

A (short) introduction to ordination with the vegan package

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SEFS9, July 5th 2015

Datasets
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Unconstrained Ordination
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Constrained Ordination
oooooooooooo

Model diagnostics / testing
oooooooooooo

Topics addressed

	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
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Unconstrained Ordination
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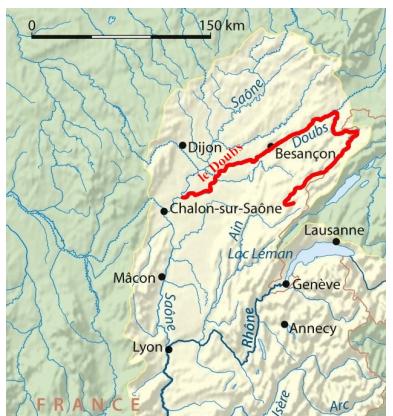
Constrained Ordination
oooooooooooo

Model diagnostics / testing
oooooooooooo

Datasets

Demonstration: Doubs river fish communities

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- ▶ Fish communities
- ▶ 30 sites along the Doubs River

Questions

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

Verneau, J. (1973) Cours d'eau de Franche-Comté (Massif du Jura). Recherches écologiques sur le réseau hydrographique du Doubs. Essai de biotypologie. These d'état, Besançon. 1–257.

Datasets



Unconstrained Ordination



Constrained Ordination



Model diagnostics / testing



Demonstration: Doubs river fish communities — Species

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```
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

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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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Demonstration: Doubs river fish communities — Environment

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```
# 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

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Unconstrained Ordination
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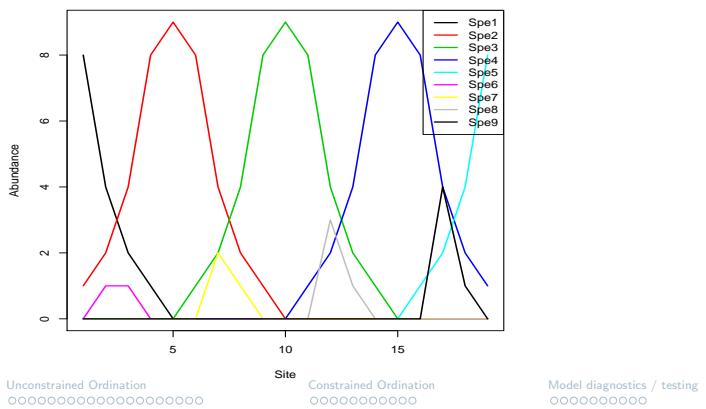
Constrained Ordination
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Model diagnostics / testing
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Exercise: Dummy abundances

