ADVANCED COMMUNITY DATA ANALYSIS USING VEGAN **PRELIMINARIES** Gavin L. Simpson ESA 2017 · Aug 6th 2017 PRELIMINARIES CONSTRAINED ORDINATION All slides and materials are online on Git hub: 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)</pre>
cca1
Call: cca(formula = varespec ~ N + P + K + Ca + Mg + S + Al + Fe +
Mn + Zn + Mo + Baresoil + Humdepth + pH, data = varechem)
           Inertia Proportion Rank
         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 \sim ., data = varechem)
```

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

Inertia Proportion Rank

Total 125.6594 1.0808

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 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 ${\it cca}($) etc some of these components will be ${\it NULL}$

\$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

9

EIGENVALUES

lise eigenvals() to extract Figenvalues from a fitted ordination object eigenvals(ccal)

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
0.0858392 0.0311408 0.0132944 0.0083644 0.0065385 0.0061563 0.0047332
CA1 CA2 CA3 CA4 CA5
0.1977645 0.1419256 0.1011741 0.0707868 0.0533034 0.0332994 0.0188676
CA8 CA9
0.0151044 0.0094876

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

12

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)

EXTRACTING AXIS SCORES

. . .

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 $\sqrt{\lambda_i}$
- \cdot 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)
```

SCALINGS...

Thankfully we can use alternative descrpitors 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

.

13

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

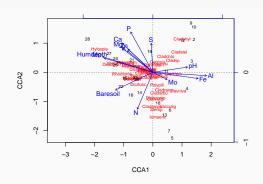
Argument Z is used for a data frame of variables to partial out

Or with the formula interface use the Condition() function

18

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(ccal)



YOUR TURN

- · 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

19

17

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> 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)

22

21

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 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 ~ Al + P + K, data = varechem)

Inertia Proportion Rank

Total 2.0832 1.0808

Constrained 0.6441 0.3892 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 expression)
```

26

STEPWISE SELECTION IN CCA

The \$anova component contains a summary of the steps involved in automatic model huilding

```
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

with the came final model

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

```
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
```

mods2 <- step(upr, scope = list(lower = formula(lwr), upper = formula(upr)), trace = 0,</pre>

27

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 \mathbb{R}^2 is due to random correlations

Can attempt to account for this bias via an adjusted R² 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 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_{adi}^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 α
 - The global R_{odi}^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

PERMUTATION TESTS IN VEGAN

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*

31

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()

- 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 _utoff = 1 = crone = NULL;

NULL strate of ect is the fifted ordination

- permutations controls what is permuted and how
- \cdot by determines what is tested; the default is to test the model

35

33

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

37

39

38

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 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>

41

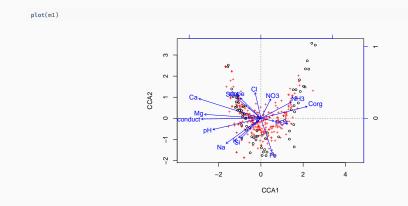
43

42

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

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

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

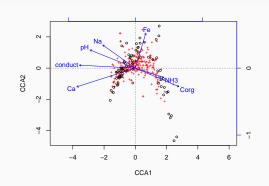


CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

```
set.seed(67)
lwr <- cca(spp ~ 1, data = env)
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
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
(Showed only 8 of all 62 unconstrained eigenvalues)
```

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

plot(m2)



46

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

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

m2\$anova

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

Alternative is RDA with a transformation

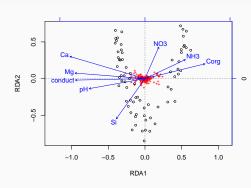
spph <- decostand(spp, method = "hellinger")
m3 <- rda(spph ~ ., data = env)
lwr <- rda(spph ~ 1, data = env)
m4 <- ordistep(lwr, scope = formula(m3), trace = FALSE)</pre>

47

45

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

plot(m4)



