.62 ...
## $ reflection : num 3.91 1.61 3.69 3 2.3 ...
                  : Factor w/ 4 levels "dry", "damp", "wet", ...: 3 4 3 3 4 4 4 3 3 3 ...
## $ soil.cat
comm[1:8, 1:8]
    Alopacce Alopcune Alopfabr Arctlute Arctperi Auloalbi Pardlugu Pardmont
##
## a
          25
                   10
                             0
## b
                                                      30
           0
                    2
                             0
                                     0
                                              0
                                                                         1
                                                                1
                   20
## c
          15
                             2
                                     2
                                              0
                                                      9
                                                                1
                                                                        29
## d
          2
                   6
                             0
                                     1
                                              0
                                                      24
                                                                1
                                                                         7
## e
          1
                   20
                            0
                                    2
                                             0
                                                      9
                                                               1
                                                                        2
                            0
                                              0
## f
           0
                   6
                                    6
                                                      6
                                                                0
                                                                        11
           2
                   7
                             0
                                    12
                                              0
                                                      16
                                                                1
                                                                        30
## g
## h
           0
                             0
                                    0
                                                      7
                                                               55
                                                                         2
                   11
```

#### 2 Basic Functions

#### 2.1 Ordination

We'll first perform a basic principle components analysis of the community with pcwOrd() and visualize the results with plot\_ord:

## **Spiders: Basic PCA**



The pca object is a list of class pcwOrd that contains a number of items. See the documentation for pcwOrd. Important ones are: 'Y\_scaled' - the community matrix after centering, scaling, and/or weighting but before further analysis; 'unconstrained' - the singular value decomosition matrices (left 'u' and right 'v') and values ('d'). All relevant information about this ordination can be calculated from these values.

#### 2.1.1 Eigenvalues

For example, if we want to calculate eigenvalues of this unconstrained ordination, we need to access the singular values (vector d) of the unconstrained solution:

#### pca\$unconstrained\$d^2

```
## [1] 251.7432705 58.7674666 25.3873166 11.7224346 5.6704074 4.1539497
## [7] 2.7649418 2.3272743 1.6733360 0.8060037 0.6634983 0.1884040
```

#### 2.1.2 Axis Variances

We can view these same data by calling ord\_variance, which summarizes the variance explained by each axis in the solution:

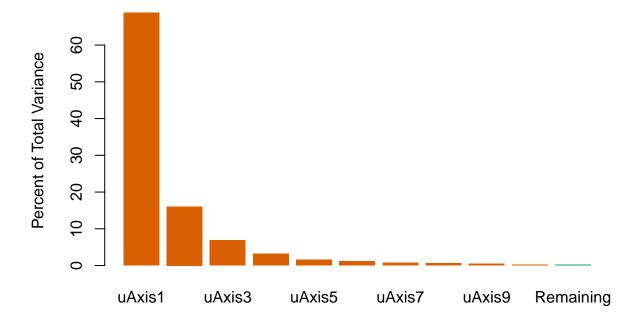
#### ord\_variance(pca)

```
## $type
## [1] "variance"
##
## $summary
##
                total partialed constrained unconstrained
## value
             365.8683
                              0
                                          0
                                                 365.8683
## pct_total 100.0000
                              0
                                          0
                                                 100.0000
##
## $total
##
                Total
            365.8683
## value
## pct_group 100.0000
## pct_total 100.0000
##
## $partialed
##
## value
## pct_group
## pct_total
##
## $constrained
##
## value
## pct_group
## pct_total
## $unconstrained
##
                uAxis1
                        uAxis2
                                   uAxis3
                                             uAxis4 uAxis5
                                                               uAxis6
                                                                         uAxis7
## value
            251.74327 58.76747 25.387317 11.722435 5.670407 4.153950 2.7649418
## pct_group 68.80707 16.06246 6.938922 3.204004 1.549849 1.135367 0.7557205
## pct total 68.80707 16.06246 6.938922 3.204004 1.549849 1.135367 0.7557205
                                                        uAxis12
##
                uAxis8
                          uAxis9
                                   uAxis10
                                             uAxis11
## value
             2.3272743 1.6733360 0.8060037 0.6634983 0.18840395
## pct_group 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
## pct_total 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
```

#### 2.1.3 Scree plots

We can also visualize variances across axes in a scree plot with ord\_scree():

