Workshop 10: Advanced multivariate analyses

QCBS R Workshop Series

Québec Centre for Biodiversity Science

About this workshop

Required packages

- Hmisc
- labdsv
- MASS
- vegan

```
install.packages(c('Hmisc', 'labdsv', 'MASS', 'vegan'))
```

Learning objectives

Use R to perform unconstrained ordinations

Introduction

Introduction

This workshop is an extension of Workshop 9, which covered the basics of unconstrained analyses:

- Distance metrics and transformations
- Hierarchical clustering
- Unconstrained ordinations (PCA, PCoA, CA, nmDS)

These identify patterns in community composition data or in descriptors, **without** exploring how environmental variables could be driving these patterns.

Introduction

In this workshop, we will focus instead on **constrained** analyses:

- Redundancy analysis (RDA)
- Partial redundancy analysis
- Variation partitioning
- Multivariate regression tree (MRT)
- Linear discriminant analysis (LDA)

These analyses allow us to **describe** and **predict** relationships between community composition data and environmental variables. (This means we can **test hypotheses**!)

Download today's script and data

All data and code can be found at qcbs.ca/wiki/r_workshop10

For this workshop, you will need:

- R script
- Data:
  - DoubsEnv data
  - DoubsSpe data
  - DoubsSpa data
  - Test data for linear discriminant analyses

Required packages

Please make sure you have downloaded, installed, and loaded these packages:

- vegan (for multivariate analyses)
- rdaTest package **(from wiki)**
- mvpart package **(from wiki)**
- MVPARTwrap package **(from wiki)**
- labdsv (to identify indicator species in the MRT)
- MASS (for LDA)

Follow along!

As always, we recommend that you:

- create your own script (or add comments to the provided script)
- avoid copy-pasting or running the code directly from the script
- remember to set the working directory to the folder in which your files are stored

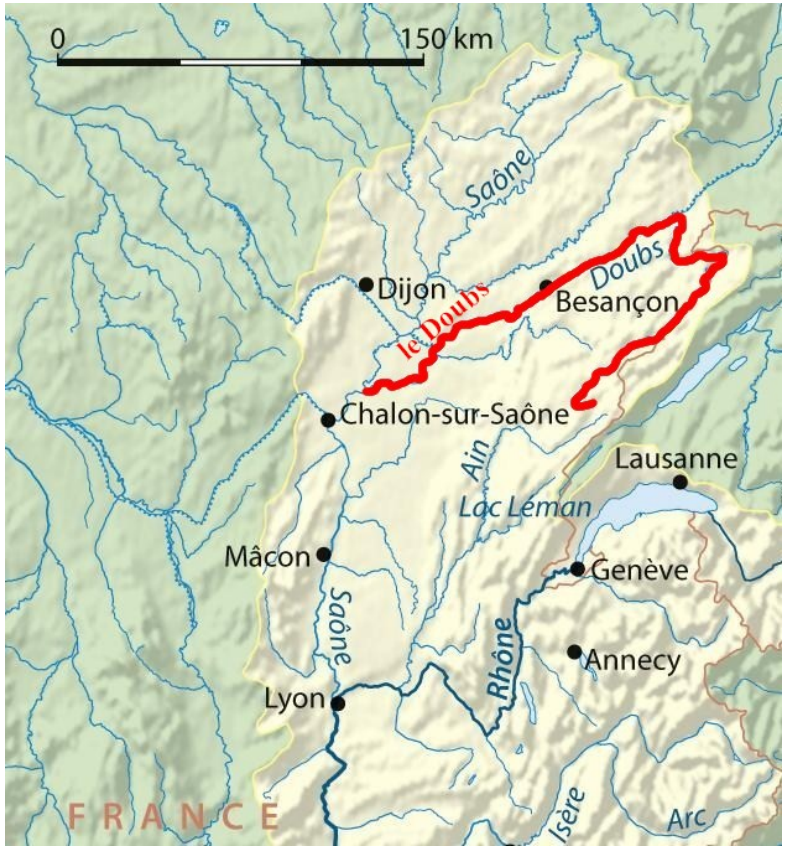
Data exploration & preparation

Today's data:

Doubs River Fish Dataset (Verneaux 1973)

Description of fish communities along the Doubs River:

- 27 species
- 30 sites
- 11 environmental variables



Load the Doubs River dataset

Make sure the datasets are in your working directory!

Load the species data (*doubsspe.csv*):

```
# Make sure the files are in your working directory!
spe ← read.csv("data/doubsspe.csv", row.names = 1)
spe ← spe[-8,] # remove site with no data
```

Load the environmental data (*doubsenv.csv*):

```
env ← read.csv("data/doubsenv.csv", row.names = 1)
env ← env[-8,] # remove site with no data
```

Note: Only execute once!

Exploring the fish community dataset

Let's briefly explore the fish community dataset:

```
names(spe) # names of objects (species)
#  [1] "CHA" "TRU" "VAI" "LOC" "OMB" "BLA" "HOT" "TOX" "VAN" "CHE" "BAR" "SPI"
# [13] "GOU" "BRO" "PER" "BOU" "PSO" "ROT" "CAR" "TAN" "BCO" "PCH" "GRE" "GAR"
# [25] "BBO" "ABL" "ANG"
dim(spe) # dataset dimensions
# [1] 29 27
```

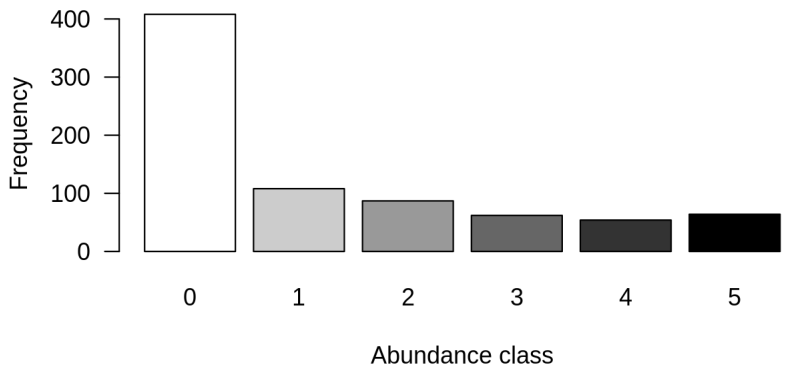
Let's take a closer look at the objects (species):

```
head(spe) # look at first 5 rows
str(spe) # structure of objects in dataset
summary(spe) # summary statistics for all objects (min, mean, max, etc.)
```

Distribution of species frequencies

Let's take a quick look at how the community is structured.

```
# Count number of species frequencies in each abundance class
ab ← table(unlist(spe))
# Plot distribution of species frequencies
barplot(ab, las = 1, # make axis labels perpendicular to axis
        xlab = "Abundance class", ylab = "Frequency", # label axes
        col = grey(5:0/5)) # 5-colour gradient for the bars
```



Notice: There are many 0s!

Distribution of species frequencies

How many 0s are in the dataset?

```
sum(spe == 0)
# [1] 408
```

What proportion of the dataset does that represent?

```
sum(spe==0)/(nrow(spe)*ncol(spe))
# [1] 0.5210728
```

Transforming the community data

Over 50% of our dataset consists of 0s, which is common in community datasets.

However, we don't want these common absences to **artificially increase** the similarity between sites.

- To avoid this, we can **transform** the community data.

Let's use the **Hellinger** transformation:

```
# The decostand() function in the vegan package makes this easy for us:
library(vegan)
spe.hel ← decostand(spe, method = "hellinger")
```

Exploring the environmental dataset

Let's briefly explore the environmental dataset:

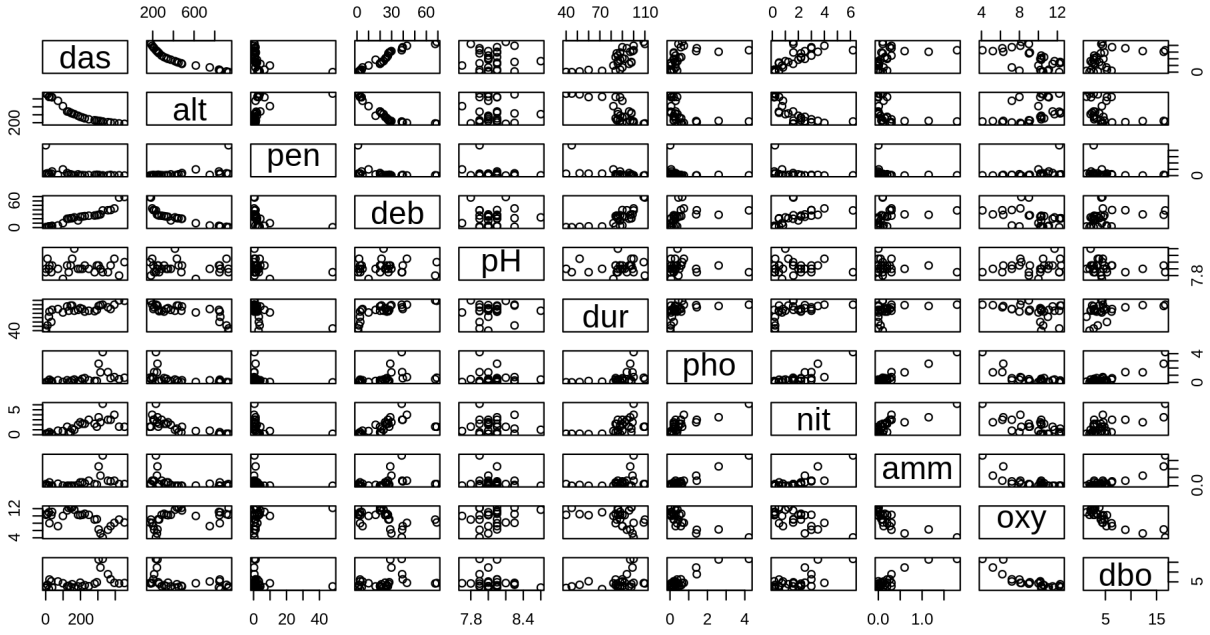
```
names(env) # names of objects (environmental variables)
#  [1] "das" "alt" "pen" "deb" "pH"  "dur" "pho" "nit" "amm" "oxy" "dbo"
dim(env) # dataset dimensions
# [1] 29 11
head(env) # look at first 5 rows
#    das alt  pen  deb  pH dur  pho  nit  amm  oxy dbo
# 1  0.3 934 48.0 0.84 7.9  45 0.01 0.20 0.00 12.2 2.7
# 2  2.2 932  3.0 1.00 8.0  40 0.02 0.20 0.10 10.3 1.9
# 3 10.2 914  3.7 1.80 8.3  52 0.05 0.22 0.05 10.5 3.5
# 4 18.5 854  3.2 2.53 8.0  72 0.10 0.21 0.00 11.0 1.3
# 5 21.5 849  2.3 2.64 8.1  84 0.38 0.52 0.20  8.0 6.2
# 6 32.4 846  3.2 2.86 7.9  60 0.20 0.15 0.00 10.2 5.3
```

For a closer look at the objects (environmental variables):

```
str(env) # structure of objects in dataset
summary(env) # summary statistics for all objects (min, mean, max, etc.)
```

Collinearity

```
# We can visually look for correlations between variables:
pairs(env)
```



Note: Some variables look correlated... (das vs. alt, das vs. deb, das vs. dur, das vs. nit, oxy vs. dbo, etc.)