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```
# 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)
```



Unconstrained Ordination

Principal Components Analysis (PCA)

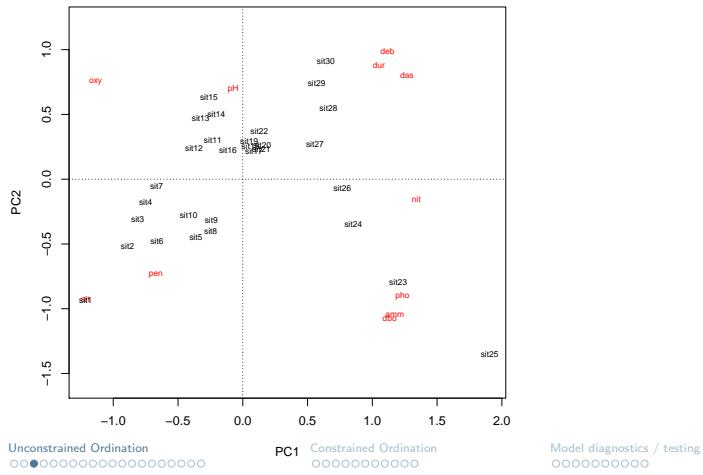
Principal coordinates analysis (PCoA)

Nonmetric Multidimensional Scaling (NMDS)

Principal Components Analysis (PCA) — How?

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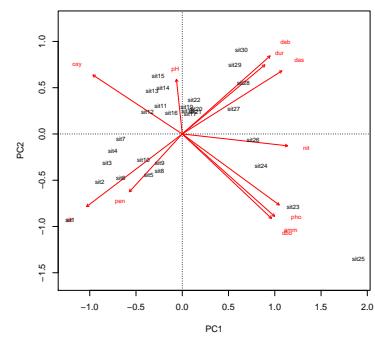
```
require(vegan)  
PCA <- rda(Denv, scale = TRUE)  
plot(PCA, scaling = 3)
```



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

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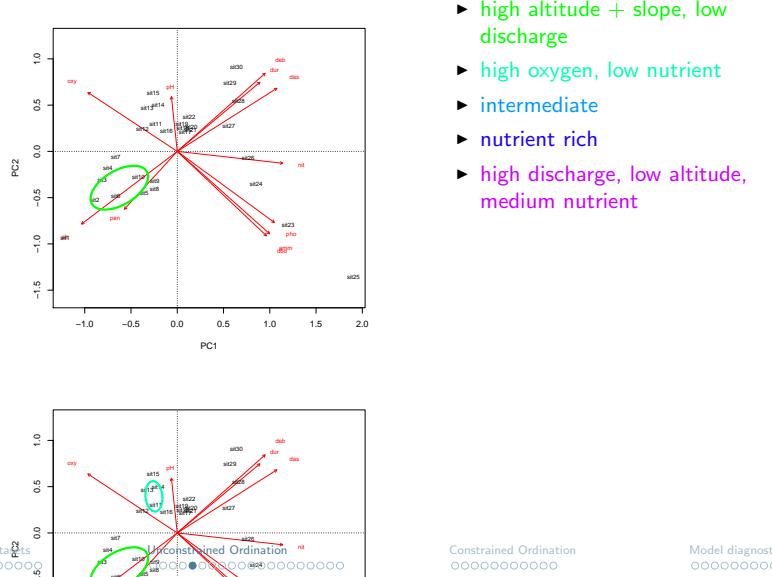
```
biplot(PCA, cex = 5, scaling = 3)
```



- ▶ 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)

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

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```
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?

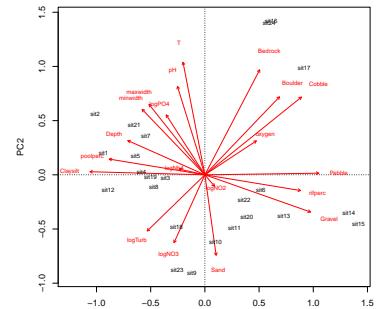
Exercise

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```
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 interpreted as *hydrological gradient*
- ▶ 2nd axis can be interpreted as *chemistry gradient*

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 the number of variables to *Principal Components*
- ▶ regress these

Datasets
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ooooooooooooModel diagnostics / testing
oooooooooooo

```
# 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

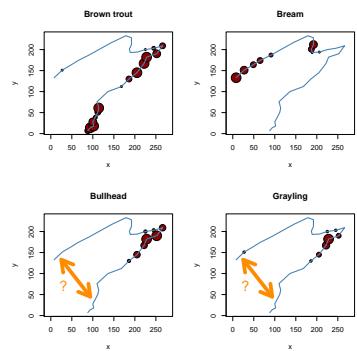
Coefficients:

	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

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

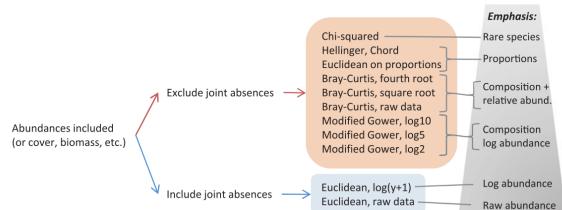
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Dissimilarity measures — Species abundance paradox

	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.