```
ord_scree(pca)
```



#### 2.1.4 Calculate scores

If you want to make your own biplots, you can calculate row, column, centeroid, and biplot scores by calling ord\_scores. Scores can be principle, standard, or contribution - with the latter being raw singular values (either 'u' or 'v'). To make plotting easy, we can also add grouping information from the envi table:

```
##
         Axis1
                   Axis2
                             Axis3 soil.cat
     7.988176 13.786265 -3.604174
                                         wet
     9.030352 -4.652883 -5.718181
                                     soaking
  c 10.606032 4.549642 -3.574303
                                         wet
  d 24.881370 -2.272295 -8.576151
                                         wet
  e 36.974719 -6.324696 19.094659
                                     soaking
     8.683557 -2.525640 -1.763815
                                     soaking
```

When plotting both standard and principle scores, often you'll need to rescale one of them for visualization:

## Rescaling standard scores by 12.63

```
## $original
##
                 Axis1
                              Axis2
                                          Axis3
## Alopacce -0.11660037 0.558436920 0.21907567
## Alopcune 0.35414730 0.243325952 0.17224233
## Alopfabr -0.15364830 -0.112002939 0.30171516
## Arctlute 0.10396386 0.008826392 0.04486536
## Arctperi -0.06877624 -0.081008292 0.12759310
## Auloalbi 0.35918227 -0.068503756 -0.42665830
##
## $rescaled
##
                Axis1
                           Axis2
                                     Axis3
## Alopacce -1.4732357 7.0558024 2.768002
## Alopcune 4.4746206 3.0744024 2.176267
## Alopfabr -1.9413330 -1.4151475 3.812145
## Arctlute 1.3135744 0.1115207 0.566870
## Arctperi -0.8689818 -1.0235328 1.612128
## Auloalbi 4.5382371 -0.8655391 -5.390791
```

#### 2.1.5 Identify top features

Finally, it's useful to identify the top features. The default scaling='contribution' gives top contribution scores - i.e. the features that contribute most to the axes chosen. Here, we identify the spider taxa that have the five highest mean contributions across the first two axes:

