

# A (brief) introduction to ordination and the vegan package

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Datasets  
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Unconstrained Ordination  
ooooooooooooooooooooooo

Constrained Ordination  
oo

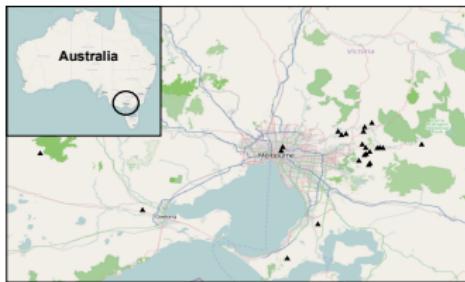
Permutation Tests  
o

End

	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

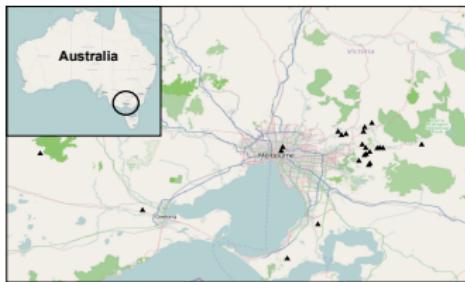
No maths today ;)

# Datasets



- ▶ Macroinvertebrates
  - ▶ 24 sites
  - ▶ covering a salinity and toxicity gradient

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.



- ▶ 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.

## 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

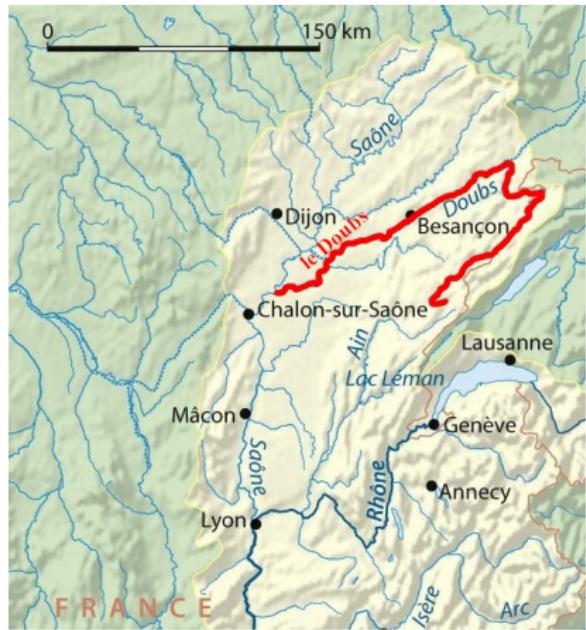
24 sites, 22 environmental variables, 75 taxa

```
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

# Demonstration: Doubs river fish communities

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- ▶ Fish
- ▶ 30 sites along the Doubs River

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Verneaux, 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



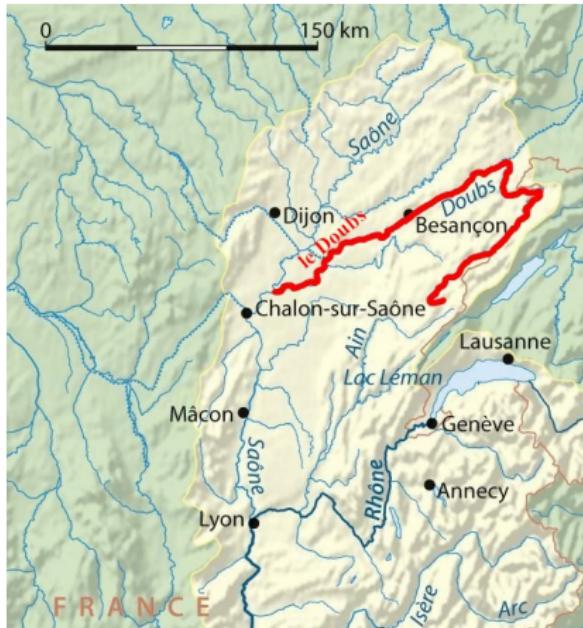
Constrained Ordination



Permutation Tests



End



- ▶ 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.

