

# Constrained Ordination

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## Introduction

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Constrained ordination is the marriage of basic ordination methods and regression

We try to extract pattern from our responses but we extract only that which can be explained by the measured covariates

We can view RDA as being the result of

- fitting a multiple regression to each species in turn using the set of covariates,
- then running PCA on the matrix of fitted values from those regressions

These are regression models so work with them as if they were a linear model

But we have little theory, so all inference is done using **permutation** tests

## Constrained Ordination

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CCA is the constrained form of CA; fitted using `cca()`.

Two interfaces for specifying models

- basic; `cca1 <- cca(X = varespec, Y = varechem)`
- formula; `cca1 <- cca(varespec ~ ., data = varechem)`

Formula interface is the more powerful — *recommended*

# Canonical Correspondence Analysis

```
cca1 <- cca(varespec ~ ., data = varechem)
cca1
```

```
Call: cca(formula = varespec ~ N + P + K + Ca + Mg + S + Al + Fe +
Mn + Zn + Mo + Baresoil + Humdepth + pH, data = varechem)
```

	Inertia	Proportion	Rank
Total	2.0832	1.0000	
Constrained	1.4415	0.6920	14
Unconstrained	0.6417	0.3080	9

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10
0.4389	0.2918	0.1628	0.1421	0.1180	0.0890	0.0703	0.0584	0.0311	0.0133
CCA11	CCA12	CCA13	CCA14						
0.0084	0.0065	0.0062	0.0047						

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9
0.19776	0.14193	0.10117	0.07079	0.05330	0.03330	0.01887	0.01510	0.00949

# Redundancy Analysis

RDA is the constrained form of PCA; fitted using `rda()`.

```
rda1 <- rda(varespec ~ ., data = varechem)
rda1
```

```
Call: rda(formula = varespec ~ N + P + K + Ca + Mg + S + Al + Fe +
Mn + Zn + Mo + Baresoil + Humdepth + pH, data = varechem)
```

	Inertia	Proportion	Rank
Total	1825.6594	1.0000	
Constrained	1459.8891	0.7997	14
Unconstrained	365.7704	0.2003	9

Inertia is variance

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7	RDA8	RDA9	RDA10	RDA11	RDA12
820.1	399.3	102.6	47.6	26.8	24.0	19.1	10.2	4.4	2.3	1.5	0.9

RDA13	RDA14
0.7	0.3

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
186.19	88.46	38.19	18.40	12.84	10.55	5.52	4.52	1.09

## The `cca.object`

- Objects of class "`cca`" are complex with many components
- Entire class described in `?cca.object`
- Depending on what analysis performed some components may be **NULL**
- Used for (C)CA, PCA, RDA, and CAP (`capscale()`)



# The `cca.object`

`cca1` has a large number of components

- `$call` how the function was called
- `$grand.total` in (C)CA sum of 'rowsum'
- `$rowsum` the row sums
- `$colsum` the column sums
- `$tot.chi` total inertia, sum of Eigenvalues
- `$pCCA` Conditioned (partial-ed out) components
- `$CCA` Constrained components
- `$CA` Unconstrained components
- `$method` Ordination method used
- `$inertia` Description of what inertia is

## The `cca` object

Depending on how one called `cca()` etc some of these components will be **NULL**

`$pCCA` is only filled in if a *partial* constrained ordination fitted

`rda()` returns objects with classes "**rda**" and "**cca**", but in most cases those objects work like those of class "**cca**"

The Eigenvalues and axis scores are now spread about the `$CA` and `$CCA` components (also `$pCCA` if a *partial* CCA)

Thankfully we can use *extractor* functions to get at such things

Use `eigenvals()` to extract Eigenvalues from a fitted ordination object

```
eigenvals(cca1)
```

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7
0.4388704	0.2917753	0.1628465	0.1421302	0.1179519	0.0890291	0.0702945
CCA8	CCA9	CCA10	CCA11	CCA12	CCA13	CCA14
0.0583592	0.0311408	0.0132944	0.0083644	0.0065385	0.0061563	0.0047332
CA1	CA2	CA3	CA4	CA5	CA6	CA7
0.1977645	0.1419256	0.1011741	0.0707868	0.0533034	0.0332994	0.0188676
CA8	CA9					
0.0151044	0.0094876					

To extract a range of scores from a fitted ordination use `scores()`

- takes an ordination object as the first argument
- `choices` — which axes? Defaults to `c(1,2)`
- `display` — which type(s) of scores to return
  - `"sites"` or `"wa"`: scores for samples in response matrix
  - `"species"`: scores for variables/columns in response
  - `"lc"`: linear combination site scores
  - `"bp"`: biplot scores (coords of arrow tip)
  - `"cn"`: centroid scores (coords of factor centroids)

## Extracting axis scores

```
str(scores(cca1, choices = 1:4, display = c("species", "sites")), max = 1)
```

List of 2

```
$ species: num [1:44, 1:4] 0.0753 -0.1813 -1.0535 -1.2774 -0.1526 ...  
..- attr(*, "dimnames")=List of 2  
$ sites : num [1:24, 1:4] 0.178 -0.97 -1.28 -1.501 -0.598 ...  
..- attr(*, "dimnames")=List of 2
```

```
head(scores(cca1, choices = 1:2, display = "sites"))
```

	CCA1	CCA2
18	0.1784733	-1.0598842
15	-0.9702382	-0.1971387
24	-1.2798478	0.4764498
27	-1.5009195	0.6521559
23	-0.5980933	-0.1840362
19	-0.1102881	0.7143142

## Scalings...

When we draw the results of many ordinations we display 2 or more sets of data

Can't display all of these and maintain relationships between the scores

*Solution* scale one set of scores relative to the other via the `scaling` argument

- `scaling = 1` — Focus on sites, scale site scores by  $\lambda_i$
- `scaling = 2` — Focus on species, scale species scores by  $\lambda_i$
- `scaling = 3` — Symmetric scaling, scale both scores by  $\sqrt{\lambda_i}$
- `scaling = -1` — As above, but
- `scaling = -2` — For `cca()` multiply results by  $\sqrt{(1/(1 - \lambda_i))}$
- `scaling = -3` — this is Hill's scaling
- `scaling < 0` — For `rda()` divide species scores by species'  $\sigma$
- `scaling = 0` — raw scores

```
scores(cca1, choices = 1:2, display = "species", scaling = 3)
```

Thankfully we can use alternative descriptors to extract scores:

- "none"
- "sites"
- "species"
- "symmetric"

Two modifiers select negative scores depending on whether the model is CCA or RDA:

- `hill = TRUE`
- `correlation = TRUE`

## Partial constrained ordinations

*Partial* constrained ordinations remove the effect of one or more variables *then* fit model of interest

Argument **Z** is used for a data frame of variables to partial out

Or with the formula interface use the **Condition()** function

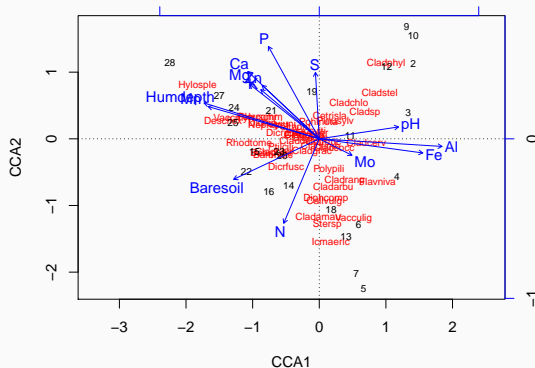
```
pcca <- cca(X = varespec,  
           Y = varechem[, "Ca", drop = FALSE],  
           Z = varechem[, "pH", drop = FALSE])  
pcca <- cca(varespec ~ Ca + Condition(pH), data = varechem) ## easier!
```



# Triplots

Triplots will generally produce a mess; we can really only display a couple of bits approximately anyway  
Trying to cram three things in is a recipe for a mess... but we can do it

```
plot(cca1)
```



## Building constrained ordination models

If we don't want to think it's easy to fit a poor model with many constraints

That's what I did with `cca1` and `rda1`

Remember, CCA and RDA are *just regression methods* — everything you know about regression applies here

A better approach is to *think* about the important variables and include only those

The formula interface allows you to create interaction or quadratic terms easily (though be careful with latter)

It also handles factor or class constraints automatically unlike the basic interface

# Building constrained ordination models

```
vare.cca <- cca(varespec ~ Al + P*(K + Baresoil), data = varechem)
vare.cca
```

```
Call: cca(formula = varespec ~ Al + P * (K + Baresoil), data =
varechem)
```

	Inertia	Proportion	Rank
Total	2.083	1.000	
Constrained	1.046	0.502	6
Unconstrained	1.038	0.498	17

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6
0.3756	0.2342	0.1407	0.1323	0.1068	0.0561

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8
0.27577	0.15411	0.13536	0.11803	0.08887	0.05511	0.04919	0.03781

(Showed only 8 of all 17 unconstrained eigenvalues)

## Building constrained ordination models

For CCA we have little choice but to do

1. Fit well-chosen set of candidate models & compare, or
2. Fit a *full* model of well-chosen variables & then do stepwise selection

But automatic approaches to model building should be used cautiously!

The standard `step()` function can be used as **vegan** provides two helper methods, `deviance()` and `extractAIC()`, used by `step()`

Vegan also provides methods for class "cca" for `add1()` and `drop1()`

## Variance inflation factors

Linear dependencies between constraints can be investigated via the *variance inflation factor* or VIF

VIF is a measure of how much the variance of  $\hat{\beta}_j$  is inflated by presence of other covariates

Lots of rules of thumb

- VIF  $\geq 20$  indicates *strong collinearity* in constraints
- VIF  $\geq 10$  potentially of concern & should be looked at

Computed via `vif.cca()`

```
vif.cca(vare.cca)
```

Al	P	K	Baresoil	P:K	P:Baresoil
1.335298	17.989518	12.891913	12.927791	41.037782	13.426405

## Stepwise selection in CCA

`step()` uses AIC which is a fudge for RDA/CCA. Alternatively use function `ordistep()`

1. Define an upper and lower model scope, say the full model and the null model
2. To step from the lower scope or null model we use

```
upr <- cca(varespec ~ ., data = varechem)
lwr <- cca(varespec ~ 1, data = varechem)
set.seed(1)
mods <- ordistep(lwr, scope = formula(upr), trace = 0)
```

`trace = 0` is used here to turn off printing of progress

Permutation tests are used (more on these later); the theory for an AIC for ordination is somewhat loose

# Stepwise selection in CCA

The object returned by `step()` is a standard "cca" object with an extra component `$anova`

The `$anova` component contains a summary of the steps involved in automatic model building

```
mods
```

```
Call: cca(formula = varespec ~ A1 + P + K, data = varechem)
```

	Inertia	Proportion	Rank
Total	2.0832	1.0000	
Constrained	0.6441	0.3092	3
Unconstrained	1.4391	0.6908	20

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3
0.3616	0.1700	0.1126

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8
0.3500	0.2201	0.1851	0.1551	0.1351	0.1003	0.0773	0.0537

(Shown only 8 of all 20 unconstrained eigenvalues)

The `$anova` component contains a summary of the steps involved in automatic model building

```
mods$anova
```

```
      Df    AIC      F Pr(>F)
+ Al   1 128.61 3.6749 0.005 **
+ P    1 127.91 2.5001 0.005 **
+ K    1 127.44 2.1688 0.035 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



# Stepwise selection in CCA

Step-wise model selection is fairly fragile; if we start from the full model we won't end up with the same final model

```
mods2 <- step(upr, scope = list(lower = formula(lwr), upper = formula(upr)), trace = 0,  
              test = "perm")  
mods2
```