```
top_scores(pca, n=5, choice='column', scaling='contribution', axes=c(1:2))
```

#### 3 More ordinations

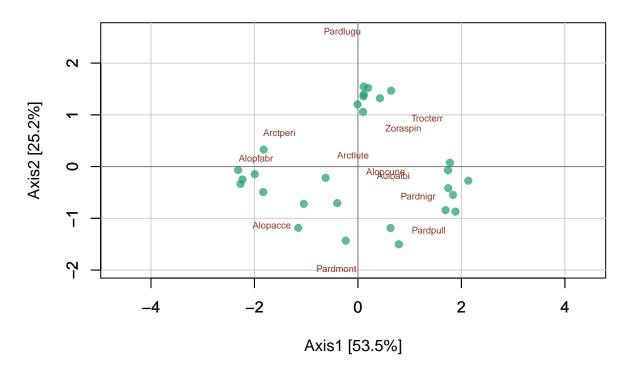
#### 3.1 Log-ratio analysis

To perform log-ratio analysis:

- 1. Remove zeros from your data. For simplicity, I'll replace zeros with a pseudocount of 0.5. Note that I am *not* adding the pseudocount as x + 0.5, as this will distort the ratios between observations.
- 2. Close the community matrix, so that all abundances are relative abundances and rowSums(x) = 1.
- 3. Perform the log-ratio transformation of your choice. The centered log-ratio (CLR), where values are centered on the geometric mean, is a common choice.
- 4. Run principle components analysis on the log-ratio transformed matrix

## Rescaling standard scores by 1.146

## **Spiders: Log Ratio Analysis**



#### 3.2 Weighted ordinations

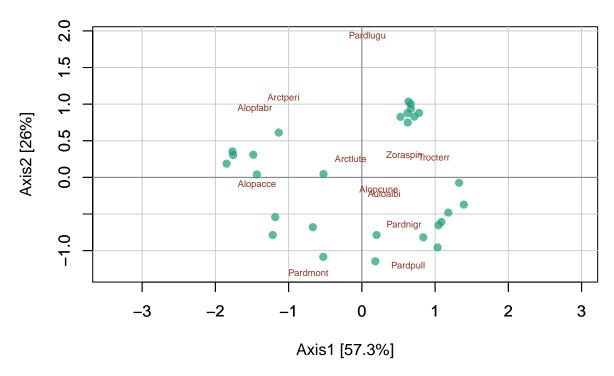
To weight either rows or columns, for example by sequencing depth or column prevalence, you have three options:

- 1. Tell pcwOrd to calculate weights automatically by setting weight\_rows=TRUE or weight\_columns=TRUE. This uses row or column masses as weights (via rowSums, for example).
- 2. Specify a vector of length nrow() or ncol() with the weights.
- 3. Input the result of easyCODA::CLR(), which returns a weighted CLR transformation and row-weights. This approach is demonstrated below:

Here is a weighted LRA:

## Rescaling standard scores by 0.9226





In this situation, the results of weighted and unweighted ordinations are approximately the same. See Greenacre and Lewi (2009) for a discussion of when weighted log-ratio analysis might be advantageous over a non-weighted log ratio analysis.

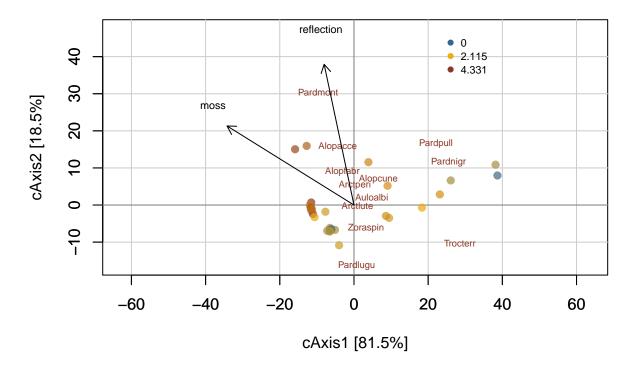
#### 3.3 Constrained ordination

We might be interested in how these spiders associate with environmental variables. For example, we might be interested in whether moss and reflection structures spider communities. A constrained ordination like redundancy analysis will do this: pcwOrd will regress the community matrix against moss and reflectivity, and then ordinate the fitted values.

## Rescaling standard scores by 13.4

## Rescaling standard scores by 48.24

## **Spiders by Moss Coverage**



In this plot, colors show moss coverage, the first column in X.

#### 3.3.1 Significance testing

To test whether spiders vary with wetness class, run a PERMANOVA on the ordination with permute\_ord():

```
pp = permute_ord(rda)
pp

## permute_on total_variance variance_after_partialing fitted residuals num_df
```

```
## permute_on total_variance variance_after_partialing fitted residuals num_df
## 1 partial 365.8683 365.8683 98.72652 267.1418 2
## denom_df F_stat p_val F_perm
## 1 25 4.619575 0.004 999
```

permute\_ord() has a number of permutation models that parallel the options in vegan::permutest() - and permute\_ord() will return the same results as vegan::permutest() with the same randomization seed. We see a p-value of 0.004 after 999 permutations.

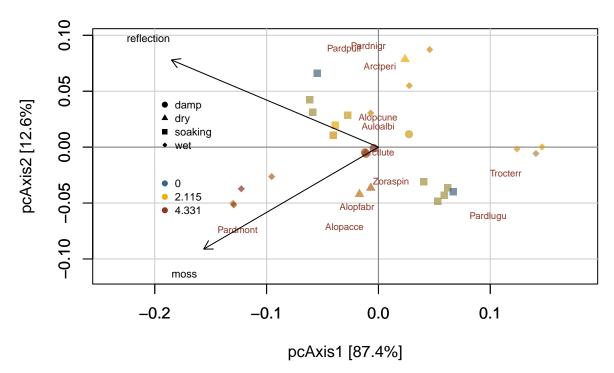
#### 3.4 Partial ordination

Say we want to look at how moass affects spider communities independently of moisture class. We can partial this out as Z:

## Rescaling standard scores by 0.05837

## Rescaling standard scores by 0.2961

## Spiders by moss and reflection, partialed by wetness



Each of the wetness categories is centered at zero, as expected due to partialing. Moss increases to the bottom left (darker red, and arrows). This model is highly significant:

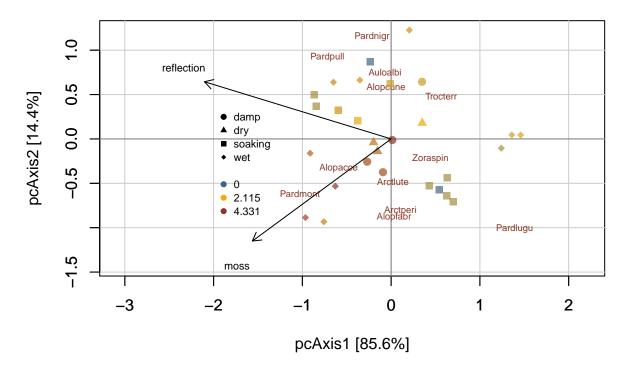
```
permute_ord(pcrda)
```

#### 3.5 Partialed, constrained, weighted ordination

The reason for this package is to perform partial, constrained, weighted ordinations. Here is a weighted logratio analysis of the spider community, constrained by moss and reflection, with moisture category partialed out:

- ## Rescaling standard scores by 0.7005
- ## Rescaling standard scores by 3.215

## Partialed, Constrained, Weighted Log-Ratio Analysis



## 4 Comparison to vegan and easyCODA

pcwOrd gives the same results as comparable vegan and easycCODA functions.

#### 4.1 Eigenvalues

Returning to eigenvalues: these eigenvalues are exactly the same as calculated by easyCODA's PCA, and solutions are the same:

