A (brief) introduction to ordination and the vegan package

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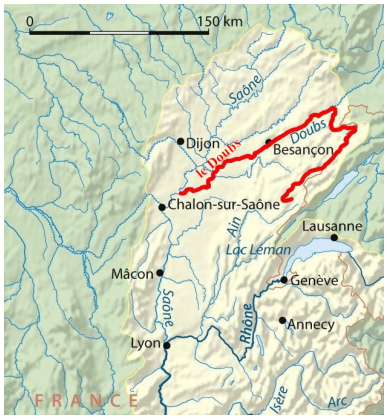
Topics adressed

	Raw data	Transformed data	Unimodal	Distance-based	Model-based
Unconstrained	PCA	tb-PCA	CA, DCA	PCoA, NMDS	MM, LVM
Constrained	RDA	tb-RDA	CCA	db-RDA	CAO, CQO
Other				Permanova, Dispersion	manyglm

(Nearly) no maths today ;)

Datasets

Demonstration: Doubs river fish communities



- Fish
- 30 sites along the Doubs River

Questions

- How does fish composition change downstream?
- Environmental drivers?

Verneaux, J. (1973) Cours d'eau de Franche-Comte (Massif du Jura). Recherches ecologiques sur le reseau hydrographique du Doubs. Essai de biotypologie. These d'etat, Besancon. 1–257.

Demonstration: Doubs river fish communities — Species


```
Dabu <- read.table('doubsAbu.csv', sep = ',', header = TRUE)
Denv <- read.table('doubsEnv.csv', sep = ',', header = TRUE)
Dspa <- read.table('doubsSpa.csv', sep = ',', header = TRUE)
```

```
dim(Dabu)

[1] 30 27
```

30 sites, 27 taxa

```
head(Dabu[ , 1:18])

  CHA TRU VAI LOC OMB BLA HOT TOX VAN CHE BAR SPI GOU BRO PER BOU PSO ROT
1   0   3   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0
2   0   5   4   3   0   0   0   0   0   0   0   0   0   0   0   0   0   0
3   0   5   5   5   0   0   0   0   0   0   0   0   0   1   0   0   0   0
4   0   4   5   5   0   0   0   0   0   1   0   0   1   2   2   0   0   0
5   0   2   3   2   0   0   0   0   5   2   0   0   2   4   4   0   0   2
6   0   3   4   5   0   0   0   0   1   2   0   0   1   1   1   0   0   0
```

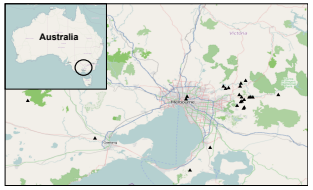
Demonstration: Doubs river fish communities — Environment


```
# Dimension and first rows of Environmental data
dim(Denv)

[1] 30 11
```

30 sites, 11 variables

```
head(Denv)

   das alt  pen  deb  pH dur  pho  nit  amm  oxy dbo
1  0.3 934 48.0 0.84 7.9  45 0.01 0.20 0.00 12.2 2.7
2  2.2 932  3.0 1.00 8.0  40 0.02 0.20 0.10 10.3 1.9
3 10.2 914  3.7 1.80 8.3  52 0.05 0.22 0.05 10.5 3.5
4 18.5 854  3.2 2.53 8.0  72 0.10 0.21 0.00 11.0 1.3
5 21.5 849  2.3 2.64 8.1  84 0.38 0.52 0.20  8.0 6.2
6 32.4 846  3.2 2.86 7.9  60 0.20 0.15 0.00 10.2 5.3
```


Exercise: Salinization and Pesticides



- Macroivertebrates
- 24 sites
- covering a salinity and toxicity gradient

Questions:

- Interaction between salinization and pesticides?
- Which species are affected?
- Other influences?

The dataset is published in: Szöcs, E., Kefford, B.J., Schäfer, R.B., 2012. Is there an interaction of the effects of salinity and pesticides on the community structure of macroinvertebrates? Science of the Total Environment 437, 121–126.

