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Section: [Ordination analysis](#)

Variable selection (constrained ordination)

Theory R functions **Examples** Exercise 

Example 1: tb-RDA with forward selection: Which chemical variables measured in fen water are important for vegetation of Carpathian wetlands?

We will use data about species composition of vascular plants in [Carpathian wetlands](#), and ask which chemical variables, measured in fen water running through these wetlands, are the most important to explain the compositional changes.

First, prepare the [data](#):

```
# Carpathian wetlands - import data
vasc <- read.delim
('https://raw.githubusercontent.com/zdealveindy/anadat-r/master/data/vasc_pl
ants.txt', row.names = 1)
chem <- read.delim
('https://raw.githubusercontent.com/zdealveindy/anadat-r/master/data/chemis
try.txt', row.names = 1)

# transform data using Hellinger transformation to prepare them for tb-RDA:
library (vegan)
vasc.hell <- decostand (vasc, 'hell')

# the last variable in the 'chem' dataset is 'slope', which is not a
chemical variable - remove it:
chem1 <- chem[,-15]
```

Then, test the global model (including all explanatory variables from which you plan to do selection) to see whether it is significant; if it is not, the forward selection should not be done.

```
tb_rda.all <- rda (vasc.hell ~ ., chem1)
anova (tb_rda.all) # 0.001 *** - it is significant
adjR2.tbrda <- RsquareAdj (tb_rda.all)$adj.r.squared # 0.2011048 - adjusted
R2 explained by all 14 variables is 20.1%
```

Option a) Use of forward.sel function

The function `forward.sel` from the package `adespatial` is elaborated forward selection approach based on linear constrained ordination (i.e. based on RDA - if you want to calculate CCA, you cannot use this function and need to resolve to use `ordiR2step` from `vegan` instead). Along to the testing significance of selected variable, this function includes also other stopping rules, published by [Blanchet et al. \(2008\)](#). The method based on significance is prone to be inflated, which may result into models with too many variables; [Blanchet et al. \(2008\)](#) came with another stopping rules along to the

significance one. First, adjusted R^2 of the model including all variables (from which the selection is made) is calculated. While selecting variables during the selection process, the selection stops if (1) the next selected variables is not significant (does not significantly improve the explained variation), and (2) if the R^2_{adj} of the model including this variable exceeds the R^2_{adj} of the global model.

```
# install.packages ('adespatial') # run this code if you haven't install  
the adespatial package before  
library (adespatial)  
sel.fs <- forward.sel (Y = vasc.hell, X = chem1, adjR2thresh = adjR2.tbrda)  
# NOTE: forward.sel calculates only RDA (or tb-RDA in case of Hell.  
transformed data)
```

```
Testing variable 1  
Testing variable 2  
Testing variable 3  
Testing variable 4  
Testing variable 5  
Testing variable 6  
Testing variable 7  
Testing variable 8  
Procedure stopped (alpha criteria): pvalue for variable 8 is 0.118000 (>  
0.050000)
```

See the table with selected variables saved in `sel.fs` object:

```
sel.fs
```

	variables	order	R2	R2Cum	AdjR2Cum	F	pval
1	Ca	1	0.13898260	0.1389826	0.1263206	10.976337	0.001
2	conduct	14	0.03248342	0.1714660	0.1467337	2.626796	0.001
3	Si	6	0.02722659	0.1986926	0.1622696	2.242529	0.001
4	NH3	10	0.02398868	0.2226813	0.1748463	2.005953	0.002
5	N03	9	0.02112004	0.2438013	0.1847233	1.787470	0.001
6	Mg	2	0.01914995	0.2629513	0.1927562	1.636862	0.003
7	pH	13	0.01744989	0.2804012	0.1991562	1.503467	0.024

Or, if you want to see only the names of selected variables, subset it from the `sel.fs` object:

```
sel.fs$variables
```

```
[1] "Ca" "conduct" "Si" "NH3" "N03" "Mg" "pH"
```

Since there is (a potentially high) number of tests of significance during the forward selection procedure, it is better to apply a correction for multiple testing issue. The most common correction (but rather conservative) is Bonferroni, which multiplies each calculated P -value by the overall number of tests. In the forward selection, the overall number of tests (which can be potentially done) equals to the number of variables from which the selection is made (i.e. not the number of selected variables), and this should be considered while applying the adjustment.