49

RESTRICTED PERMUTATION TESTS

CONSTRAINED ORDINATION WORKED EXAMPLE | SPRING MEADOW VEGETATION

Stepwise using R_{adi}^2

```
m5 <- ordiR2step(lwr, scope = formula(m3), trace = FALSE)</pre>
m5$anova
               R2.adj Df AIC
                                    F Pr(>F)
              0.12588 1 -41.779 10.9370 0.002 **
+ Ca
              0.14628 1 -42.468 2.6242 0.002 **
+ NH3
              0.16322 1 -42.925 2.3570 0.002 **
+ conduct
              0.17711 1 -43.164 2.1136 0.002 **
+ Si
+ Corg
              0.18518 1 -42.940 1.6442 0.006 **
              0.19257 1 -42.680 1.5853 0.018 *
+ NO3
             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

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...

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

51

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
- \cdot 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

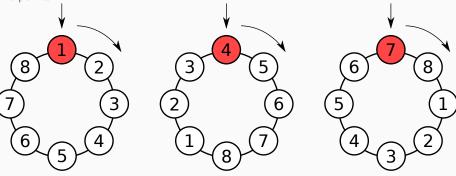
Using blocks you can achieve what the **strata** argument used to in **vegan**; needs to be a factor variable

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

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

58

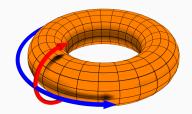
RESTRICTED PERMUTATIONS WITH PERMUTE | SPATIAL GRIDS

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

matrix(perm, ncol = 3)

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



Source: Dave Burke, Wikimedia 🐵 🕦

59

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

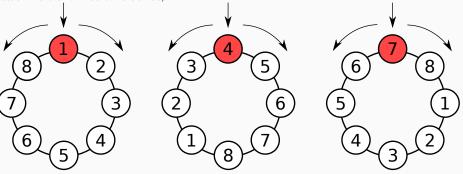
If the split-plots are parallel time series & time is an autocorrelated error component affecting all series then the same cyclic shift can be applied to each time series (within each whole-plot) (constant = TRUE)

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)



62

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

- 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

65

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)
```

67

RESTRICTED PERMUTATIONS WITH PERMUTE | TIME SERIES EXAMPLE I

Time series within 3 nlots 10 observation each plt <- gl(3, 10)

69

OHRAZ CASE STUDY

RESTRICTED PERMUTATIONS WITH PERMUTE | TIME SERIES EXAMPLE II

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

70

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

71

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

```
Analyse the Ohraz data Case study 5 of Lens & Smilauer

## load vegan

## load the data

spc - read.csv(*data/ohraz-spc.csv*, header = TRUE, row.names = 1)

env - read.csv(*data/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</pre>
```

74

76

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

RESTRICTED PERMUTATIONS WITH PERMUTE | WORKED EXAMPLE WITH VEGAN

75

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

77

78

HIERARCHICAL ANALYSIS OF CRAYFISH

HIERARCHICAL ANALYSIS OF CRAYFISH — UNCONSTRAINED

Number of samples have 0 cravfish which excludes unimodal methods m.pca <- rda(crayfish)

summary(eigenvals(m.pca))

Importance of components:

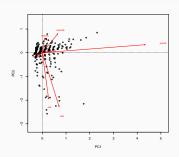
| PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC7

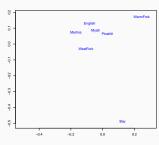
PC8 PC9 PC10
Eigenvalue 0.21225 0.20528 0.004881
Proportion Explained 0.02268 0.02194 0.000520
Cumulative Proportion 0.97754 0.99948 1.000000

79

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>
m.ws
Call: rda(formula = crayfish ~ Watershed, data = design)
            Inertia Proportion Rank
             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
```

82

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
set.seed(1)
ctrl <- how(nperm = 499, within = Within(type = "none"),</pre>
           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
```

HIERARCHICAL ANALYSIS OF CRAYFISH — STREAM SCALE

```
m.str <- rda(cravfish ~ 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
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
                 0.006326 0.003042 0.002894 0.0001546
Proportion Explained 0.005510 0.002650 0.002520 0.0001300
  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)
       14 1.1478 6.9477 0.004 **
Model
Residual 546 6.4433
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
                                                                                                                           85
```

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"),
           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 = 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
                                                                                                                                    87
```

HIERARCHICAL ANALYSIS OF CRAYFISH — RUN SCALE

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

90

DIAGNOSTICS FOR CONSTRAINED ORDINATIONS

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

- · goodness()
- · inertcomp()
- \cdot 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

head(goodness(mods)) CCA1 CCA2 CCA3 Callvulg 0.0062471656 0.318907619 0.8254657 Empenigr 0.1164701677 0.137604994 0.1953245 Rhodtome 0.0999089739 0.169697909 0.1824153 Vaccmyrt 0.2361482843 0.246916323 0.2406730 Vaccviti 0.1523704591 0.156502301 0.2110550 Pinusulv a BABOQZALO33 A BABARDA7A a BABARDAGA head(goodness(mods, summarize = TRUE)) Callvulg Empenigr Rhodtome Vaccmyrt Vaccviti Pinusylv

0.8254657 0.1953245 0.1824153 0.2406730 0.2110550 0.0060096

93

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
```

94

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)

Retter models can have nonrer 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
intersector(mods)

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

95

LINKS

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

RE-USE

Copyright ©(2015–16) Gavin L. Simpson *Some Rights Reserved*Unless indicated otherwise, this slide deck is licensed under a Creative Commons
Attribution 4.0 International License.



REFERENCES

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