Exercise: Salinization and Pesticides


```
# setwd('3-Ordination/data/')
abu <- read.table('melbourneAbu.csv', sep = ';', header = TRUE)
env <- read.table('melbourneEnv.csv', sep = ';', header = TRUE)
```

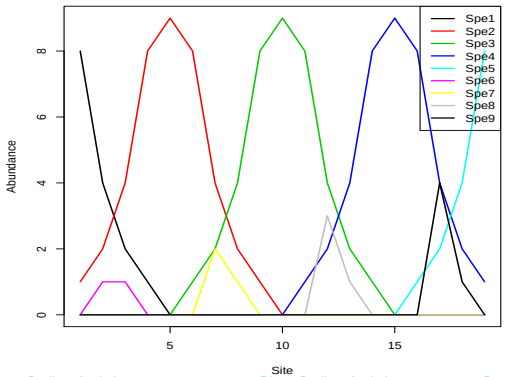
```
# dimensions of data.frame
dim(env)

[1] 24 23

dim(abu)

[1] 24 76
```

24 sites, 22 environmental variables, 75 taxa

```
head(env[ , 1:10])

    ID    T   pH oxygen Depth maxwidth minwidth rifperc poolperc Bedrock
1 1-11 16.8 7.67   80.1   0.9       15     12.0       0      100       0
2 2-11 16.5 7.29   83.0   0.9       30     15.0       0      100       0
3 3-11 17.3 7.20   77.9   0.4        4      2.5       0      100       0
4 4-11 15.6 7.84   72.0   0.7        8      2.5       0      100       0
5 5-11 17.2 6.97   69.9   0.9        7      4.0       0      100       0
6 6-11 15.5 7.26   80.0   0.2        3      2.0       5       95       0
```


Exercise: Dummy abundances

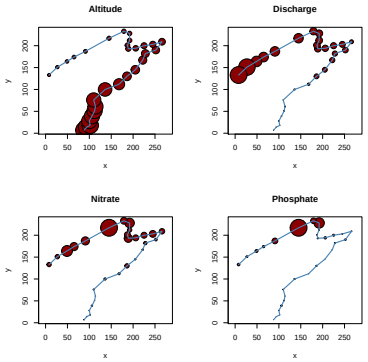

```
# Load dummy data
dummy <- read.table('dummydata.csv', header = TRUE, sep = ';')
# plot dummy data
matplot(dummy[ , -1], type = 'l', xlab = 'Site', ylab = 'Abundance',
        lty = 'solid', lwd = 2, col = 1:9)
legend('topright', legend = colnames(dummy)[-1],
        col = 1:9, lty = 'solid', lwd = 2)
```



Indirect Gradient Analysis

Principal Components Analysis (PCA)
Principal coordinates analysis (PCoA)
Nonmetric Multidimensional Scaling (NMDS)

Principal Components Analysis (PCA) — Why?



- 11 variables

Questions:

- Which variables are correlated?
- Which sites have similar conditions?
- How do conditions change downstream?

Solutions?

- pairwise comparisons
- 3D possible
- more than 3 dimensions?

Principal Components Analysis (PCA) — What?

- *"Look from another angle on the data"*
- PCA is just a rotation of the coordinate system
- The rotation is done so that the first axis contains as much variation as possible
- Second axis than most of remaining variation

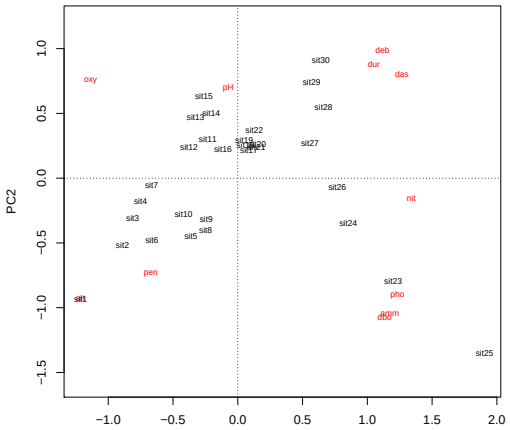
Short Demo.