```
Call: cca(formula = varespec ~ P + K + Mg + S + Mn + Mo + Baresoil  
+ Humdepth, data = varechem)
```

	Inertia	Proportion	Rank
Total	2.0832	1.0000	
Constrained	1.1165	0.5360	8
Unconstrained	0.9667	0.4640	15

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8
0.4007	0.2488	0.1488	0.1266	0.0875	0.0661	0.0250	0.0130

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9
0.25821	0.18813	0.11927	0.10204	0.08791	0.06085	0.04461	0.02782	0.02691
CA10	CA11	CA12	CA13	CA14	CA15			
0.01646	0.01364	0.00823	0.00655	0.00365	0.00238			

## Adjusted $R^2$ for *linear* models

As with ordinary  $R^2$ , that of an RDA is biased for the same reasons as for a linear regression

- adding a variable to constraints will increase  $R^2$
- the larger the number of constraints in the model the larger  $R^2$  is due to random correlations

Can attempt to account for this bias via an *adjusted*  $R^2$  measure

$$R_{adj}^2 = 1 - \frac{n - 1}{n - m - 1} (1 - R^2)$$

- $n$  is number of samples  $m$  is number of constraints (model degrees of freedom)
- Can be used up to  $\sim M > n/2$  before becomes too conservative
- Can be negative
- Compute using `RsquareAdj()`

## Stepwise selection via adjusted $R^2$

The problems with stepwise selection in regression models are myriad. Affects RDA, CCA, etc as well

Blanchet, Legendre, and Borcard (2008) proposed a two-step solution for models where  $R_{adj}^2$  makes sense

- *Global test* of all constraints
  - Proceed **only** if this test is significant
  - Helps prevent inflation of overall type I error
- Proceed with forward selection, but with *two* stopping rules
  - Usual significance threshold  $\alpha$
  - The global  $R_{adj}^2$
  - Stop if next candidate model is non-significant or if  $R_{adj}^2$  exceeds the global  $R_{adj}^2$

Available in `ordiR2step()`

## Permutation tests

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RDA has lots of theory behind it, CCA not as much. However, ecological/environmental data invariably violate what little theory we have

Instead we use permutation tests to assess the *importance* of fitted models — the data are shuffled in some way and the model refitted to derive a Null distribution under some hypothesis of *no effect*

What *is* shuffled and *how* is of **paramount** importance for the test to be valid

- No conditioning (partial) variables then rows of the species data are permuted
- With conditioning variables, two options are available, both of which *permute residuals* from model fits
  - The *full model* uses residuals from model  $Y = X + Z + \epsilon$
  - The *reduced model* uses residuals from model  $Y = X + Z + \epsilon$
- In **vegan** which is used can be set via argument `model` with "**direct**", "~~full~~", and "**reduced**" respectively
- In current **vegan** option `method = "full"` is disabled

A test statistic is required, computed for observed model & each permuted model

**vegan** uses a pseudo- $F$  statistic

$$F = \frac{\chi^2_{model}/df_{model}}{\chi^2_{resid}/df_{resid}}$$

Evaluate whether  $F$  is unusually large relative to the null (permutation) distribution of  $F$

## Permutation tests in vegan: `anova()`

- The main user function is the `anova()` method
- It is an interface to the lower-level function `permutest.cca()`
- At its most simplest, the `anova()` method tests whether the “model” as a whole is significant

$$F = \frac{1.4415/14}{0.6417/9} = 1.4441$$

```
set.seed(42)
(perm <- anova(cca1))
```

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

```
Model: cca(formula = varespec ~ N + P + K + Ca + Mg + S + Al + Fe + Mn + Zn + Mo + Baresoil + Humdepth + pH, data = varechem)
```

	Df	ChiSquare	F	Pr(>F)
Model	14	1.44148	1.4441	0.041 *
Residual	9	0.64171		

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



## Permutation tests in vegan: `anova()`

- `anova.cca()` has a number of arguments

```
args(anova.cca)
```

```
function (object, ..., permutations = how(nperm = 999), by = NULL,  
      model = c("reduced", "direct", "full"), parallel = getOption("mc.cores"),  
      strata = NULL, cutoff = 1, scope = NULL)  
NULL
```

- `object` is the fitted ordination
- `permutations` controls what is permuted and how
- `by` determines what is tested; the default is to test the model

## Types of permutation test in vegan

A number of types of test can be envisaged

- Testing the overall significance of the model
- Testing constrained (canonical) axes
- Testing individual model terms *sequentially*
- The *marginal* effect of a single variable

The first is the default in `anova()`

The other three can be selected via the argument `by`

## Permutation tests | testing canonical axes

- The constrained (canonical) axes can be individually tests by specifying `by = "axis"`
- The first axis is tested in terms of variance explained compared to residual variance
- The second axis is tested after partialling out the first axis... and so on

```
set.seed(1)
anova(mods, by = "axis")
```

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

```
Model: cca(formula = varespec ~ A1 + P + K, data = varechem)
```

	Df	ChiSquare	F	Pr(>F)
CCA1	1	0.36156	5.0249	0.001 ***
CCA2	1	0.16996	2.3621	0.011 *
CCA3	1	0.11262	1.5651	0.124
Residual	20	1.43906		

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Permutation tests | testing terms sequentially

- The individual terms in the model can be tested using `by = "terms"`
- The terms are assessed in the order they were specified in the model, sequentially from first to last
- Test is of the additional variance explained by adding the  $k$ th variable to the model
- **Ordering of the terms** will affect the results

```
set.seed(5)
anova(mods, by = "terms")
```

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

```
Model: cca(formula = varespec ~ A1 + P + K, data = varechem)
```

	Df	ChiSquare	F	Pr(>F)
A1	1	0.29817	4.1440	0.001 ***
P	1	0.18991	2.6393	0.011 *
K	1	0.15605	2.1688	0.024 *
Residual	20	1.43906		

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Permutation tests | testing terms marginal effects

- The marginal *effect* of a model term can be assessed using `by = "margin"`
- The marginal *effect* is the effect of a particular term when all other model terms are included in the model

```
set.seed(10)
anova(mods, by = "margin")
```

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

```
Model: cca(formula = varespec ~ A1 + P + K, data = varechem)
```

	Df	ChiSquare	F	Pr(>F)
A1	1	0.31184	4.3340	0.001 ***
P	1	0.16810	2.3362	0.012 *
K	1	0.15605	2.1688	0.025 *
Residual	20	1.43906		

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Your turn - Spring meadows

---

Example & data taken from Leps & Smilauer, Case Study 2

Spring fen meadow vegetation in westernmost Carpathian mountains

```
## load vegan
library("vegan")

## load the data
spp <- read.csv("../00-data-sets/meadow-spp.csv", header = TRUE, row.names = 1)
env <- read.csv("../00-data-sets/meadow-env.csv", header = TRUE, row.names = 1)
```

## Constrained ordination worked example | spring meadow vegetation

CCA a reasonable starting point as the gradient is long here (check with `decorana()` if you want)

