

A (short) introduction to ordination with the vegan package

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Datasets
oooooooo

Unconstrained Ordination
oooooooooooooooooooo

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
oooooooo

Unconstrained Ordination
oooooooooooooooooooo

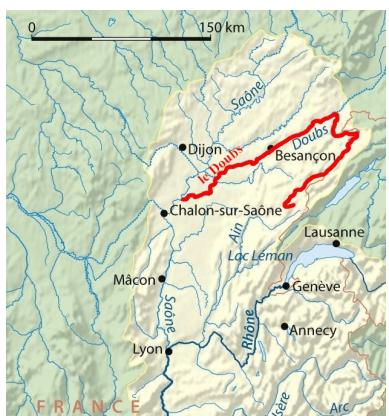
Constrained Ordination
oooooooooooo

Model diagnostics / testing
oooooooooooo

Datasets

Demonstration: Doubs river fish communities

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- ▶ Fish
 - ▶ 30 sites along the Doubs River

Questions

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

Verneau, 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

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```
Dabu <- read.table('doubtsAbu.csv', sep = ',', header = TRUE)
Denv <- read.table('doubtsEnv.csv', sep = ',', header = TRUE)
Dspa <- read.table('doubtsSpa.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
2	0	5	4	3	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	1	0	0	0	0
4	0	4	5	5	0	0	0	0	0	1	0	0	1	2	2	0	0
5	0	2	3	2	0	0	0	0	5	2	0	0	2	4	4	0	2
6	0	3	4	5	0	0	0	0	1	2	0	0	1	1	1	0	0

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

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)`

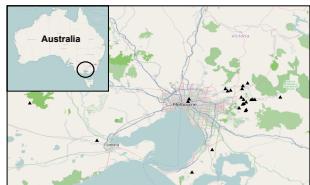
	das	alt	pen	deb	pH	dur	pho	nit	amm	oxy	dbo
1	0.3	934	48.0	0.84	7.9	45	0.01	0.20	0.00	12.2	2.7
2	2.2	932	3.0	1.00	8.0	40	0.02	0.20	0.10	10.3	1.9
3	10.2	914	3.7	1.80	8.3	52	0.05	0.22	0.05	10.5	3.5
4	18.5	854	3.2	2.53	8.0	72	0.10	0.21	0.00	11.0	1.3
5	21.5	849	2.3	2.64	8.1	84	0.38	0.52	0.20	8.0	6.2
6	32.4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

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing



- ▶ Macroinvertebrates
 - ▶ 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.

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing
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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