```
easy_pca = PCA(comm, weight=FALSE)
rbind(pcwOrd = pca$unconstrained$d^2,
      easyCODA = easy_pca$sv^2
##
                          [,2]
                                   [,3]
                                             [,4]
                                                      [,5]
                                                               [,6]
                                                                        [,7]
                                                                                 [,8]
                 [,1]
            251.7433 58.76747 25.38732 11.72243 5.670407 4.15395 2.764942 2.327274
## easyCODA 251.7433 58.76747 25.38732 11.72243 5.670407 4.15395 2.764942 2.327274
                 [,9]
                          [,10]
                                    [,11]
                                              [,12]
            1.673336 0.8060037 0.6634983 0.188404
## pcwOrd
## easyCODA 1.673336 0.8060037 0.6634983 0.188404
```

Eigenvalues are not identical to vegan eigenvalues, because pcwOrd (and easyCODA) weights the initial community matrix rows by 1/sqrt(nrow), while vegan weights rows by 1/sqrt(nrow-1). You can access vegan-style weighting, which will agree with vegan results:

```
##
               PC1
                        PC2
                                 PC3
                                          PC4
                                                   PC5
                                                            PC6
                                                                     PC7
                                                                              PC8
## pcwOrd 3132.805 731.3285 315.9311 145.8792 70.56507 51.6936 34.40816 28.96164
## vegan 3132.805 731.3285 315.9311 145.8792 70.56507 51.6936 34.40816 28.96164
##
               PC9
                       PC10
                                PC11
## pcwOrd 20.82374 10.03027 8.256867 2.344583
## vegan 20.82374 10.03027 8.256867 2.344583
```

These weightings don't change relative variance or relative distances, only absolute variances and distances. Generally, we're concerned with relative variances and distances, so the choice of (equal) weighting doesn't matter.

```
list(
  coda_weightings = ord_variance(pca)$unconstrained,
  vegan_weightings = ord_variance(veganlike_pca)$unconstrained
)

## $coda_weightings
## uAxis1 uAxis2 uAxis3 uAxis4 uAxis5 uAxis6 uAxis7
```

```
## value 251.74327 58.76747 25.387317 11.722435 5.670407 4.153950 2.7649418
## pct_group 68.80707 16.06246 6.938922 3.204004 1.549849 1.135367 0.7557205
## pct_total 68.80707 16.06246 6.938922 3.204004 1.549849 1.135367 0.7557205
## value 2.3272743 1.6733360 0.8060037 0.6634983 0.18840395
## pct group 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
```

```
## pct total 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
##
## $vegan_weightings
##
                         uAxis2
                                   uAxis3
                                               uAxis4
                                                        uAxis5
                uAxis1
                                                                  uAxis6
## value
           3132.80514 731.32847 315.931051 145.879186 70.565070 51.693597
## pct group 68.80707 16.06246
                                6.938922 3.204004 1.549849 1.135367
              68.80707 16.06246
                                  6.938922
                                             3.204004 1.549849 1.135367
## pct_total
##
                uAxis7
                          uAxis8
                                     uAxis9
                                               uAxis10 uAxis11
                                                                   uAxis12
## value
            34.4081650 28.9616352 20.8237366 10.0302687 8.2568673 2.34458251
## pct_group 0.7557205 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
## pct_total 0.7557205 0.6360962 0.4573602 0.2202989 0.1813489 0.05149502
```

#### 4.2 Scores

## [1,] 0.5034643 1.7983663 ## [2,] 0.5691487 -0.6069510