Standardizing the environmental variables

You cannot compare the effects of variables with different units.
Before moving on to further analyses, **standardizing** your environmental variables is therefore crucial.

```
# Scale and center variables
env.z ← decostand(env, method = "standardize")

# Variables are now centered around a mean of 0:
round(apply(env.z, 2, mean), 1)
# das alt pen deb  pH dur pho nit amm oxy dbo
#   0   0   0   0   0   0   0   0   0   0   0

# and scaled to have a standard deviation of 1
apply(env.z, 2, sd)
# das alt pen deb  pH dur pho nit amm oxy dbo
#   1   1   1   1   1   1   1   1   1   1   1
```

Canonical analyses

Canonical analyses

Canonical analyses allow us to:

- identify relationships between a response matrix and explanatory matrix/matrices
- test hypotheses about these relationships
- make predictions

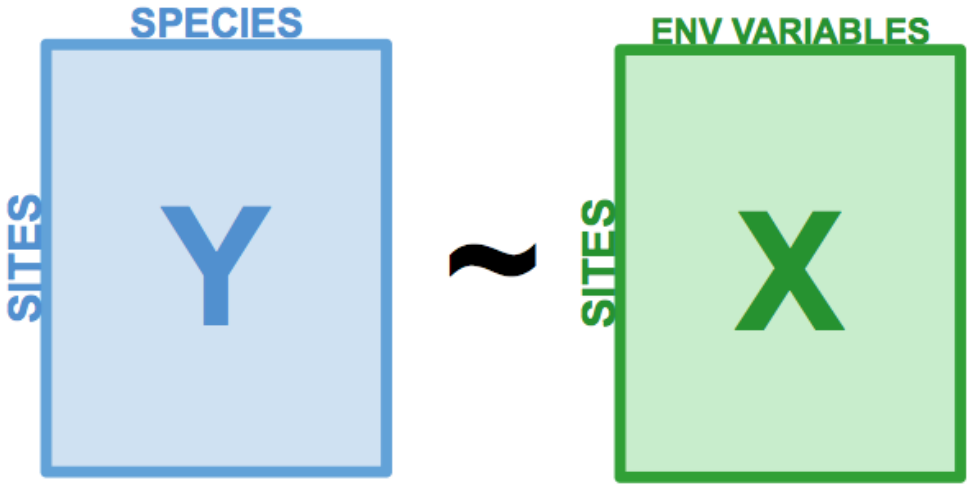
Canonical analyses

Redundancy analysis (RDA)

Redundancy analysis (RDA)

RDA is constrained ordination.

- RDA is a direct extension of multiple regression.
- RDA models the effect of an explanatory matrix on a response matrix *(instead of a single response variable)*.



Variables can be quantitative, qualitative, or binary (0/1).

- **transform** and/or **standardize** them prior to running an RDA.

Running an RDA in R

Prepare the data

```
# We'll use our standardized environmental data
# But we will remove 'das', which was correlated with many other variables:
env.z ← subset(env.z, select = -das)
```

Run the RDA

```
# Model the effect of all environmental variables on fish community composition
spe.rda ← rda(spe.hel ~ ., data = env.z)
```

Extract key results of the RDA

```
summary(spe.rda, display = NULL)
```

RDA output in R

```
Call:
rda(formula = spe.hel ~ alt + pen + deb + pH + dur + pho + nit +
amm + oxy + dbo, data = env.z)

Partitioning of variance:
              Inertia Proportion
Total          0.5025     1.0000
Constrained    0.3689     0.7341
Unconstrained  0.1336     0.2659
```

- **Constrained Proportion**: variance of Y explained by X **(73.41%)**
- **Unconstained Proportion**: unexplained variance in Y **(26.59%)**

How would you report these results?

- *The included environmental variables explain **73.41%** of the variation in fish community composition across sites.*

Selecting variables

Using **forward selection**, we can select the explanatory variables that are statistically "important".

Which variables significantly contribute to our model's explanatory power?

```
# Forward selection of variables:
fwd.sel ← ordiR2step(rda(spe.hel ~ 1, data = env.z), # lower model limit (simple
                scope = formula(spe.rda), # upper model limit (the "full" model)
                direction = "forward",
                R2scope = TRUE, # can't surpass the "full" model's R2
                pstep = 1000,
                trace = FALSE) # change to TRUE to see the selection process!
```

Here, we are essentially adding one variable at a time, and retaining it if it significantly increases the model's adjusted R2.

Selecting variables

- Which variables are retained by the forward selection?

```
# Check the new model with forward-selected variables
fwd.sel$call
# rda(formula = spe.hel ~ alt + oxy + dbo, data = env.z)
```

- What is the adjusted R2 of the RDA with the selected variables?

```
# Write our new model
spe.rda.signif ← rda(spe.hel ~ alt + oxy + dbo, data = env.z)
# check the adjusted R2
RsquareAdj(spe.rda.signif)
# $r.squared
# [1] 0.5894243
#
# $adj.r.squared
# [1] 0.5401552
```

Significance testing

Use **anova.cca()** to test the significance of your RDA.

```
anova.cca(spe.rda.signif, step = 1000)
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(formula = spe.hel ~ alt + oxy + dbo, data = env.z)
#          Df Variance      F Pr(>F)
# Model     3  0.29619 11.963  0.001 ***
# Residual 25  0.20632
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

You can also test the significance of each variable!

```
anova.cca(spe.rda.signif, step = 1000, by = "term")
```

RDA plot

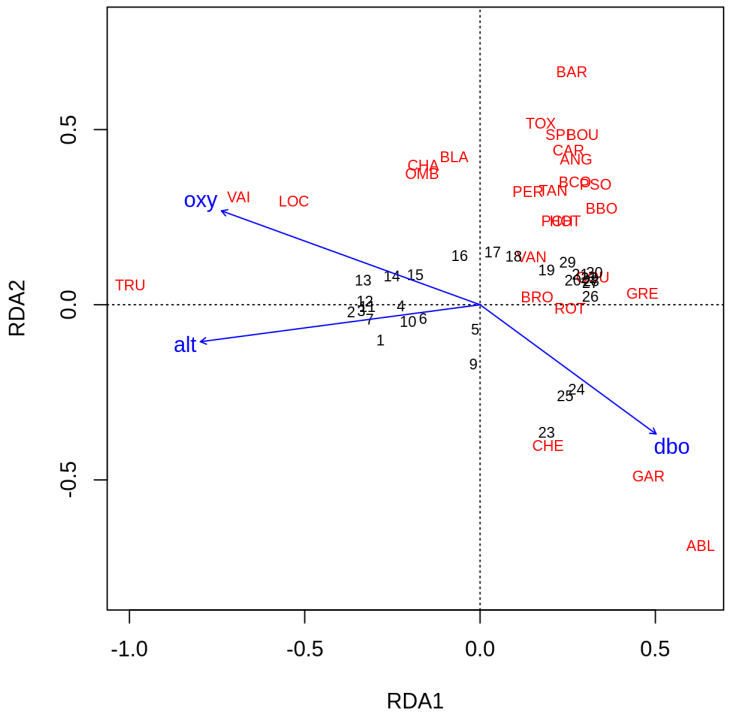
One of the most powerful aspects of RDA is the **simultaneous visualization** of your response and explanatory variables *(i.e. species and environmental variables)*.

There are 2 scaling options:

Type 1	Type 2
distances among objects reflect their similarities	angles between variables reflect their correlation

RDA plot: Type 1

```
ordiplot(spe.rda.signif,
         scaling = 1,
         type = "text")
```

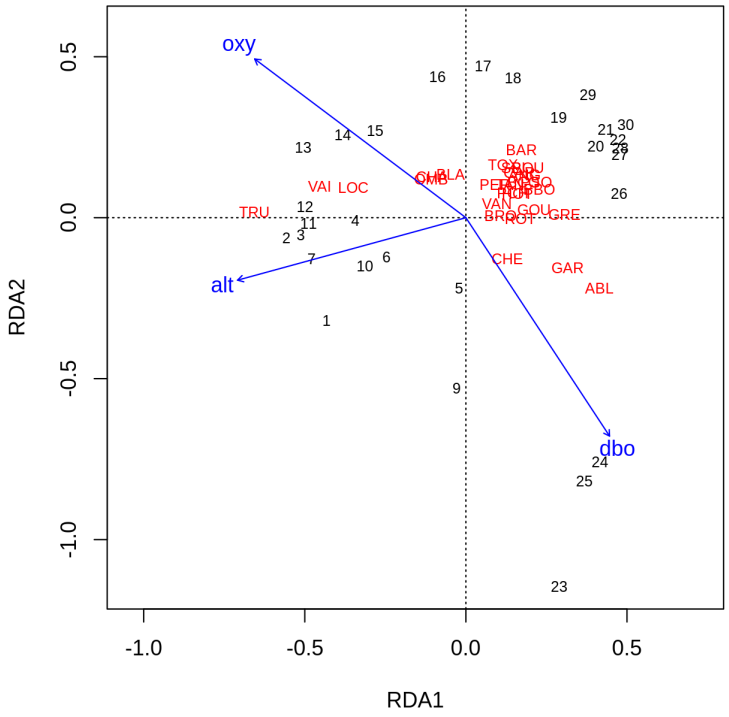


Scaling 1 shows similarities between objects in the *response matrix*.

- Sites (numbers) that are **closer together** have more similar communities.
- Species that are **closer together** occupy more sites in common.

RDA plot: Type 2

```
ordiplot(spe.rda.signif,
         scaling = 2,
         type = "text")
```

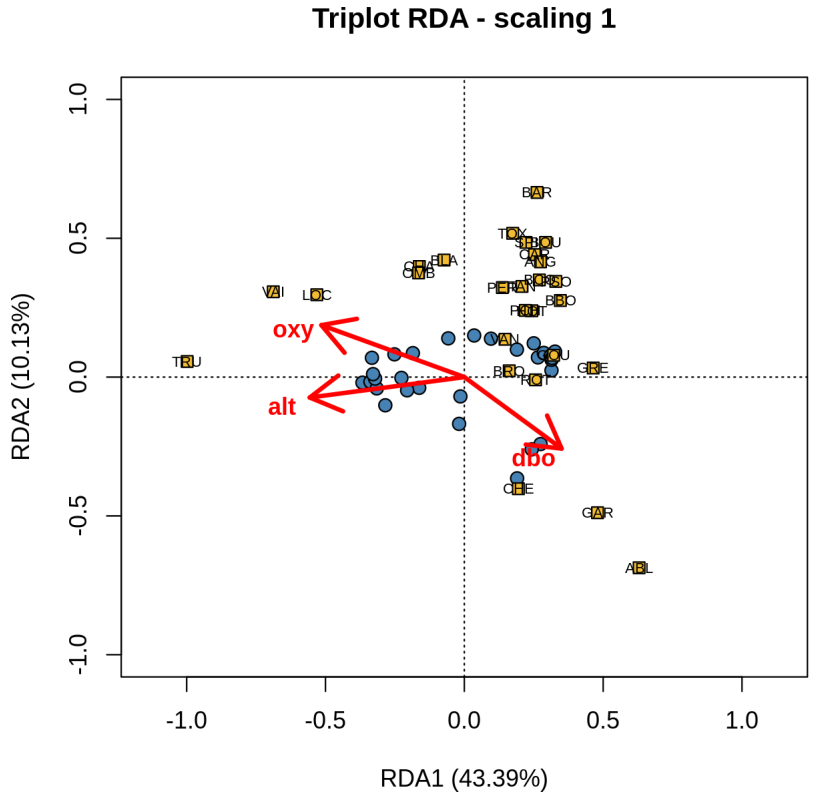


Scaling 2 shows the effects of *explanatory variables*.

- **Longer** arrows mean this variable strongly drives the variation in the community matrix.
- Arrows pointing in **opposite directions** have a negative relationship
- Arrows pointing in the **same direction** have a positive relationship

Customizing RDA plots

Both plot() and ordiplot() make quick and simple ordination plots, but you can customize your plots by manually setting the aesthetics of points, text, and arrows.



See the wiki page for more details!

Challenge 1

Run an RDA to model the effects of environmental variables on mite species abundances.

First, load the mite data:

```
# Load mite species abundance data
data("mite")

# Load environmental data
data("mite.env")
```

Recall some useful functions:

```
decostand()
rda()
ordiR2step()
anova.cca()
ordiplot()
```

Challenge 1: Solution

Step 1: Prepare the data

```
# Hellinger transform the community data
mite.spe.hel ← decostand(mite, method = "hellinger")

# Standardize quantiative environmental data
mite.env$SubsDens ← decostand(mite.env$SubsDens, method = "standardize")
mite.env$WatrCont ← decostand(mite.env$WatrCont, method = "standardize")
```

Challenge 1: Solution

Step 2: Select environmental variables

```
# Initial RDA with ALL of the environmental data
mite.spe.rda ← rda(mite.spe.hel ~ ., data = mite.env)

# Forward selection of environmental variables
fwd.sel ← ordiR2step(rda(mite.spe.hel ~ 1, data = mite.env),
                     scope = formula(mite.spe.rda),
                     direction = "forward",
                     R2scope = TRUE, pstep = 1000, trace = FALSE)
fwd.sel$call
# rda(formula = mite.spe.hel ~ WatrCont + Shrub + Substrate + Topo,
#     data = mite.env)
```

Challenge 1: Solution

Step 3: Run RDA and check adjusted R2

```
# Re-run the RDA with the significant variables
mite.spe.rda.signif ← rda(mite.spe.hel ~ WatrCont + Shrub +
                          Substrate + Topo + SubsDens,
                          data = mite.env)

# Find the adjusted R2 of the model with the retained env variables
RsquareAdj(mite.spe.rda.signif)$adj.r.squared
# [1] 0.4367038
```

Challenge 1: Solution

Step 4: Test model significance

```
anova.cca(mite.spe.rda.signif, step = 1000)
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(formula = mite.spe.hel ~ WatrCont + Shrub + Substrate + Topo + SubsD
#          Df Variance     F Pr(>F)
# Model    11  0.20759 5.863  0.001 ***
# Residual 58  0.18669
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

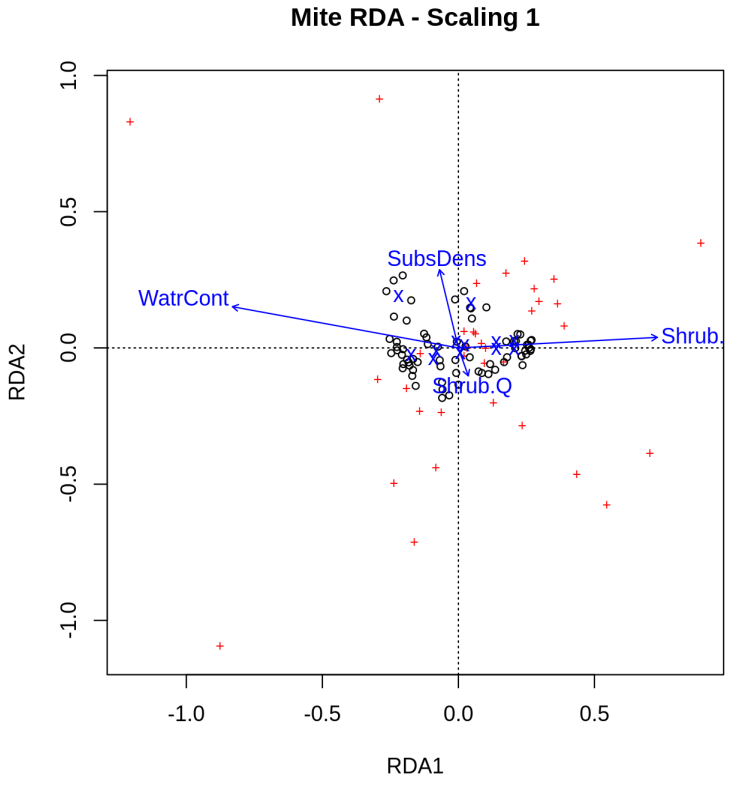
The selected environmental variables significantly explain **43.7% (p = 0.001)** of the variation in mite species abundances.

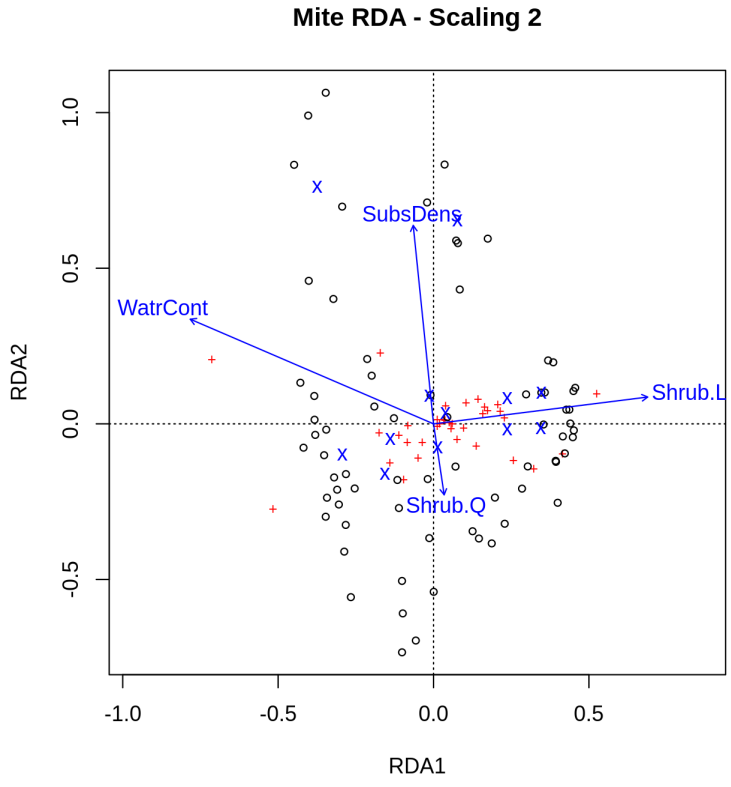
Challenge 1: Solution

Step 5: Plot the RDA!

```
ordiplot(mite.spe.rda.signif,
         scaling = 1,
         main = "Mite RDA - Scaling 1
```

```
ordiplot(mite.spe.rda.signif,
         scaling = 2,
         main = "Mite RDA - Scaling 2
```



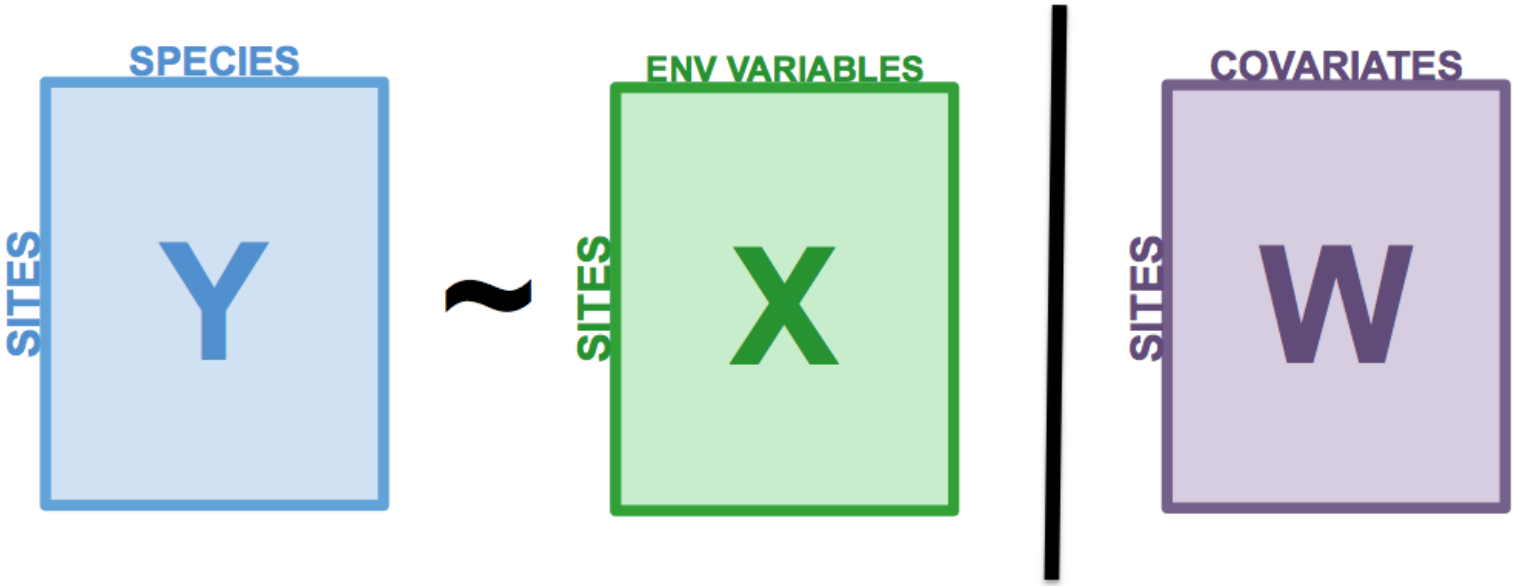


Canonical analyses

Partial RDA

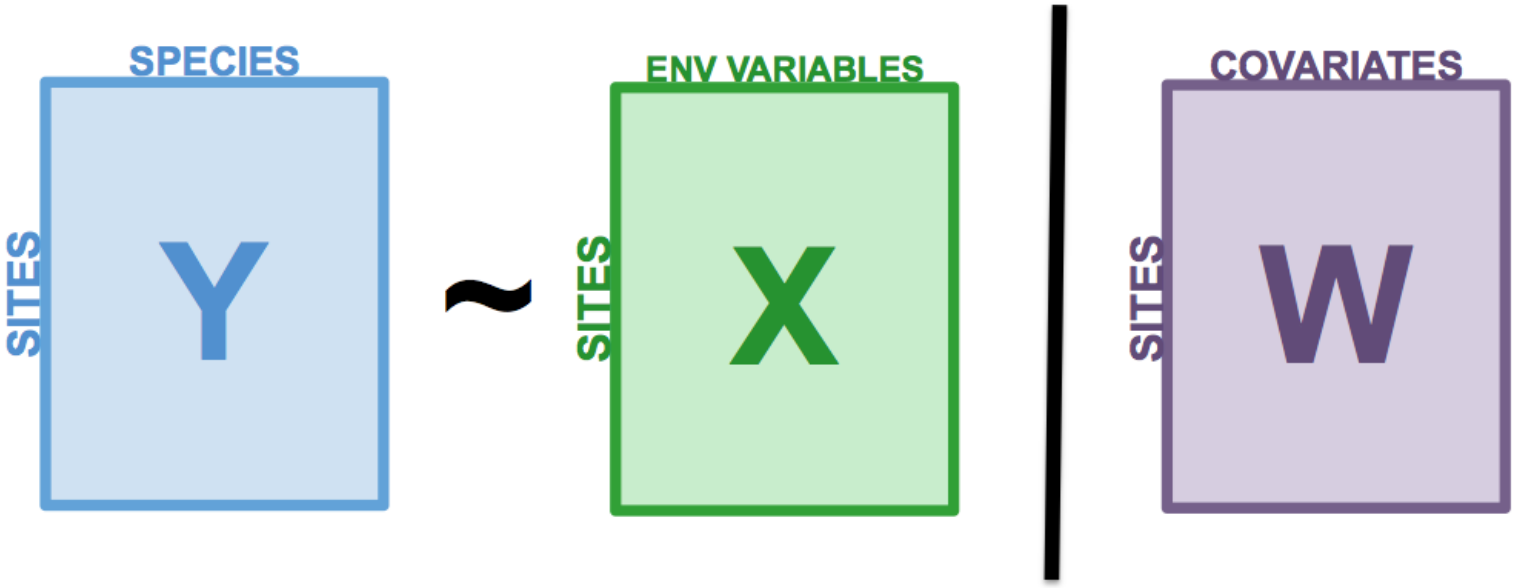
Partial RDA

- Special case of RDA involving covariates
  - Models the linear effects of matrix X on matrix Y while controling for a matrix W of **covariates**.



