

Community ecology - Computer lab III - AB332

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Computer lab III

First load the necessary packages.

Load data from previous lab (if it is not already in the environment)

```
load("AB332_lab_II.RData")
```

Analyses using environmental variation

The aim is to investigate how the environmental variation may explain community variance.

Read table with environmental data:

```
isa.metadata<-read_tsv("https://raw.githubusercontent.com/krabberod/UNIS_AB332_2023/main/computer_lab/d
```

```
## Rows: 82 Columns: 26
## -- Column specification -----
## Delimiter: "\t"
## chr  (3): Sample_Name, date, seasons
## dbl (23): year, season_nr, month, day, DOY, running_day, template, depth, de...
##
## i Use 'spec()' to retrieve the full column specification for this data.
## i Specify the column types or set 'show_col_types = FALSE' to quiet this message.
```

```
isa.metadata
```

```
## # A tibble: 82 x 26
##   Sample_Name date      year season_nr seasons month   day   DOY running_day
##   <chr>      <chr>    <dbl>    <dbl> <chr>   <dbl> <dbl> <dbl>    <dbl>
## 1 Isa_111214 14-Dec-11 2011      1 winter    12    14    348      348
## 2 Isa_120117 17-Jan-12 2012      1 winter     1    17     17      382
## 3 Isa_120128 28-Jan-12 2012      1 winter     1    28     28      393
## 4 Isa_120209 09-Feb-12 2012      1 winter     2     9     40      405
## 5 Isa_120216 16-Feb-12 2012      1 winter     2    16     47      412
## 6 Isa_120223 23-Feb-12 2012      1 winter     2    23     54      419
## 7 Isa_120301 01-Mar-12 2012      2 spring     3     1     61      426
## 8 Isa_120308 08-Mar-12 2012      2 spring     3     8     68      433
## 9 Isa_120320 20-Mar-12 2012      2 spring     3    20     80      445
## 10 Isa_120321 21-Mar-12 2012      2 spring     3    21     81      446
```

```
## # i 72 more rows
## # i 17 more variables: template <dbl>, depth <dbl>, declination <dbl>,
## #   daylength <dbl>, nitrate <dbl>, phosphate <dbl>, silicate <dbl>, N_P <dbl>,
## #   watermass <dbl>, temp_25 <dbl>, sal_25 <dbl>, F_25 <dbl>, chla_GFF <dbl>,
## #   chla_10 <dbl>, chl_small <dbl>, bacteria <dbl>, virus <dbl>
```

```
isa.metadata <- column_to_rownames(isa.metadata, var = "Sample_Name")
```

Check the variables available in the metadata:

```
colnames(isa.metadata)
```

```
## [1] "date"          "year"          "season_nr"     "seasons"       "month"
## [6] "day"           "DOY"           "running_day"   "template"       "depth"
## [11] "declination"   "daylength"     "nitrate"       "phosphate"      "silicate"
## [16] "N_P"           "watermass"     "temp_25"       "sal_25"        "F_25"
## [21] "chla_GFF"      "chla_10"       "chl_small"     "bacteria"       "virus"
```

Select the environmental variables for the same samples as we have been using for the previous labs.

```
isa.metadata.simp <- isa.metadata[6:30,]
isa.metadata.simp
```

```
##           date year season_nr seasons month day DOY running_day template
## Isa_120223 23-Feb-12 2012      1 winter    2  23  54      419         1
## Isa_120301 01-Mar-12 2012      2 spring    3   1  61      426         1
## Isa_120308 08-Mar-12 2012      2 spring    3   8  68      433         1
## Isa_120320 20-Mar-12 2012      2 spring    3  20  80      445         1
## Isa_120321 21-Mar-12 2012      2 spring    3  21  81      446         1
## Isa_120322 22-Mar-12 2012      2 spring    3  22  82      447         1
## Isa_120323 23-Mar-12 2012      2 spring    3  23  83      448         1
## Isa_120329 29-Mar-12 2012      2 spring    3  29  89      454         1
## Isa_120403 03-Apr-12 2012      2 spring    4   3  94      459         1
## Isa_120411 11-Apr-12 2012      2 spring    4  11 102      467         1
## Isa_120416 16-Apr-12 2012      2 spring    4  16 107      472         1
## Isa_120419 19-Apr-12 2012      3 summer    4  19 110      475         1
## Isa_120423 23-Apr-12 2012      3 summer    4  23 114      479         1
## Isa_120426 26-Apr-12 2012      3 summer    4  26 117      482         1
## Isa_120430 30-Apr-12 2012      3 summer    4  30 121      486         1
## Isa_120503 03-May-12 2012      3 summer    5   3 124      489         1
## Isa_120507 07-May-12 2012      3 summer    5   7 128      493         1
## Isa_120508 08-May-12 2012      3 summer    5   8 129      494         1
## Isa_120509 09-May-12 2012      3 summer    5   9 130      495         1
## Isa_120510 10-May-12 2012      3 summer    5  10 131      496         1
## Isa_120516 16-May-12 2012      3 summer    5  16 137      502         1
## Isa_120524 24-May-12 2012      3 summer    5  24 145      510         1
## Isa_120621 21-Jun-12 2012      4 autumn    6  21 173      538         1
## Isa_120706 06-Jul-12 2012      4 autumn    7   6 188      553         1
## Isa_120806 06-Aug-12 2012      4 autumn    8   6 219      584         1
##           depth declination daylength nitrate phosphate silicate N_P
## Isa_120223    25      -9.92         5    6.85      0.42    3.94 16.4
## Isa_120301    25      -7.66         7    6.38      0.50    3.20 12.7
```