```
m1 <- cca(spp ~ ., data = env)
set.seed(32)
anova(m1)
```

Permutation test for cca under reduced model

Permutation: free

Number of permutations: 999

Model: cca(formula = spp ~ Ca + Mg + Fe + K + Na + Si + S04 + P04 + N03 + NH3 + Cl + Corg + pH + conduct + slope, data = env)

	Df	ChiSquare	F	Pr(>F)
Model	15	1.5597	1.497	0.001 ***
Residual	54	3.7509		

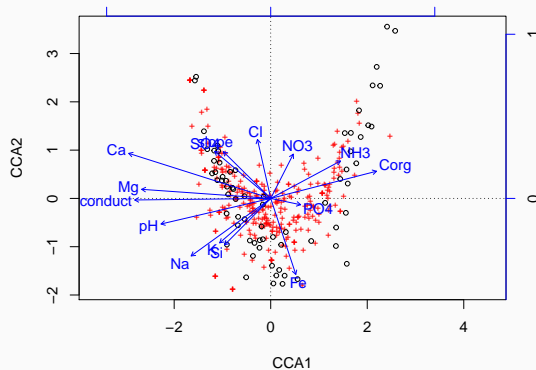
---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



## Constrained ordination worked example | spring meadow vegetation

```
plot(m1)
```



# Constrained ordination worked example | spring meadow vegetation

```
set.seed(67)
lwr <- cca(spp ~ 1, data = env)
m2 <- ordistep(lwr, scope = formula(m1), trace = FALSE)
```

m2

Call: cca(formula = spp ~ Ca + conduct + Corg + Na + NH3 + Fe + pH, data = env)

	Inertia	Proportion	Rank
Total	5.3107	1.0000	
Constrained	0.9899	0.1864	7
Unconstrained	4.3208	0.8136	62

Inertia is mean squared contingency coefficient

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7
0.4268	0.1447	0.1116	0.0936	0.0760	0.0719	0.0652

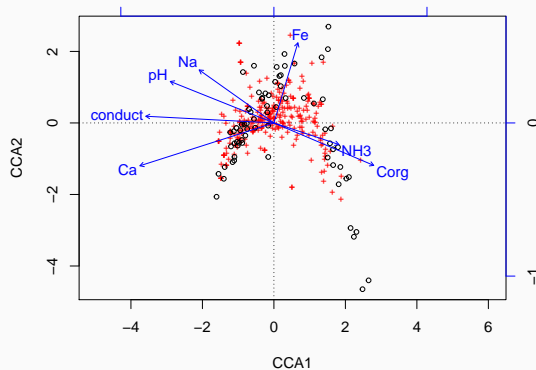
Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8
0.27251	0.19518	0.16703	0.14993	0.14606	0.14168	0.13292	0.12154

(Shown only 8 of all 62 unconstrained eigenvalues)

## Constrained ordination worked example | spring meadow vegetation

```
plot(m2)
```



## Constrained ordination worked example | spring meadow vegetation

```
m2$anova
```

	Df	AIC	F	Pr(>F)	
+ Ca	1	453.14	4.7893	0.005	**
+ conduct	1	453.29	1.7915	0.005	**
+ Corg	1	453.61	1.6011	0.005	**
+ Na	1	453.93	1.5827	0.005	**
+ NH3	1	454.36	1.4507	0.020	*
+ Fe	1	454.89	1.3386	0.015	*
+ pH	1	455.46	1.2756	0.015	*

---

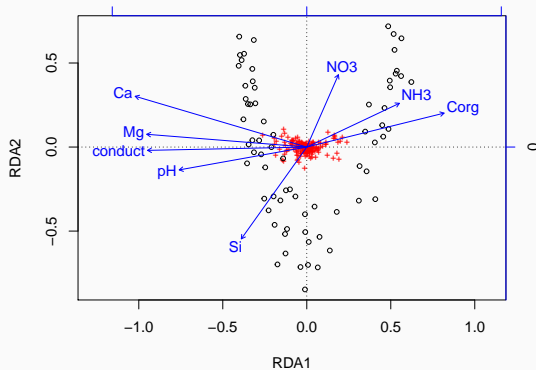
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Alternative is RDA with a transformation

```
spph <- decostand(spp, method = "hellinger")  
m3 <- rda(spph ~ ., data = env)  
lwr <- rda(spph ~ 1, data = env)  
m4 <- ordistep(lwr, scope = formula(m3), trace = FALSE)
```

## Constrained ordination worked example | spring meadow vegetation

```
plot(m4)
```



## Constrained ordination worked example | spring meadow vegetation

Stepwise using  $R^2_{adj}$

