

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","F_25","chla_GFF")
```

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
```

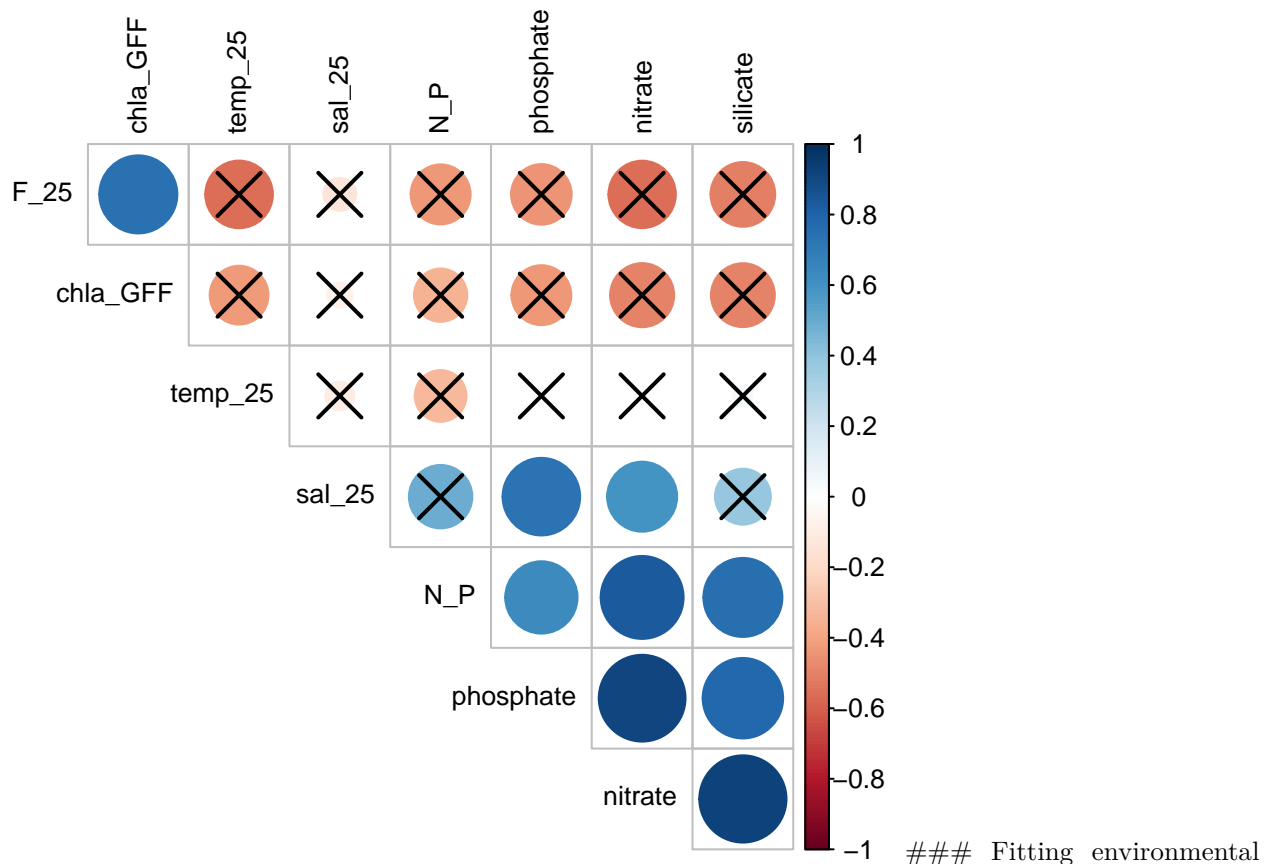
```
##
## Pairwise two-sided p-values:
##      nitrate phosphate silicate N_P    temp_25 sal_25 F_25    chla_GFF
## nitrate      <.0001    <.0001    <.0001 0.9642 0.0017 0.0041 0.0110
## phosphate <.0001      <.0001    0.0006 0.8739 <.0001 0.0250 0.0288
## silicate <.0001 <.0001      <.0001 0.8250 0.0604 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,
```