Datasets

Unconstrained Ordination

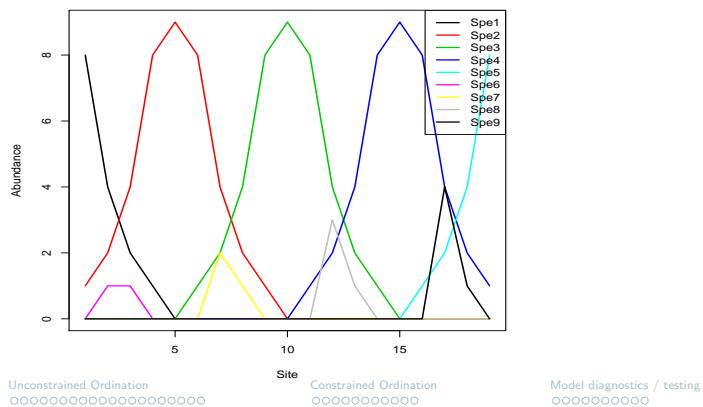
Constrained Ordination

Model diagnostics / testing

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)
```

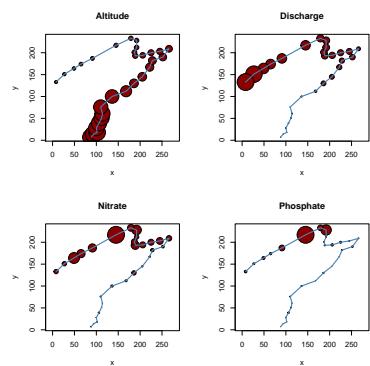


Unconstrained Ordination

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

Principal Components Analysis (PCA) — Why?

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- ## ► 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?

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

Principal Components Analysis (PCA) — What?

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- ▶ "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

Datasets

Unconstrained Ordination

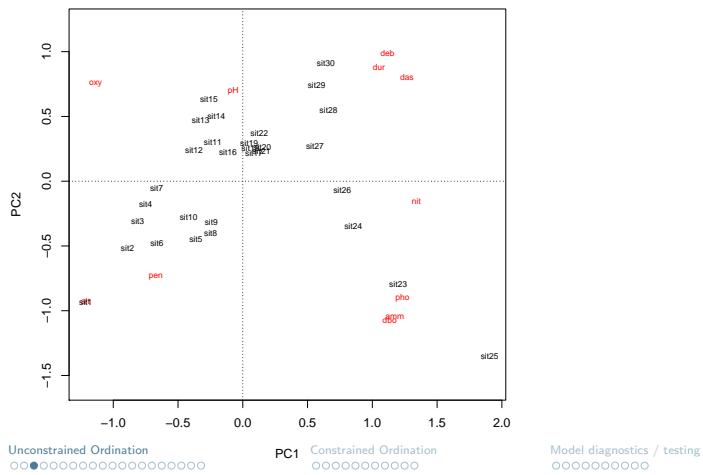
Constrained Ordination

Model diagnostics / testing

Principal Components Analysis (PCA) — How?

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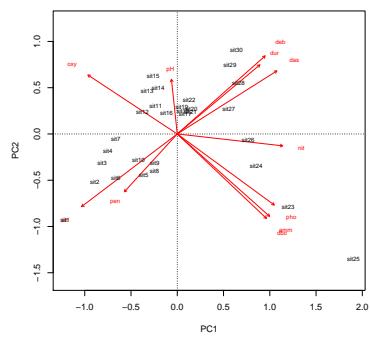
```
require(vegan)
PCA <- rda(Denv, scale = TRUE)
plot(PCA, scaling = 3)
```



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

Datasets



Unconstrained Ordination

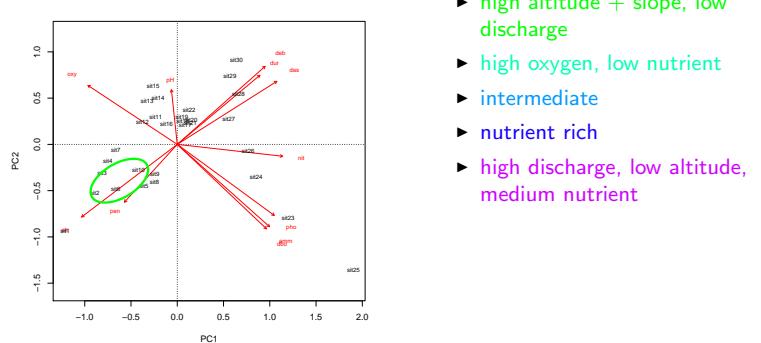
Constrained Ordination

Model diagnostics / testing

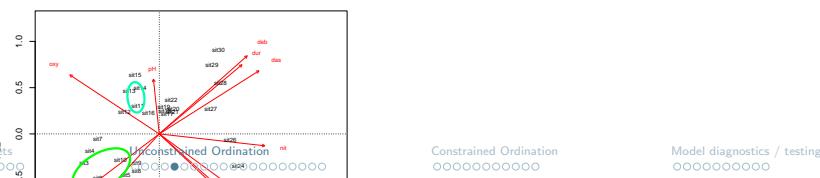


Principal Components Analysis (PCA) — Interpretation? (II)

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



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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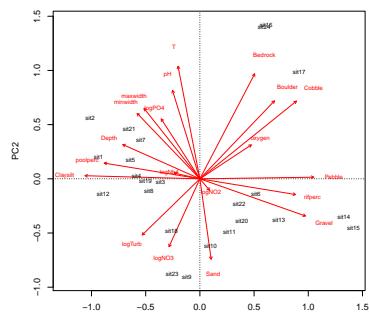
```

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

Datasets
ooooooooUnconstrained Ordination
oooooooo●ooooooooooooConstrained Ordination
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