```
m5 <- ordiR2step(lwr, scope = formula(m3), trace = FALSE)
m5$anova
```

	R2.adj	Df	AIC	F	Pr(>F)	
+ Ca	0.12588	1	-41.779	10.9370	0.002	**
+ NH3	0.14628	1	-42.468	2.6242	0.002	**
+ conduct	0.16322	1	-42.925	2.3570	0.002	**
+ Si	0.17711	1	-43.164	2.1136	0.002	**
+ Corg	0.18518	1	-42.940	1.6442	0.006	**
+ NO3	0.19257	1	-42.680	1.5853	0.018	*
+ pH	0.19966	1	-42.417	1.5583	0.010	**
<All variables>	0.20332					
---						
Signif. codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '	1

## Restricted permutation tests

---



## Restricted permutation tests

What *is* shuffled and *how* is of **paramount** importance for the test to be valid

Complete randomisation (default in **vegan**) assumes a null hypothesis where all observations are *independent*

Ecological / environmental data often aren't independent

- Temporal or spatial correlation
- Clustering, repeated measures
- Nested sampling designs (Split-plots designs)
- Blocks
- ...

Permutation *must* give null distribution of the test statistic whilst preserving the *dependence* between observations

Trick is to shuffle the data whilst preserving that dependence

## Restricted permutations

Canoco has had restricted permutations for a *long* time. **vegan** has only recently caught up & we're not (quite) there yet

**vegan** used to only know how to completely randomise data or completely randomise within blocks (via **strata** in **vegan**)

The newish package **permute** grew out of initial code in the **vegan** repository to generate the sorts of restricted permutations available in Canoco

We have now fully integrated **permute** into **vegan**...

**vegan** depends on **permute** so it will already be installed & loaded when using **vegan**

**permute** follows Canoco closely — at the chiding of Cajo ter Braak when it didn't do what he wanted!

Samples can be thought of as belonging to three levels of a hierarchy

- the *sample* level; how are individual samples permuted
- the *plot* level; how are samples grouped at an intermediate level
- the *block* level; how are samples grouped at the outermost level

Blocks define groups of plots, each of which can contain groups of samples

## Restricted permutations with permute

Blocks are *never* permuted; if defined, only plots or samples *within* the blocks get shuffled & samples are **never** swapped between blocks

Plots or samples within plots, or both can be permuted following one of four simple permutation types

1. Free permutation (randomisation)
2. Time series or linear transect, equal spacing
3. Spatial grid designs, equal regular spacing
4. Permutation of plots (groups of samples)
5. Fixed (no permutation)

Multiple plots per block, multiple samples per plot; plots could be arranged in a spatial grid and samples within each of the plots form a time series

## Restricted permutations with `permute` | `blocks`

Blocks are a random factor that does not interact with factors that vary within blocks

Blocks form groups of samples that are never permuted between blocks, only within blocks

Using blocks you can achieve what the `strata` argument used to in `vegan`; needs to be a factor variable

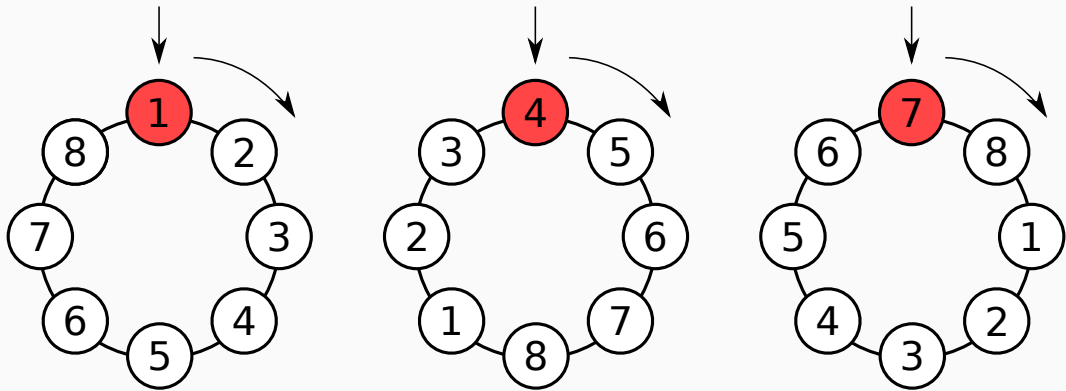
The variation *between* blocks should be excluded from the test; `permute` doesn't do this for you!

Use `+ Condition(blocks)` in the model formula where `blocks` is a factor containing the block membership for each observation

## Restricted permutations with permute | time series & linear transects

Can link *randomly* starting point of one series to any time point of another series if series are stationary under null hypothesis that the series are unrelated

Achieve this via cyclic shift permutations — wrap series into a circle by joining start and end points



Works OK if there are no trends or cyclic pattern — autocorrelation structure only broken at the end points *if* series are stationary

Can detrend to make series stationary but not if you want to test significance of a trend

```
shuffle(10, control = how(within = Within(type = "series")))
```

```
[1] 9 10 1 2 3 4 5 6 7 8
```

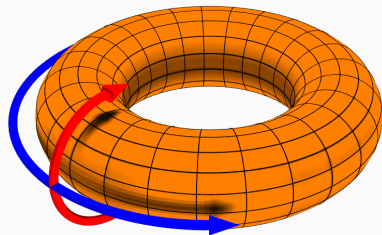
## Restricted permutations with permute | spatial grids

The trick of cyclic shifts can be extended to two dimensions for a regular spatial grid arrangement of points

Now shifts are *toroidal* as we join the end point in the x direction together and in the y direction together

```
matrix(perm, ncol = 3)
```

	[,1]	[,2]	[,3]
[1,]	6	9	3
[2,]	4	7	1
[3,]	5	8	2



Source: Dave Burke, Wikimedia 



Split-plot designs are hierarchical with two levels of units

1. **whole-plots** , which contain
2. **split-plots** (the samples)

Can permute one or both of these but whole-plots must be of equal size

Essentially allows more than one error stratum to be analyzed

Test effect of constraints that vary *between* whole plots by permuting the whole-plots whilst retaining order of split-plots (samples) within the whole-plots

Test effect of constraints that vary *within* whole-plots by permuting the split-plots within whole-plots without permuting the whole-plots

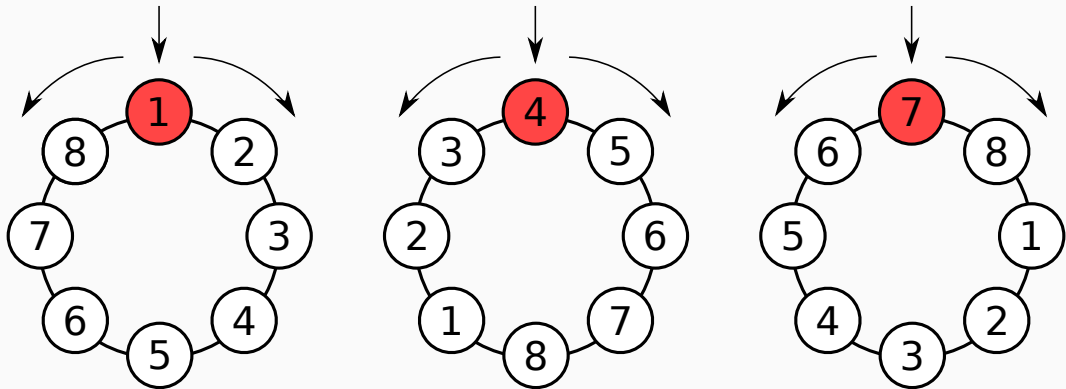
Whole-plots or split-plots can be time series, linear transects or rectangular grids in which case the appropriate restricted permutation is used

If the split-plots are parallel time series & **time** is an autocorrelated error component affecting all series then the same cyclic shift can be applied to each time series (within each whole-plot) (**constant** = **TRUE**)

## Restricted permutations with permute | mirroring

Mirroring in restricted permutations allows for isotropy in dependencies by reflecting the ordering of samples in time or spatial dimensions

For a linear transect, technically the autocorrelation at lag  $h$  is equal to that at lag  $-h$  (also in a trend-free time series)



Hence the series (1, 2, 3, 4) and (4, 3, 2, 1) are equivalent from this point of view & we can draw permutations from either version

Similar argument can be made for spatial grids

Using `mirror = TRUE` then can double (time series, linear transects) or quadruple (spatial grids) the size of the set of permutations

## Restricted permutations with permute | the set of permutations

Using restricted permutations can severely reduce the size of the set of allowed permutations

As the minimum  $p$  value obtainable is  $1/np$  where  $np$  is number of allowed permutations (including the observed) this can impact the ability to detect signal/pattern

If we don't want mirroring

- in a time series of 20 samples the minimum  $p$  is  $1/20$  (0.05)
- in a time series of 100 samples the minimum  $p$  is  $1/100$  (0.01)
- in a data set with 10 time series each of 20 observations (200 total), if we assume an autocorrelated error component over all series (**constant = TRUE**) then there are only 20 permutations of the data and minimum  $p$  is 0.05

When the set of permutations is small it is better to switch to an exact test & evaluate all permutations in the set rather than randomly sample from the set

In `permute`, we set up a permutation scheme with `how()`

We sample from the permutation scheme with

- `shuffle()`, which gives a single draw from scheme, or
- `shuffleSet()`, which returns a set of `n` draws from the scheme

`allPerms()` can generate the entire set of permutations — **note** this was designed for small sets of permutations & is slow if you request it for a scheme with many thousands of permutations!

`how()` has three main arguments

1. `within` — takes input from helper `Within()`
2. `plots` — takes input from helper `Plots()`
3. `blocks` — takes a factor variable as input