The function available for adjusting the P -values is `p.adjust`. It can be applied on a single P -value or

value 1/50000 = 0.00002).

```
sel.fs <- forward.sel (vasc.hell, chem1, nperm = 49999)
pval.adj <- p.adjust (sel.fs$pval, method = 'holm', n = ncol (chem1))
sel.fs$pval.adj <- pval.adj
sel.fs$pval.adj.stars <- gtools::stars.pval (pval.adj)
sel.fs
```

	variables	order	R2	R2Cum	AdjR2Cum	F	pval	pval.adj
	pval.adj.stars							
1	Ca	1	0.13898260	0.1389826	0.1263206	10.976337	0.00002	0.00028

2	conduct	14	0.03248342	0.1714660	0.1467337	2.626796	0.00004	0.00052

3	Si	6	0.02722659	0.1986926	0.1622696	2.242529	0.00016	0.00192
**								
4	NH3	10	0.02398868	0.2226813	0.1748463	2.005953	0.00080	0.00880
**								
5	N03	9	0.02112004	0.2438013	0.1847233	1.787470	0.00304	0.03040
*								
6	Mg	2	0.01914995	0.2629513	0.1927562	1.636862	0.00824	0.07416
.								
7	pH	13	0.01744989	0.2804012	0.1991562	1.503467	0.02094	0.16752

Since the `forward.sel` function is not optimised, the calculation with 49,999 permutation take some time, but the reward is the *P*-values (after correction) below 0.001 in case of the first two variables.

You can see that `forward.sel` function selected (after the correction) five environmental variables, which together explain 18.5% of the variance (adjusted R2, the column `AdjR2Cum` in the table above showing cumulative adjusted R2 of all variables). This is just slightly less than 20.1% which is explained by global model with 14 variables (see the result of `RsquareAdj (rda.all)$adj.r.squared` above).

In this case, the results of `forward.sel` function are the same as `ordiR2step` and `ordistep` functions below (option b and c), with seven included environmental variables (if calculated without correction for multiple testing).

Option b) Use of `ordiR2step` function

The `vegan`'s function `ordiR2step` does similar job as `forward.sel` from `adespatial`, but it's use is a bit more complex. On the other side, the method is more general - it allows to use also CCA or db-RDA methods, while `forward.sel` is based purely on linear constrained ordination (RDA, and tb-RDA in the case that the species composition data are pre-transformed e.g. by Hellinger transformation). In the default setting the criteria for including the variable is based on both significance of the newly selected variables, and the comparison of adjusted variation (R^2_{adj}) explained by the selected variables to R^2_{adj} explained by the global model (with all variables); if the new variable is not significant *or* the R^2_{adj} of the model including this new variable would exceed the R^2_{adj} of the global model, the selection will be stopped.

To use the `ordiR2step` function, you need to specify two models - the “empty” model containing only intercept, and the “full” model including all variables (aka global model). Also, the argument `direction` allows for selecting the selection direction (forward, backward, both, with the default to both):

```
tb_rda.vasc.0 <- rda (vasc.hell ~ 1, data = chem1) # model containing only
species matrix and intercept
tb_rda.vasc.all <- rda (vasc.hell ~ ., data = chem1) # model including all
variables from matrix chem1 (the dot after tilde (~) means "include all from
data")
```

The function `ordiR2step` then uses the empty model and the scope (variables) from the full model to proceed the selection. Set `direction = 'forward'` to ensure that forward instead of stepwise selection is done, and increase the number of permutations to 999 (instead of default 499) to make results comparable to `forward.sel` (which by default is based on 999 permutations):

```
sel.osR2 <- ordiR2step (tb_rda.vasc.0, scope = formula (tb_rda.vasc.all),
R2scope = adjR2.tbrda, direction = 'forward', permutations = 999)
```

