Permutation tests

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Welcome

Today's topics

- Restricted permutation tests
- PERMANOVA
- Distance-based RDA
- Diagnostics

Permutation tests

RDA has lots of theory behind it, CCA bit less. 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
 - \circ The full model uses residuals from model Y=X+Z+arepsilon
 - \circ The *reduced model* uses residuals from model Y=Z+arepsilon
- In vegan which is used can be set via argument model with "direct", "full", and "reduced" respectively

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

 \mathbf{vegan} uses a pseudo F statistic

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

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

```
cca1 ← cca(varespec ~ ., data = varechem)
pstat ← permustats(anova(cca1))
summary(pstat)
```

```
##

## statistic SES mean lower median upper Pr(perm)

## Model 1.4441 2.2865 1.0158 1.0011 1.3634 0.021 *

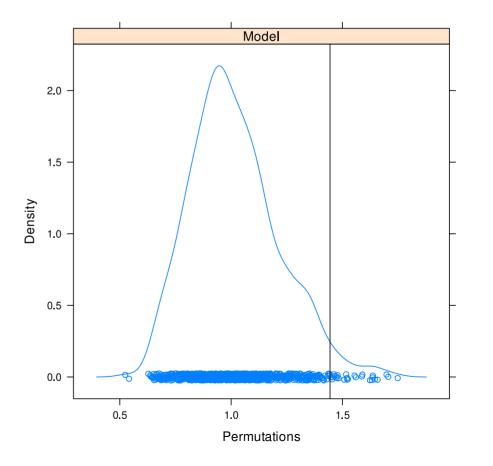
## ---

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

##

## (Interval (Upper - Lower) = 0.95)
```

densityplot(pstat)



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

Permutation tests in vegan: anova()

$$F = rac{1.4415/14}{0.6417/9} = 1.4441$$

```
set.seed(42)
(perm ← anova(cca1))
```

Restricted permutation tests

Restricted permutation tests

What is shuffled and how is of **paramount** importance for a valid test

Complete randomisation assumes a null hypothesis where all observations are *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 **permute** package 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 (friendly!) 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 & samples within plots form time series

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

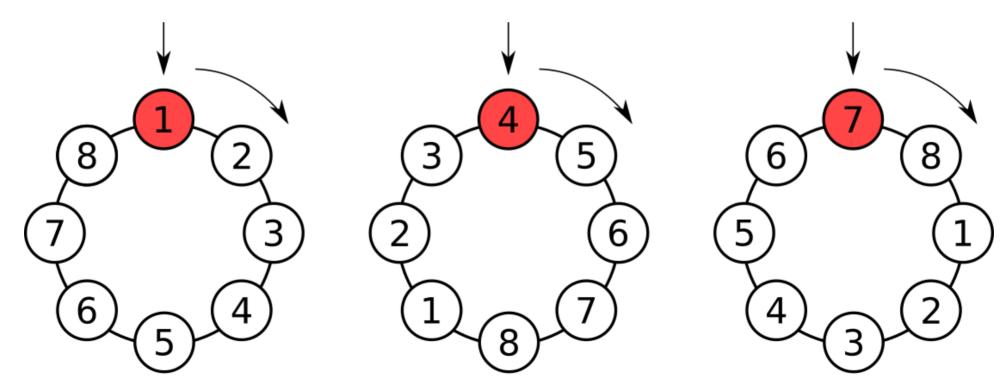
The variation *between* blocks should be excluded from the test; **permute** doesn't do this for you!

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

Time series & linear transects

Can link randomly starting point of one series to any time point of another series if series are stationary under H_0 that series are unrelated

Achieve this via cyclic shift permutations — wrap series into a circle



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

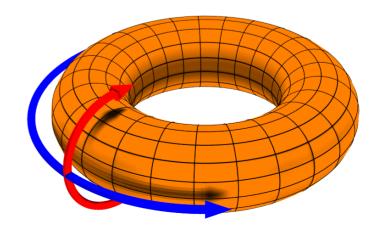
Spatial grids

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 CC BY

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



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)

Permute one or both, 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

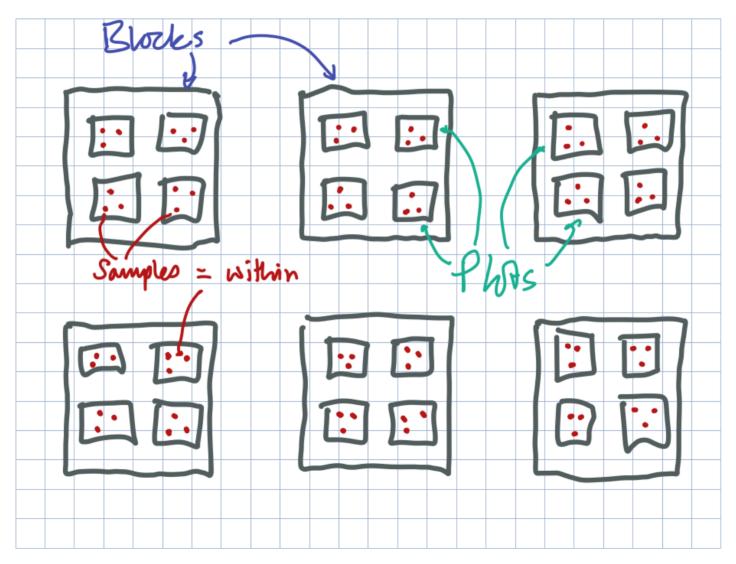
Test effect of constraints that vary within whole-plots by permuting the split-plots within whole-plots without permuting the whole-plots