| | | | | | | | | |
|----|------------|-----------|------------|-----------|------------|------------|-------------|------|
| ## | Isa_120308 | 25 | -4.52 | 9 | 3.98 | 0.33 | 1.95 | 12.2 |
| ## | Isa_120320 | 25 | 0.32 | 12 | 9.39 | 0.66 | 5.50 | 14.3 |
| ## | Isa_120321 | 25 | 0.72 | 12 | 4.82 | 0.47 | 2.30 | 10.3 |
| ## | Isa_120322 | 25 | 1.12 | 13 | 4.26 | 0.39 | 1.90 | 10.8 |
| ## | Isa_120323 | 25 | 1.52 | 13 | 8.23 | 0.54 | 4.97 | 15.2 |
| ## | Isa_120329 | 25 | 3.92 | 14 | 7.59 | 0.86 | 3.52 | 8.9 |
| ## | Isa_120403 | 25 | 5.88 | 16 | 4.87 | 0.54 | 2.35 | 9.0 |
| ## | Isa_120411 | 25 | 8.91 | 18 | 9.02 | 0.70 | 4.10 | 12.9 |
| ## | Isa_120416 | 25 | 10.72 | 20 | 5.06 | 0.59 | 2.20 | 8.6 |
| ## | Isa_120419 | 25 | 11.76 | 22 | 7.66 | 0.64 | 3.30 | 11.9 |
| ## | Isa_120423 | 25 | 13.10 | 24 | 3.24 | 0.42 | 0.82 | 7.7 |
| ## | Isa_120426 | 25 | 14.06 | 24 | 4.49 | 0.38 | 1.65 | 11.8 |
| ## | Isa_120430 | 25 | 15.29 | 24 | 3.41 | 0.35 | 1.27 | 9.6 |
| ## | Isa_120503 | 25 | 16.16 | 24 | 1.55 | 0.26 | 0.64 | 5.9 |
| ## | Isa_120507 | 25 | 17.26 | 24 | 1.86 | 0.18 | 1.21 | 10.4 |
| ## | Isa_120508 | 25 | 17.52 | 24 | 1.65 | 0.20 | 1.28 | 8.2 |
| ## | Isa_120509 | 25 | 17.77 | 24 | 0.83 | 0.21 | 1.03 | 4.0 |
| ## | Isa_120510 | 25 | 18.03 | 24 | 1.54 | 0.24 | 0.28 | 6.5 |
| ## | Isa_120516 | 25 | 19.42 | 24 | 1.34 | 0.15 | 1.33 | 8.9 |
| ## | Isa_120524 | 25 | 20.96 | 24 | 0.23 | 0.24 | 1.27 | 1.0 |
| ## | Isa_120621 | 25 | 23.28 | 24 | 0.00 | 0.07 | 0.37 | 0.0 |
| ## | Isa_120706 | 25 | 22.46 | 24 | 0.21 | 0.08 | 0.43 | 2.6 |
| ## | Isa_120806 | 25 | 16.50 | 24 | 0.25 | 0.12 | 1.42 | 2.0 |
| ## | | watermass | temp_25 | sal_25 | F_25 | chl_a_GFF | chl_a_10 | |
| ## | Isa_120223 | 5 | -0.3365000 | 34.34000 | 0.00000000 | 0.01501667 | 0.005950000 | |
| ## | Isa_120301 | 3 | 1.3284000 | 34.62000 | 0.00000000 | 0.02096667 | 0.010341667 | |
| ## | Isa_120308 | NA | 1.1212000 | 34.61750 | 0.00000000 | 0.01501667 | 0.008500000 | |
| ## | Isa_120320 | 5 | 0.9140000 | 34.61500 | 0.00000000 | 0.01317500 | 0.010058333 | |
| ## | Isa_120321 | 2 | 1.3662500 | 34.71000 | 0.00000000 | 0.01671667 | 0.008925000 | |
| ## | Isa_120322 | 2 | 1.9827500 | 34.77250 | 0.00000000 | 0.02054167 | 0.009491667 | |
| ## | Isa_120323 | 3 | 1.2695000 | 34.68500 | 0.00000000 | 0.01877083 | 0.010695834 | |
| ## | Isa_120329 | 2 | 1.9096667 | 34.79000 | 0.00000000 | 0.01700000 | 0.011900000 | |
| ## | Isa_120403 | 2 | 1.2520000 | 34.73500 | 0.00000000 | 0.04774167 | 0.024791667 | |
| ## | Isa_120411 | 2 | 1.4846667 | 34.83667 | 0.01500000 | 0.11928333 | 0.060916667 | |
| ## | Isa_120416 | 2 | 1.4995000 | 34.84500 | 0.04000000 | 0.22695000 | 0.106108333 | |
| ## | Isa_120419 | NA | 1.1692500 | 34.81000 | 0.28000000 | 1.41950000 | NA | |
| ## | Isa_120423 | 5 | 0.8390000 | 34.77500 | 0.52000000 | 2.58541667 | 1.865750000 | |
| ## | Isa_120426 | 5 | -0.2305000 | 34.56250 | 0.71500000 | 1.41950000 | 1.260833333 | |
| ## | Isa_120430 | 5 | -0.2266667 | 34.51667 | 0.72000000 | 1.69716667 | 1.188583333 | |
| ## | Isa_120503 | 5 | -0.2345000 | 34.54000 | 0.54750000 | 2.21991667 | 1.735416667 | |
| ## | Isa_120507 | 5 | 0.0210000 | 34.53750 | 0.25750000 | 1.71700000 | 1.114916667 | |
| ## | Isa_120508 | 5 | 0.4458750 | 34.58625 | 1.10000000 | 5.05466667 | 2.402666667 | |
| ## | Isa_120509 | 5 | 0.2825000 | 34.57167 | 1.21000000 | 7.26325000 | 4.658000000 | |
| ## | Isa_120510 | 5 | 0.3088000 | 34.57000 | 1.04000000 | 3.79383333 | 3.163416667 | |
| ## | Isa_120516 | 5 | 0.6255000 | 34.60750 | 0.87500000 | 2.28791667 | 1.861500000 | |
| ## | Isa_120524 | 5 | 0.1610000 | 34.47333 | 1.70000000 | 1.10122222 | 0.630888889 | |
| ## | Isa_120621 | 3 | 3.2950000 | 34.35000 | 0.08666667 | 0.36266667 | 0.032158333 | |
| ## | Isa_120706 | 3 | 2.1282500 | 34.24000 | 0.07000000 | 0.44908333 | 0.038816667 | |
| ## | Isa_120806 | 3 | 2.9423333 | 34.12333 | 0.08666667 | 1.04040000 | 0.024480000 | |
| ## | | chl_small | bacteria | virus | | | | |
| ## | Isa_120223 | 0.009000 | 25569416 | 160216949 | | | | |
| ## | Isa_120301 | 0.011000 | 35347574 | 236494102 | | | | |
| ## | Isa_120308 | 0.007000 | 460490 | 14842982 | | | | |
| ## | Isa_120320 | 0.003000 | 3067808 | 122004027 | | | | |

```
## Isa_120321 0.008000 361644 3937919
## Isa_120322 0.011000 293220 5671576
## Isa_120323 0.008075 624099 18447977
## Isa_120329 0.005000 334140 3851871
## Isa_120403 0.023000 328804 4615040
## Isa_120411 0.058000 43950699 137057832
## Isa_120416 0.121000 13880010 97026943
## Isa_120419 NA 633668 5177999
## Isa_120423 0.720000 10714463 153115961
## Isa_120426 0.159000 10027454 83069530
## Isa_120430 0.509000 2298629 9911242
## Isa_120503 0.484000 2678603 13635701
## Isa_120507 0.602000 1764379 47981392
## Isa_120508 2.652000 17984464 171485701
## Isa_120509 2.605000 2393951 9129285
## Isa_120510 0.630000 2206537 9700788
## Isa_120516 0.426000 3903607 13827876
## Isa_120524 0.470000 4604404 21391153
## Isa_120621 0.331000 4613273 26213561
## Isa_120706 0.410000 890168 14265496
## Isa_120806 1.016000 1182793 11967962
```

Check that the samples are correct, i.e. that the same names are in the OTU-table and the metadata:

```
identical(colnames(otu.tab.red),rownames(isa.metadata.simp))
```

```
## [1] TRUE
```