Step: R2.adj= 0

Call: `vasc.hell ~ 1`

	R2.adjusted
<All variables>	0.201104784
+ Ca	0.126320581
+ conduct	0.109418731
+ Mg	0.106926067
+ Corg	0.076260217
+ pH	0.074169805
+ Na	0.043030978
+ NH3	0.031875777
+ Si	0.022863900
+ S04	0.018929801
+ K	0.015918981
+ N03	0.008112568
+ Fe	0.004863012
+ P04	0.002933349
<none>	0.000000000
+ Cl	-0.002347612

	Df	AIC	F	Pr(>F)
+ Ca	1	-47.149	10.976	0.001 ***

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

Step: R2.adj= 0.1263206

Call: `vasc.hell ~ Ca`

	R2.adjusted
<All variables>	0.2011048

```
+ conduct      0.1467337
+ NH3          0.1417066
+ Na           0.1403837
+ Si           0.1403827
+ pH           0.1403289
+ Mg           0.1386000
+ Corg         0.1375824
+ N03          0.1340217
+ K            0.1302515
+ Fe           0.1299960
+ S04          0.1266017
<none>        0.1263206
+ Cl           0.1254155
+ P04          0.1235944
```

```
          Df    AIC      F Pr(>F)
+ conduct  1 -47.841 2.6268 0.001 ***
```

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

Step: R2.adj= 0.1467337
Call: vasc.hell ~ Ca + conduct

```
          R2.adjusted
<All variables> 0.2011048
+ Si            0.1622696
+ NH3           0.1592776
+ N03           0.1572584
+ Mg            0.1565375
+ Corg          0.1538422
+ Na            0.1533884
+ Fe            0.1517167
+ pH            0.1513672
+ K             0.1511453
+ S04           0.1495485
+ Cl            0.1470011
<none>         0.1467337
+ P04           0.1444723
```

```
          Df    AIC      F Pr(>F)
+ Si    1 -48.18 2.2425 0.002 **
```

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

Step: R2.adj= 0.1622696
Call: vasc.hell ~ Ca + conduct + Si

```
          R2.adjusted
<All variables> 0.2011048
+ NH3           0.1748463
```



```

+ N03      0.1739710
+ Mg       0.1708815
+ Corg     0.1689184
+ pH       0.1667252
+ S04      0.1666206
+ Na       0.1661906
+ Fe       0.1655162
+ K        0.1653789
<none>    0.1622696
+ Cl       0.1620351
+ P04      0.1599773

```

```

      Df      AIC      F Pr(>F)
+ NH3  1 -48.308 2.006  0.001 ***

```

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

Step: R2.adj= 0.1748463

Call: vasc.hell ~ Ca + conduct + Si + NH3

```

                R2.adjusted
<All variables> 0.2011048
+ N03           0.1847233
+ Mg            0.1835775
+ S04           0.1799079
+ pH            0.1798906
+ Corg          0.1793391
+ Fe            0.1786200
+ K             0.1766585
+ Na            0.1756240
<none>         0.1748463
+ Cl            0.1737180
+ P04           0.1725072

```

```

      Df      AIC      F Pr(>F)
+ N03  1 -48.236 1.7875  0.005 **

```

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

Step: R2.adj= 0.1847233

Call: vasc.hell ~ Ca + conduct + Si + NH3 + N03

```

                R2.adjusted
<All variables> 0.2011048
+ Mg            0.1927562
+ pH            0.1907475
+ Corg          0.1890699
+ S04           0.1876281
+ Fe            0.1869468
+ K             0.1868746
+ Na            0.1848251

```

```
<none>          0.1847233
+ Cl             0.1834910
+ P04           0.1826904

      Df      AIC      F Pr(>F)
+ Mg  1 -48.032 1.6369 0.011 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Step: R2.adj= 0.1927562
Call: vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg

```
                R2.adjusted
<All variables> 0.2011048
+ pH            0.1991562
+ Corg          0.1963215
+ S04           0.1958876
+ K             0.1956246
+ Fe            0.1953612
<none>         0.1927562
+ Na           0.1922729
+ Cl           0.1915194
+ P04          0.1907648
```