Whole-plots & split-plots II

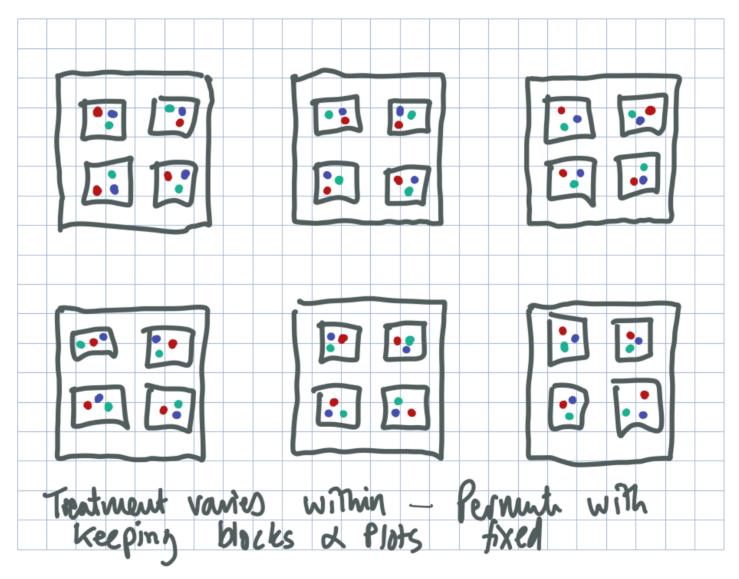
Whole-plots or split-plots, or both, can be time series, linear transects or rectangular grids in which case the appropriate restricted permutation is used

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)

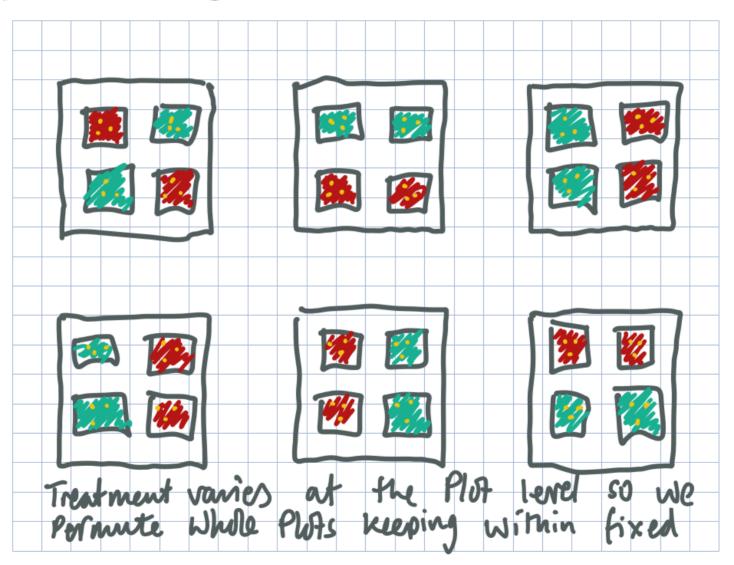
Split plot designs



Split plot designs



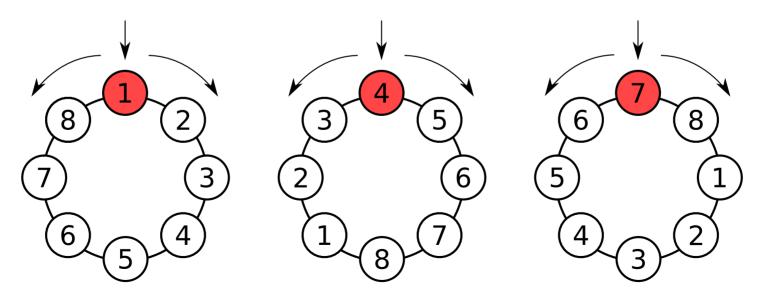
Split plot designs



Mirrored permutations

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)



Mirrored permutations

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

Sets of permutations — no free lunch

Restricted severely reduce the size of the set of 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

Sets of permutations — no free lunch

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

```
Use complete = TRUE in the call to how() — perhaps also increase maxperm
```

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!