```
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	
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	0	1	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	0	
6	0	3	4	5	0	0	0	0	1	2	0	0	1	1	1	0	0	

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

[1] 30 11

30 sites, 11 variables

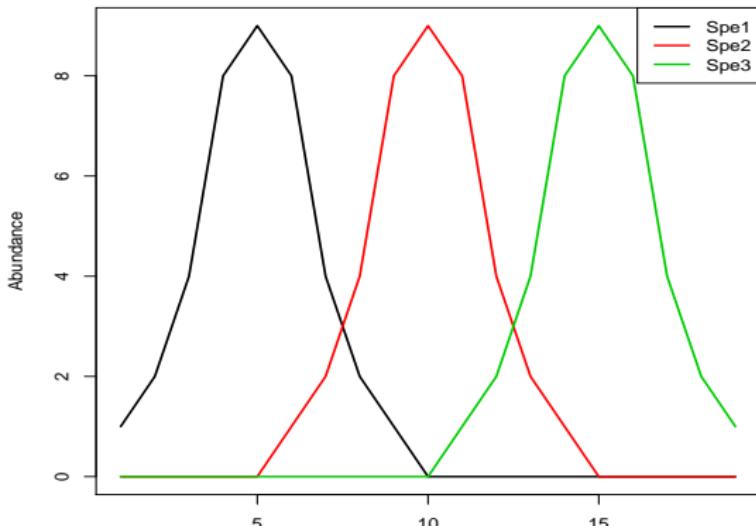
**head(Denv)**

	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: 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)
legend('topright', legend = colnames(dummy)[-1],
       col = 1:3, lty = 'solid', lwd = 2)
```



Datasets

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Unconstrained Ordination

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Site

Constrained Ordination

oo

Permutation Tests

o

End