For this exercise we will use a selection of the continuous variables as metadata.
This line will extract 8 of the variables, based on the column names:

```
isa.metadata.cont <- isa.metadata.simp %>% dplyr::select("nitrate","phosphate","silicate","N_P","temp_25","sal_25")
```

We transform variables using z-scores to have comparable ranges of variation.

```
isa.metadata.cont.zscores <- scale(isa.metadata.cont, center = T, scale = T)
isa.metadata.cont.zscores[1:5,]
```

```
##          nitrate phosphate silicate    N_P temp_25 sal_25
## Isa_120223 0.97203545 0.1823123 1.28060579 1.7160683 -1.3943371 -1.3743479
## Isa_120301 0.81458554 0.5621295 0.76858489 0.8726235 0.3262221 0.1453681
## Isa_120308 0.01058599 -0.2449821 -0.09631528 0.7586444 0.1120952 0.1317992
## Isa_120320 1.82293496 1.3217639 2.36000121 1.2373564 -0.1020317 0.1182303
## Isa_120321 0.29198583 0.4196980 0.14585677 0.3255241 0.3653375 0.6338482
##          F_25 chla_GFF
## Isa_120223 -0.7573225 -0.7194154
## Isa_120301 -0.7573225 -0.7161295
## Isa_120308 -0.7573225 -0.7194154
## Isa_120320 -0.7573225 -0.7204324
## Isa_120321 -0.7573225 -0.7184765
```

Let's check if the environmental variables are correlated to each other. We Calculate correlations and p-values:

```
(env.corr.signif.adjust <- rcorr.adjust(as.matrix(isa.metadata.cont.zscores)))
```

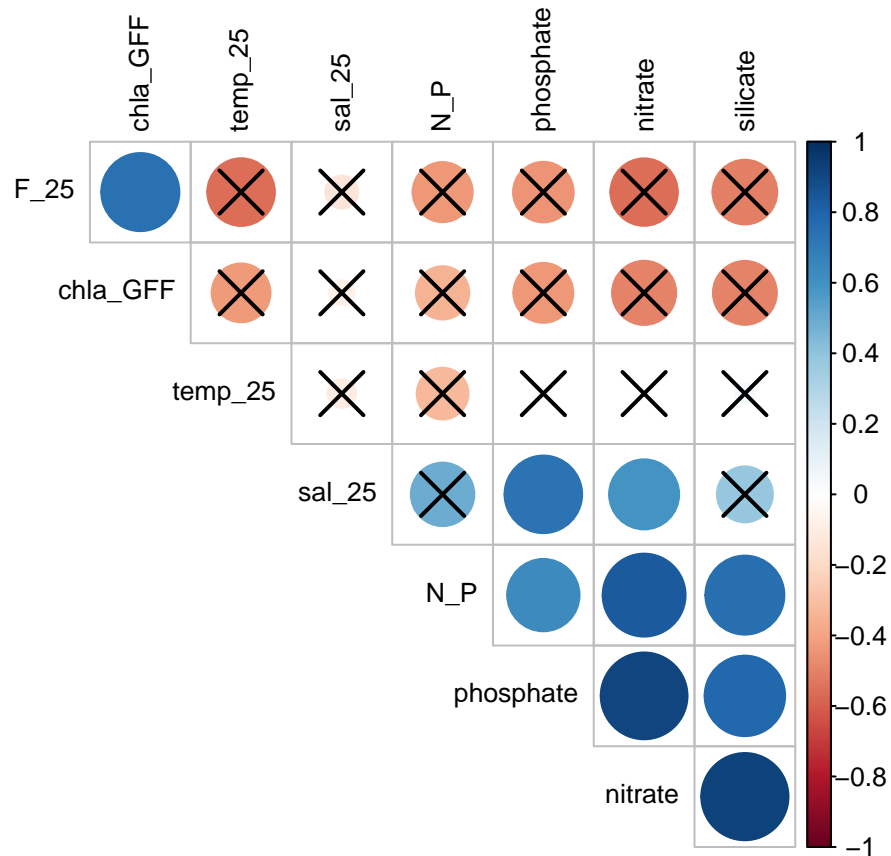
```
##
## Pearson correlations:
##      nitrate phosphate silicate   N_P temp_25 sal_25   F_25 chla_GFF
## nitrate    1.0000    0.9155    0.9250  0.8363 -0.0095  0.5964 -0.5533 -0.4996
## phosphate  0.9155    1.0000    0.7876  0.6361  0.0334  0.7318 -0.4471 -0.4372
## silicate   0.9250    0.7876    1.0000  0.7588  0.0466  0.3808 -0.5093 -0.4968
## N_P        0.8363    0.6361    0.7588  1.0000 -0.3256  0.4901 -0.4386 -0.3460
## temp_25    -0.0095    0.0334    0.0466 -0.3256  1.0000 -0.1002 -0.5564 -0.4205
## sal_25     0.5964    0.7318    0.3808  0.4901 -0.1002  1.0000 -0.1328 -0.0779
## F_25       -0.5533   -0.4471   -0.5093 -0.4386 -0.5564 -0.1328  1.0000  0.7413
## chla_GFF   -0.4996   -0.4372   -0.4968 -0.3460 -0.4205 -0.0779  0.7413  1.0000
##
## Number of observations: 25
##
## Pairwise two-sided p-values:
##      nitrate phosphate silicate N_P   temp_25 sal_25 F_25   chla_GFF
## nitrate                <.0001    <.0001    <.0001  0.9642  0.0017  0.0041  0.0110
## phosphate <.0001                <.0001    0.0006  0.8739  <.0001  0.0250  0.0288
## silicate  <.0001    <.0001                <.0001  0.8250  0.0604  0.0093  0.0115
## N_P       <.0001    0.0006    <.0001                0.1122  0.0129  0.0283  0.0902
## temp_25   0.9642  0.8739    0.8250  0.1122                0.6335  0.0039  0.0363
## sal_25    0.0017  <.0001    0.0604  0.0129  0.6335                0.5268  0.7114
## F_25      0.0041  0.0250    0.0093  0.0283  0.0039  0.5268                <.0001
## chla_GFF  0.0110  0.0288    0.0115  0.0902  0.0363  0.7114  <.0001
##
## Adjusted p-values (Holm's method)
##      nitrate phosphate silicate N_P   temp_25 sal_25 F_25   chla_GFF
## nitrate                <.0001    <.0001    <.0001  1.0000  0.0330  0.0741  0.1759
## phosphate <.0001                <.0001    0.0133  1.0000  0.0007  0.3256  0.3397
## silicate  <.0001    <.0001                0.0003  1.0000  0.5434  0.1583  0.1759
## N_P       <.0001    0.0133    0.0003                0.7852  0.1804  0.3397  0.7218
## temp_25   1.0000  1.0000    1.0000  0.7852                1.0000  0.0735  0.3634
## sal_25    0.0330  0.0007    0.5434  0.1804  1.0000                1.0000  1.0000
## F_25      0.0741  0.3256    0.1583  0.3397  0.0735  1.0000                0.0005
## chla_GFF  0.1759  0.3397    0.1759  0.7218  0.3634  1.0000  0.0005
```