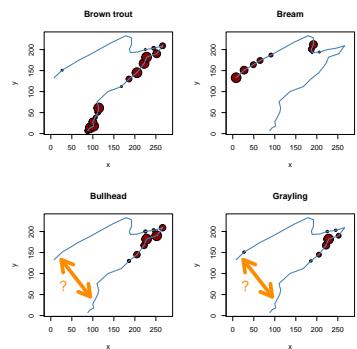
Coefficients:

	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

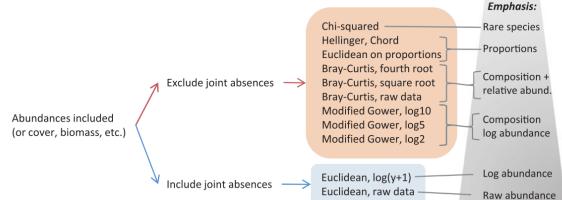
Datasets
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Dissimilarity measures — Species abundance paradox

	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.

Datasets
○○○○○Unconstrained Ordination
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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

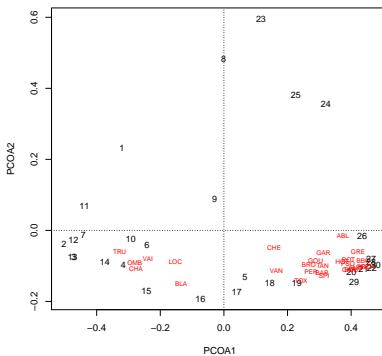
```
# Distance matrix
Dabu_dist <- vegdist(Dabu, method = 'bray')

# 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

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

Nonmetric Multidimensional Scaling (NMDS)

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

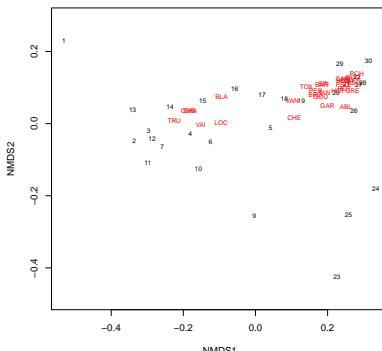
# NMDS
NMDS <- metaMDS(Dabu_dist, k = 2, trace = 0)

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

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

# Stress value
NMDS$stress

[1] 0.07429467
```



Datasets



Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing
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Your turn!

Using the artificial dummy dataset.

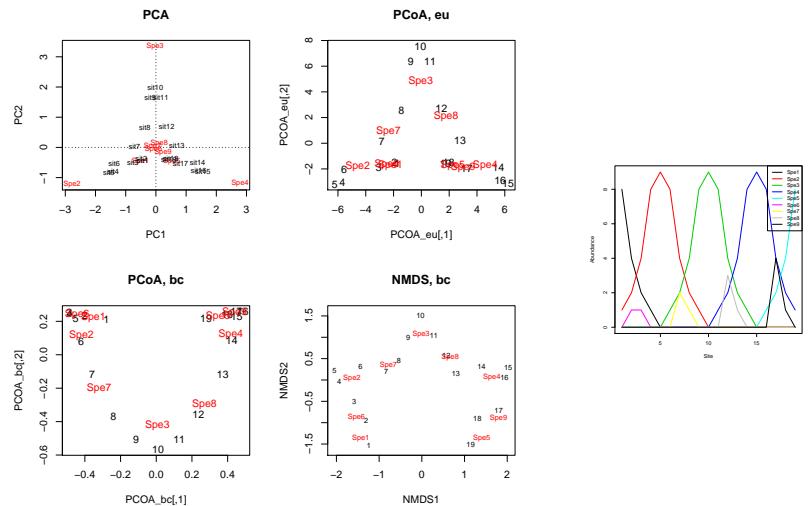
Run:

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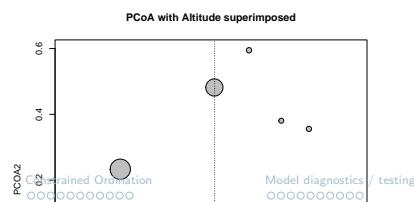
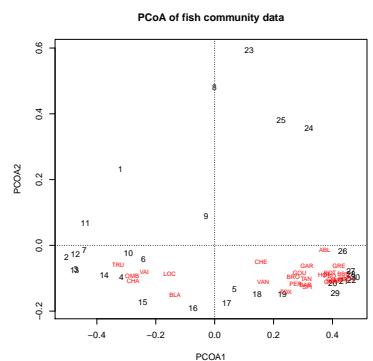
- This ordination is **only** driven by fish community data

Question:

- How can we interpret the gradients in community composition?