```
plt <- gl(3, 10)
h <- how(within = Within(type = "series"), plots = Plots(strata = plt))
```

## Restricted permutations with permute | designing permutation schemes

Helper functions make it easy to change one or a few aspects of permutation scheme, rest left at defaults

```
args(Within)
```

```
function (type = c("free", "series", "grid", "none"), constant = FALSE,  
  mirror = FALSE, ncol = NULL, nrow = NULL)  
NULL
```

```
args(Plots)
```

```
function (strata = NULL, type = c("none", "free", "series", "grid"),  
  mirror = FALSE, ncol = NULL, nrow = NULL)  
NULL
```



## Restricted permutations with permute | designing permutation schemes

`how()` has additional arguments, many of which control the heuristics that kick in to stop you shooting yourself in the foot and demanding 9999 permutations when there are only 10

- **complete** should we enumerate the entire set of permutations?
- **minperm** lower bound on the size of the set of permutations at & below which we turn on complete enumeration

```
args(how)
```

```
function (within = Within(), plots = Plots(), blocks = NULL,  
  nperm = 199, complete = FALSE, maxperm = 9999, minperm = 5040,  
  all.perms = NULL, make = TRUE, observed = FALSE)  
NULL
```

## Restricted permutations with permute | time series example I

Time series within 3 plots, 10 observation each

```
plt <- gl(3, 10)
h <- how(within = Within(type = "series"),
         plots = Plots(strata = plt))
set.seed(4)
p <- shuffle(30, control = h)
do.call("rbind", split(p, plt)) ## look at perms in context
```

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]
1	7	8	9	10	1	2	3	4	5	6
2	12	13	14	15	16	17	18	19	20	11
3	24	25	26	27	28	29	30	21	22	23

## Restricted permutations with permute | time series example II

Time series within 3 plots, 10 observation each, same permutation within each

```
plt <- gl(3, 10)
h <- how(within = Within(type = "series", constant = TRUE),
         plots = Plots(strata = plt))
set.seed(4)
p <- shuffle(30, control = h)
do.call("rbind", split(p, plt)) ## look at perms in context
```

	[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]	[,8]	[,9]	[,10]
1	7	8	9	10	1	2	3	4	5	6
2	17	18	19	20	11	12	13	14	15	16
3	27	28	29	30	21	22	23	24	25	26

## Ohraz Case Study

---

Now we've seen how to drive **permute**, we can use the same **how( )** commands to set up permutation designs within **vegan** functions

Analyse the Ohraz data Case study 5 of Leps & Smilauer

Repeated observations of composition from an experiment

- Factorial design (3 replicates)
- Treatments: fertilisation, mowing, *Molinia* removal

Test 1 of the hypotheses

*There are no directional changes in species composition in time that are common to all treatments or specific treatments*

Analyse the Ohraz data Case study 5 of Leps & Smilauer

```
## load vegan
library("vegan")

## load the data
spp <- read.csv("../00-data-sets/ohraz-spp.csv", header = TRUE, row.names = 1)
env <- read.csv("../00-data-sets/ohraz-env.csv", header = TRUE, row.names = 1)
molinia <- spp[, 1]
spp <- spp[, -1]

## Year as numeric
env <- transform(env, year = as.numeric(as.character(year)))
```

## Restricted permutations with permute | worked example with vegan

```
c1 <- rda(spp ~ year + year:mowing + year:fertilizer + year:removal + Condition(plotid), data = env)
(h <- how(within = Within(type = "none"), plots = Plots(strata = env$plotid, type = "free")))
```

Permutation Design:

Blocks:

Defined by: none

Plots:

Plots: env\$plotid

Permutation type: free

Mirrored?: No

Within Plots:

Permutation type: none

Permutation details:

Number of permutations: 199

Max. number of permutations allowed: 9999

Evaluate all permutations?: No. Activation limit: 5040

## Restricted permutations with permute | worked example with vegan

```
set.seed(42)
anova(c1, permutations = h, model = "reduced")
```

Permutation test for rda under reduced model

Plots: env\$plotid, plot permutation: free

Permutation: none

Number of permutations: 199

Model: rda(formula = spp ~ year + year:mowing + year:fertilizer + year:removal + Condition(plotid), data = env)

	Df	Variance	F	Pr(>F)
Model	4	158.85	6.4247	0.005 **
Residual	90	556.30		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



## Restricted permutations with permute | worked example with vegan

```
set.seed(24)
anova(c1, permutations = h, model = "reduced", by = "axis")
```

Permutation test for rda under reduced model

Marginal tests for axes

Plots: env\$plotid, plot permutation: free

Permutation: none

Number of permutations: 199

Model: rda(formula = spp ~ year + year:mowing + year:fertilizer + year:removal + Condition(plotid), data = env)

	Df	Variance	F	Pr(>F)	
RDA1	1	89.12	14.4173	0.005	**
RDA2	1	34.28	5.5458	0.005	**
RDA3	1	26.52	4.2900	0.010	**
RDA4	1	8.94	1.4458	0.650	
Residual	90	556.30			

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Hierarchical analysis of crayfish

---

Variation in communities may exist at various scales, sometimes hierarchically

A first step in understanding this variation is to test for its existence

In this example from Leps & Smilauer (2014) uses crayfish data from Spring River, Arkansas/Missouri, USA, collected by Dr.~Camille Flinders.

567 records of 5 species (each sub-divided into *Large* & *Small* individuals on basis of carapace length)

## Hierarchical analysis of crayfish

```
## load data
crayfish <- head(read.csv("crayfish-spp.csv"), -1, -1)
design <- read.csv("crayfish-design.csv", skip = 1)[-1, -1]