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



Principal coordinates analysis (PCoA) 24 / 53

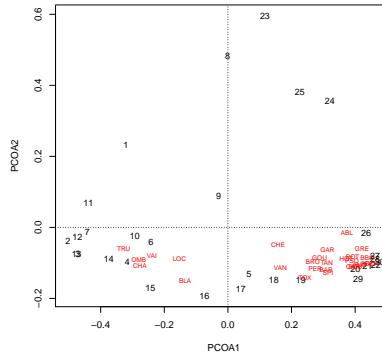
```
# Distance matrix
Dabu_dist <- vegdist(Dabu, method = 'bray')

# 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)

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

[1] 49.24914 15.95758



Nonmetric Multidimensional Scaling (NMDS)

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- ▶ 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

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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Nonmetric Multidimensional Scaling (NMDS)

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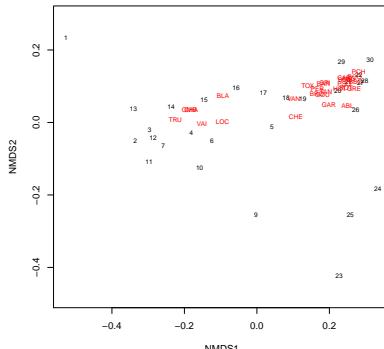
```
# Distance matrix
Dabu_0 <- Dabu[!rowSums(Dabu) == 0, ]
Dabu_dist <- vegdist(Dabu_0, method = 'bray')

# 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)

# Stress value
NMDS$stress
[1] 0.07376347
```



Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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Your turn!

Using the artificial dummy dataset.

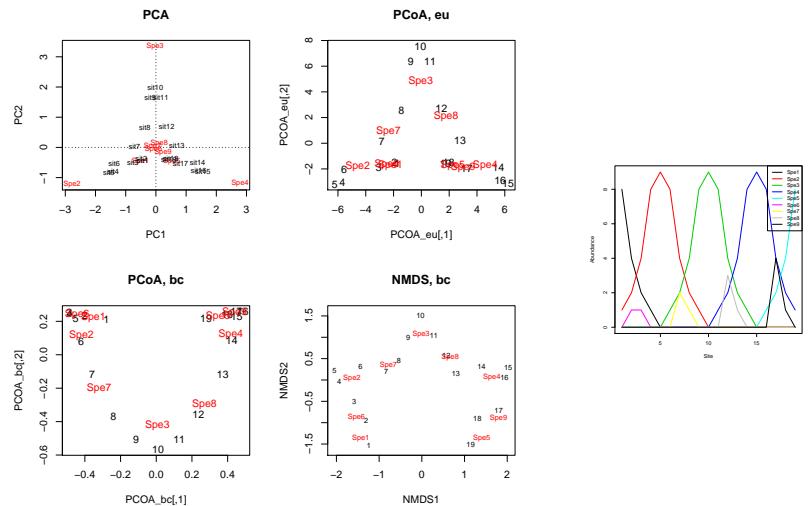
Run:

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

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Datasets



Unconstrained Ordination



Constrained Ordination



Model diagnostics / testing



Fit environmental variables to ordination (I)

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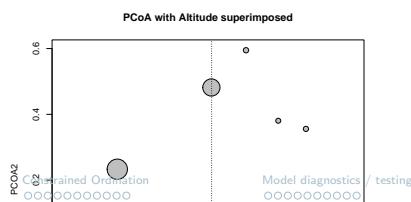
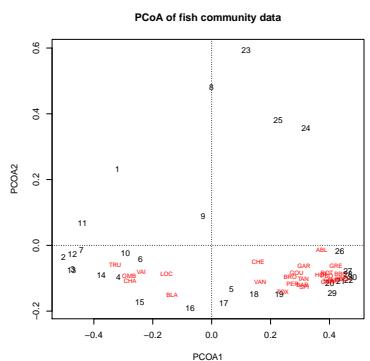
- This ordination is **only** driven by fish community data

Question:

- How can we interpret the gradients in community composition?

A solution:

- Superimpose environmental variables



Fit environmental variables to ordination (II)

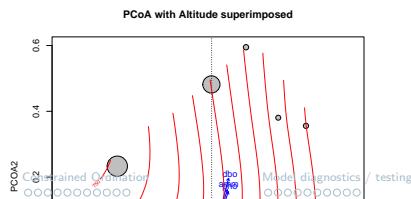
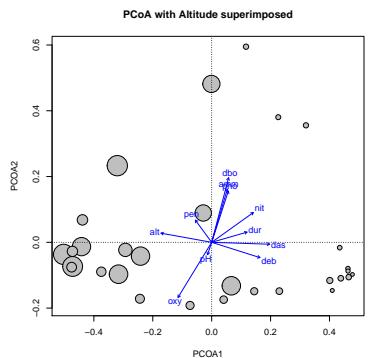
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```
# PCoA of fish community data
plot(PCOA$points,
  xlab = 'PCoA1', ylab = 'PCoA2',
  cex = 5*Denv$alt / max(Denv$alt),
  main = 'PCoA with Altitude',
  bg = 'grey75', pch = 21)
abline(h = 0 , lty = 'dotted')
abline(v = 0 , lty = 'dotted')

# 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

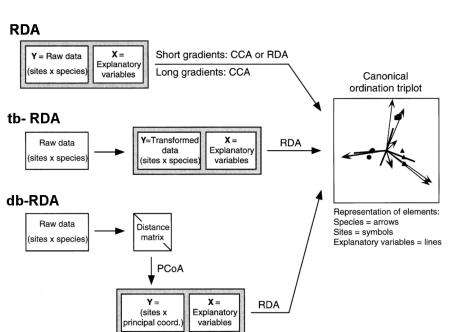


Constrained Ordination

Constrained Ordination

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- ▶ 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.

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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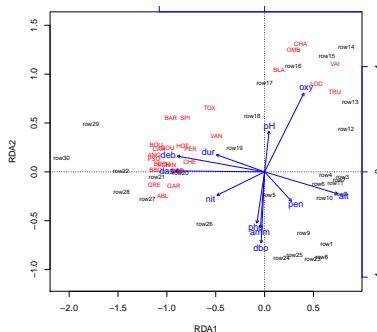
- ▶ Associates both environmental and community data at once
- ▶ Combination of regression and PCA:
 1. Regress explanatory variables on community data
 2. Run PCA on fitted values
- ▶ Can test hypothesis about relationships



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



transformation-based RDA

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- 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:

$$y'_{ij} = \sqrt{\frac{y_{ij}}{\sum_{j=1}^p y_{ij}}}$$

mat

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