# Unconstrained Ordination

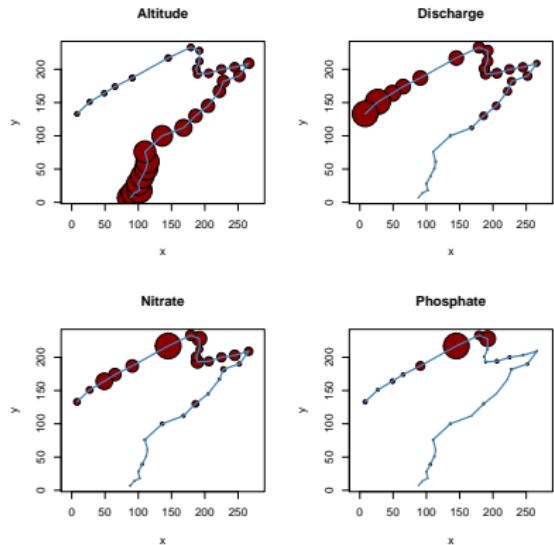
Principal Components Analysis (PCA)

Principal coordinates analysis (PCoA)

Nonmetric Multidimensional Scaling (NMDS)

# Principal Components Analysis (PCA) — Why?

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- ▶ 11 variables

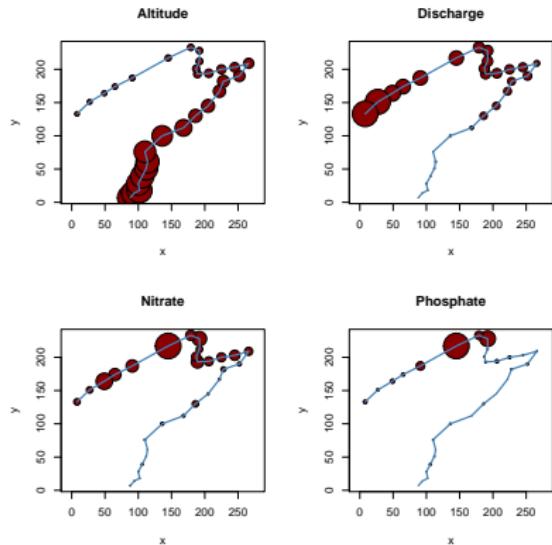
## Questions:

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

## Solutions?

# Principal Components Analysis (PCA) — Why?

12 / 37



- ▶ 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?

- ▶ *"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.

- ▶ *"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

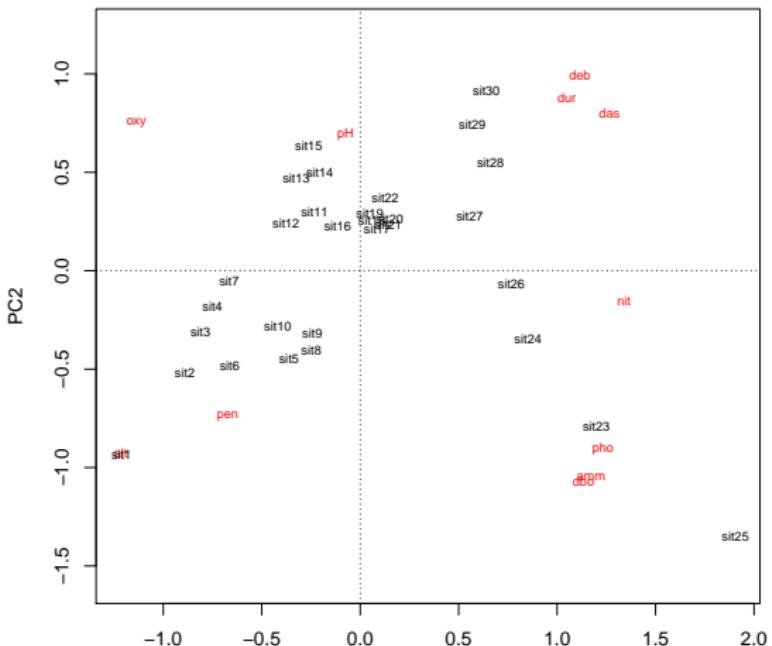
## Short Demo.

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)
```