A solution:

- Superimpose environmental variables



Fit environmental variables to ordination (II)

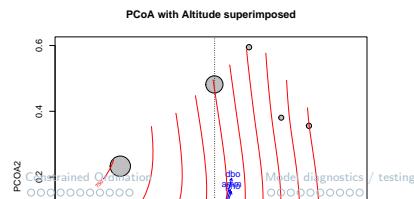
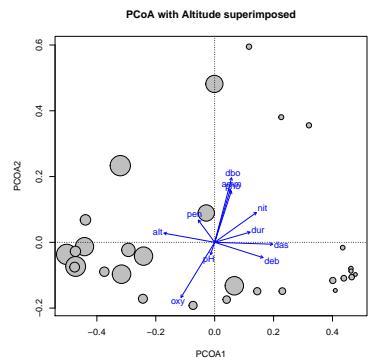
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```
# PCoA of fish community data
plot(PCOA$points,
  xlab = 'PCoA1', ylab = 'PCoA2',
  cex = 5*Denv$alt / max(Denv$alt),
  main = 'PCoA with Altitude',
  bg = 'grey75', pch = 21)
abline(h = 0 , lty = 'dotted')
abline(v = 0 , lty = 'dotted')

# 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

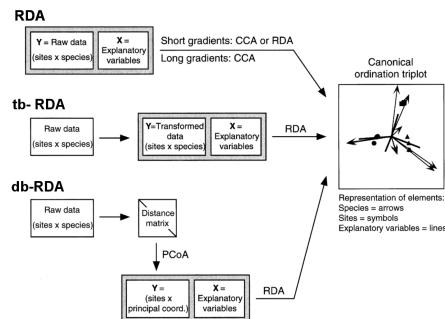


Constrained Ordination

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.

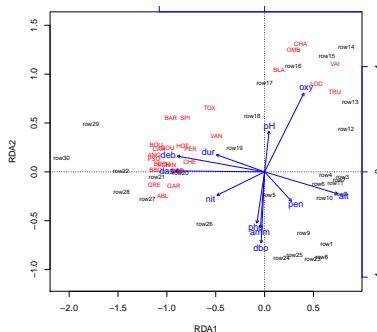
- ▶ Associates both environmental and community data at once
- ▶ Combination of regression and PCA:
 1. Regress explanatory variables on community data
 2. Run PCA on fitted values
- ▶ Can test hypothesis about relationships



Redundancy analysis (RDA) — How?

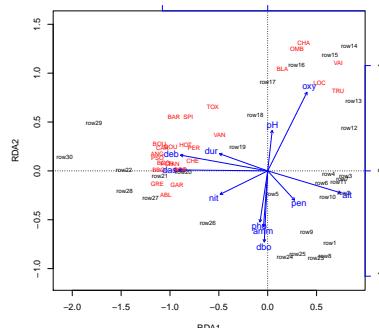
```
RDA <- rda(Dabu ~ ., data = Denv,  
            scale = TRUE)  
plot(RDA, scaling = 3)
```

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



Redundancy analysis (RDA) — Interpretation? (I)

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

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

Redundancy analysis (RDA) — Interpretation? (II)