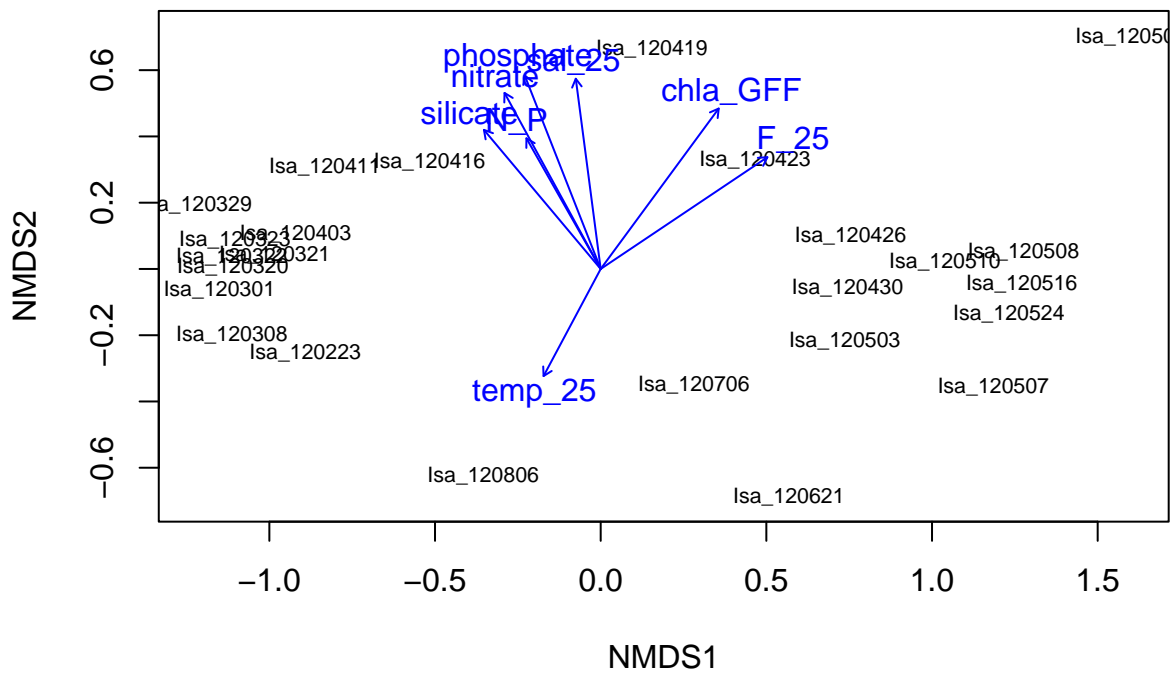
variables to ordinations `envfit` will fit the environmental variables to the NMDS ordination as vectors
First we fit the variables to the OTU-table that was rarified:

```
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  -0.47942  0.87758 0.7294 0.001 ***
## phosphate -0.36541  0.93085 0.7835 0.001 ***
## silicate  -0.64291  0.76595 0.5976 0.001 ***
## N_P       -0.49425  0.86932 0.4093 0.003 **
## temp_25   -0.47060 -0.88235 0.2671 0.045 *
## sal_25    -0.12979  0.99154 0.6670 0.001 ***
## F_25      0.83061  0.55685 0.7302 0.001 ***
## chla_GFF  0.59268  0.80544 0.7190 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

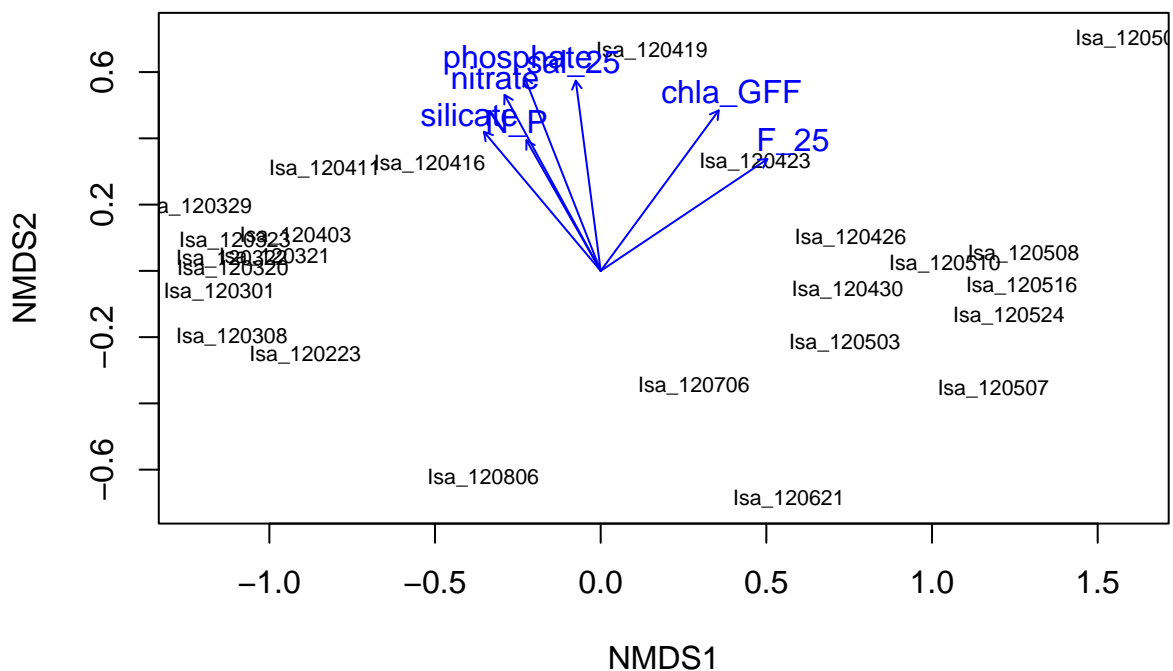
```
plot(otu.tab.trans.ss.nozero.bray.nm.ds, type="t", display="sites") # plot the samples
plot(otu.tab.trans.ss.nozero.bray.nm.ds.envfit) # plot all environmental vectors
```



The

plotting only the vectors with $p < 0.01$.