```
ord_scores(pca, 'row', 'principle')
##
          Axis1
                     Axis2
## a
      7.988176 13.7862654
     9.030352 -4.6528827
## c 10.606032 4.5496420
## d 24.881370 -2.2722951
## e 36.974719 -6.3246958
      8.683557 -2.5256396
## g 40.458746 2.6835088
## h -6.167524 -5.6887597
## i -11.395552 3.0854298
## j -11.973728 2.0464630
## k -10.765634 22.9846309
## 1 -7.483546 23.5898261
## m 27.409257 2.0678738
## n 20.354007 -0.8378813
## o -7.687032 -4.8091247
## p -6.740881 -4.8547748
## q -8.058080 -4.6416878
     -7.769638 -4.7163087
## s -8.108993 -4.9559103
## t -8.264546 -4.9285167
## u -8.866147 -4.6802406
## v -12.383293 -3.5219133
## w -12.469082 -2.0121482
## x -12.652253 -3.0858499
## y -8.360181 -1.2930062
## z -12.312196 -4.2679844
## A -12.721757 -2.7161932
## B -12.206151 -2.0078266
easy_pca$rowcoord[, 1:2]
##
               [,1]
                          [,2]
```

```
[3,] 0.6684578 0.5934836
##
   [4,] 1.5681780 -0.2964123
  [5,] 2.3303757 -0.8250327
## [6,] 0.5472915 -0.3294601
   [7,] 2.5499606 0.3500536
## [8,] -0.3887156 -0.7420772
## [9,] -0.7182182 0.4024827
## [10,] -0.7546585 0.2669534
## [11,] -0.6785169 2.9982583
## [12,] -0.4716594 3.0772038
## [13,] 1.7275011 0.2697463
## [14,] 1.2828355 -0.1092984
## [15,] -0.4844843 -0.6273322
## [16,] -0.4248521 -0.6332871
## [17,] -0.5078701 -0.6054906
## [18,] -0.4896907 -0.6152247
## [19,] -0.5110789 -0.6464798
## [20,] -0.5208828 -0.6429064
## [21,] -0.5587995 -0.6105197
## [22,] -0.7804718 -0.4594203
## [23,] -0.7858788 -0.2624771
## [24,] -0.7974233 -0.4025375
## [25,] -0.5269104 -0.1686678
## [26,] -0.7759908 -0.5567424
## [27,] -0.8018039 -0.3543171
## [28,] -0.7693072 -0.2619134
```

#### 4.3 Permutation Tests

vegan::permutest() gives the same as pcwOrd::permute\_ord(), if you set the same seed.

```
## $vegan
##
## Permutation test for rda under reduced model
##
## Permutation: free
## Number of permutations: 999
##
## Model: rda(X = comm, Y = X)
## Permutation test for all constrained eigenvalues
```