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```

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

```

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

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 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.

Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

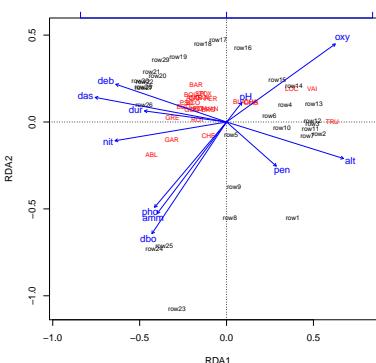
transformation-based RDA

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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 minnow (VAI) found at high sites with high oxygen
 - ▶ Bleak (ABL) is found a low oxygen and high nutrients
 - ▶ Other species in similar environments



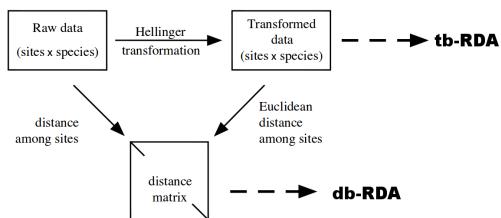
Datasets

Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

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

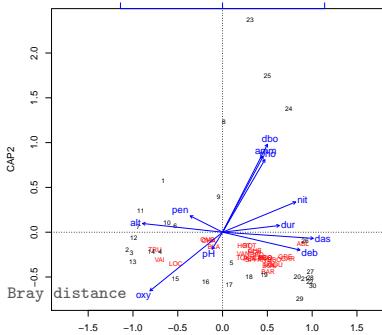


modified from: Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280.



distance-based RDA

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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

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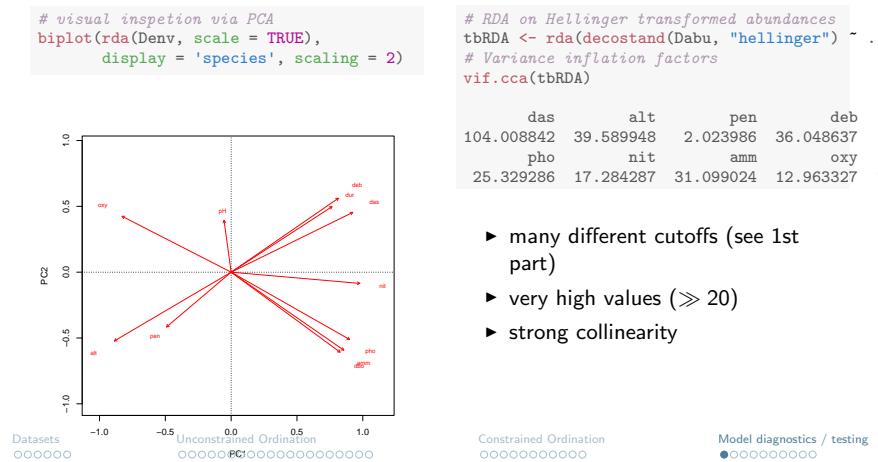
See Demo.

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!*



- ▶ 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

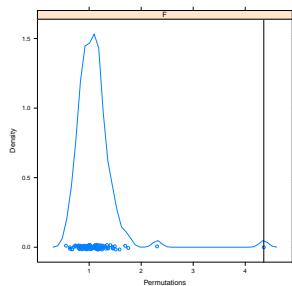
Model diagnostics / testing

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.

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



Datasets



Unconstrained Ordination

Constrained Ordination

Model diagnostics / testing

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

Constrained Ordination

Model diagnostics / testing

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 + das + nit + pho, data
          Df Variance   F Pr(>F)
Model    6  0.31490 6.4397  0.001 ***
Residual 23  0.18745
---
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 + das + nit + pho, data
          Df Variance   F Pr(>F)
RDA1   1  0.217577 26.6970  0.001 ***
RDA2   1  0.047459 5.8233  0.001 ***
RDA3   1  0.030110 3.6946  0.001 ***
RDA4   1  0.012113 1.4863  0.143
RDA5   1  0.005714 0.7012  0.716
RDA6   1  0.001923 0.2359  0.995
Residual 23  0.187447
---
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
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Constrained Ordination
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Model diagnostics / testing
oooooooo●oooo

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 + das + nit + pho, data
        Df Variance      F Pr(>F)
alt      1 0.153953 18.8903 0.001 ***
oxy      1 0.085314 10.4682 0.001 ***
pH       1 0.004285 0.5258 0.755
das      1 0.031761 3.8971 0.009 **
nit      1 0.007244 0.8889 0.433
pho      1 0.032338 3.9680 0.008 **
Residual 23 0.187447
---
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
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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 + das + nit + pho, data
        Df Variance      F Pr(>F)
alt      1 0.023369 2.8674 0.031 *
oxy      1 0.037891 4.6493 0.003 **
pH       1 0.004184 0.5134 0.773
das      1 0.027131 3.3290 0.020 *
nit      1 0.014190 1.7411 0.118
pho      1 0.032338 3.9680 0.010 **
Residual 23 0.187447
---
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