## Datasets

## Unconstrained Ordination

UNCONSTRAINED STIMULATION

Constrained Ordination

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## Permutation Tests

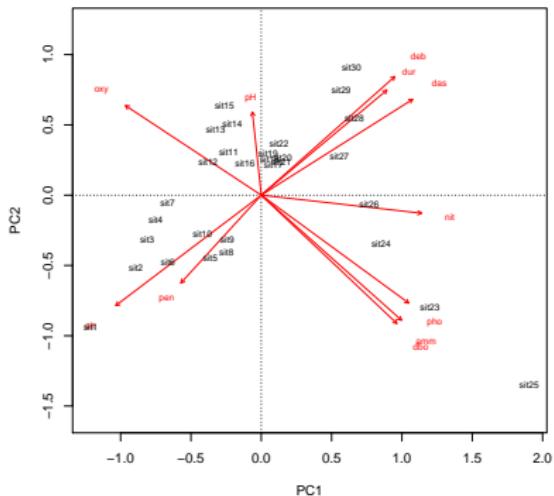
•

End

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

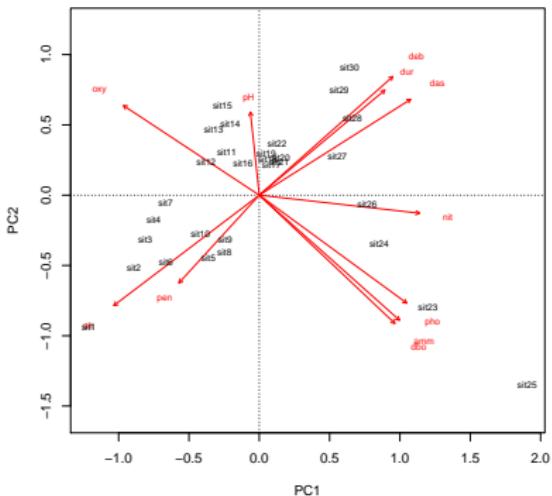
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```
biplot(PCA, cex = 5, scaling = 3)
```



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

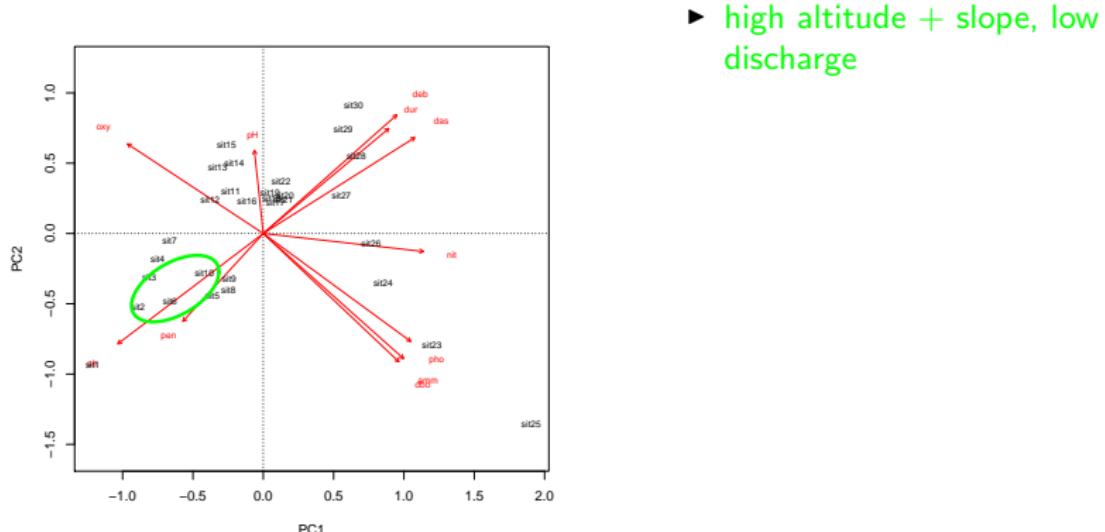
```
biplot(PCA, cex = 5, scaling = 3)
```



- ▶ 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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## Datasets

## Unconstrained Ordination

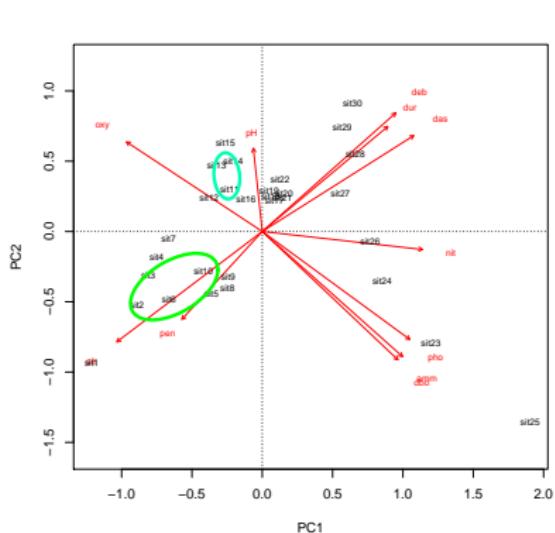
## Constrained Ordination

## Permutation Tests

End

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

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- ▶ high altitude + slope, low discharge
  - ▶ high oxygen, low nutrient

## Datasets

## Unconstrained Ordination

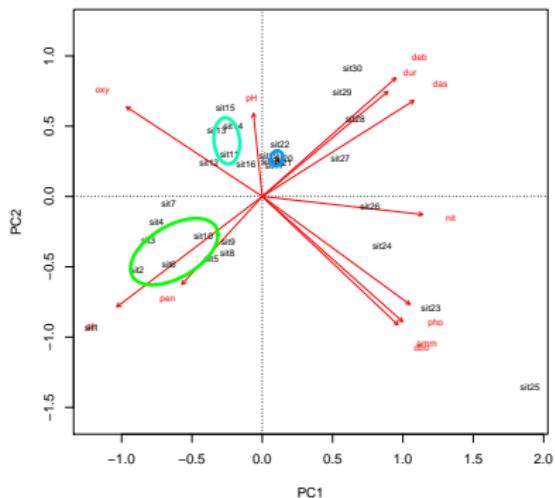
## Constrained Ordination

## Permutation Tests

End

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

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