## fixup the names
names(crayfish) <- gsub("\\\\.", "", names(crayfish))
names(design) <- c("Watershed", "Stream", "Reach", "Run",
                  "Stream.Nested", "ReachNested", "Run.Nested")
```

## Hierarchical analysis of crayfish — Unconstrained

A number of samples have 0 crayfish, which excludes unimodal methods — CA would just delete these rows as they contain no data

Fit unconstrained PCA

```
m.pca <- rda(crayfish)
summary(eigenvals(m.pca))
```

Importance of components:

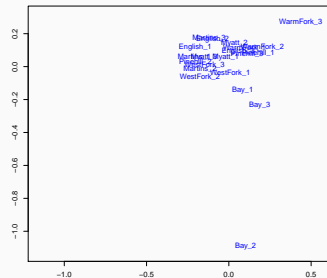
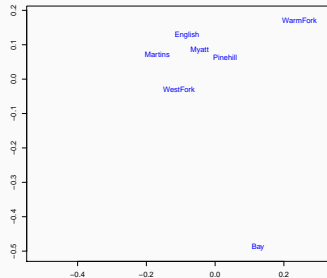
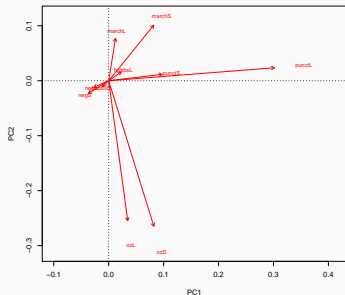
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	3.5728	1.8007	1.1974	0.9012	0.79337	0.38886	0.28132
Proportion Explained	0.3818	0.1924	0.1280	0.0963	0.08478	0.04155	0.03006
Cumulative Proportion	0.3818	0.5742	0.7022	0.7985	0.88325	0.92480	0.95486

	PC8	PC9	PC10
Eigenvalue	0.21225	0.20528	0.004881
Proportion Explained	0.02268	0.02194	0.000520
Cumulative Proportion	0.97754	0.99948	1.000000

# Hierarchical analysis of crayfish — Unconstrained

```
set.seed(23)
layout(matrix(1:3, ncol = 3))
biplot(m.pca, type = c("text", "none"), display = "species", scaling = "species", correlation = TRUE)
plot(envfit(m.pca ~ Watershed, data = design, scaling = "species", correlation = TRUE), add = FALSE,
     labels = levels(design$Watershed))
ev.pca <- envfit(m.pca ~ Stream, data = design, scaling = "species", correlation = TRUE)
plot(ev.pca, labels = levels(design$Stream), add = FALSE)
layout(1)
```



# Hierarchical analysis of crayfish — Watershed scale

```
m.ws <- rda(crayfish ~ Watershed, data = design)
m.ws
```

```
Call: rda(formula = crayfish ~ Watershed, data = design)
```

	Inertia	Proportion	Rank
Total	9.3580	1.0000	
Constrained	1.7669	0.1888	6
Unconstrained	7.5911	0.8112	10

Inertia is variance

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6
0.7011	0.5540	0.3660	0.1064	0.0381	0.0013

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
3.0957	1.2109	0.9717	0.7219	0.5333	0.3838	0.2772	0.2040	0.1879	0.0048

# Hierarchical analysis of crayfish — Watershed scale

```
summary(eigenvals(m.ws, constrained = TRUE))
```

Importance of components:

	RDA1	RDA2	RDA3	RDA4	RDA5	RDA6
Eigenvalue	0.7011	0.5540	0.3660	0.1064	0.03814	0.001279
Proportion Explained	0.3968	0.3135	0.2072	0.0602	0.02159	0.000720
Cumulative Proportion	0.3968	0.7103	0.9175	0.9777	0.99928	1.000000

```
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "none"),
           plots = with(design, Plots(strata = Stream, type = "free")))
(sig.ws <- anova(m.ws, permutations = ctrl))
```

Permutation test for rda under reduced model  
Plots: Stream, plot permutation: free  
Permutation: none  
Number of permutations: 499

Model: rda(formula = crayfish ~ Watershed, data = design)

	Df	Variance	F	Pr(>F)
Model	6	1.7669	21.724	0.002 **
Residual	560	7.5911		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1



## Hierarchical analysis of crayfish — Stream scale

```
m.str <- rda(crayfish ~ Stream + Condition(Watershed), data = design)
m.str
```

```
Call: rda(formula = crayfish ~ Stream + Condition(Watershed), data
= design)
```

	Inertia	Proportion	Rank
Total	9.3580	1.0000	
Conditional	1.7669	0.1888	6
Constrained	1.1478	0.1227	10
Unconstrained	6.4433	0.6885	10

Inertia is variance

Some constraints were aliased because they were collinear (redundant)

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7	RDA8	RDA9	RDA10
0.4928	0.2990	0.2058	0.0782	0.0372	0.0224	0.0063	0.0030	0.0029	0.0002

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
2.7853	0.8528	0.7737	0.6317	0.5144	0.2808	0.2517	0.1923	0.1559	0.0046

# Hierarchical analysis of crayfish — Stream scale

```
summary(eigenvals(m.str, constrained = TRUE))
```

Importance of components:

	RDA1	RDA2	RDA3	RDA4	RDA5	RDA6
Eigenvalue	0.4928	0.2990	0.2058	0.07824	0.03719	0.02235
Proportion Explained	0.4293	0.2605	0.1793	0.06816	0.03240	0.01947
Cumulative Proportion	0.4293	0.6898	0.8691	0.93731	0.96971	0.98918

	RDA7	RDA8	RDA9	RDA10
Eigenvalue	0.006326	0.003042	0.002894	0.0001546
Proportion Explained	0.005510	0.002650	0.002520	0.0001300
Cumulative Proportion	0.994690	0.997340	0.999870	1.0000000

```
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "none"),
  plots = with(design, Plots(strata = Reach, type = "free")),
  blocks = with(design, Watershed))
(sig.str <- anova(m.str, permutations = ctrl))
```

Permutation test for rda under reduced model

Blocks: with(design, Watershed)

Plots: Reach, plot permutation: free

Permutation: none

Number of permutations: 499

Model: rda(formula = crayfish ~ Stream + Condition(Watershed), data = design)

	Df	Variance	F	Pr(>F)
Model	14	1.1478	6.9477	0.004 **
Residual	546	6.4433		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Hierarchical analysis of crayfish — Reach scale