```
decostand(mat, 'hellinger')
Spe1      Spe2      Spe3
sit1 0.5773503 0.8164966
sit2 0.7071068 0.7071068
sit3 1.0000000 0.0000000
attr("decostand")
[1] "hellinger"
```

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

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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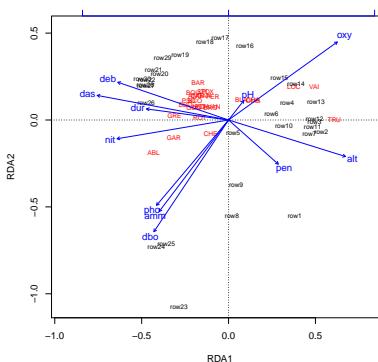
transformation-based RDA

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```
# 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 at low oxygen and high nutrients
- Other species in similar environments



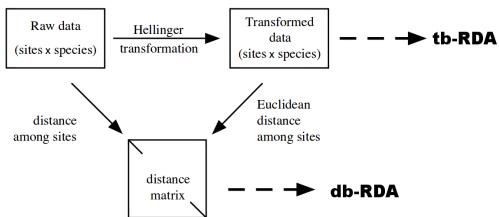
Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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- db-RDA is a related method
- hellinger transformation can also be expressed as distance matrix
- *Constrained 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.

Datasets



Unconstrained Ordination



Constrained Ordination



Model diagnostics / testing



distance-based RDA

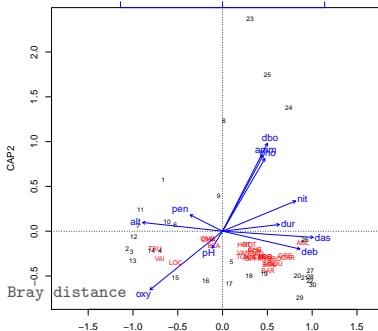
```
# dbRDA
dbRDA <- capscale(Dabu ~ ., data = Denv,
                     distance = 'bray')
plot(dbRDA, type = 't')
```

