ADVANCED COMMUNITY DATA ANALYSIS USING VEGAN **PRELIMINARIES** Gavin L. Simpson ESA 2016 · Aug 9th 2016 PRELIMINARIES All slides and materials are online on Git hub: CONSTRAINED ORDINATION https://github.com/gavinsimpson/esa-advanced-vegan-2016

CANONICAL CORRESPONDENCE ANALYSIS

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

Two interfaces for specifying models

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

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
Figenvalues 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
```

6

REDUNDANCY ANALYSIS

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

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

```
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 RD2.1 393, 102.6 4.7.6 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

10

EIGENVALUES

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
 0.0047332
 0.0047332
 0.0047332

YOUR TURN

- Fit a CCA model to the lichen pasture data. The model should include, N, P, and K only.
- · Save the model in object mycca1
- How much variance is explained by this model?
- Extract the eigenvalues, how many constrained axes are there?

```
library("vegan")
data(varechem, varespec)
## ...your code here...
```

11

EXTRACTING AXIS SCORES

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)

13

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 λ_i
- scaling = 2 Focus on species, scale species scores by λ_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' σ
- scaling = 0 raw scores

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

EXTRACTING AXIS SCORES

SCALINGS...

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

15

YOUR TURN

• Using the CCA model you fitted, extract the site scores for axes 2 and 3 with Hill's scaling

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

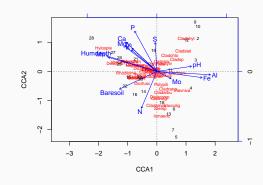
Or with the formula interface use the Condition() function

40

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)



YOUR TURN

17

- · Using mycca, draw a triplot of axes 2 and 3 with sites scaling
- Use the help file **?plot.cca** to help you work out how to do this

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)</pre>
vare.cca
Call: cca(formula = varespec ~ Al + P * (K + Baresoil), data =
            Inertia Proportion Rank
             2.083
Total
                      1.000
Constrained 1.046
                      0.502
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
0.27577 0.15411 0.13536 0.11803 0.08887 0.05511 0.04919 0.03781
(Showed only 8 of all 17 unconstrained eigenvalues)
```

22

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 >= 20 indicates strong collinearity in constraints
- · VIF >= 10 potentially of concern & should be looked at

Computed via vif.cca()

23

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)</pre>
```

trace = 0 is used her to turn off printing of progress

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

25

STEPWISE SELECTION IN CCA

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

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

```
Thertia 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
(Showed only 8 of all 20 unconstrained eigenvalues)
```

26

STEPWISE SELECTION IN CCA

mods2

test = "perm")

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,</pre>

ADJUSTED r^2 FOR LINEAR MODELS

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

- \cdot adding a variable to constraints will increase R^2
- \cdot the larger the number of constraints in the model the larger \mathbb{R}^2 is due to random correlations

Can attempt to account for this bias via an adjusted \mathbb{R}^2 measure

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

- \cdot 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 $\it r^2$

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^2_{adi} 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 α
 - The global R_{adi}^2
 - · Stop if next candidate model is non-significant or if R_{adi}^2 exceeds the global R_{adi}^2

Available in ordiR2step()

PERMUTATION TESTS IN VEGAN

29

30

PERMUTATION TESTS

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 $no\ effect$

PERMUTATION TESTS IN VEGAN

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 $Y = X + Z + \varepsilon$
 - The reduced model uses residuals from model $Y = X + Z + \varepsilon$
- 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

PERMUTATION TESTS IN VEGAN

A test statistic is required, computed for observed model & each permuted model vegan uses a pseudo-F statistic

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

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

34

PERMUTATION TESTS IN VEGAN: anova()

set seed(42)

- · The main user function is the anova() method
- \cdot It is an interface to the lower-level function <code>permutest.cca()</code>
- At its most simplest, the anova() method tests whether the "model" as a whole is significant

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

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)
```

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

35

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

37

39

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 kth variable to the model
- · Ordering of the terms will affect the results

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

38

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

YOUR TURN - SPRING MEADOWS

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

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("data/meadow-spp.csv", header = TRUE, row.names = 1)
env <- read.csv("data/meadow-env.csv", header = TRUE, row.names = 1)</pre>
```