Maths:

- The covariance (or correlation) matrix is decomposed into its Eigenvectors and Eigenvalues.
- The Eigenvectors give the rotation needed
- The Eigenvalues stretch the axes

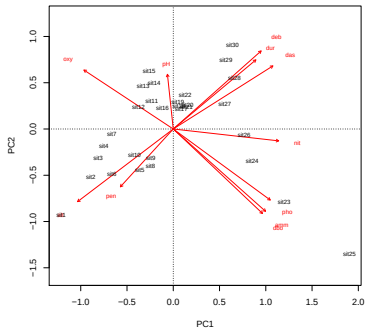
Principal Components Analysis (PCA) — How?


```
require(vegan)
PCA <- rda(Denv, scale = TRUE)
plot(PCA, scaling = 3)
```



Principal Components Analysis (PCA) — Interpretation? (I)

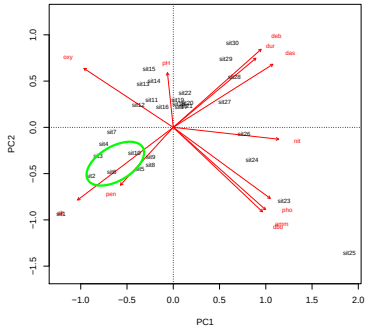

```
biplot(PCA, cex = 5, scaling = 3)
```

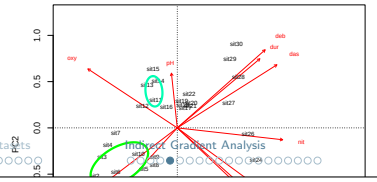


- angle between variables **approx.** their correlation
- distance between sites **approx.** their euclidean distance
- projecting a site on a variable **approx.** the relative value

- `scaling = 1` - to interpret (only) distances between sites
- `scaling = 2` - to interpret (only) correlations between variables

Principal Components Analysis (PCA) — Interpretation? (II)





- high altitude + slope, low discharge
- high oxygen, low nutrient
- intermediate
- nutrient rich
- high discharge, low altitude, medium nutrient

Principal Components Analysis (PCA) — Interpretation? (III)

```
summary(PCA, display = NULL, scaling = 3)


Call:
rda(X = Denv, scale = TRUE)

Partitioning of correlations:
              Inertia Proportion
Total              11          1
Unconstrained      11          1

Eigenvalues, and their contribution to the correlations

Importance of components:
                          PC1    PC2     PC3     PC4     PC5     PC6    PC7
Eigenvalue             5.9687 2.1638 1.06516 0.73873 0.40027 0.33565 0.1727
Proportion Explained   0.5426 0.1967 0.09683 0.06716 0.03639 0.03051 0.0157
Cumulative Proportion  0.5426 0.7393 0.83616 0.90331 0.93970 0.97022 0.9859
                           PC8     PC9    PC10     PC11
Eigenvalue             0.10821 0.02368 0.01707 0.005993
Proportion Explained   0.00984 0.00215 0.00155 0.000540
Cumulative Proportion  0.99575 0.99790 0.99946 1.000000

Scaling 3 for species and site scores
* Both sites and species are scaled proportional to eigenvalues
 on all dimensions
* General scaling constant of scores:
```


Your turn!

Load the Melbourne dataset (only environmental variables).

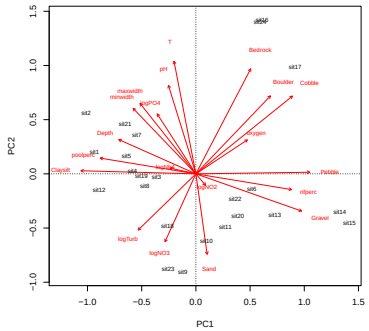
Exclude the variables ID, logCond and logmaxTU.
Perform a PCA.

Which variables are correlated?
How much variance is explained by the first 2 axes?
How could the two PCA axes be interpreted?

Exercise


```
take <- env[ , !names(env) %in% c('ID', 'logCond', 'logmaxTU')]
PCA <- rda(take, scale = TRUE)
cumsum(PCA$CA$eig / PCA$tot.chi)[1:2]

      PC1       PC2
0.2839873 0.4537452
```

```
biplot(PCA, scaling = 3)
```



- multiple variables interrelated
- 1st axis can be intepreted as *hydrological gradient*
- 2nd axis can be intepreted as *chemistry gradient*

Excursus — Principal component regression (PCR)

Question:

- How is diversity related to salinity, pesticides and other variables?