Applications of partial RDA

- Assess effects of environmental variables on community composition while **accounting for variation** that isn't the focus of the study.
- **Isolate** effect of one or more groups of explanatory variables



Partial RDA on Doubs River data

Let's assess the effect of water chemistry on fish species abundances while controling for the effect of topography.

```
# Subset environmental data into topography variables and chemistry variables
env.topo ← subset(env.z, select = c(alt, pen, deb))
env.chem ← subset(env.z, select = c(pH, dur, pho, nit, amm, oxy, dbo))

# Partial RDA
spe.partial.rda ← rda(spe.hel, env.chem, env.topo)
```

Note: Alternative syntax for the partial RDA:

```
spe.partial.rda ← rda(spe.hel ~ pH + dur + pho + nit + amm + oxy + dbo +
                      Condition(alt + pen + deb),
                      data = env.z)
```

Partial RDA output in R

```
summary(spe.partial.rda, display = NULL)
```

```
Call:
rda(X = spe.hel, Y = env.chem, Z = env.topo)

Partitioning of variance:
              Inertia Proportion
Total          0.5025     1.0000
Conditioned    0.2087     0.4153
Constrained    0.1602     0.3189
Unconstrained  0.1336     0.2659
```

- **Conditioned Proportion**: variance of Y explained by W **(41.53%)**
- **Constrained Proportion**: variance of Y explained by X **(31.89%)**
- **Unconstained Proportion**: unexplained variance in Y **(26.59%)**

How would you report these results? *Water chemistry explains 31.9% of the variation in fish community composition across sites, while topography explains 41.5% of this variation.*

Significance testing

First, let's extract the model's adjusted R2.

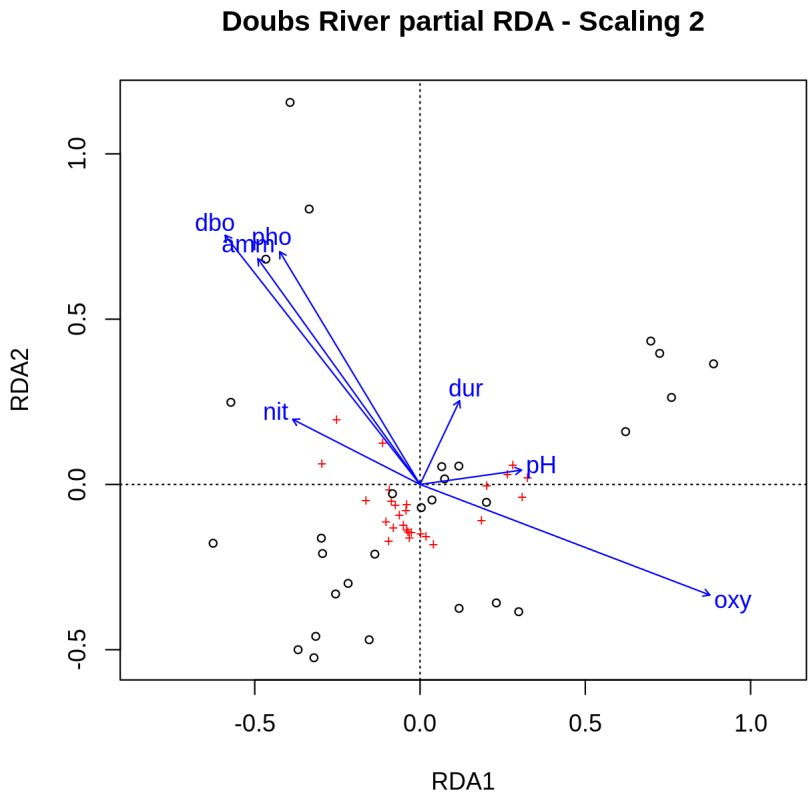
```
RsquareAdj(spe.partial.rda)$adj.r.squared
# [1] 0.2413464
```

Then, let's test whether the model is statistically significant.

```
anova.cca(spe.partial.rda, step = 1000)
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = spe.hel, Y = env.chem, Z = env.topo)
#          Df Variance      F Pr(>F)
# Model     7  0.16024 3.0842  0.001 ***
# Residual 18  0.13360
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Plot the partial RDA

```
ordiplot(spe.partial.rda, scaling = 2,
         main = "Doubs River partial RDA - Scaling 2")
```



Challenge 2

Run a partial RDA to model the effects of environmental variables on mite species abundances, while controling for substrate variables (SubsDens, WatrCont, and Substrate).

- What is the variance explained by substrate variables?
- Is the model significant?
- Which axes are significant?

Recall some useful functions:

```
rda()
summary()
RsquareAdj()
anova.cca() # hint: see the 'by' argument in ?anova.cca
```

Challenge 2: Solution

Our species abundance and environmental datasets have already been transformed and standardized.

So, we can start with the partial RDA:

```
# Compute partial RDA
mite.spe.subs ← rda(mite.spe.hel ~ Shrub + Topo
                    + Condition(SubsDens + WatrCont + Substrate),
                    data = mite.env)

# Check summary
summary(mite.spe.subs, display = NULL)
```

Shrub and Topo explain **9.8%** of the variation in mite species abundances, while substrate covariables explain **42.8%** of this variation.

Challenge 2: Solution

- What is the variance explained by substrate variables?

```
RsquareAdj(mite.spe.subs)$adj.r.squared
# [1] 0.08327533
```

- Is the model significant?

```
anova.cca(mite.spe.subs, step = 1000)
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(formula = mite.spe.hel ~ Shrub + Topo + Condition(SubsDens + WatrCon
#          Df Variance     F Pr(>F)
# Model     3 0.038683 4.006  0.001 ***
# Residual 58 0.186688
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Challenge 2: Solution