## Datasets

## Unconstrained Ordination

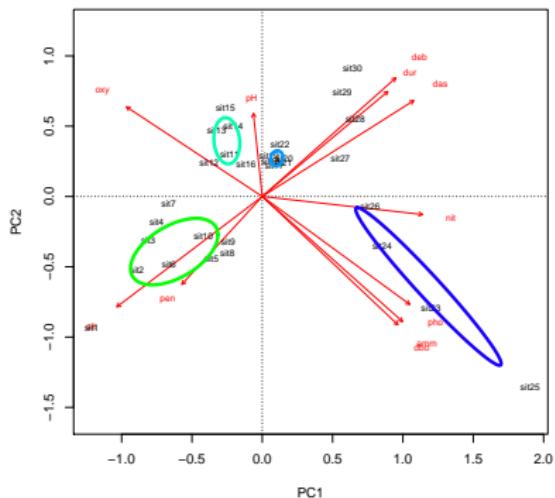
## Constrained Ordination

## Permutation Tests

End

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

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

## Datasets

## Unconstrained Ordination

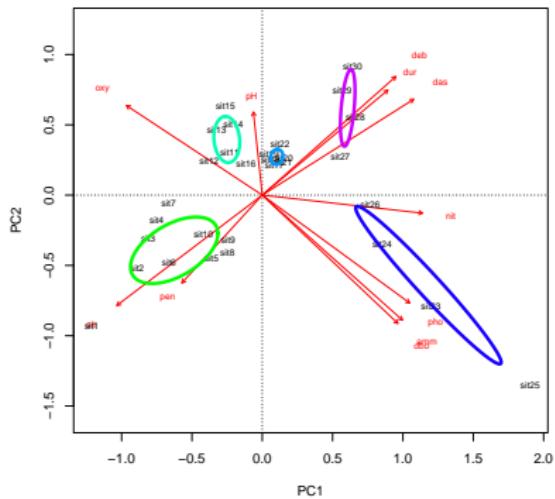
## Constrained Ordination

## Permutation Tests

End

## 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

## Datasets

## Unconstrained Ordination

## Constrained Ordination oo

## Permutation Tests

End

# 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?

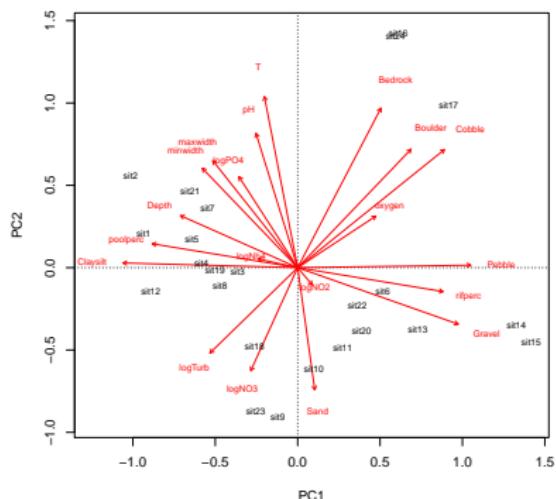
How could the two PCA axes be interpreted?

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

## Question:

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

## Datasets

## Unconstrained Ordination

## Constrained Ordination

## Permutation Tests

End

## Question:

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

## Problem:

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

### A Solution:

## Question:

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

## Problem:

- ▶ Only 24 sites
  - ▶ but 22 (partly 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)