Problem:

- Only 24 sites
- but 22 (potentially correlated) explanatory variables
- strong hypotheses about salinity and pesticides

A Solution:

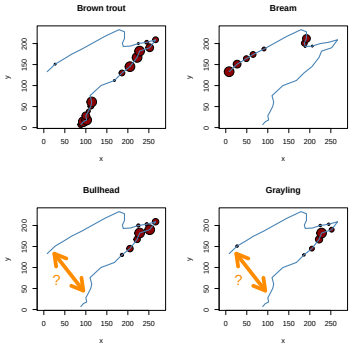
- Reduce number of variables to *Principal Components*
- regress these

Excursus — principal component regression (PCR)


```r
# calculate shannon diversity index
div <- diversity(abu[ , -1], index = 'shannon')
pc <- scores(PCA, choices = c(1, 2), scaling = 1, display = 'sites')
model_data <- data.frame(div, pc, logCond = env$logCond, logmaxTU = env$logmaxTU)
model <- lm(div ~ PC1 + PC2 + logCond + logmaxTU, data = model_data)
summary(model)
```

```
Call:
lm(formula = div ~ PC1 + PC2 + logCond + logmaxTU, data = model_data)

Residuals:
     Min       1Q   Median       3Q      Max
-0.64415 -0.15688  0.02063  0.18219  0.57929

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.83079    0.43429   4.216 0.000468 ***
PC1          0.01971    0.16691   0.118 0.907262
PC2          0.02192    0.19570   0.112 0.911996
logCond     -0.20942    0.13050  -1.605 0.125049
logmaxTU    -0.12572    0.07316  -1.718 0.101994
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3645 on 19 degrees of freedom
Multiple R-squared:  0.2682, Adjusted R-squared:  0.1141
F-statistic: 1.741 on 4 and 19 DF,  p-value: 0.1827
```


Abundances — The Problem with zeros



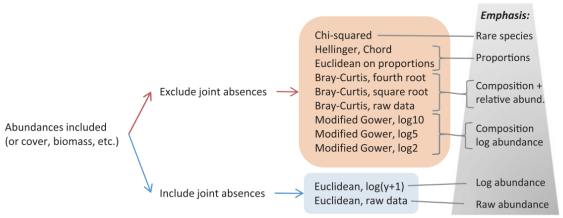
- Species may be absent due to different factors (too high flow, too saline, etc.)
- *Absence* contains less information then *Presence*
- PCA preserves the euclidean distance between sites
- Need another measure of similarity for (raw) abundances

Dissimilarity measures — Species abundance paradox


```
     Spe1 Spe2 Spe3
sit1    0    4    8
sit2    0    1    1
sit3    1    0    0
```

```
vegdist(mat, method = 'euclidean')

         sit1     sit2
sit2 7.615773
sit3 9.000000 1.732051
```

```
vegdist(mat, method = 'bray')

          sit1      sit2
sit2 0.7142857
sit3 1.0000000 1.0000000
```



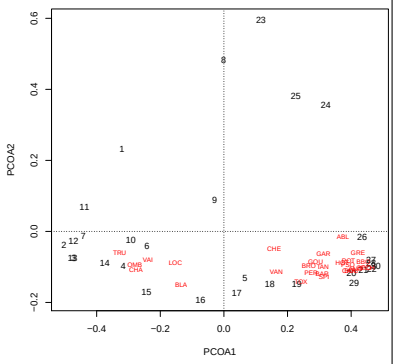
from: Anderson, M.J., Crist, T.O., et al. , 2011. Navigating the multiple meanings of beta diversity: a roadmap for the practicing ecologist. Ecology Letters 14, 19–28.

Principal coordinates analysis (PCoA)

- Works on distance matrices
- Species can be added as *weighted averages*
- Eigenvalue based
- PCoA with euclidean distance == PCA

Principal coordinates analysis (PCoA)


```r
# Distance matrix
Dabu_dist <- vegdist(Dabu, method = 'bray')

# PCoA
PCOA <- cmdscale(Dabu_dist, eig = TRUE)

# Create plot
plot(PCOA$points, type = 'n',
     xlab = 'PCOA1', ylab = 'PCOA2')
text(PCOA$points,
     labels = rownames(Dabu), cex = 0.9)
abline(h = 0 , lty = 'dotted')
abline(v = 0 , lty = 'dotted')
# Add species as weighted averages
wa <- wascores(PCOA$points, Dabu)
text(wa, labels = colnames(Dabu),
     col = 'red', cex = 0.7)
```




```r
# explained variance
(PCOA$eig / sum(PCOA$eig))[1:2] * 100

[1] 49.24914 15.95758
```


Nonmetric Multidimensional Scaling (NMDS)