```
      Df      AIC      F Pr(>F)
+ pH  1 -47.709 1.5035 0.028 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Step: R2.adj= 0.1991562
Call: vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg + pH

```
                R2.adjusted
+ Corg          0.2023822
+ Fe            0.2015249
+ K             0.2014550
+ S04           0.2012822
<All variables> 0.2011048
<none>         0.1991562
+ Na           0.1988753
+ P04          0.1974424
+ Cl           0.1974018
```

sel.osR2

Call: rda(formula = vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg + pH,
data = chem1)

```
                Inertia Proportion Rank
Total          0.5674      1.0000
```

```

Constrained    0.1591    0.2804    7
Unconstrained 0.4083    0.7196   62
Inertia is variance

Eigenvalues for constrained axes:
  RDA1   RDA2   RDA3   RDA4   RDA5   RDA6   RDA7
0.09590 0.01904 0.01205 0.01027 0.00871 0.00718 0.00595

Eigenvalues for unconstrained axes:
  PC1    PC2    PC3    PC4    PC5    PC6    PC7    PC8
0.03601 0.03279 0.02383 0.01908 0.01687 0.01593 0.01504 0.01358
(Shown only 8 of all 62 unconstrained eigenvalues)

```

The summary table is part of the `ordiR2step` function output, stored in the component `$anova`:

```
sel.osR2$anova
```

```

          R2.adj Df      AIC      F Pr(>F)
+ Ca          0.12632  1 -47.149 10.9763 0.001 ***
+ conduct     0.14673  1 -47.841  2.6268 0.001 ***
+ Si          0.16227  1 -48.180  2.2425 0.002 **
+ NH3         0.17485  1 -48.308  2.0060 0.001 ***
+ NO3         0.18472  1 -48.236  1.7875 0.005 **
+ Mg          0.19276  1 -48.032  1.6369 0.011 *
+ pH          0.19916  1 -47.709  1.5035 0.028 *
<All variables> 0.20111
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

The result is exactly the same as results of `forward.sel` function and also as results of `ordistep` method based on P-values and AIC criteria (below). P-values can be adjusted for multiple testing (e.g. by Holm's correction) as follows:

```

sel.osR2_adj <- sel.osR2
sel.osR2_adj$anova$`Pr(>F)` <- p.adjust (sel.osR2$anova$`Pr(>F)` , method =
'holm', n = ncol (chem1))
sel.osR2_adj$anova

```

```

          R2.adj Df      AIC      F Pr(>F)
+ Ca          0.12632  1 -47.149 10.9763 0.014 *
+ conduct     0.14673  1 -47.841  2.6268 0.014 *
+ Si          0.16227  1 -48.180  2.2425 0.022 *
+ NH3         0.17485  1 -48.308  2.0060 0.014 *
+ NO3         0.18472  1 -48.236  1.7875 0.050 *
+ Mg          0.19276  1 -48.032  1.6369 0.099 .
+ pH          0.19916  1 -47.709  1.5035 0.224
<All variables> 0.20111
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

You may consider to increase the number of permutations (argument `permutations` in `ordiR2step` to reach lower P-values; in the `forward.sel` example above we increased the permutations to 49,999 to make sure that even after adjustment of P-values the most significant one could be smaller than 0.001.

Option c) Use of `ordistep` function

Function `ordistep` performs step-wise selection of environmental variables based two criteria: if their inclusion into the model leads to significant increase of explained variance (the same as in `forward.sel` and `ordiR2step`), and if the AIC of the new model is lower than AIC of the more simple model. In contrary to previous functions, the function does not consider as criteria whether the adjusted R^2 of the model exceeds the adjusted R^2 of the global model. The use of `ordistep` function has the same logic as `ordiR2step`.

```
rda.vasc.0 <- rda (vasc.hell ~ 1, data = chem1) # model containing only
species matrix and intercept
rda.vasc.all <- rda (vasc.hell ~ ., data = chem1) # model including all
variables from matrix chem1 (the dot after tilde (~) means ALL!)
sel.os <- ordistep (rda.vasc.0, scope = formula (rda.vasc.all), direction =
'forward')
```