- Which axes are significant?

```
anova.cca(mite.spe.subs, step = 1000, by = "axis")
# Permutation test for rda under reduced model
# Forward tests for axes
# Permutation: free
# Number of permutations: 999
#
# Model: rda(formula = mite.spe.hel ~ Shrub + Topo + Condition(SubsDens + WatrCon
#          Df Variance      F Pr(>F)
# RDA1      1 0.027236 8.4618  0.001 ***
# RDA2      1 0.008254 2.5643  0.024 *
# RDA3      1 0.003193 0.9919  0.430
# Residual 58 0.186688
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

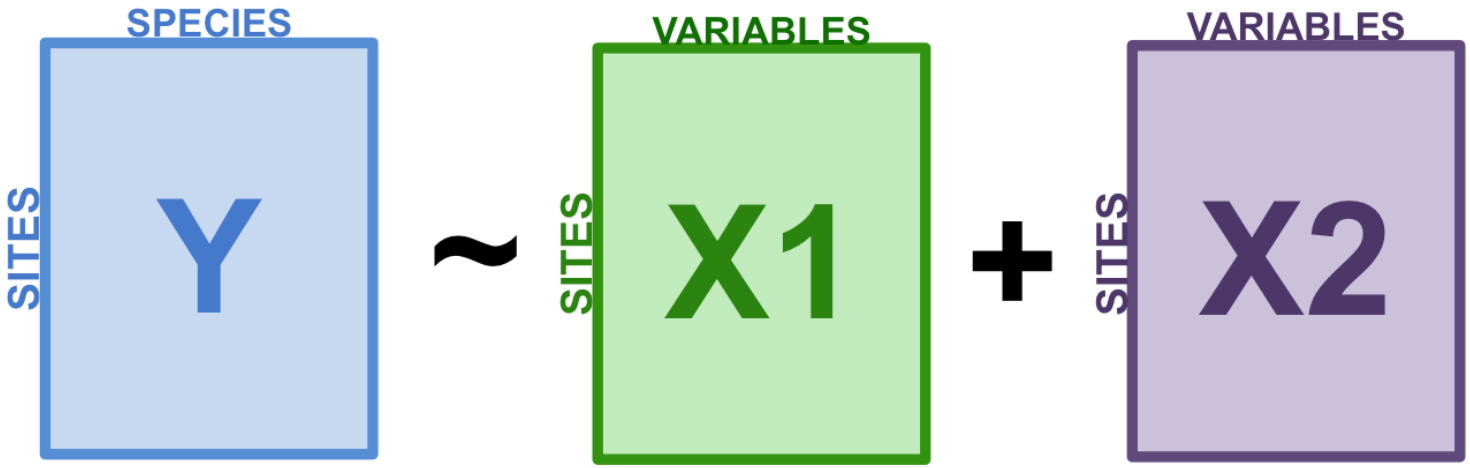
Canonical analyses

Variation partitioning

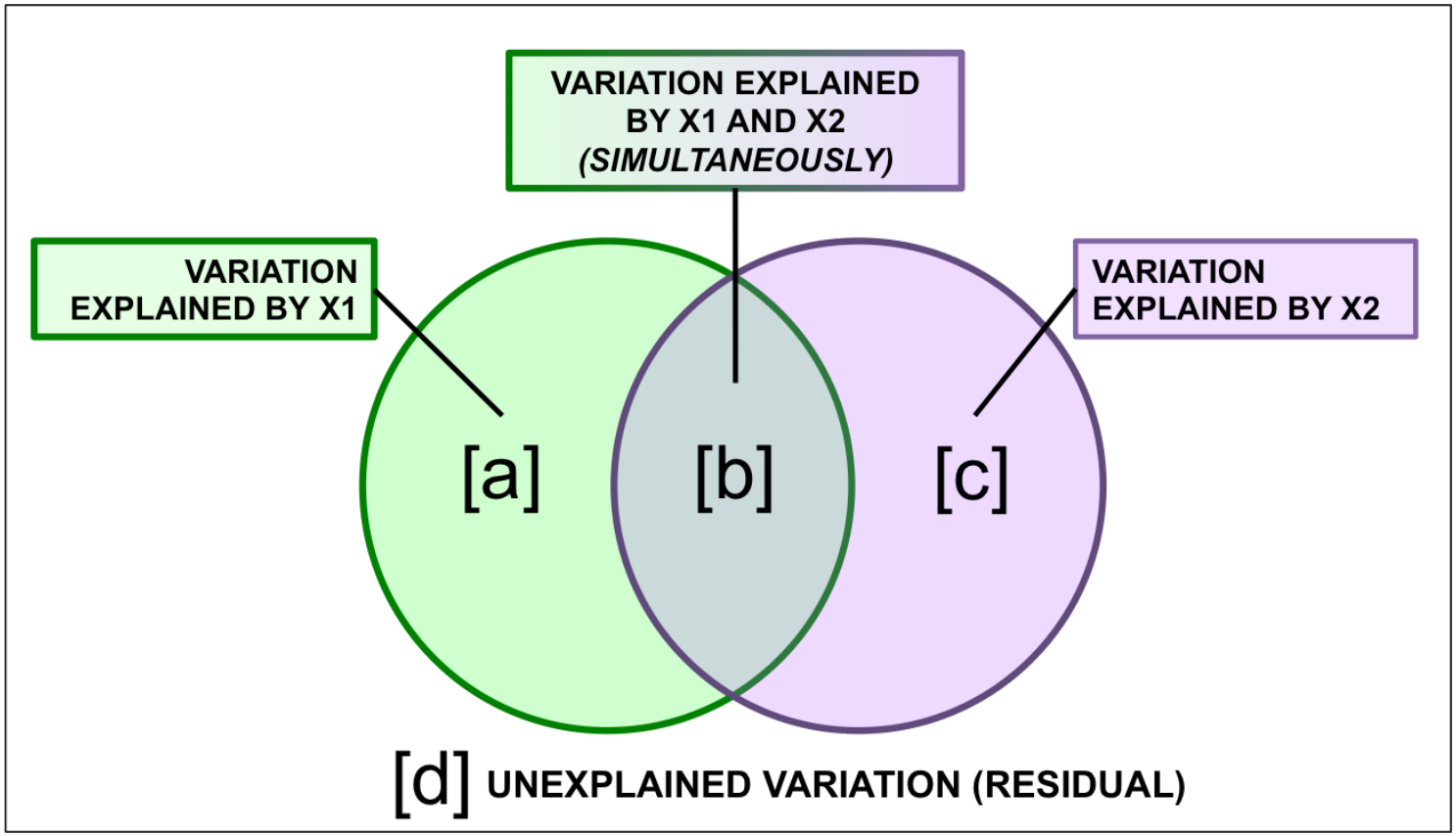
Variation partitioning

Partitions the variation of response variables among 2, 3, or 4 explanatory datasets.

- e.g. large-scale and small-scale
- e.g. abiotic and biotic



Variation partitioning



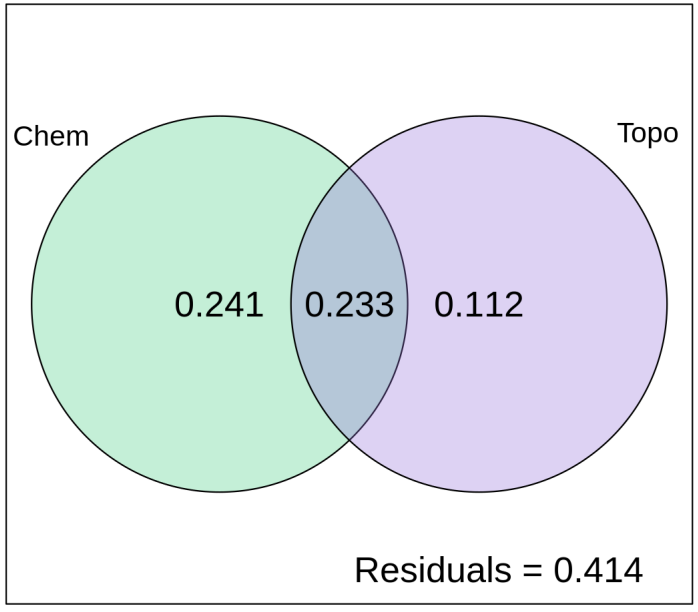
Variation partitioning in R

Note: Make sure you've loaded the *vegan* package!

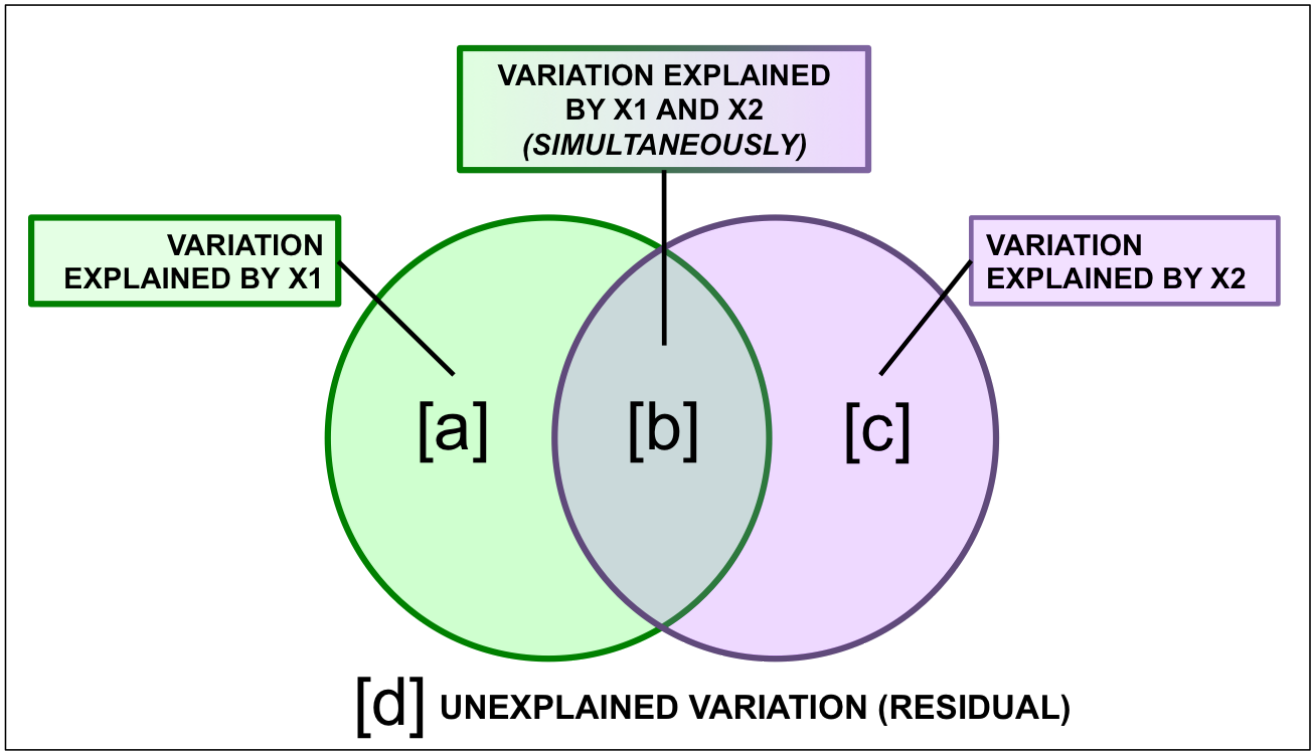
```
spe.part.all ← varpart(spe.hel, env.chem, env.topo)
spe.part.all$part # access results!
# No. of explanatory tables: 2
# Total variation (SS): 14.07
#             Variance: 0.50251
# No. of observations: 29
#
# Partition table:
#                     Df R.squared Adj.R.squared Testable
# [a+b] = X1           7   0.60579       0.47439     TRUE
# [b+c] = X2           3   0.41526       0.34509     TRUE
# [a+b+c] = X1+X2     10   0.73414       0.58644     TRUE
# Individual fractions
# [a] = X1|X2          7                 0.24135     TRUE
# [b]                  0                 0.23304    FALSE
# [c] = X2|X1          3                 0.11205     TRUE
# [d] = Residuals                        0.41356    FALSE
# ---
# Use function 'rda' to test significance of fractions of interest
```

Variation partitioning plot

```
plot(spe.part.all,
     Xnames = c("Chem", "Topo"), # name the partitions
     bg = c("seagreen3", "mediumpurple"), alpha = 80, # colour the circles
     digits = 2, # only show 2 digits
     cex = 1.5)
```

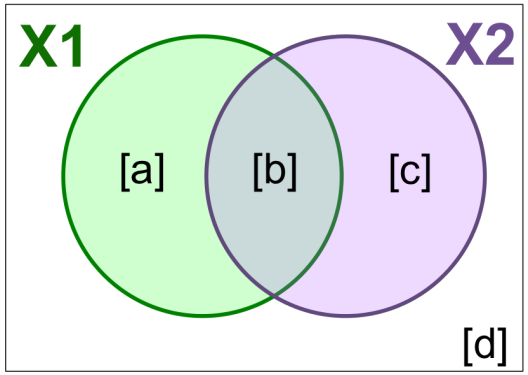


Significance testing



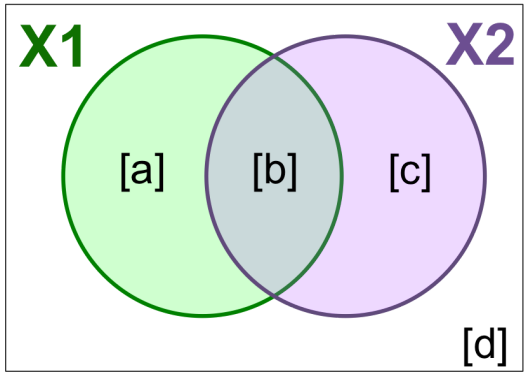
- The shared fraction [b] **cannot** be tested for significance.
- But, we can test the significance of the remaining fractions!

Significance testing: X1 [a+b]



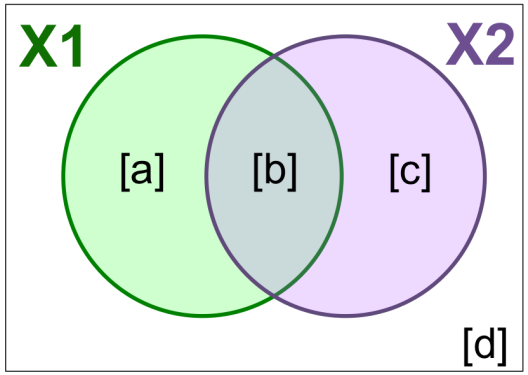

```
# [a+b] Chemistry without controlling for topography
anova.cca(rda(spe.hel, env.chem))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = spe.hel, Y = env.chem)
#          Df Variance      F Pr(>F)
# Model     7  0.30442 4.6102  0.001 ***
# Residual 21  0.19809
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Significance testing: X2 [b+c]




```
# [b+c] Topography without controlling for chemistry
anova.cca(rda(spe.hel, env.topo))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = spe.hel, Y = env.topo)
#          Df Variance     F Pr(>F)
# Model     3  0.20867 5.918  0.001 ***
# Residual 25  0.29384
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

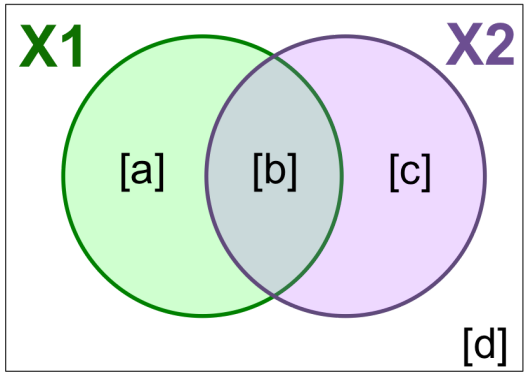
Significance testing: Individual fractions




```
# [a] Chemistry alone
anova.cca(rda(spe.hel, env.chem, env.topo))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = spe.hel, Y = env.chem, Z = env.topo)
#          Df Variance      F Pr(>F)
# Model     7  0.16024 3.0842  0.001 ***
# Residual 18  0.13360
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Note: Recognize this? It's a partial RDA!