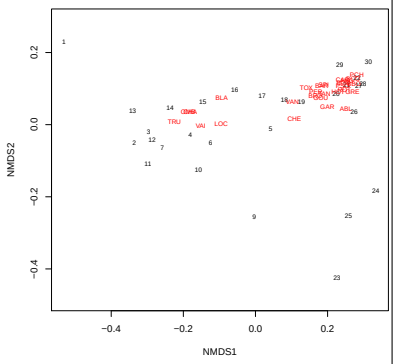
- Similar to PCoA
- Does not preserve exact distances between objects
- Possibly better representation in low dimensions
- **Not** eigenvalue based, iterative algorithm
- Axes have no meaning, just the relative distances

Nonmetric Multidimensional Scaling (NMDS)


```r
# Distance matrix
Dabu_0 <- Dabu[!rowSums(Dabu) == 0, ]
Dabu_dist <- vegdist(Dabu_0, method = 'bray')

# NMDS
NMDS <- metaMDS(Dabu_dist, k = 2, trace = 0)

# Plot
plot(NMDS, type = 't')

# Add species as weighted averages
wa <- wascores(NMDS$points, Dabu_0)
text(wa, labels = colnames(Dabu),
     col = 'red', cex = 0.7)
```

```r
# Stress value
NMDS$stress
```

```
[1] 0.07429467
```



Your turn!

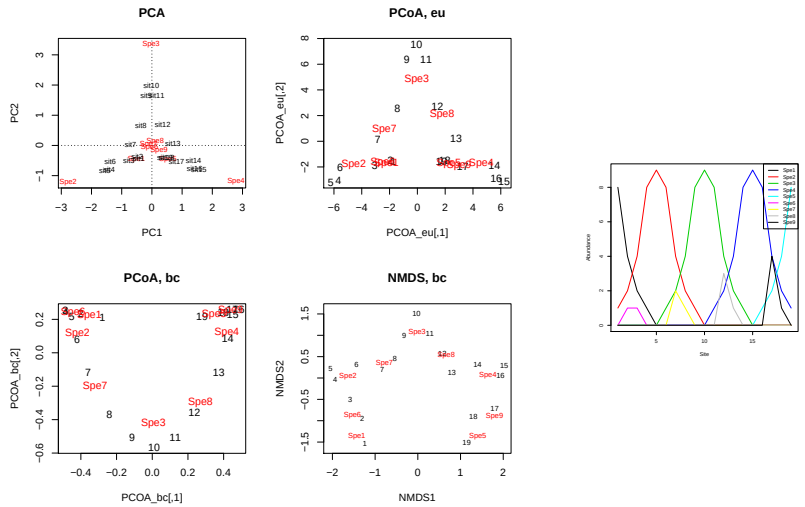
Using the artificial dummy dataset.

Run:

1. PCA
2. PCoA with euclidean distance
3. PCoA with Bray-Curtis dissimilarity
4. NMDS with Bray-Curtis dissimilarity

What are the differences between ordinations?
Which represent better the underlying gradient?

Exercise



Fit environmental variables to ordination (I)

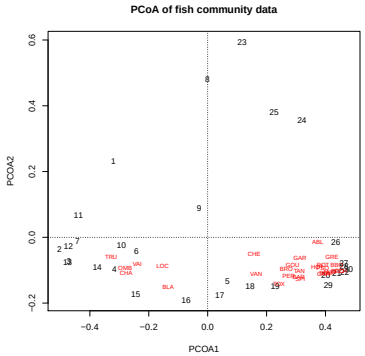
- This ordination is **only** driven by fish community data

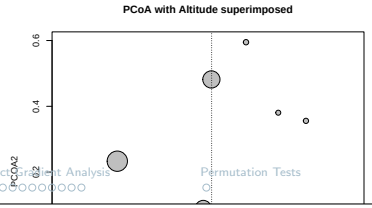
Question:

- How can we interpret the gradients in community composition?