Start: `vasc.hell ~ 1`

	Df	AIC	F	Pr(>F)	
+ Ca	1	-47.149	10.9763	0.005	**
+ conduct	1	-45.808	9.4775	0.005	**
+ Mg	1	-45.612	9.2612	0.005	**
+ Corg	1	-43.249	6.6964	0.005	**
+ pH	1	-43.091	6.5277	0.005	**
+ Na	1	-40.775	4.1026	0.005	**
+ NH3	1	-39.964	3.2718	0.005	**
+ Si	1	-39.316	2.6145	0.005	**
+ S04	1	-39.034	2.3314	0.005	**
+ K	1	-38.820	2.1162	0.010	**
+ N03	1	-38.267	1.5643	0.060	.
+ Fe	1	-38.038	1.3372	0.105	
+ P04	1	-37.902	1.2030	0.150	
+ Cl	1	-37.532	0.8384	0.700	

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

Step: `vasc.hell ~ Ca`

	Df	AIC	F	Pr(>F)	
+ conduct	1	-47.841	2.6268	0.005	**
+ NH3	1	-47.430	2.2190	0.005	**
+ pH	1	-47.318	2.1081	0.005	**
+ Na	1	-47.322	2.1125	0.010	**
+ Si	1	-47.322	2.1124	0.010	**

```

+ Mg      1 -47.177 1.9694 0.010 **
+ Corg    1 -47.095 1.8880 0.010 **
+ N03     1 -46.806 1.6047 0.035 *
+ K       1 -46.502 1.3073 0.110
+ Fe      1 -46.482 1.2873 0.115
+ S04     1 -46.209 1.0219 0.435
+ Cl      1 -46.114 0.9296 0.590
+ P04     1 -45.968 0.7885 0.890

```

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

Step: vasc.hell ~ Ca + conduct

```

      Df      AIC      F Pr(>F)
+ Si    1 -48.180 2.2425 0.005 **
+ NH3   1 -47.931 1.9997 0.005 **
+ N03   1 -47.763 1.8367 0.005 **
+ Mg    1 -47.703 1.7788 0.005 **
+ Corg  1 -47.480 1.5629 0.020 *
+ Na    1 -47.442 1.5266 0.035 *
+ K     1 -47.257 1.3482 0.040 *
+ pH    1 -47.275 1.3658 0.050 *
+ S04   1 -47.125 1.2218 0.085 .
+ Fe    1 -47.304 1.3936 0.095 .
+ Cl    1 -46.916 1.0210 0.420
+ P04   1 -46.709 0.8229 0.810

```

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

Step: vasc.hell ~ Ca + conduct + Si

```

      Df      AIC      F Pr(>F)
+ NH3   1 -48.308 2.0060 0.005 **
+ N03   1 -48.234 1.9350 0.005 **
+ Mg    1 -47.972 1.6855 0.020 *
+ Corg  1 -47.807 1.5280 0.025 *
+ S04   1 -47.614 1.3446 0.055 .
+ pH    1 -47.622 1.3529 0.075 .
+ Na    1 -47.577 1.3104 0.095 .
+ Fe    1 -47.521 1.2568 0.105
+ K     1 -47.509 1.2459 0.130
+ Cl    1 -47.229 0.9815 0.470
+ P04   1 -47.058 0.8199 0.805

```

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

Step: vasc.hell ~ Ca + conduct + Si + NH3

```

      Df      AIC      F Pr(>F)
+ N03   1 -48.236 1.7875 0.005 **
+ Mg    1 -48.138 1.6951 0.005 **

```

```
+ S04  1 -47.824 1.4012  0.035 *  
+ pH   1 -47.822 1.3998  0.045 *  
+ Corg 1 -47.775 1.3558  0.045 *  
+ Fe   1 -47.714 1.2986  0.125  
+ K    1 -47.547 1.1431  0.235  
+ Na   1 -47.459 1.0613  0.370  
+ Cl   1 -47.298 0.9112  0.665  
+ P04  1 -47.195 0.8163  0.805
```