Significance testing: Individual fractions




```
# [c] Topography alone
anova.cca(rda(spe.hel, env.topo, env.chem))
# Permutation test for rda under reduced model
# Permutation: free
# Number of permutations: 999
#
# Model: rda(X = spe.hel, Y = env.topo, Z = env.chem)
#          Df Variance      F Pr(>F)
# Model     3 0.064495 2.8965  0.001 ***
# Residual 18 0.133599
# ---
# Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Challenge 3

Partition the variation in the mite species data according to substrate variables (SubsDens, WatrCont) and significant spatial variables.

- What proportion of the variation is explained by substrate variables? By space?
- Which individual fractions are significant?
- Plot your results!

Load the spatial variables:

```
data("mite.pcnm")
```

Recall some useful functions:

```
ordiR2step()
varpart()
anova.cca(rda())
plot()
```

Challenge 3: Solution

Step 1: Forward selection of significant spatial variables

```
# Write full RDA model with all variables
full.spat ← rda(mite.spe.hel ~ ., data = mite.pcnm)

# Forward selection of spatial variables
spat.sel ← ordiR2step(rda(mite.spe.hel ~ 1, data = mite.pcnm),
               scope = formula(full.spat),
               R2scope = RsquareAdj(full.spat)$adj.r.squared,
               direction = "forward",
               trace = FALSE)
spat.sel$call
# rda(formula = mite.spe.hel ~ V2 + V3 + V8 + V1 + V6 + V4 + V9 +
#     V16 + V7 + V20, data = mite.pcnm)
```

Challenge 3: Solution

Step 2: Create variable groups

```
# Subset environmental data to retain only substrate variables
mite.subs ← subset(mite.env, select = c(SubsDens, WatrCont))

# Subset to keep only selected spatial variables
mite.spat ← subset(mite.pcnm,
                   select = names(spat.sel$terminfo$ordered))
                   # a faster way to access the selected variables!
```

Challenge 3: Solution

Step 3: Variation partitioning

```
mite.part ← varpart(mite.spe.hel, mite.subs, mite.spat)
mite.part$part$indfract # access results!
#                 Df R.squared Adj.R.squared Testable
# [a] = X1|X2      2        NA    0.05901929     TRUE
# [b]              0        NA    0.24765221    FALSE
# [c] = X2|X1     10        NA    0.19415929     TRUE
# [d] = Residuals NA        NA    0.49916921    FALSE
```

- What proportion of the variation is explained by substrate variables?
  - **5.9%**
- What proportion of the variation is explained by spatial variables?
  - **19.4%**

Challenge 3: Solution

Step 4: Which individual fractions are significant?

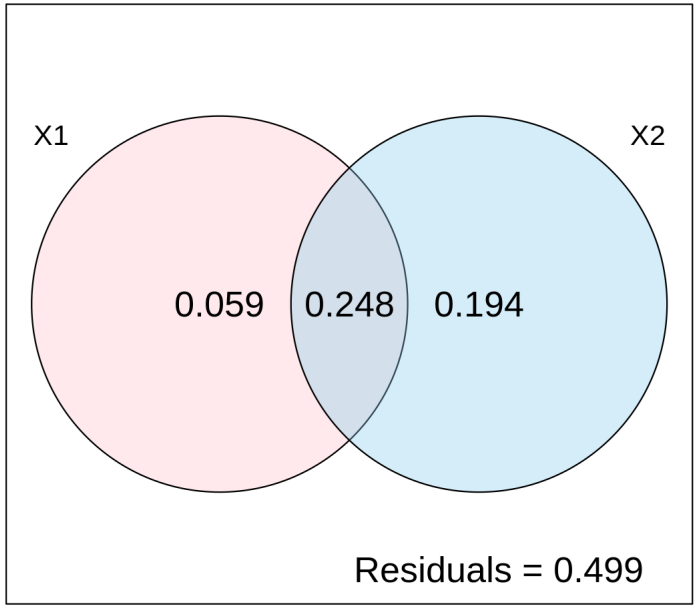
```
# [a]: Substrate only
anova.cca(rda(mite.spe.hel, mite.subs, mite.spat))
# p = 0.001 ***

# [c]: Space only
anova.cca(rda(mite.spe.hel, mite.spat, mite.subs))
# p = 0.001 ***
```

So, what can you say about the effects of substrate and space on mite species abundances?

Challenge 3: Solution

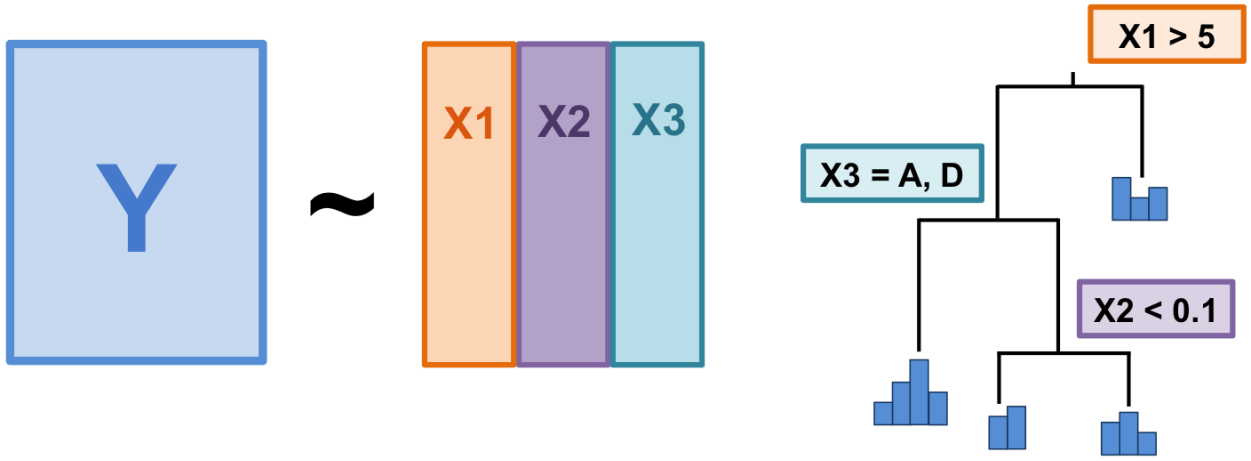
Step 5: Plot the variation partitioning results

```
plot(mite.part, digits = 2, cex = 1.5,
     bg = c("pink", "skyblue"), alpha = 90) # add colour!
```



Multivariate regression tree (MRT)

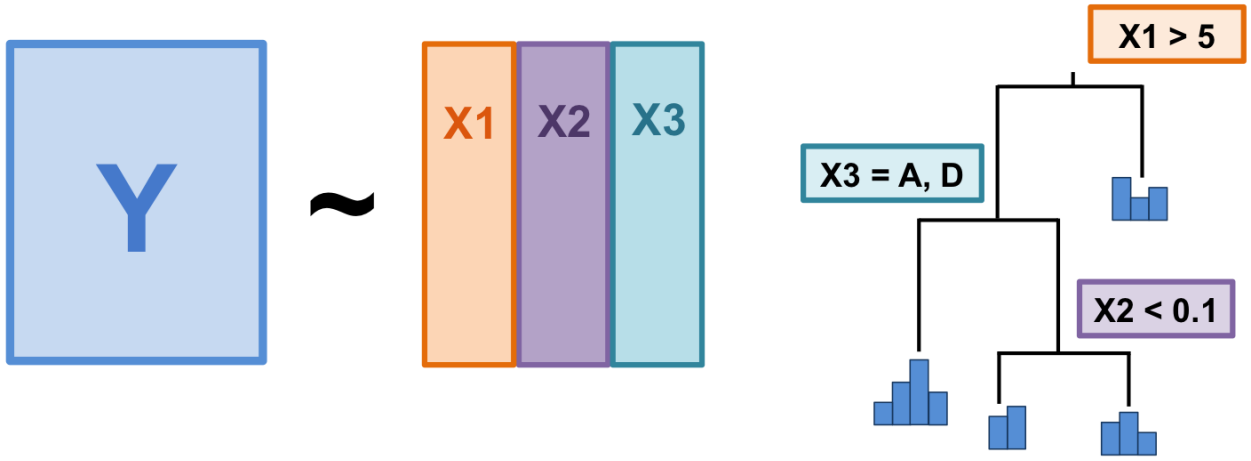
Multivariate regression tree (MRT)



MRT is a constrained clustering technique.

- Splits a response matrix (Y) into clusters based on thresholds of explanatory variables (X)

Multivariate regression tree (MRT)



An MRT consists of:

- **Branch**: each group formed by a split
- **Node**: splitting point (threshold value of an explanatory variable)
- **Leaf**: terminal group of sites

Multivariate regression tree (MRT)

MRT has many advantages:

- Doesn't assume a linear relationship between Y and X matrices
- Results are easy to visualize (it's a tree!)
- Clearly identifies importance of explanatory variables
- Robust (missing values, collinearity)
- Can handle raw explanatory variables

MRT: Tree selection

When you run an MRT, 2 things happen:

1. Constrained partitioning of the data
2. **Cross-validation** to identify best predictive tree

The "best" tree varies depending on your study goals. Usually you want a tree:

- that is *parsimonious*
- but still has an *informative* number of groups
- Basically: what makes sense for your question?

MRT in R

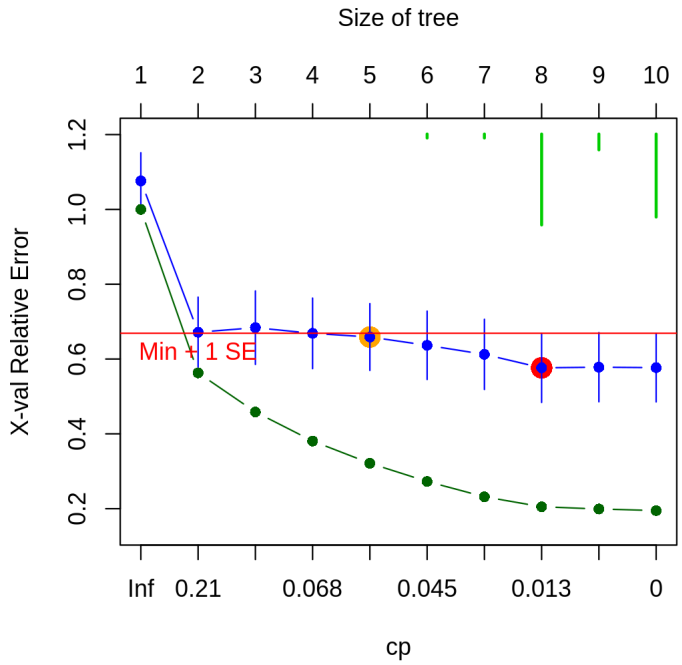
In what follows we will be using `mvpart` **that is currently archived on CRAN**. We install it from GitHub using the package remotes:

```
remotes::install_github("cran/mvpart")
library(mvpart)
```

MRT in R

```
# First, remove the "distance from source" variable
env ← subset(env, select = -das)