```
(m.re <- rda(crayfish ~ Reach + Condition(Stream), data = design))
```

```
Call: rda(formula = crayfish ~ Reach + Condition(Stream), data =  
design)
```

	Inertia	Proportion	Rank
Total	9.3580	1.0000	
Conditional	2.9148	0.3115	20
Constrained	1.4829	0.1585	10
Unconstrained	4.9603	0.5301	10

Inertia is variance

Some constraints were aliased because they were collinear (redundant)

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7	RDA8	RDA9	RDA10
0.6292	0.2706	0.2146	0.1414	0.1123	0.0467	0.0344	0.0270	0.0064	0.0003

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
2.1635	0.6080	0.5605	0.5166	0.3749	0.2212	0.2052	0.1588	0.1477	0.0040

## Hierarchical analysis of crayfish — Reach scale

```
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "none"),
           plots = with(design, Plots(strata = Run, type = "free")),
           blocks = with(design, Stream))
(sig.re <- anova(m.re, permutations = ctrl))
```

Permutation test for rda under reduced model

Blocks: with(design, Stream)

Plots: Run, plot permutation: free

Permutation: none

Number of permutations: 499

Model: rda(formula = crayfish ~ Reach + Condition(Stream), data = design)

	Df	Variance	F	Pr(>F)
Model	42	1.4829	3.5875	0.002 **
Residual	504	4.9603		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## Hierarchical analysis of crayfish — Run scale

```
(m.run <- rda(crayfish ~ Run + Condition(Reach), data = design))
```

```
Call: rda(formula = crayfish ~ Run + Condition(Reach), data =  
design)
```

	Inertia	Proportion	Rank
Total	9.3580	1.0000	
Conditional	4.3977	0.4699	62
Constrained	1.8225	0.1948	10
Unconstrained	3.1378	0.3353	10

Inertia is variance

Some constraints were aliased because they were collinear (redundant)

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7	RDA8	RDA9	RDA10
0.8541	0.3141	0.1679	0.1393	0.1328	0.0835	0.0474	0.0429	0.0390	0.0016

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
1.3137	0.4165	0.3832	0.2759	0.2378	0.1725	0.1215	0.1130	0.1016	0.0021

## Hierarchical analysis of crayfish — Run scale

```
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "free"),
           blocks = with(design, Reach))
(sig.run <- anova(m.run, permutations = ctrl))
```

Permutation test for rda under reduced model

Blocks: with(design, Reach)

Permutation: free

Number of permutations: 499

Model: rda(formula = crayfish ~ Run + Condition(Reach), data = design)

	Df	Variance	F	Pr(>F)
Model	126	1.8225	1.7425	0.002 **
Residual	378	3.1378		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Component	Explained %	DF	'Mean Square'	p value
Watershed	18.9	6	3.150	0.002
Stream	12.3	14	0.879	0.008
Reach	15.8	42	0.376	0.002
Run	19.5	126	0.155	0.002
Residual	33.5	378	0.089	NA
Total	100.0	566	0.177	NA

Other stuff

---



`vegan` provides a series of diagnostics to help assess the model fit

- `goodness()`
- `inertcomp()`
- `spenvcor()`
- `intersetcor()`
- `vif.caa()`

`goodness()` computes two goodness of fit statistics for species or sites

Which goodness of fit measure is returned is controlled by argument `statistic`

- `statistic = "explained"` (default) gives the cumulative proportion of variance explained by each axis
- `statistic = "distance"` gives the residual distance between the “fitted” location in constrained ordination space and the location in the full dimensional space

## Diagnostics for constrained ordinations | goodness of fit

```
head(goodness(mods))
```

	CCA1	CCA2	CCA3
Callvulg	0.0062471656	0.318907619	0.8254657
Empenigr	0.1164701677	0.137604904	0.1953245
Rhodtome	0.0999089739	0.169697909	0.1824153
Vaccmyrt	0.2361482843	0.240516323	0.2406730
Vaccviti	0.1523704591	0.156502301	0.2110550
Pinusylv	0.0009244423	0.004802076	0.0060096

```
head(goodness(mods, summarize = TRUE))
```

Callvulg	Empenigr	Rhodtome	Vaccmyrt	Vaccviti	Pinusylv
0.8254657	0.1953245	0.1824153	0.2406730	0.2110550	0.0060096

## Diagnostics for constrained ordinations | inertia decomposition

`inertcomp()` decomposes the variance in samples or species in partial, constrained, and unconstrained components

Same two types of measure available by argument `statistic`

- `statistic = "explained"` (default) gives the decomposition in terms of variance
- `statistic = "distance"` gives decomposition in terms of the the residual distance
- Can output as proportions of total via `proportion = TRUE`

```
head(inertcomp(mods, proportional = TRUE))
```

	CCA	CA
Callvulg	0.8254657	0.1745343
Empenigr	0.1953245	0.8046755
Rhodtome	0.1824153	0.8175847
Vaccmyrt	0.2406730	0.7593270
Vaccviti	0.2110550	0.7889450
Pinusylv	0.0060096	0.9939904

## Diagnostics for constrained ordinations | species-environment correlations

`spenvcor()` returns the species-environment correlation

The (weighted) correlation between the weighted average-based and the linear combination-based sets of site scores

A *poor* measure of goodness of fit. Sensitive to

- outliers (like all correlations)
- overfitting (using too many constraints)

Better models can have poorer species-environment correlations

```
spenvcor(mods)
```

CCA1	CCA2	CCA3
0.8554808	0.8132873	0.8792797

## Diagnostics for constrained ordinations | interset correlations

`intersetcor()` returns the interset correlations; the (weighted) correlation between the weighted average-based site scores and each constraint variable

Another *poor* diagnostic

- correlation based
- focuses on a single constraint–axis combination at a time

Vector fitting (`envfit()`) or biplot scores (`scores(model, display = "bp")`) are better alternatives

```
intersetcor(mods)
```

	CCA1	CCA2	CCA3
Al	0.7356445	-0.1304293	0.4260453
P	-0.3588931	-0.6109601	0.4478786
K	-0.3767902	-0.1339051	0.7759566

I have several **vegan**-related posts on my blog. For a list of posts see  
<http://www.fromthebottomoftheheap.net/blog/>

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Blanchet, F Guillaume, Pierre Legendre, and Daniel Borcard. 2008. "Forward Selection of Explanatory Variables." *Ecology* 89 (9). Eco Soc America: 2623–32.