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

Step: vasc.hell ~ Ca + conduct + Si + NH3 + N03

```
      Df    AIC      F Pr(>F)  
+ Mg   1 -48.032 1.6369  0.010 **  
+ pH   1 -47.858 1.4764  0.040 *  
+ Corg 1 -47.713 1.3430  0.040 *  
+ S04  1 -47.588 1.2288  0.135  
+ Fe   1 -47.530 1.1750  0.170  
+ K    1 -47.523 1.1693  0.210  
+ Na   1 -47.347 1.0080  0.480  
+ Cl   1 -47.233 0.9034  0.600  
+ P04  1 -47.164 0.8408  0.785
```

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

Step: vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg

```
      Df    AIC      F Pr(>F)  
+ pH   1 -47.709 1.5035  0.040 *  
+ S04  1 -47.424 1.2453  0.095 .  
+ Corg 1 -47.462 1.2795  0.100 .  
+ Fe   1 -47.378 1.2040  0.115  
+ K    1 -47.401 1.2247  0.135  
+ Na   1 -47.110 0.9623  0.580  
+ Cl   1 -47.044 0.9036  0.650  
+ P04  1 -46.979 0.8450  0.775
```

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

Step: vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg + pH

```
      Df    AIC      F Pr(>F)  
+ Corg 1 -47.130 1.2508  0.140  
+ Fe   1 -47.054 1.1839  0.150  
+ K    1 -47.048 1.1785  0.175  
+ S04  1 -47.033 1.1650  0.185  
+ Na   1 -46.823 0.9783  0.595  
+ P04  1 -46.697 0.8676  0.715  
+ Cl   1 -46.694 0.8645  0.735
```

Results are stored in `sel.os` object:

```
sel.os
```

```
Call: rda(formula = vasc.hell ~ Ca + conduct + Si + NH3 + N03 + Mg + pH,
data = chem1)
```

	Inertia	Proportion	Rank
Total	0.5674	1.0000	
Constrained	0.1591	0.2804	7
Unconstrained	0.4083	0.7196	62

Inertia is variance

Eigenvalues for constrained axes:

RDA1	RDA2	RDA3	RDA4	RDA5	RDA6	RDA7
0.09590	0.01904	0.01205	0.01027	0.00871	0.00718	0.00595

Eigenvalues for unconstrained axes:

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
0.03601	0.03279	0.02383	0.01908	0.01687	0.01593	0.01504	0.01358

(Showed only 8 of all 62 unconstrained eigenvalues)

Selected variables are Ca, conduct, Si, NH3, N03, Mg and pH.

Example 2: CCA with forward selection on data from Carpathian wetlands

This example is using the same data as the Example 1 above, namely composition of vascular plants in [Carpathian wetlands](#) and chemical variables measured in fen water running through them. While in Example 1 we analysed the data using tb-RDA (RDA applied on data after Hellinger transformation), here we do this using CCA (note: no Hellinger transformation is done). Since the function `forward.sel` from `adespatial` package is using only RDA algorithm, we cannot use it here; instead, we will do the calculation using `ordiR2step` from `vegan`, which also implements variable selection with double stopping criteria: the selection is finished if the new variable to be selected is not significant at certain alpha level, or if the adjusted R^2 explained by the model with this variable exceeds the adjusted R^2 of the global model.

```
# Carpathian wetlands - import data
vasc <- read.delim
('https://raw.githubusercontent.com/zdealveindy/anadat-r/master/data/vasc_pl
ants.txt', row.names = 1)
chem <- read.delim
('https://raw.githubusercontent.com/zdealveindy/anadat-r/master/data/chemis
try.txt', row.names = 1)

# the last variable in the 'chem' dataset is 'slope', which is not a
chemical variable - remove it:
chem1 <- chem[, -15]
```

We do similar steps as in the option b) of Example 1 above: create global model with CCA (including

all variables) and test it's significance, and if it is significant, we will create also an empty model with only intercept and use the `ordiR2step` function with appropriate arguments:

```
cca.all <- cca (vasc ~ ., data = chem1)
anova (cca.all) # P < 0.001 - yes, significant!
adjRsqr <- RsquareAdj (cca.all)$adj.r.square # adjR2 as stopping criteria
used in ordiR2step
cca.0 <- cca (vasc ~ 1, data = chem1) # empty model only with intercept
```