# Create multivariate regression tree
# library(mvpart)
doubs.mrt ← mvpart(as.matrix(spe.hel) ~ ., data = env,
                   xv = "pick", # interactively select best tree
                   xval = nrow(spe.hel), # number of cross-validations
                   xvmult = 100, # number of multiple cross-validations
                   which = 4, # plot both node labels
                   legend = FALSE, margin = 0.01, cp = 0)
```

MRT in R: Tree selection

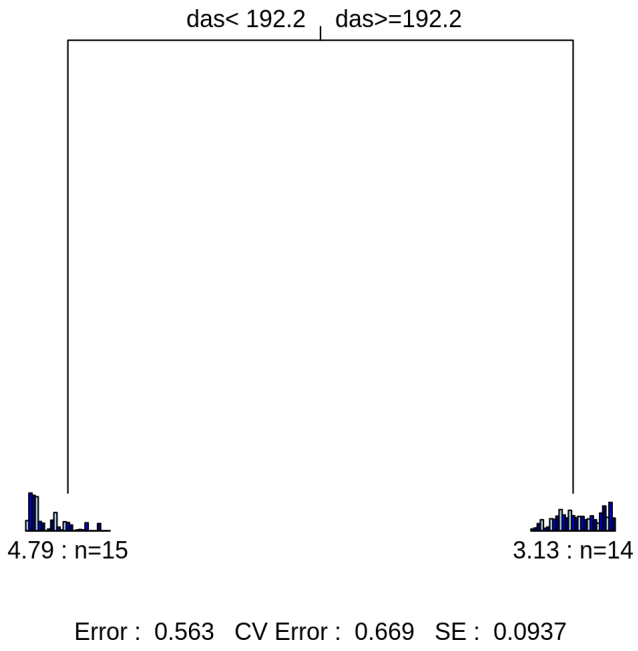


- Green points: Relative error
- Blue points: Cross-validated relative error (CVRE)
- Red dot: Which tree has the smallest CVRE
- Orange dot: Smallest tree within one standard error of the CVRE
- Lime green bars: # of times each tree size was chosen

MRT in R: Tree selection

- Pick the "best" tree by clicking on a blue dot that corresponds to your chosen tree size!
- We don't have an *a priori* expectation about how to partition this data, so we'll select the *smallest tree within 1 standard error of the overall best-fit tree* (i.e. the orange dot).

MRT in R: Tree plot

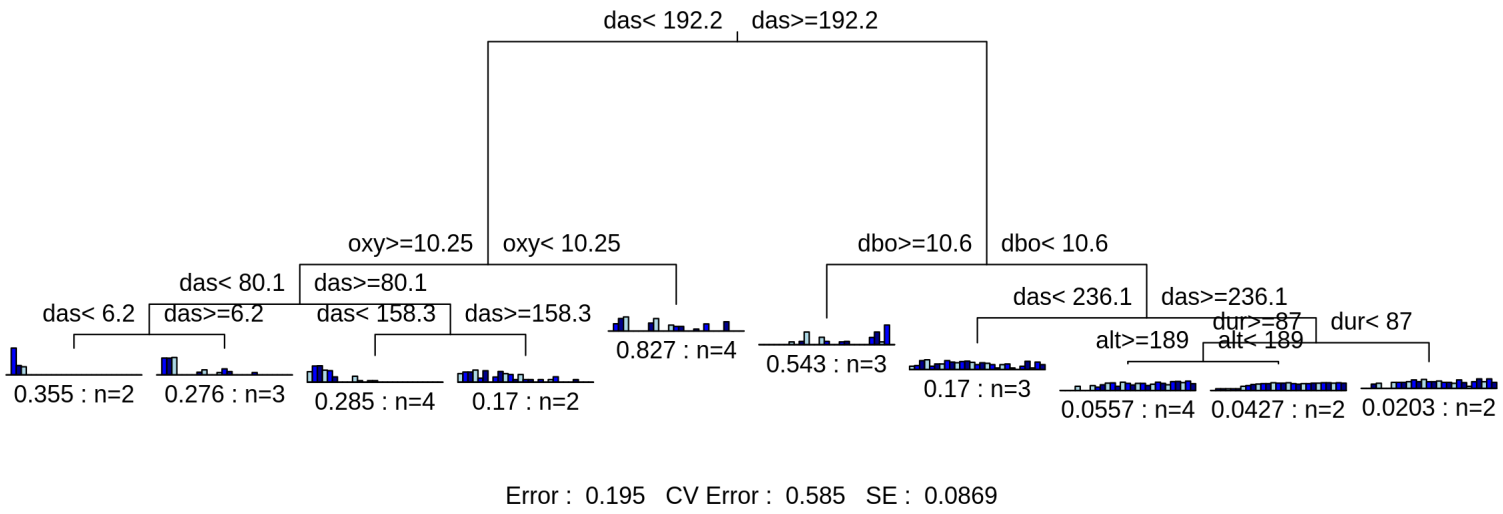


- The species matrix is partitioned according to an **altitude threshold (361.5)**
  - Barplots: species abundances in the sites included in each group
- Residual error = 0.563, which means the model's R2 is **43.7%**

MRT in R: Comparing trees

We can also compare solutions, to help us chose the best tree.

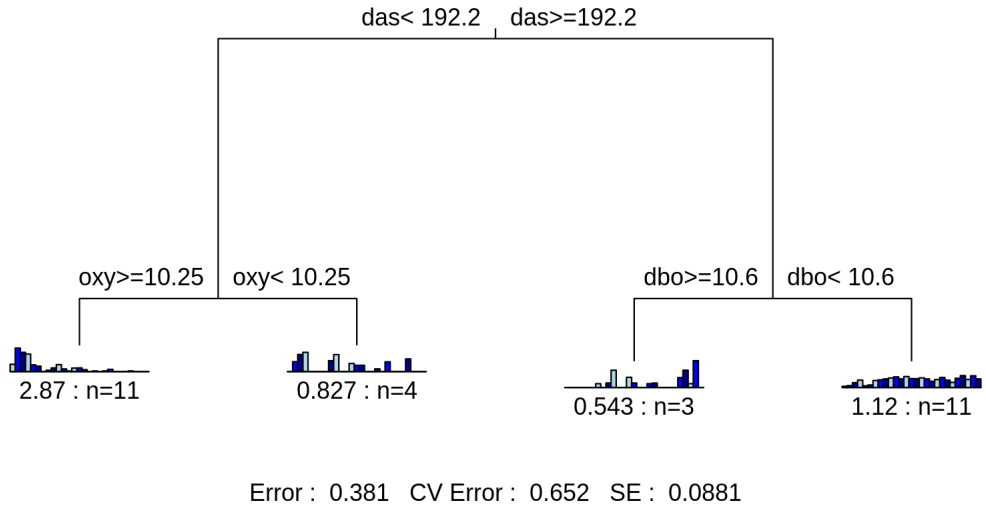
For example, let's look at a 10-group solution!



- This is much **harder to interpret** (so many groups!)
- Higher explanatory power, **BUT** predictive power (CV Error = 0.671) is basically the same as the previous solution (CV Error = 0.673).

MRT in R: Comparing trees

Let's look at a solution with fewer (4) groups!



- This is easier to interpret!
- Higher explanatory power **(lower Error)** than our original solution
- **Higher predictive power** than both previous solutions (CV Error)

MRT in R: Complexity parameter

To find out how much variance is explained by each node in the tree, we need to look at the complexity parameter (CP).

```
doubs.mrt$cptable
#          CP nsplit rel error    xerror       xstd
# 1 0.4369561      0 1.0000000 1.0752730 0.07518210
# 2 0.1044982      1 0.5630439 0.6694153 0.09368615
```

- CP @ nsplit 0 = R2 of the whole tree
- CP at subsequent nodes = R2 of each node (see full summary to see which node corresponds to which variable threshold)

MRT in R: Summary output

We can access more information about the tree (such as which node corresponds to which variable threshold):

```
summary(doubs.mrt)
# Call:
# mvpart(form = as.matrix(spe.hel) ~ ., data = env, xv = "1se",
#     xval = nrow(spe.hel), xvmult = 100, xvse = 1, margin = 0.01,
#     which = 4, legend = FALSE, prn = FALSE, cp = 0)
#   n= 29
#
#          CP nsplit rel error    xerror       xstd
# 1 0.4369561      0 1.0000000 1.0752730 0.07518210
# 2 0.1044982      1 0.5630439 0.6694153 0.09368615
#
# Node number 1: 29 observations,    complexity param=0.4369561
#   Means=0.07299,0.2472,0.2581,0.2721,0.07133,0.06813,0.06897,0.07664,0.1488,0.2
#   left son=2 (15 obs) right son=3 (14 obs)
#   Primary splits:
#       das < 192.2 to the left,  improve=0.4369561, (0 missing)
#       alt < 361.5 to the right, improve=0.4369561, (0 missing)
#       deb < 23.65 to the left,  improve=0.4369561, (0 missing)
#       amm < 0.06  to the left,  improve=0.3529830, (0 missing)
#       nit < 1.415 to the left,  improve=0.3513335, (0 missing)
#
```

MRT in R: Discriminant species

You might also want to know which species are contributing most to the explained variance at each split (i.e. **discriminant species**), or which sites are included within each leaf (group).

To do this, we have the *MVPARTwrap* package.

MVPARTwrap is archived, we use the package remotes to install it from GitHub:

```
remotes::install_github("cran/MVPARTwrap")
library(MVPARTwrap)
```

MRT in R: Discriminant species

```
# Generate a nicer and more informative output
doubs.mrt.wrap ← MRT(doubs.mrt, percent = 10, species = colnames(spe.hel))

# Access the full output:
summary(doubs.mrt.wrap)
```

MRT in R: Discriminant species

To see each species' contribution to explained variance at each node:

```
summary(doubs.mrt.wrap)
# Portion (%) of deviance explained by species for every particular node
#
# ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
#                       --- Node 1 ---
#  Complexity(R2) 43.69561
#  das< 192.2 das ⩾ 192.2
#
# ~ Discriminant species :
#                               TRU         VAI         ABL
# % of expl. deviance 20.03148905 13.16272887 13.3114611
# Mean on the left     0.44630603  0.41943941  0.0000000
# Mean on the right    0.03390824  0.08514225  0.3361805
#
# ~ INDVAL species for this node: : left is 1, right is 2
#     cluster indicator_value probability
# TRU       1          0.8674       0.001
# VAI       1          0.7758       0.001
# LOC       1          0.7042       0.002
# ABL       2          1.0000       0.001
# HOT       2          0.8571       0.001
# GRE       2          0.8571       0.001
```

MRT in R: Indicator species

You might also be interested in finding out which species are significant **indicator species** for each grouping of sites.

```
library(labdsv)

# Calculate indicator values (indval) for each species
doubs.mrt.indval ← indval(spe.hel, doubs.mrt$where)

# Extract the significant indicator species (and which node they represent)
doubs.mrt.indval$maxcls[which(doubs.mrt.indval$pval ⩽ 0.05)]
# TRU VAI LOC HOT TOX BAR SPI GOU BRO PER BOU PSO ROT CAR TAN BCO PCH GRE GAR BBO
#   1   1   1   2   2   2   2   2   2   2   2   2   2   2   2   2   2   2   2   2
# ABL ANG
#   2   2

# Extract their indicator values
doubs.mrt.indval$indcls[which(doubs.mrt.indval$pval ⩽ 0.05)]
#       TRU       VAI       LOC       HOT       TOX       BAR       SPI       GOU
# 0.8674301 0.7758443 0.7042392 0.8571429 0.6185282 0.6363569 0.7347359 0.6442950
#       BRO       PER       BOU       PSO       ROT       CAR       TAN       BCO
# 0.5533235 0.5449488 0.7857143 0.8070918 0.6352865 0.7307582 0.5115135 0.6428571
#       PCH       GRE       GAR       BBO       ABL       ANG
# 0.5000000 0.8571429 0.7726181 0.7142857 1.0000000 0.7857143
```

Challenge 4

Create a multivariate regression tree for the mite data.

- Select the smallest tree within 1 SE of the CVRE.
- What is the proportion of variance (R2) explained by this tree?
- How many leaves does it have?
- What are the top 3 discriminant species?

Remember to load the mite data:

```
data("mite")
data("mite.env")
```

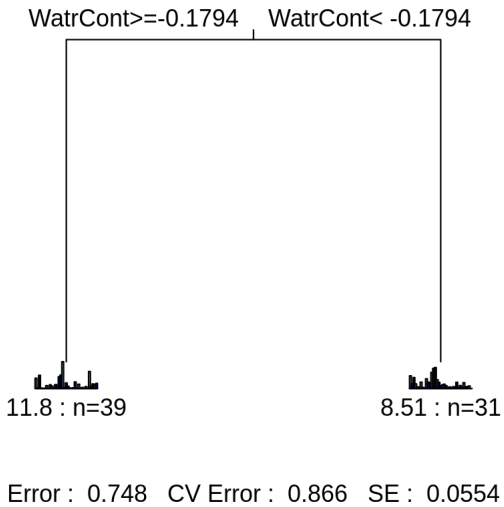
Recall some useful functions:

```
?mvpart() # hint: pay attention to the 'xv' argument!
?MRT()
summary()
```

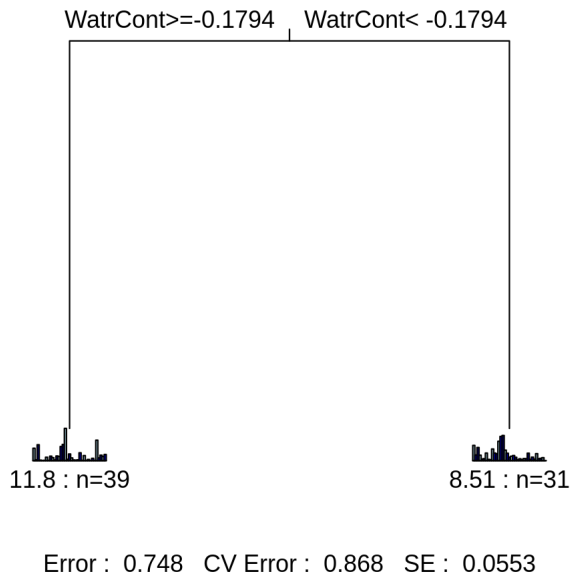
Challenge 4: Solution

Step 1: Create the multivariate regression tree

```
mite.mrt ← mvpart(as.matrix(mite.spe.hel) ~ ., data = mite.env,
                  xv = "1se", # choose smallest tree within 1 SE
                  xval = nrow(mite.spe.hel),
                  xvmult = 100,
                  which = 4, legend = FALSE, margin = 0.01, cp = 0,
                  prn = FALSE)
```



Challenge 4: Solution



- What is the proportion of variance (R2) explained by this tree?
  - 1 - Error = 0.252, so the tree explains **25.2%** of the variance in the species matrix.
- How many leaves does it have?
  - 2 leaves

Challenge 4: Solution

What are the top 3 discriminant species **for node 1**?