The p-values are corrected for multiple inference using Holm's method (see `p.adjust`). More info on: https://en.wikipedia.org/wiki/Multiple_comparisons_problem Holm corrected values for multiple comparisons

```
env.corr.signif.r <- env.corr.signif.adjust$R$r
env.corr.signif.p <- env.corr.signif.adjust$P
# Edit the object to replace any "<" by "0" using the function "gsub"
env.corr.signif.p <- gsub("<","0", env.corr.signif.p)
# We modify the object to be numeric datatype.
# NB: the transformation is done so the matrix of p values can be read as numeric!
env.corr.signif.p <- apply(env.corr.signif.p, 2 ,as.numeric)
rownames(env.corr.signif.p) <- colnames(env.corr.signif.p)
```

Plot the correlation plot:

```
corrplot(env.corr.signif.r , type="upper", order="hclust", p.mat = env.corr.signif.p, sig.level = 0.05,
```



Fitting environmental variables to ordinations `envfit` will fit the environmental variables to the NMDS ordination as vectors

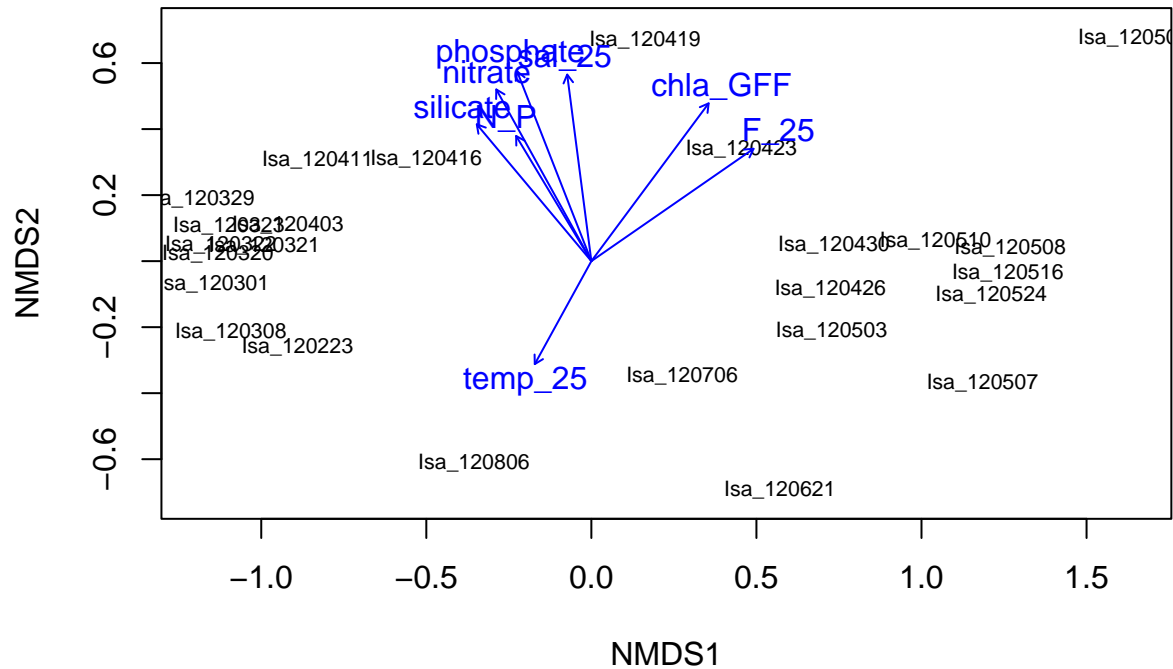
First we fit the variables to the OTU-table that was rarified:

```
otu.tab.trans.ss.nozero.bray.nm.ds.envfit <- envfit(otu.tab.trans.ss.nozero.bray.nm.ds,
                                                    as.data.frame(isa.metadata.cont.zscores), permu=999, na.rm=TRUE)
otu.tab.trans.ss.nozero.bray.nm.ds.envfit
```

```
##
## ***VECTORS
##
##          NMDS1    NMDS2    r2 Pr(>r)
## nitrate  -0.48446  0.87482 0.7234 0.001 ***
## phosphate -0.36431  0.93128 0.7800 0.001 ***
## silicate  -0.64214  0.76659 0.5981 0.001 ***
## N_P       -0.51519  0.85708 0.4006 0.006 **
## temp_25   -0.48166 -0.87636 0.2593 0.048 *
## sal_25    -0.12845  0.99172 0.6645 0.001 ***
## F_25       0.82196  0.56955 0.7312 0.001 ***
## chla_GFF  0.59651  0.80261 0.7259 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

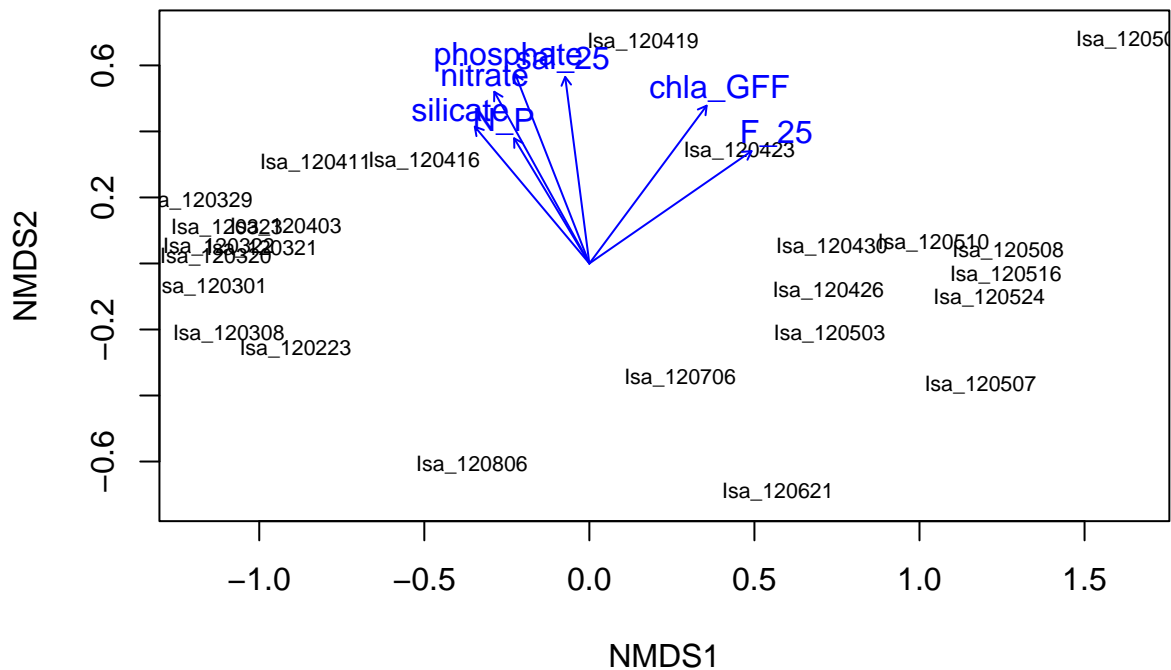
The two last columns indicate the squared correlation coefficient and the associated p-value. We plot the vectors of the significant correlations.