```
summary(dbRDA)
```

```
...
Partitioning of squared Bray distance:
   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
```

	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



Datasets



Unconstrained Ordination



Constrained Ordination



Model diagnostics / testing



Your turn!

Using the artificial dummy dataset and Site as only containing 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

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See Demo.

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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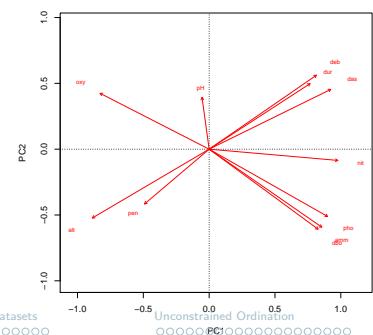
Model diagnostics and testing

Collinearity

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- RDA et al. are *regression methods* - everything you know applies also here
- Collinearity of predictors may lead to wrong conclusions.
- Many methods available (see ref.). Additional: *Use your ecological knowledge!*

```
# visual inspection via PCA
biplot(rda(Denv, scale = TRUE),
       display = 'species', scaling = 2)
```



```
# RDA on Hellinger transformed abundances
tbRDA <- rda(decostand(Dabu, "hellinger") ~ .,
# Variance inflation factors
vif.cca(tbRDA)
```

	das	alt	pen	deb
104.008842	39.589948	2.023986	36.048637	1
pho	nit	amm	oxy	
25.329286	17.284287	31.099024	12.963327	17

- many different cutoffs (see 1st part)
- very high values ($\gg 20$)
- strong collinearity

Model diagnostics / testing

- ▶ cumulative variance explained by constraints
- ▶ available for *sites* or *species*
- ▶ `summarize = TRUE` gives the accumulated total variance (= last column)

```
# GOF for each species on  
goodness(tbRDA)[ , 1:3]
```

	RDA1	RDA2	RDA3
CHA	0.16597466	0.28222282	0.72531373
TRU	0.68728597	0.68728738	0.75205565
VAI	0.66278142	0.76121936	0.77215444
LOC	0.50167024	0.63319561	0.63433575
OMB	0.16050386	0.25551654	0.64853919
BLA	0.04766735	0.17402885	0.47152162
...			

```
# total var explained by constraints  
goodness(tbRDA, summarize = TRUE)[1:6]
```

	CHA	TRU	VAI	LOC	OMB	BLA
	0.8010478	0.8798422	0.8676846	0.8223702	0.7889173	0.5669931

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

Inertia decomposition

- ▶ can decompose variance into constrained (CCA) and unconstrained (CA) parts

```
# variance explained by constrained (CCA) and unconstrained (CA) axes  
inertcomp(tbRDA, proportional = TRUE)
```

	CCA	CA
CHA	0.8010478	0.1989522
TRU	0.8798422	0.1201578
VAI	0.8676846	0.1323154
LOC	0.8223702	0.1776298
OMB	0.7889173	0.2110827
BLA	0.5669931	0.4330069
...		

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

Permutation Tests (I)

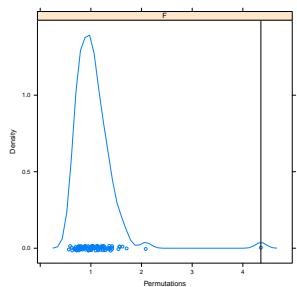
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- ▶ Cannot use parametric test theory

- ▶ Use of permutation tests :

1. Shuffle the data (H_0 : No effect)
2. Fit model to shuffled data
3. Compute (pseudo-) F statistic for each model (Null distribution if H_0 is true)
- 4.

$$p = \frac{(\text{No. of } F_{\text{perm}} \geq F) + 1}{\text{Total No. of } F_{\text{perm}} + 1}$$



Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
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Permutation Tests (II)

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A number of different test can be applied to RDA:

- ▶ Test overall significance of model
- ▶ Test RDA axes
- ▶ Test terms:
 - ▶ sequential
 - ▶ marginal

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
oooo●oooo

Test overall model

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I omit some variables due to collinearity:

```
# RDA on Hellinger transformed abundances
tbRDA <- rda(decostand(Dabu, "hellinger") ~ alt + oxy + pH + nit + pho,
               data = Denv)
vif.cca(tbRDA)

      alt      oxy      pH      nit      pho
2.929759 2.198671 1.047617 6.577783 4.164368

# Tests if the overall model is significant
anova(tbRDA)

Permutation test for rda under reduced model
Permutation: free
Number of permutations: 999

Model: rda(formula = decostand(Dabu, "hellinger") ~ alt + oxy + pH + nit + pho, data = Den
          Df Variance   F Pr(>F)
Model    5  0.28776 6.4372  0.001 ***
Residual 24  0.21458
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Datasets
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Unconstrained Ordination
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Constrained Ordination
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Model diagnostics / testing
oooooooo●oooo

Test of RDA axes

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```
# Tests RDA axes
anova(tbRDA, by = 'axis')

Permutation test for rda under reduced model
Marginal tests for axes
Permutation: free
Number of permutations: 999

Model: rda(formula = decostand(Dabu, "hellinger") ~ alt + oxy + pH + nit + pho, data = Den
          Df Variance   F Pr(>F)
RDA1    1  0.212483 23.7658  0.001 ***
RDA2    1  0.046671  5.2201  0.001 ***
RDA3    1  0.020291  2.2695  0.029 *
RDA4    1  0.006385  0.7141  0.675
RDA5    1  0.001935  0.2165  0.998
Residual 24  0.214578
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Legendre, P., Oksanen, J. and ter Braak, C.J.F. (2011). Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution* 2, 269–277.

Datasets
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Unconstrained Ordination
oooooooooooooooooooo

Constrained Ordination
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Model diagnostics / testing
oooooooo●ooo

Sequential test of variables

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- ▶ Variables are tested in the order they were specified (first to last)
- ▶ Test of additional variance explained by adding the variable to the model
- ▶ Order matters!

```
# Tests RDA axes
anova(tbRDA, by = 'terms')

Permutation test for rda under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 999

Model: rda(formula = decostand(Dabu, "hellinger") ~ alt + oxy + pH + nit + pho, data = Den)
        Df Variance      F Pr(>F)
alt      1 0.153953 17.2193 0.001 ***
oxy      1 0.085314 9.5422 0.001 ***
pH       1 0.004285 0.4793 0.814
nit      1 0.007642 0.8547 0.489
pho      1 0.036571 4.0904 0.006 **
Residual 24 0.214578
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Datasets
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Unconstrained Ordination
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Constrained Ordination
oooooooooooo

Model diagnostics / testing
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Marginal test of variables

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- ▶ Test explained variance of variable when all other variable are included in the model
- ▶ Order has no influence

```
# Tests RDA axes
anova(tbRDA, by = 'margin')

Permutation test for rda under reduced model
Marginal effects of terms
Permutation: free
Number of permutations: 999

Model: rda(formula = decostand(Dabu, "hellinger") ~ alt + oxy + pH + nit + pho, data = Den)
        Df Variance      F Pr(>F)
alt      1 0.031774 3.5539 0.020 *
oxy      1 0.067640 7.5653 0.001 ***
pH       1 0.003905 0.4367 0.862
nit      1 0.011760 1.3153 0.238
pho      1 0.036571 4.0904 0.010 **
Residual 24 0.214578
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Datasets
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Unconstrained Ordination
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Constrained Ordination
oooooooooooo

Model diagnostics / testing
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Multivariate topics not covered here:

- ▶ Model selection (be careful with automatic methods!)
- ▶ Distance-based hypothesis testing ((PER-)MANOVA, SIMPER, ANOSIM)
- ▶ Dispersion measures (β -Diversity, Functional diversity)
- ▶ Consensus RDA, RLQ (traits), ...
- ▶ Model-based multivariate framework (See work of David Warton et al.)
- ▶ manymore