42

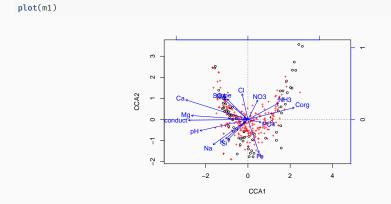
CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

m1 <- cca(spp ~ ., data = env)

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

43

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

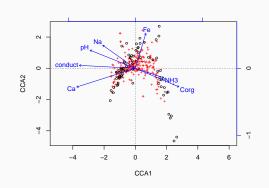


CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

```
lwr <- cca(spp ~ 1, data = env)</pre>
m2 <- ordistep(lwr, scope = formula(m1), trace = FALSE)</pre>
Call: cca(formula = spp ~ Ca + conduct + Corg + Na + NH3 + Fe +
pH, data = env)
            Inertia Proportion Rank
             5.3107 1.0000
Constrained 0.9899
                       0.1864
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
(Showed only 8 of all 62 unconstrained eigenvalues)
                                                                                                                              45
```

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

plot(m2)



46

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
```

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

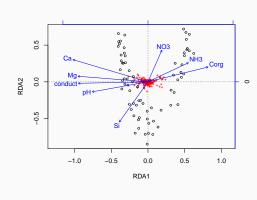
Alternative is RDA with a transformation

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

47

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

plot(m4)



CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

Stepwise using R_{adi}^2

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

```
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
```

50

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
- ٠..

49

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

RESTRICTED PERMUTATIONS WITH PERMUTE

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

53

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

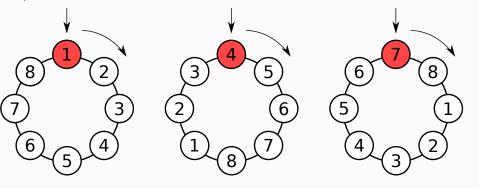
The variation *between* blocks should be excluded from the test; **permute** doesn't do this for you!

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



RESTRICTED PERMUTATIONS WITH PERMUTE | TIME SERIES & LINEAR TRANSECTS

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

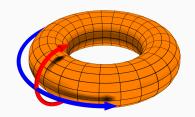
[1] 9 10 1 2 3 4 5 6 7 8

58

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



Source: Dave Burke, Wikimedia @:

RESTRICTED PERMUTATIONS WITH PERMUTE | WHOLE-PLOTS & SPLIT-PLOTS I

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-splots (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

RESTRICTED PERMUTATIONS WITH PERMUTE | WHOLE-PLOTS & SPLIT-PLOTS II

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

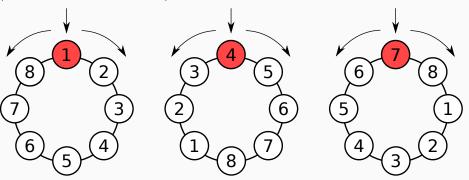
If the split-plots are parallel time series & time is an autocorrelated error component affecting all series then the same cyclic shift can be applied to each time series (within each whole-plot) (constant = TRUE)

61

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)



6

RESTRICTED PERMUTATIONS WITH PERMUTE | MIRRORING

Hence the series (1, 2, 3, 4) and (4, 3, 2, 1) are equivalent fom 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

RESTRICTED PERMUTATIONS WITH PERMUTE | DESIGNING PERMUTATION SCHEMES

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 generated 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!

RESTRICTED PERMUTATIONS WITH PERMUTE | DESIGNING PERMUTATION SCHEMES

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))</pre>
```

66

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

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
```

67

RESTRICTED PERMUTATIONS WITH PERMUTE | TIME SERIES EXAMPLE I

Time series within 3 plots, 10 observation each

RESTRICTED PERMUTATIONS WITH PERMUTE | TIME SERIES EXAMPLE II

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

69

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

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

OHRAZ CASE STUDY

72

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

Analyse the Ohraz data Case study 5 of Leps & Smilauer