The function `ordiR2step` needs to know the variables which are being selected (this is defined by the empty model and the scope taken from the global model), the direction of the selection (forward, backward, both - default is both, which means combined forward and backward), the threshold of adjusted R² which should be used as stopping criteria (`R2scope`), number of permutations for the Monte Carlo permutation test applied on each variable (default is 499). Additionally, I set also argument `trace = FALSE` (default is TRUE), which limits the verbal output of the function (as you can see above, the function is rather talkative).

```
sel.osR2 <- ordiR2step (tb_rda.vasc.0, scope = formula (tb_rda.vasc.all),
R2scope = adjR2.tbrda, direction = 'forward', permutations = 999, trace =
FALSE)
sel.osR2
```

```
Call: cca(formula = vasc ~ Ca + conduct + Si + NH3 + N03 + S04 + Corg, data
= chem1)
```

	Inertia	Proportion	Rank
Total	3.0238	1.0000	
Constrained	0.6772	0.2240	7
Unconstrained	2.3466	0.7760	62

Inertia is scaled Chi-square

Eigenvalues for constrained axes:

CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7
0.3388	0.0942	0.0618	0.0569	0.0485	0.0418	0.0352

Eigenvalues for unconstrained axes:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8
0.17567	0.16047	0.12242	0.10303	0.09427	0.08952	0.08455	0.07697

(Showing 8 of 62 unconstrained eigenvalues)

```
sel.osR2.cca$anova
```

	R2.adj	Df	AIC	F	Pr(>F)	
+ Ca	0.082170	1	385.90	7.1738	0.001	***
+ conduct	0.095775	1	385.81	2.0268	0.001	***
+ Si	0.108412	1	385.76	1.9704	0.003	**
+ NH3	0.118073	1	385.96	1.6923	0.005	**
+ N03	0.125542	1	386.27	1.5578	0.009	**
+ S04	0.131015	1	386.73	1.4028	0.029	*
+ Corg	0.136553	1	387.17	1.3959	0.027	*


```
<All variables> 0.142700
```

The first five variables (Ca, conductivity, Si, NH3 and NO3) are the same as in tb-RDA analysis above, the last two differ. If we increase the number of permutations to 49,000 (to reduce the lowest P-value we can obtain - but this takes quite some time!) and adjust the P-values with Holm's correction for multiple testing issue, only the first four variables will be selected (compared to the first five in the case of tb-RDA):

```
sel.osR2.cca <- ordiR2step (cca.0, scope = formula (cca.all), direction =
'forward', R2scope = adjRsqr.cca, permutations = 49999, trace = F)
sel.osR2.cca_adj <- sel.osR2.cca
sel.osR2.cca_adj$anova$`Pr(>F)` <- p.adjust (sel.osR2.cca$anova$`Pr(>F)` ,
method = 'holm', n = ncol (chem1))
sel.osR2.cca_adj$anova
```

	R2.adj	Df	AIC	F	Pr(>F)	
+ Ca	0.082144	1	385.90	7.1738	0.00028	***
+ conduct	0.095628	1	385.81	2.0268	0.00156	**
+ Si	0.108806	1	385.76	1.9704	0.00336	**
+ NH3	0.118293	1	385.96	1.6923	0.04752	*
+ NO3	0.125796	1	386.27	1.5578	0.10720	
+ SO4	0.130940	1	386.73	1.4028	0.30276	
+ Mg	0.136065	1	387.19	1.3789	0.30276	
+ Corg	0.141488	1	387.65	1.3550	0.30276	
<All variables>	0.142191					

You may have noticed that the use of `ordiR2step` function with CCA takes considerably longer than when applied on RDA (or tb-RDA as above). This is because in the case of CCA, [adjusted R2 needs to be calculated using the permutation method](#) introduced by [Peres-Neto et al. \(2006\)](#), while in the case of RDA the adjusted R² can be calculated analytically by Ezekiel's formula. This also means that the resulting adjusted R² values will slightly change between calculations.

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