```
# 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:

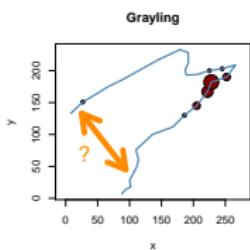
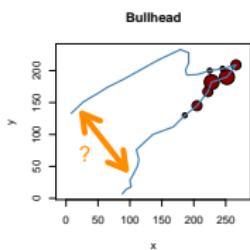
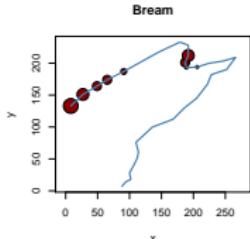
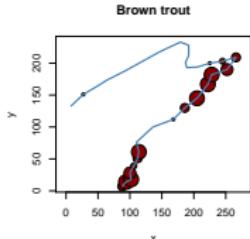
	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	1.83079	0.43429	4.216	0.000468 ***
PC1	0.01971	0.16691	0.118	0.907262
PC2	0.02192	0.19570	0.112	0.911996
logCond	-0.20942	0.13050	-1.605	0.125049
logmaxTU	-0.12572	0.07316	-1.718	0.101994
---				

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



- ▶ 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

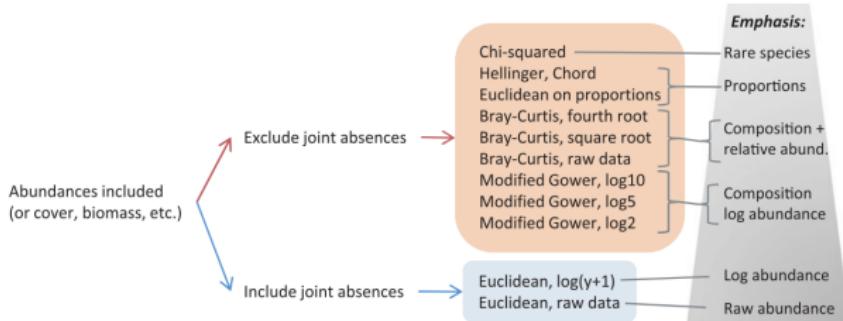
## Unconstrained Ordination

## Constrained Ordination

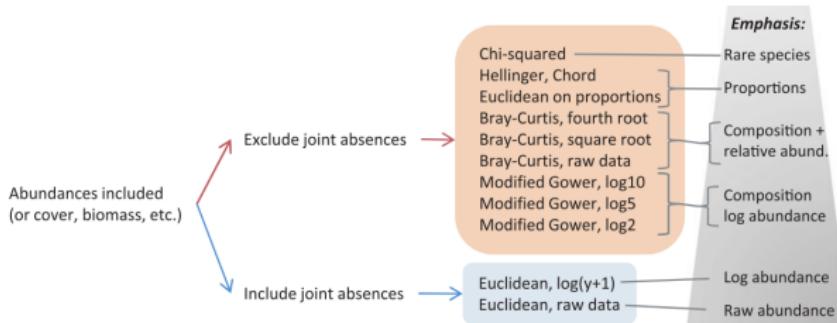
## Permutation Tests

End

# Dissimilarity measures



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.



	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
```

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.

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

## Principal coordinates analysis (PCoA)

```

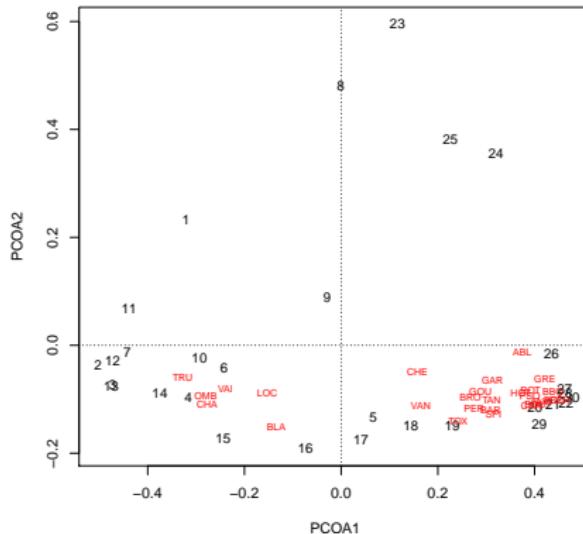
# Distance matrix
Dabu_dist <- vegdist(Dabu, method = 'bray')

# 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
```



- ▶ 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)

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

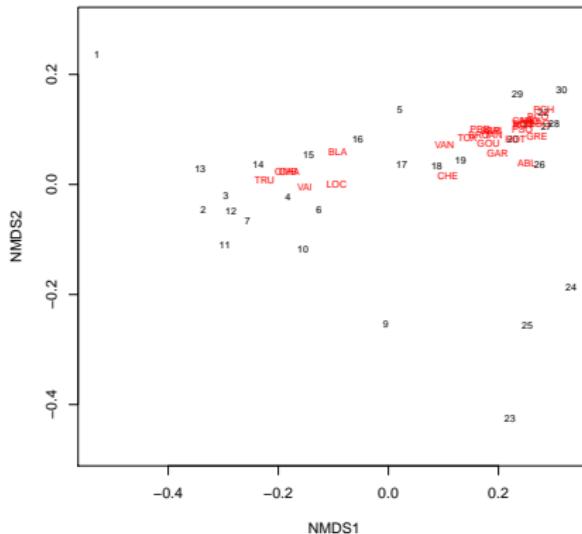
# NMDS
NMDS <- metaMDS(Dabu_dist, k = 2, trace = 0)

# 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] 0.07477834
```



# Your turn!

Using the artificial dummy dataset.