```
plot(otu.tab.trans.ss.nozero.bray.nm.ds, type="t", display="sites") # plot the samples
plot(otu.tab.trans.ss.nozero.bray.nm.ds.envfit) # plot all environmental vectors
```



The plotting only the vectors with $p < 0.01$.

```
plot(otu.tab.trans.ss.nozero.bray.nm.ds, type="t", display="sites") # plot the samples
plot(otu.tab.trans.ss.nozero.bray.nm.ds.envfit, p.max=0.01)
```



Can you see any difference?

Constrained Ordination

Distance-based redundancy analysis (dbRDA) is an ordination method similar to Redundancy Analysis (rda), but it allows non-Euclidean dissimilarity indices, such as Manhattan or Bray–Curtis distance. Selection of the most important (i.e. significant) variables for dbRDA is done by comparing a null model to the full model and doing a stepwise selection of significant variables.

Start with a model containing only species matrix and intercept:

```
mod0.rarefaction <- capscale(otu.tab.trans.ss.nozero.bray ~ 1, as.data.frame(isa.metadata.cont.zscores))
mod0.rarefaction
```

```
## Call: capscale(formula = otu.tab.trans.ss.nozero.bray ~ 1, data =
## as.data.frame(isa.metadata.cont.zscores))
##
##               Inertia Rank
## Total          5.60657
## Unconstrained  5.63679   22
## Imaginary      -0.03021    2
## Inertia is squared Bray distance
##
## Eigenvalues for unconstrained axes:
##   MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8
## 3.0926 0.6618 0.4602 0.3567 0.3298 0.1752 0.1413 0.1062
## (Showing 8 of 22 unconstrained eigenvalues)
```


Now make a model including all variables from env matrix (the dot after tilde (~) means ALL!)

```
mod1.rarefaction <- capscale(otu.tab.trans.ss.nozero.bray ~ ., as.data.frame(isa.metadata.cont.zscores))
mod1.rarefaction
```

```
## Call: capscale(formula = otu.tab.trans.ss.nozero.bray ~ nitrate +
## phosphate + silicate + N_P + temp_25 + sal_25 + F_25 + chla_GFF, data =
## as.data.frame(isa.metadata.cont.zscores))
##
##              Inertia Proportion Rank
## Total          5.606574    1.000000
## Constrained    3.915023    0.698292    8
## Unconstrained  1.721764    0.307097   16
## Imaginary      -0.030213   -0.005389    2
## Inertia is squared Bray distance
##
## Eigenvalues for constrained axes:
##   CAP1   CAP2   CAP3   CAP4   CAP5   CAP6   CAP7   CAP8
## 2.6518 0.4522 0.3213 0.2152 0.1257 0.1018 0.0269 0.0200
##
## Eigenvalues for unconstrained axes:
##   MDS1   MDS2   MDS3   MDS4   MDS5   MDS6   MDS7   MDS8   MDS9   MDS10  MDS11
## 0.6291 0.4016 0.1845 0.1281 0.0865 0.0739 0.0517 0.0407 0.0371 0.0286 0.0177
##   MDS12  MDS13  MDS14  MDS15  MDS16
## 0.0147 0.0101 0.0096 0.0052 0.0026
```

NB here you might get an error if you have missing values. Missing values can be dealt with in different ways depending on the situation. Sometimes it is easiest to drop the sample, sometimes you can input the values of the missing data. The default in the capscale (na.fail) is to stop with missing values. Choices na.omit and na.exclude delete rows with missing values, but differ in representation of results. With na.omit only non-missing site scores are shown, but na.exclude gives NA for scores of missing observations.

Finally do the stepwise selection of variables:

```
ordistep(mod0.rarefaction, scope = formula(mod1.rarefaction), perm.max = 1000, direction="forward")
```

```
##
## Start: otu.tab.trans.ss.nozero.bray ~ 1
##
##              Df      AIC      F Pr(>F)
## + F_25         1 34.859 13.2212 0.005 **
## + silicate     1 36.979 10.2750 0.005 **
## + nitrate      1 37.009 10.2353 0.005 **
## + chla_GFF     1 37.941  9.0198 0.005 **
## + phosphate    1 38.027  8.9090 0.005 **
## + N_P          1 40.914  5.4298 0.005 **
## + sal_25       1 42.913  3.2449 0.030 *
## + temp_25      1 42.605  3.5698 0.035 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: otu.tab.trans.ss.nozero.bray ~ F_25
```

```
##
##           Df      AIC      F Pr(>F)
## + phosphate 1 31.668 5.0769 0.005 **
## + sal_25    1 32.720 3.9607 0.005 **
## + silicate  1 32.046 4.6697 0.010 **
## + nitrate   1 32.225 4.4803 0.010 **
## + N_P       1 33.729 2.9337 0.020 *
## + chla_GFF  1 35.116 1.5882 0.160
## + temp_25   1 35.641 1.0982 0.285
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Step: otu.tab.trans.ss.nozero.bray ~ F_25 + phosphate
##
##           Df      AIC      F Pr(>F)
## + silicate  1 31.536 1.8691 0.105
## + N_P       1 31.854 1.5803 0.125
## + chla_GFF  1 31.677 1.7403 0.135
## + temp_25   1 32.070 1.3857 0.170
## + sal_25    1 32.240 1.2339 0.335
## + nitrate   1 32.764 0.7726 0.585