```
# Generate nicer MRT output
mite.mrt.wrap ← MRT(mite.mrt,
                    percent = 10,
                    species = colnames(mite.spe.hel))

# Look at discriminant species table from MRT output
summary(mite.mrt.wrap)
```

Challenge 4: Solution

What are the top 3 discriminant species **for node 1**?

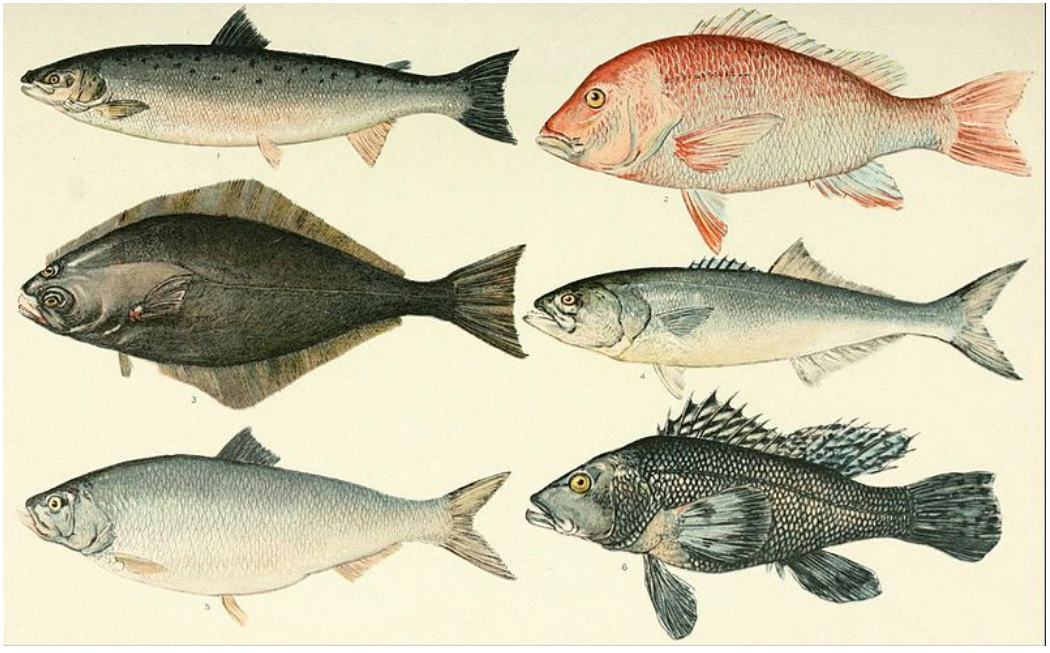
- LCIL, LRUG, Ceratoz1

```
~ INDVAL species for this node: : left is 1, right is 2
         cluster indicator_value probability
LCIL           1          0.7152       0.001
LRUG           1          0.6683       0.001
Ceratoz1       1          0.4745       0.023
Trhypch1       1          0.4546       0.005
NCOR           1          0.4540       0.005
Trimalc2       1          0.4359       0.002
Ceratoz3       1          0.3963       0.015
TVIE           1          0.3793       0.005
TVEL           2          0.7412       0.001
HMIN           2          0.6421       0.001
FSET           2          0.6361       0.001
ONOV           2          0.6312       0.001
HMIN2          2          0.6193       0.001
SUCT           2          0.6088       0.001
Oribatl1       2          0.5978       0.001
Galumna1       2          0.5974       0.001
MEGR           2          0.5770       0.001
RARD           2          0.5585       0.001
PHTH           2          0.5318       0.001
Stgncrs2       2          0.3898       0.001
Protopl        2          0.2518       0.007
SSTR           2          0.2257       0.007
SLAT           2          0.2249       0.006
Lepidzts       2          0.2108       0.006
Miniglmn       2          0.1880       0.023
MPRO           2          0.1568       0.037
```

Linear discriminant analysis (LDA)

Linear discriminant analysis (LDA)

- Determine how well your descriptor variables explain an *a priori* grouping of your response variable
- Make predictions about how to classify new data
  - e.g. classifying whether a fish comes from a lake or ocean population, based on morphology



LDA in R: Doubs fish dataset

We know that environmental variables generally change with latitude.

If we classify our Doubs River sites according to latitude, how well do environmental variables explain these groupings?

- We can use an LDA to answer this question.

LDA in R: Doubs fish dataset

Let's begin by loading spatial coordinates for the Doubs sites:

```
# load spatial data for Doubs sites
spa ← read.csv("data/doubsspa.csv", row.names = 1)
spa$site ← 1:nrow(spa) # add site numbers
spa ← spa[-8,] # remove site 8
```

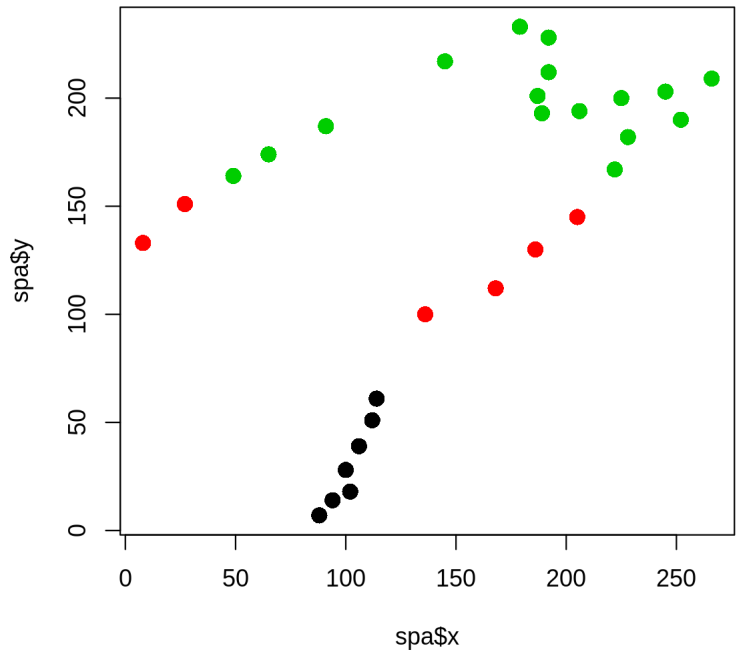
We can then assign sites to 3 latitude groups:

```
# group sites based on latitude
spa$group ← NA # create "group" column
spa$group[which(spa$y < 82)] ← 1
spa$group[which(spa$y > 82 & spa$y < 156)] ← 2
spa$group[which(spa$y > 156)] ← 3
```

LDA in R: Latitude groups

Let's quickly plot the latitude groupings to see if they make sense:

```
plot(spa$x, spa$y, col = spa$group, pch = 16, cex = 1.5)
```



LDA in R

Note: Usually we would check the multivariate homogeneity of within-group variances before proceeding (see Borcard et al. 2011).

For the purposes of this workshop, we will move straight to doing the LDA:

```
# load required library
library(MASS)

# run the LDA grouping sites into latitude groups based on env data
LDA ← lda(env, spa$group)
```

LDA in R: Grouping accuracy

We can then determine how sites were grouped, and whether this grouping is accurate.

```
# Classification of the objects based on the LDA
spe.class ← predict(LDA)$class

# Posterior probabilities that the objects belong to those groups
spe.post ← predict(LDA)$posterior

# Table of prior vs. predicted classifications
(spe.table ← table(spa$group, spe.class))
#    spe.class
#      1  2  3
#   1  7  0  0
#   2  0  6  0
#   3  0  0 16

# Proportion of corrected classification
diag(prop.table(spe.table, 1))
# 1 2 3
# 1 1 1
```

All sites were correctly classified into the latitude groups based on environmental variables.

LDA in R: Predictions

We can now use this relationship to *classify new sites into latitude groups*.

Let's **predict the grouping** of 5 new sites using our LDA results:

```
# Load the new site data
classify.me ← read.csv("data/classifyme.csv", header = TRUE)
# classify.me ← classify.me[,-1] # remove das variable

# Predict grouping of new sites
predict.group ← predict(LDA, newdata = classify.me)

# View site classification
predict.group$class
# [1] 1 1 1 3 3
# Levels: 1 2 3
```

Challenge 5

Create 4 latitude groups in the *mite.xy* dataset. Then, run an LDA to classify mite sites into latitude groupings based on environmental variables (*SubsDens* and *WatrCont*).

- What proportion of sites was correctly classified in group1? in group2?

Load *mite.xy* data:

```
data(mite.xy)
```

Recall some useful functions:

```
lda()
predict()
table()
diag()
```

Challenge 5: Solution

Step 1: Create 4 latitude groups

```
# assign numbers to sites
mite.xy$site ← 1:nrow(mite.xy)

# find latitudinal range for each group
(max(mite.xy[,2])-min(mite.xy[,2]))/4
# [1] 2.4

# group sites into 4 latitude groups
# group sites based on latitude
mite.xy$group ← NA # create "group" column
mite.xy$group[which(mite.xy$y < 2.5)] ← 1
mite.xy$group[which(mite.xy$y ⩾ 2.5 & mite.xy$y < 4.9)] ← 2
mite.xy$group[which(mite.xy$y ⩾ 4.9 & mite.xy$y < 7.3)] ← 3
mite.xy$group[which(mite.xy$y ⩾ 7.3)] ← 4
```

Step 2: Run the LDA

```
LDA.mite ← lda(mite.env[,1:2], mite.xy$group)
```

Challenge 5: Solution

Step 3: Check whether the groupings are correct

```
# classification of the objects based on LDA
mite.class ← predict(LDA.mite)$class
# table of prior versus predicted classifications
(mite.table ← table(mite.xy$group, mite.class))
#    mite.class
#      1  2  3  4
#   1  9  4  2  0
#   2  2 11  4  0
#   3  1  2 14  2
#   4  0  0  3 16
# proportion of correct classification
diag(prop.table(mite.table, 1))
#         1         2         3         4
# 0.6000000 0.6470588 0.7368421 0.8421053
```

What proportion of sites was correctly classified in group1? in group2?

- **60%** were correctly classified into group1, and **64.7%** were classified into group2.

Thank you for attending this workshop!