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))
```

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
```

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
```

Time series example I

Time series within 3 plots, 10 observation each

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

Time series example II

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

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

Ohraz Case Study

Restricted permutations | Ohraz

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 \( \tau \text{read.csv("data/ohraz-spp.csv", header = TRUE, row.names = 1)} \)
env \( \tau \text{read.csv("data/ohraz-env.csv", header = TRUE, row.names = 1)} \)
molinia \( \tau \text{spp[, 1]} \)
spp \( \tau \text{spp[, -1]} \)

## Year as numeric
env \( \tau \text{transform(env, year = as.numeric(as.character(year)))} \)
```

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

```
## Permutation Design:
## Blocks:
    Defined by: none
##
## Plots:
    Plots: env$plotid
    Permutation type: none
    Mirrored?: No
## Within Plots:
    Permutation type: free
## Permutation details:
    Number of permutations: 199
    Max. number of permutations allowed: 9999
     Evaluate all permutations?: No. Activation limit: 5040
```

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

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

```
## Permutation test for rda under reduced model
## Forward 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 0.025 *
## RDA4 1 8.94 1.4458 0.485
## Residual 90 556.30
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Hierarchical analysis of crayfish

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 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

Hierarchical analysis of crayfish

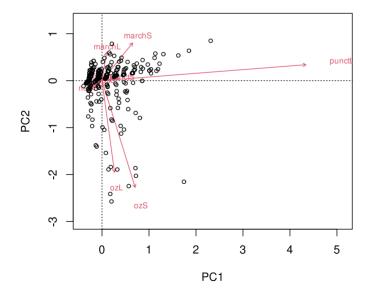
Crayfish — Unconstrained

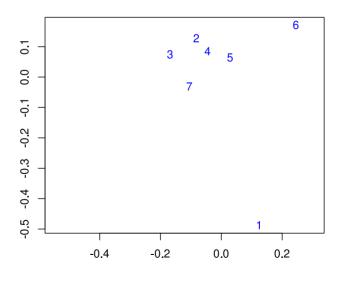
A number of samples have 0 crayfish, which excludes unimodal methods

```
m.pca \leftarrow rda(crayfish)
 summary(eigenvals(m.pca))
## Importance of components:
                           PC1
                                  PC2
                                                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
                                    PC9
                                             PC10
###
                            PC8
## Eigenvalue
               0.21225 0.20528 0.0048809
## Proportion Explained 0.02268 0.02194 0.0005216
## Cumulative Proportion 0.97754 0.99948 1.0000000
```

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)
```





Crayfish — Watershed scale

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

```
## Call: rda(formula = cravfish ~ 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
                                                                   PC10
## 3.0957 1.2109 0.9717 0.7219 0.5333 0.3838 0.2772 0.2040 0.1879 0.0048
```

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 0.0012791

## Proportion Explained 0.3968 0.3135 0.2072 0.0602 0.02159 0.0007239

## Cumulative Proportion 0.3968 0.7103 0.9175 0.9777 0.99928 1.0000000
```

Crayfish — Watershed scale

```
## 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 '**' 0.05 '.' 0.1 ' ' 1
```

Crayfish — Stream scale

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

```
## Call: rda(formula = cravfish ~ 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 or conditions were aliased because they were 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
                                                                   PC10
###
                                               PC7
                                                      PC8
## 2.7853 0.8528 0.7737 0.6317 0.5144 0.2808 0.2517 0.1923 0.1559 0.0046
```

Crayfish — **Stream scale**

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

```
## Importance of components:
                                                         RDA5
                                                                          RDA7
                           RDA1
                                  RDA2
                                         RDA3
                                                 RDA4
                                                                 RDA6
## Eigenvalue
                        0.4928 0.2990 0.2058 0.07824 0.03719 0.02235 0.006326
## Proportion Explained 0.4293 0.2605 0.1793 0.06816 0.03240 0.01947 0.005511
## Cumulative Proportion 0.4293 0.6898 0.8691 0.93731 0.96971 0.98918 0.994694
                                      RDA9
                                               RDA10
###
                             RDA8
## Eigenvalue
                       0.003042 0.002894 0.0001546
## Proportion Explained 0.002651 0.002521 0.0001347
## Cumulative Proportion 0.997344 0.999865 1.0000000
```

Crayfish — Stream scale

Crayfish — Reach scale

```
(m.re \leftarrow rda(crayfish \sim Reach + Condition(Stream), data = design))
```

```
## Call: rda(formula = cravfish ~ 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 or conditions were aliased because they were 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
                                                                    PC10
## 2.1635 0.6080 0.5605 0.5166 0.3749 0.2212 0.2052 0.1588 0.1477 0.0040
```

Crayfish — Reach scale

Crayfish — Run scale

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

```
## Call: rda(formula = cravfish ~ 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 or conditions were aliased because they were redundant
##
## Eigenvalues for constrained axes:
     RDA1
            RDA2
                  RDA3
                          RDA4
                                RDA5
                                       RDA6
                                              RDA7
                                                                  RDA10
###
                                                     RDA8
                                                            RDA9
### 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
                                                                   PC10
## 1.3137 0.4165 0.3832 0.2759 0.2378 0.1725 0.1215 0.1130 0.1016 0.0021
```

Crayfish — Run scale