```
plot(otu.tab.trans.ss.nozero.bray.nmnds, type="t", display="sites") # plot the samples
plot(otu.tab.trans.ss.nozero.bray.nmnds.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          5.6040
## Unconstrained  5.6328   22
## Imaginary     -0.0288    2
## Inertia is squared Bray distance
##
## Eigenvalues for unconstrained axes:
##   MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8
## 3.1000 0.6641 0.4584 0.3506 0.3246 0.1755 0.1416 0.1076
## (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          5.603981   1.000000
## Constrained     3.915949   0.698780    8
## Unconstrained   1.716829   0.306359   16
## Imaginary      -0.028797  -0.005139    2
## Inertia is squared Bray distance
##
## Eigenvalues for constrained axes:
##   CAP1  CAP2  CAP3  CAP4  CAP5  CAP6  CAP7  CAP8
## 2.6596 0.4516 0.3198 0.2105 0.1282 0.0988 0.0270 0.0205
##
## Eigenvalues for unconstrained axes:
##   MDS1  MDS2  MDS3  MDS4  MDS5  MDS6  MDS7  MDS8  MDS9  MDS10  MDS11
## 0.6284 0.4014 0.1796 0.1281 0.0898 0.0758 0.0490 0.0393 0.0367 0.0267 0.0198
##   MDS12  MDS13  MDS14  MDS15  MDS16
## 0.0148 0.0107 0.0086 0.0054 0.0029
```

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 34.840 13.2228 0.005 **
## + silicate   1 36.900 10.3566 0.005 **
## + nitrate    1 36.945 10.2968 0.005 **
## + chla_GFF   1 37.919  9.0252 0.005 **
## + phosphate  1 37.971  8.9589 0.005 **
## + N_P        1 40.869  5.4599 0.005 **
## + sal_25     1 42.894  3.2456 0.025 *
## + temp_25    1 42.589  3.5678 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 31.633 5.0941 0.005 **
## + nitrate    1 32.179 4.5083 0.005 **
## + sal_25     1 32.713 3.9487 0.005 **
## + silicate   1 31.977 4.7232 0.010 **
## + N_P        1 33.692 2.9522 0.025 *
## + chla_GFF   1 35.111 1.5746 0.175
## + temp_25    1 35.620 1.0996 0.290
## ---
## 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 31.489 1.8801 0.095 .
## + N_P        1 31.822 1.5771 0.125
## + chla_GFF   1 31.667 1.7181 0.135
## + temp_25    1 32.029 1.3916 0.160
## + sal_25     1 32.220 1.2210 0.350
## + nitrate    1 32.731 0.7714 0.570
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Call: capscale(formula = otu.tab.trans.ss.nozero.bray ~ F_25 +
## phosphate, data = as.data.frame(isa.metadata.cont.zscores))
##
##           Inertia Proportion Rank
## Total      5.603981  1.000000
## Constrained 2.728646  0.486912  2
## Unconstrained 2.904132  0.518227 22
## Imaginary   -0.028797 -0.005139  2
## Inertia is squared Bray distance
##
## Eigenvalues for constrained axes:
##   CAP1  CAP2
## 2.4127 0.3160
##