```
## Df Inertia F Pr(>F)
## Model 2 1228.6 4.6196 0.004 **
## Residual 25 3324.4
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## $pcwOrd
## fitted residuals num_df denom_df F_stat p_val
## 1 1228.597 3324.431 2 25 4.619575 0.004
```

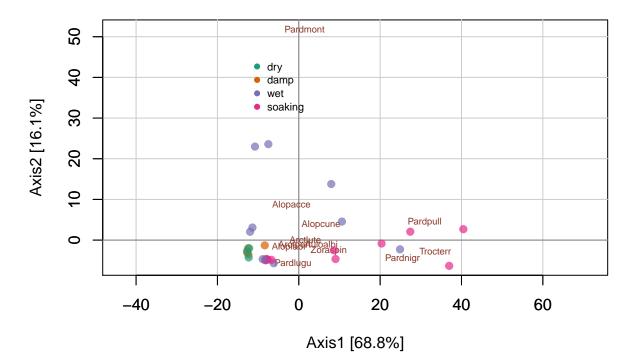
## 5 Additional plotting options

#### 5.1 Added categories

Add groupings to any ordination. They can be continuous.

## Rescaling standard scores by 15.45

## PCA with groupings by soil moisture class

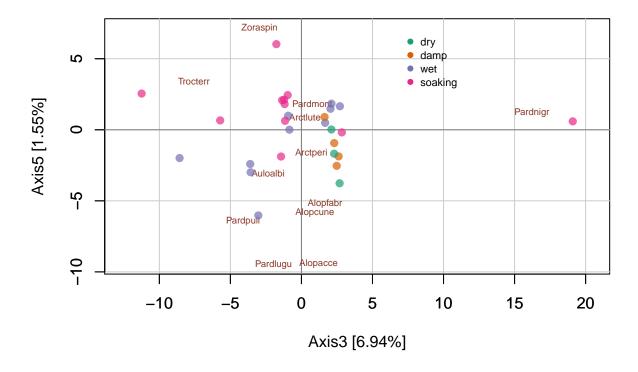


#### 5.2 Different axes

Plot different axes

## Rescaling standard scores by 5.487

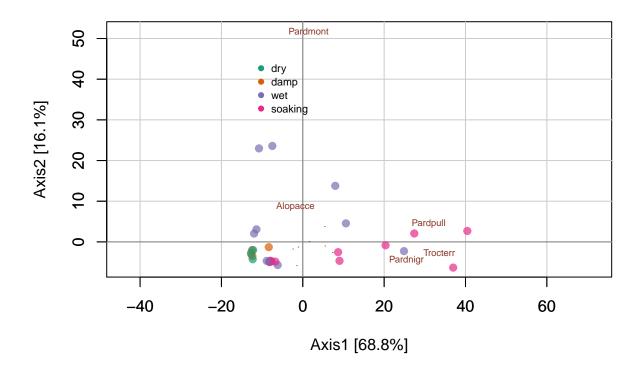
#### PCA axes 3 and 5



#### 5.3 Limit column labels

Reduce clutter by only labeling the top columns (by plotted score)

## Rescaling standard scores by 15.45

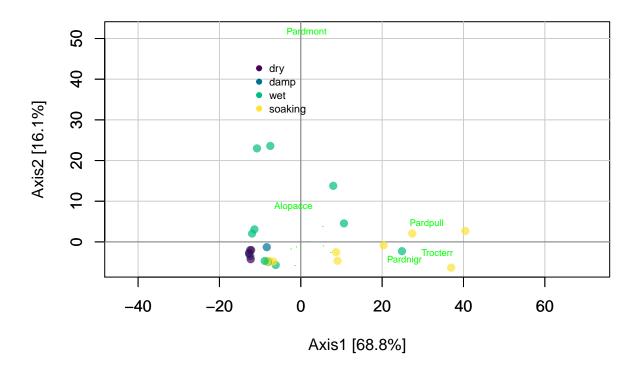


## 5.4 colors and shapes

Change the colors of text, groupings, arrows, and change shapes.

## Rescaling standard scores by 15.45

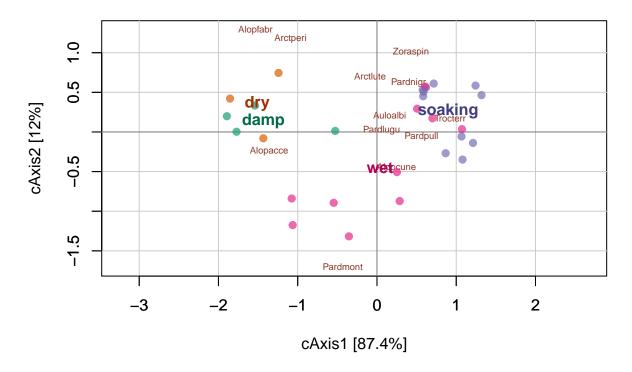
## **Fluorescent PCA**



If you constrain an ordination with categories, the plot function will automatically recognize these and plot them as named centeroids:

## Rescaling standard scores by 0.8593

## **Spiders wLRA with Centeroids**



## 5.5 Screeplots as grobs

Screeplots can be produced as grobs (via ggplot2) for downstream manipulation, saving as R objects, and arranging with gridExtra. If you want to do this with ordinations, you're on your own!

```
ord_scree(cat_cwLRA, main='ggplot2 Scree Plot', as_grob=TRUE) +
    theme_dark()
```

## Loading required package: ggplot2