## Call: capscale(formula = otu.tab.trans.ss.nozero.bray ~ F_25 +
## phosphate, data = as.data.frame(isa.metadata.cont.zscores))
##
##           Inertia Proportion Rank
## Total          5.606574   1.000000
## Constrained     2.728613   0.486681    2
## Unconstrained   2.908174   0.518708   22
## Imaginary      -0.030213  -0.005389    2
## Inertia is squared Bray distance
##
## Eigenvalues for constrained axes:
##   CAP1   CAP2
## 2.4094 0.3192
##
## Eigenvalues for unconstrained axes:
##   MDS1   MDS2   MDS3   MDS4   MDS5   MDS6   MDS7   MDS8
## 1.0560 0.4935 0.3567 0.2095 0.1797 0.1432 0.1266 0.0808
## (Showing 8 of 22 unconstrained eigenvalues)
```

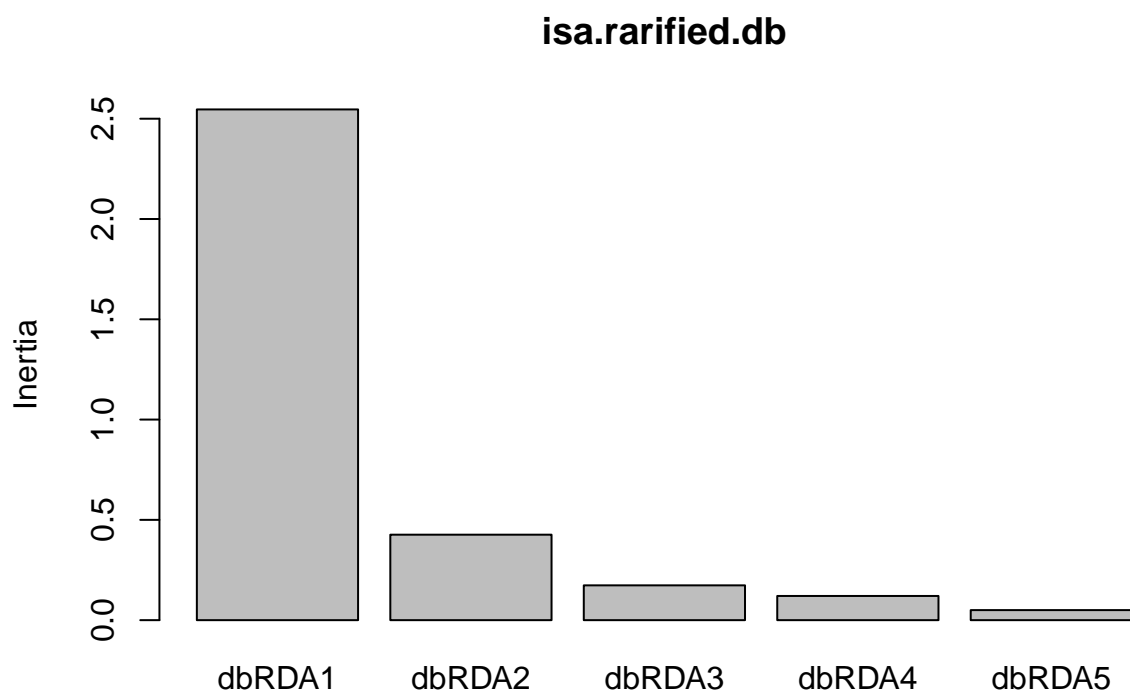
Can you see which variables were selected?

When doing a stepwise building of models you can this either “forward” (as in the example), “backwards”, or “both”. Try different methods and see if the end result is any different.

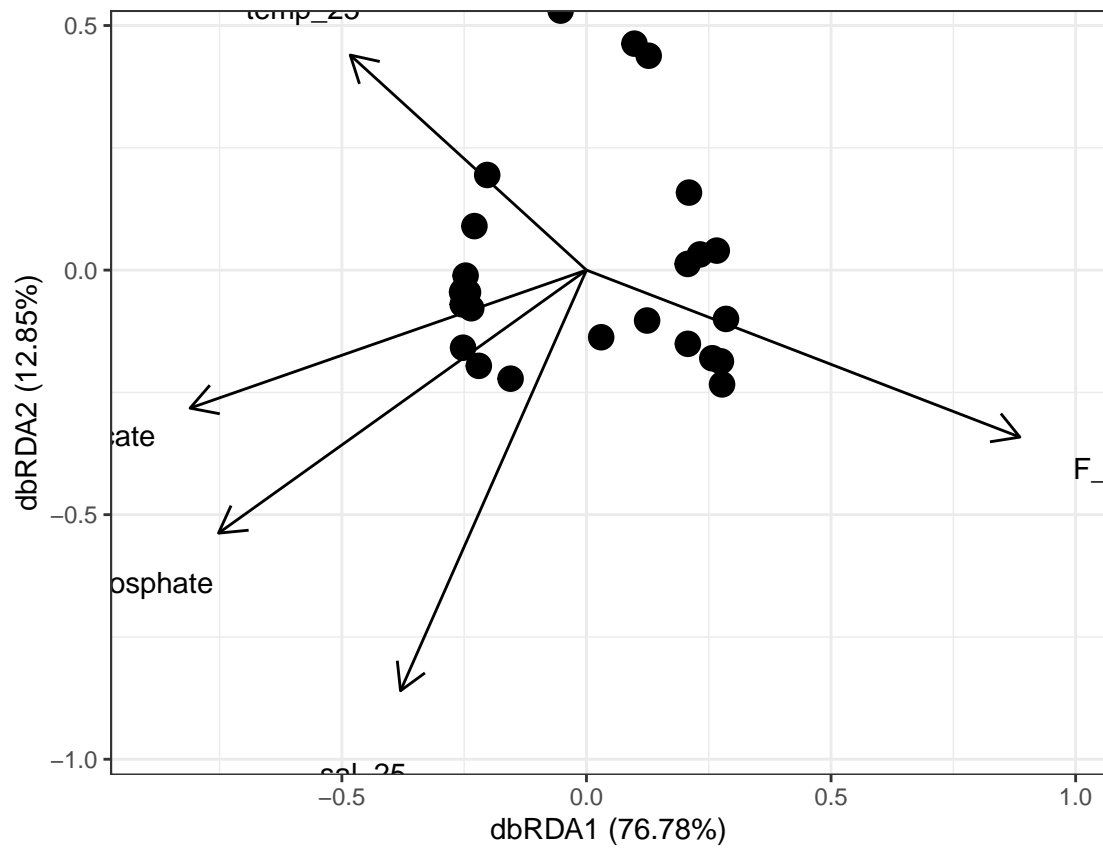
Plot the ordination

In the following sections we will use `ggord` for more control of the ordination plot. `ggord` is a packages that makes use of `ggplot2`. It can take many different parameters. See `?ggord` for details.

```
isa.rarified.db <- dbrda(formula = otu.tab.trans.ss.nozero.bray ~ silicate+temp_25+sal_25+F_25+phosphat
stats::screeplot(isa.rarified.db)
```

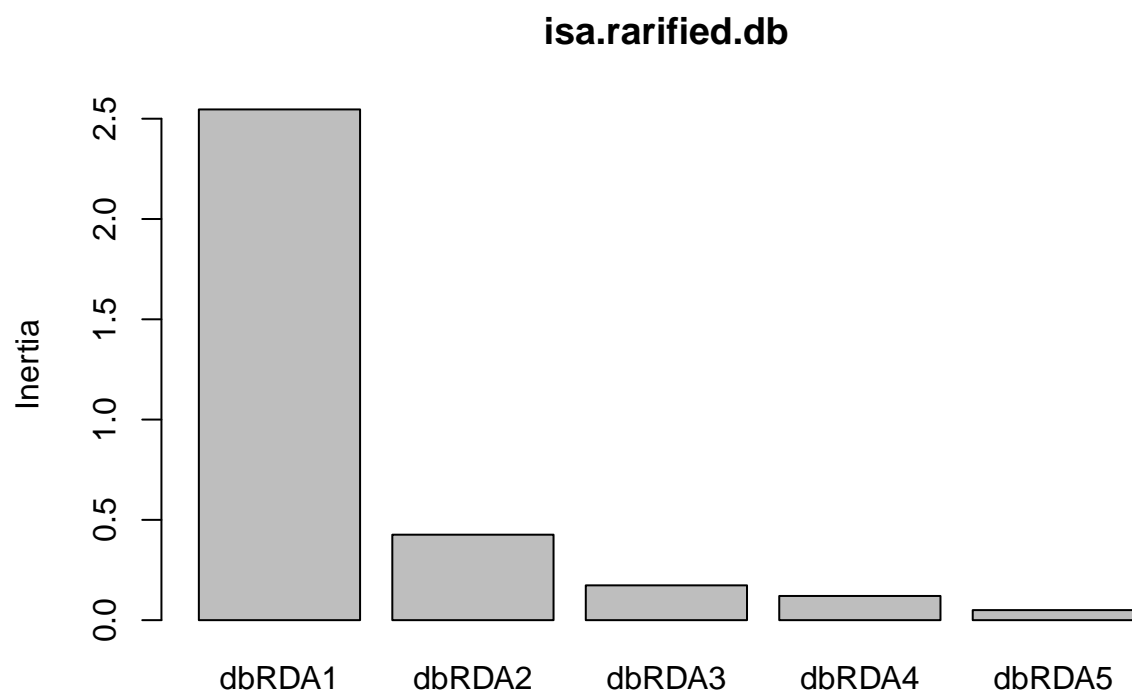