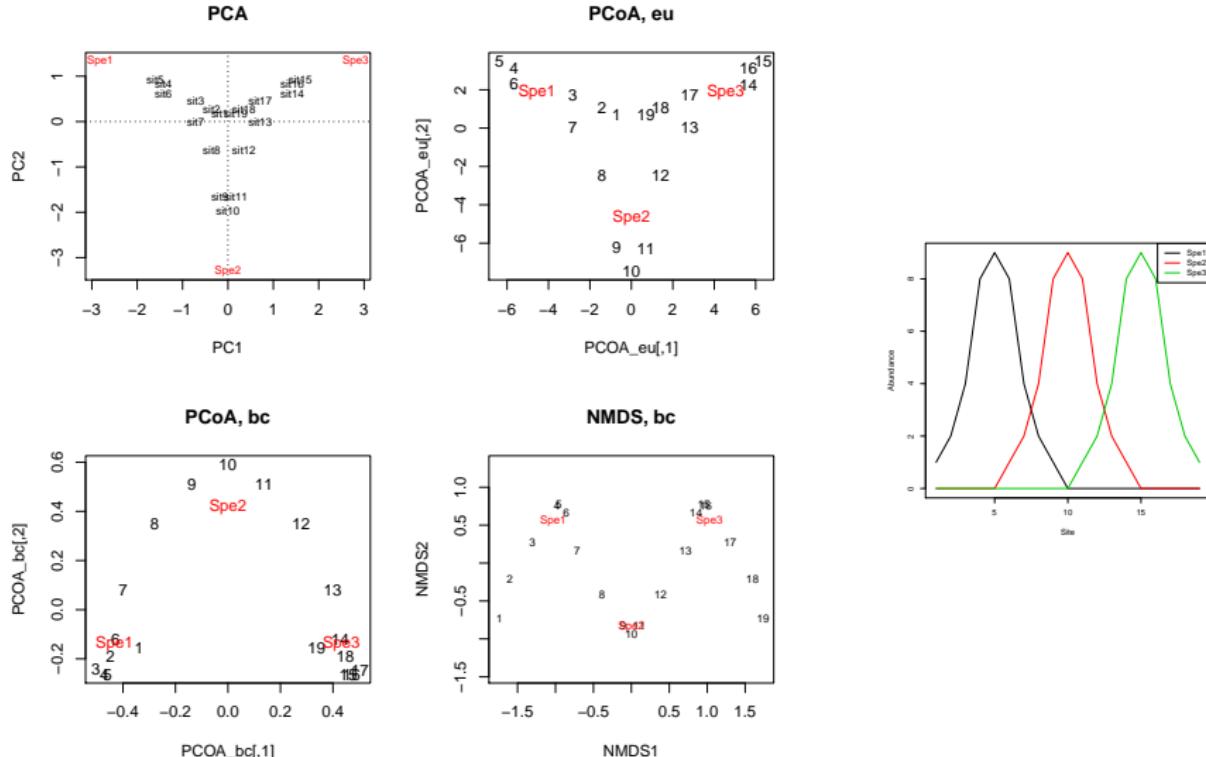
Run:

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  
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Unconstrained Ordination  
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Constrained Ordination  
○○

Permutation Tests  
○

End

## Datasets

oooooo

## Unconstrained Ordination

## Constrained Ordination oo

## Permutation Tests

End

# Your turn!

Using the melbourne data.

# Constrained Ordination

- ▶ Redundancy analysis (RDA)
- ▶ Transformation-based RDA
- ▶ Distance-based RDA

# Your turn!

Using the melbourne data.

# Permutation Tests

# Your turn!

Using the melbourne data.

- ▶ Distance-based hypothesis testing ((PER-)MANOVA, SIMPER, ANOSIM)
- ▶ Dispersion measures ( $\beta$ -Diversity, Functional diversity)
- ▶ Model-based ordination / hypothesis testing (See work of David Warton et al.)