A solution:

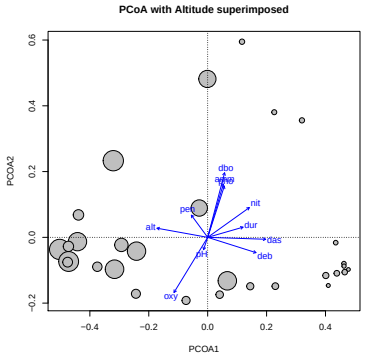
- Superimpose environmental variables



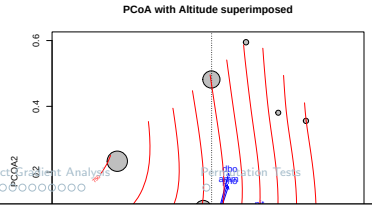


Fit environmental variables to ordination (II)


```
# PCoA of fish community data
plot(PCOA$points,
     xlab = 'PCOA1', ylab = 'PCOA2',
     cex = 5*Denv$alt / max(Denv$alt),
     main = 'PCoA with Altitude',
     bg = 'grey75', pch = 21)
abline(h = 0 , lty = 'dotted')
abline(v = 0 , lty = 'dotted')

# Fit Altitude to site-scores
ef <- envfit(PCOA, Denv)
plot(ef)
ef    # summary

# Fit GAM
ordisurf(PCOA, Denv$alt, add = TRUE)
```

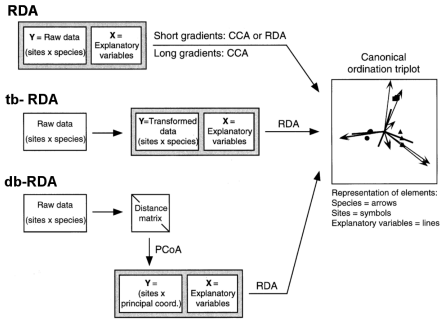


- *Post hoc* method
- non-linearity?
- be careful with `summary`
- Constrained ordination a better alternative



Direct Gradient Analysis

- Redundancy analysis (RDA)
- Transformation-based RDA (tb-RDA)
- Distance-based RDA (db-RDA)



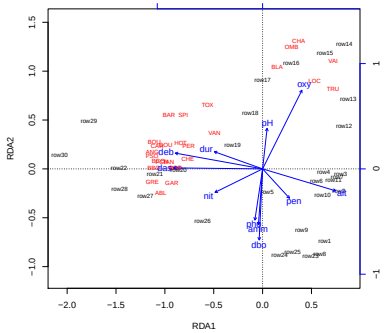
Adapted from: Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.

- Associates both environmental and community data at once
- Combination of regression and PCA:
  1. Regress explanatory variables on community data
  2. Run PCA on fitted values
- Can test hypothese about relationships

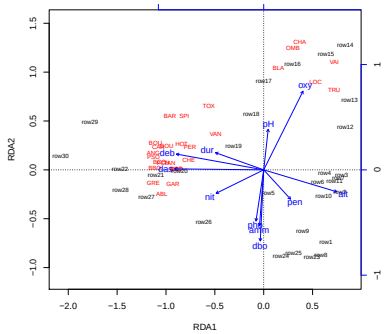
Redundancy analysis (RDA) — How?


```
RDA <- rda(Dabu ~ ., data = Denv,
           scale = TRUE)
plot(RDA, scaling = 3)
```

- Formula interface
  - Left side: Response **matrix**
  - Right side: Response variables from `Denv`



Redundancy analysis (RDA) — Interpretation? (I)



- Similar to PCA
- Projecting a site on response or explanatory variable **approx.** the value
- angles between response and expl. variables **approx.** their correlations

Redundancy analysis (RDA) — Interpretation? (II)


```
...
Partitioning of correlations:
              Inertia Proportion
Total          27.000     1.0000
Constrained    20.177     0.7473
Unconstrained   6.823     0.2527

Eigenvalues, and their contribution to the correlations

Importance of components:
                         RDA1   RDA2   RDA3    RDA4    RDA5    RDA6    RDA7
Eigenvalue            14.714 2.6433 1.1341 0.76821 0.33807 0.28135 0.09356
Proportion Explained   0.545 0.0979 0.0420 0.02845 0.01252 0.01042 0.00347
Cumulative Proportion  0.545 0.6429 0.6849 0.71333 0.72585 0.73627 0.73974
                         RDA8    RDA9   RDA10   RDA11     PC1    PC2
Eigenvalue            0.08411 0.07592 0.02314 0.02129 2.44703 1.4094
Proportion Explained  0.00312 0.00281 0.00086 0.00079 0.09063 0.0522
Cumulative Proportion 0.74285 0.74566 0.74652 0.74731 0.83794 0.8901
...
```


transformation-based RDA

- RDA (as PCA) preserves the euclidean distance.