```
## load vegan
library("vegan")
## load the data
spp <- read.csv("data/ohraz-spp.csv", header = TRUE, row.names = 1)</pre>
env <- read.csv("data/ohraz-env.csv", header = TRUE, row.names = 1)</pre>
molinia <- spp[, 1]</pre>
spp <- spp[, -1]
## Year as numeric
env <- transform(env, year = as.numeric(as.character(year)))</pre>
```

73

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

```
c1 <- rda(spp ~ year + year:mowing + year:fertilizer + year:removal + Condition(plotid), data = env)</pre>
(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
                                                                                                   74
```

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 =
        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 =
        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

HIERARCHICAL ANALYSIS OF CRAYFISH

Variation in communities may exist at various scales, sometimes hierarchically

A firt step in understanding this variation is to test for its exisistence

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

78

HIERARCHICAL ANALYSIS OF CRAYFISH

HIERARCHICAL ANALYSIS OF CRAYFISH — UNCONSTRAINED

Number of samples have 0 crayfish, which excludes unimodal methods

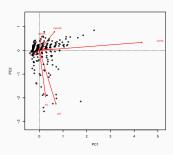
```
m.pca <- rda(crayfish)
summary(eigenvals(m.pca))</pre>
```

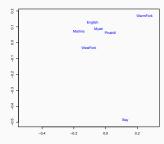
Importance of components:

/

HIERARCHICAL ANALYSIS OF CRAYFISH — UNCONSTRAINED

```
layout(matrix(1:2, ncol = 2))
biplot(m.pca, type = c("text", "points"), scaling = "species")
set.seed(23)
ev.pca <- envfit(m.pca ~ Watershed, data = design, scaling = "species")
plot(ev.pca, labels = levels(design$Watershed), add = FALSE)
layout(1)</pre>
```





81

HIERARCHICAL ANALYSIS OF CRAYFISH — WATERSHED SCALE

```
m.ws <- rda(crayfish ~ Watershed, data = design)</pre>
Call: rda(formula = crayfish ~ Watershed, data = design)
            Inertia Proportion Rank
Total
             9.3580 1.0000
Constrained 1.7669
                      0.1888
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 — STREAM SCALE

```
m.str <- rda(crayfish ~ Stream + Condition(Watershed), data = design)</pre>
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 — WATERSHED SCALE

```
summary(eigenvals(m.ws, constrained = TRUE))
Importance of components:
                      RDA1 RDA2 RDA3 RDA4 RDA5 RDA6
                    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))</pre>
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)
          6 1.7669 21.724 0.002 **
Residual 560 7.5911
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
                                                                                                                                   83
```

84

HIERARCHICAL ANALYSIS OF CRAYFISH — STREAM SCALE

```
summary(eigenvals(m.str, constrained = TRUE))
Importance of components:
                    RDA1 RDA2 RDA3 RDA4 RDA5 RDA6
                   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
                   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))</pre>
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
                                                                                                                                 85
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))</pre>
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
                                                                                         86
```

HIERARCHICAL ANALYSIS OF CRAYFISH — REACH SCALE

```
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "none"),</pre>
            plots = with(design, Plots(strata = Run, type = "free")),
           blocks = with(design, Stream))
(sig.re <- anova(m.re, permutations = ctrl))</pre>
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 = cravfish ~ Reach + Condition(Stream), data = design)
                        F Pr(>F)
        Df Variance
Model 42 1.4829 3.5875 0.002 **
Residual 504 4.9603
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
                                                                                                 87
```

HIERARCHICAL ANALYSIS OF CRAYFISH — RUN SCALE

```
(m.run <- rda(cravfish ~ 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

OTHER STUFF

89

DIAGNOSTICS FOR CONSTRAINED ORDINATIONS

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

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

DIAGNOSTICS FOR CONSTRAINED ORDINATIONS | GOODNESS OF FIT

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

91

DIAGNOSTICS FOR CONSTRAINED ORDINATIONS | GOODNESS OF FIT

```
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
```

93

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

intersetcor(mods)

· focuses on a single constraint-axis combination at a time

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

```
CCA1 CCA2 CCA3
Al 0.7356151 -0.1302334 0.4259623
P -0.3589749 -0.6110772 0.4478933
K -0.3768462 -0.1339192 0.7760441
```

LINKS

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

RE-USE

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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.