```
ggord(isa.rarified.db)
```

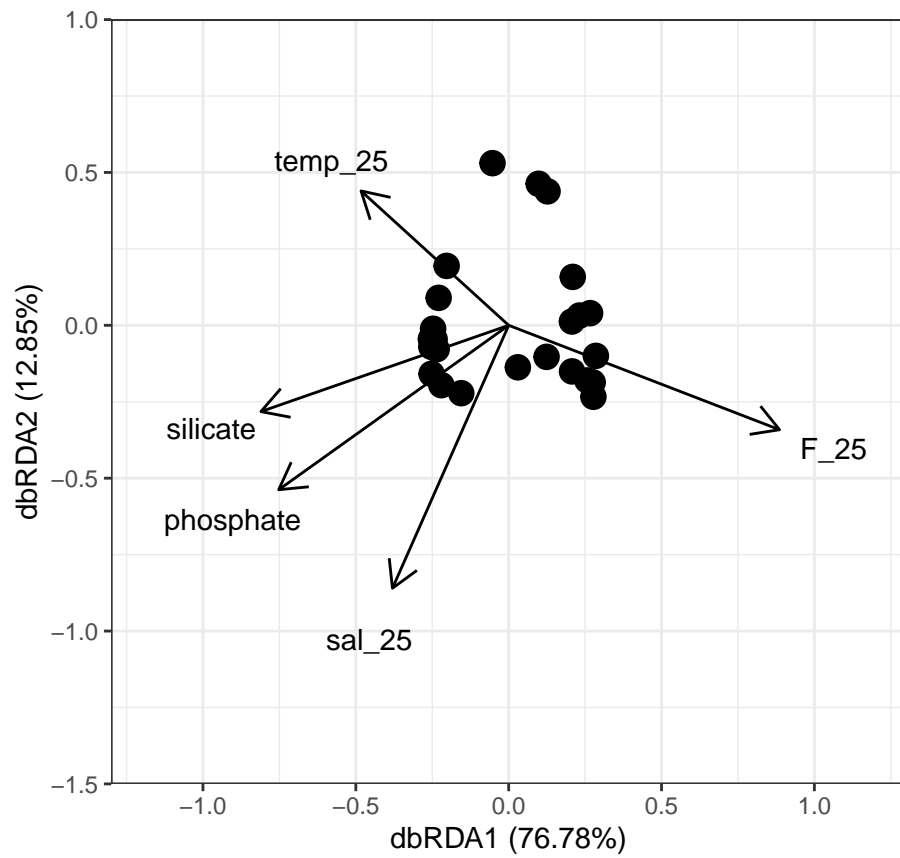


The plot wasn't very pretty. Specifying limits to the plot might help:

```
isa.rarified.db <- dbrda(formula = otu.tab.trans.ss.nozero.bray ~ silicate+temp_25+sal_25+F_25+phosphate,
stats::screeplot(isa.rarified.db))
```

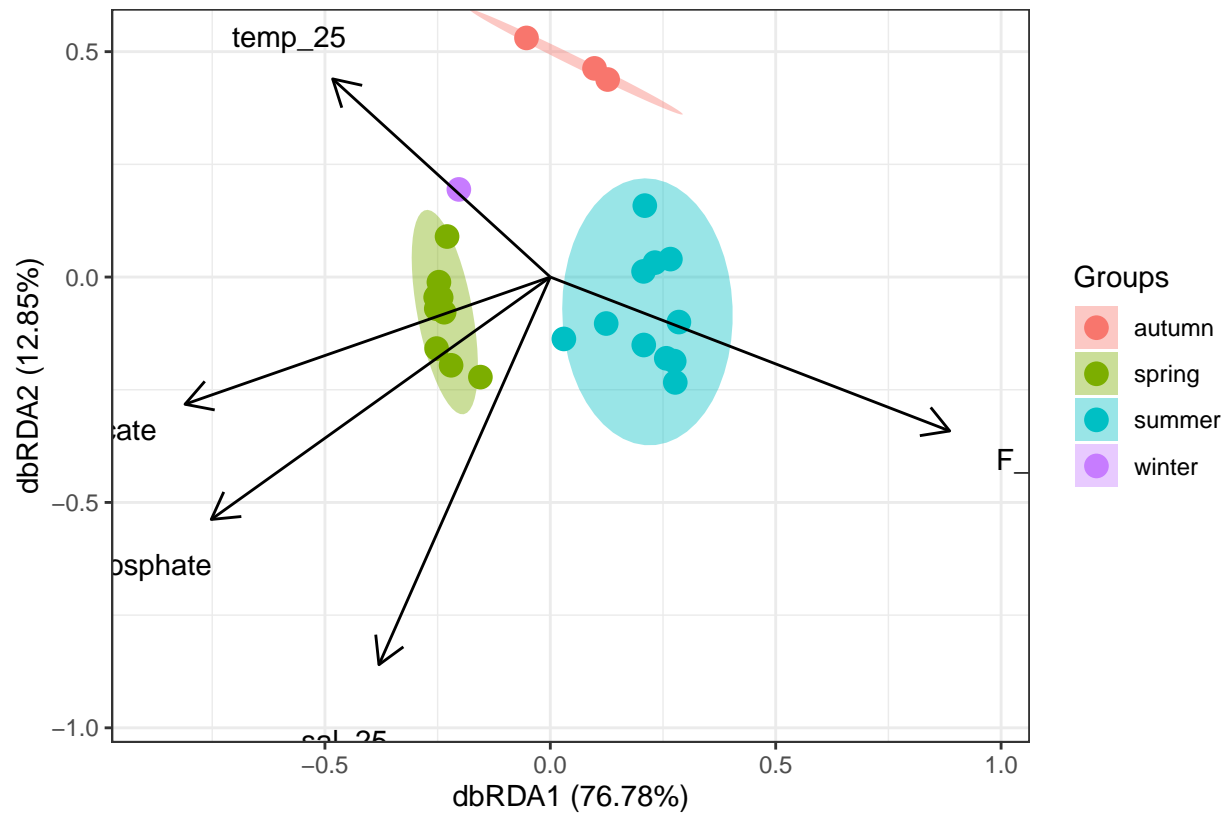