  What about the species abundance paradox?

- Can transform data to use with euclidean distance
  - Remove differences in total abundance, while keeping the variations relative abundance
- Chord and Hellinger transformations useful.

Hellinger:

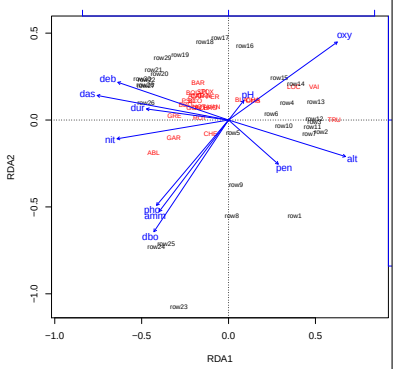
$$y'_{ij} = \sqrt{\frac{y_{ij}}{y_{i+}}}$$

```
mat

     Spe1 Spe2 Spe3
sit1    0    4    8
sit2    0    1    1
sit3    1    0    0

decostand(mat, 'hellinger')

     Spe1      Spe2      Spe3
sit1    0 0.5773503 0.8164966
sit2    0 0.7071068 0.7071068
sit3    1 0.0000000 0.0000000
attr(,"decostand")
[1] "hellinger"
```

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.

transformation-based RDA

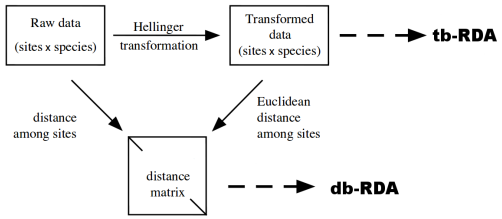

```
# Hellinger transformation
Dabu_h <- decostand(Dabu, 'hellinger')
# RDA on Hellinger transformed abundances
tbRDA <- rda(Dabu_h ~ ., data = Denv)

# Plot
plot(tbRDA, type = 't')
```



- `alt`, oxy and nutrients important
- Trout (TRU) and minow (VAI) found at high sites with high oxygen
- Bleak (ABL) is found a low oxygen and high nutrients
- Other species in similar environments

distance-based RDA

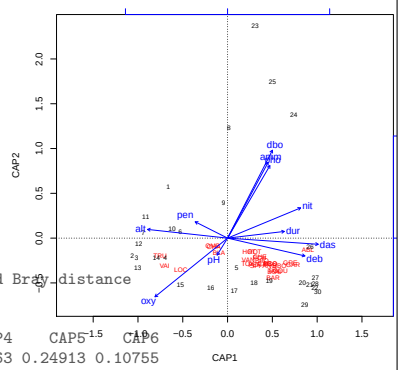
- db-RDA is a related method
- hellinger transformation can also be expressed as distance matrix
- *Constained PCoA*
- Can use every distance metric



modified from: Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.

distance-based RDA

```
# dbRDA
dbRDA <- capscale(Dabu ~ ., data = Denv,
                  distance = 'bray')
plot(dbRDA, type = 't')
```




```
summary(dbRDA)

...
              Inertia Proportion
Total           7.802     1.0000
Constrained     5.715     0.7325
Unconstrained   2.087     0.2675

Eigenvalues, and their contribution to the squared Bray distance

Importance of components:
                          CAP1   CAP2    CAP3    CAP4    CAP5    CAP6
Eigenvalue              3.2627 1.0283 0.53633 0.37863 0.24913 0.10755
Proportion Explained    0.4182 0.1318 0.06874 0.04853 0.03193 0.01379
Cumulative Proportion   0.4182 0.5500 0.61875 0.66729 0.69922 0.71300
...
```


Your turn!

Using the artificial dummy dataset and `Site` as only contraining variable

Run:

1. RDA
2. tbRDA (Hellinger)
3. dbRDA with Bray-Curtis
4. dbRDA with $x^{0.25}$ transformed abundances and Bray-Curtis

What ordination presents best the gradient?
What method explains most of variance?

Exercise

See Demo.

Permutation Tests

Your turn!

Using the melbourne data.

Usefull topics not covered here

- Distance-based hypothesis testing ((PER-)MANOVA, SIMPER, ANOSIM)
- Dispersion measures (β-Diversity, Functional diversity)
- Consensus RDA, RLQ (traits), ...
- Model-based ordination / hypothesis testing (See work of David Warton et al.)