```
ggord(isa.rarified.db,xlims=c(-1.3,1.3), ylims=c(-1.5,1))
```



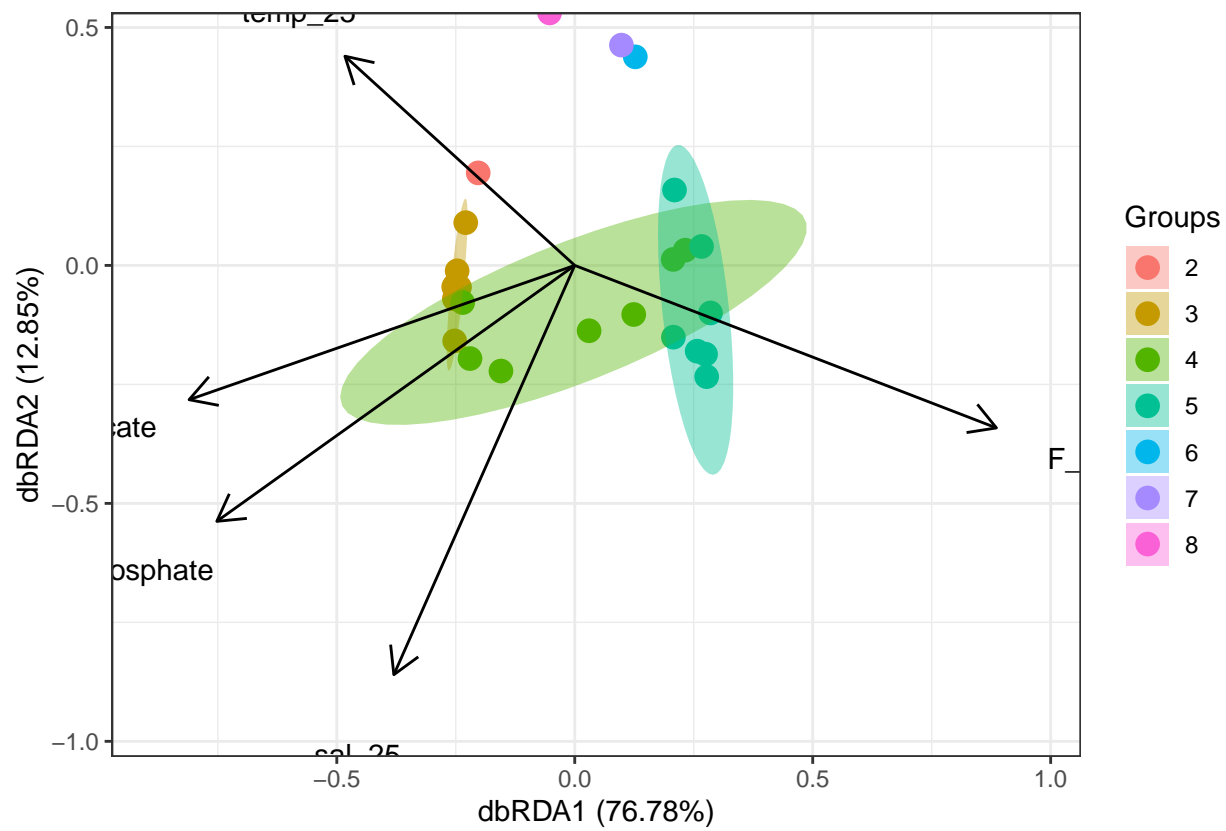
Ggord can take several parameters, for instance the seasons from the metadata:

```
ggord(isa.rarified.db, isa.metadata.simp$seasons)
```



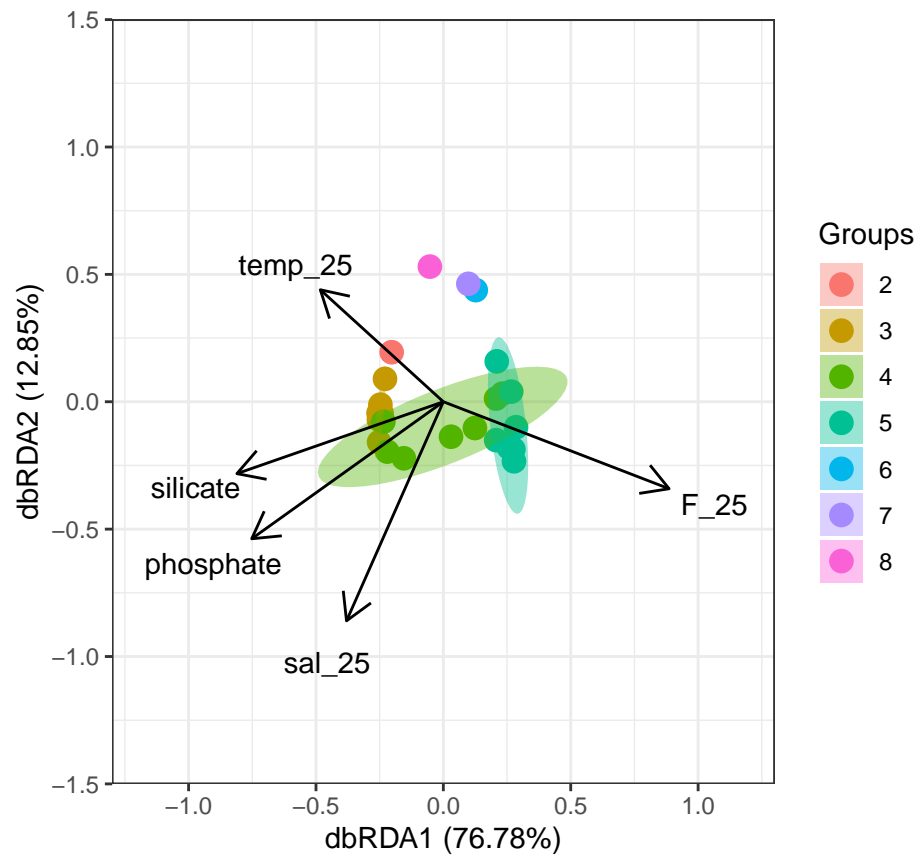
See if you can modify the plot and make the text visible!
Or the months:

```
ggord(isa.rarified.db, as.factor(isa.metadata.simp$month))
```



And you can specify the limits of the axes if the plot does not look nice:

```
ggord(isa.rarified.db, grp_in=as.factor(isa.metadata.simp$month), xlims=c(-1.3,1.3), ylims=c(-1.5,1.5))
```

Finally save!

```
save.image("AB332_lab_III.RData")
```