```

```
## Eigenvalues for unconstrained axes:
##   MDS1   MDS2   MDS3   MDS4   MDS5   MDS6   MDS7   MDS8
## 1.0612 0.4918 0.3523 0.2068 0.1800 0.1423 0.1246 0.0839
## (Showing 8 of 22 unconstrained eigenvalues)
```

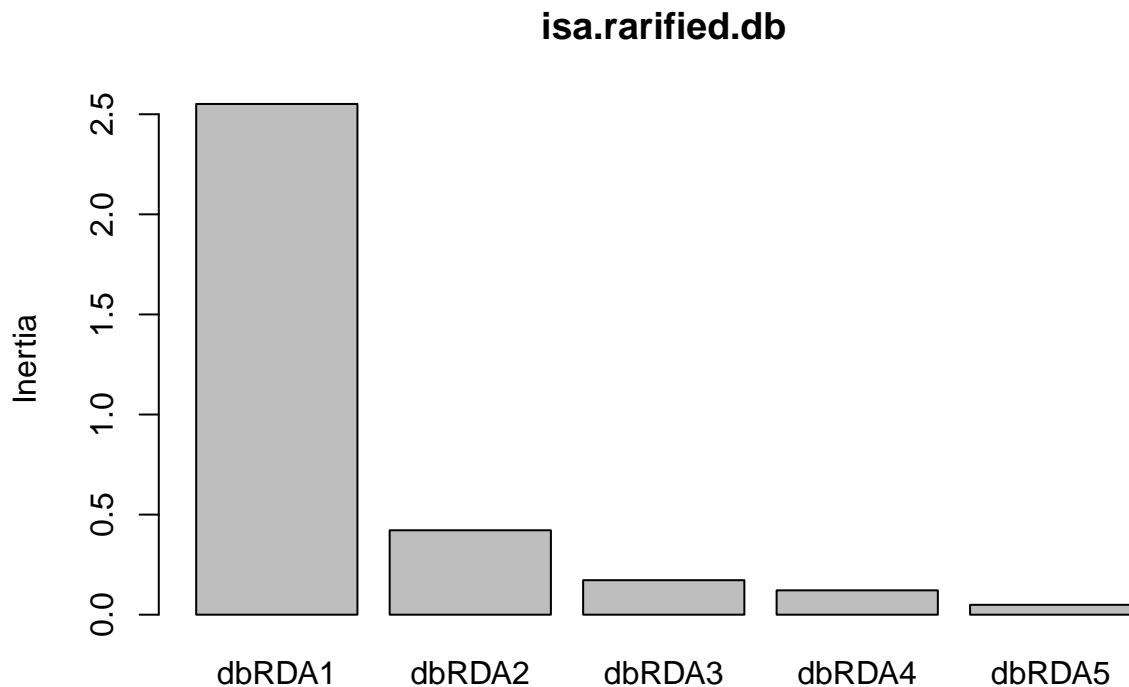
Can you see which variables were selected?

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.

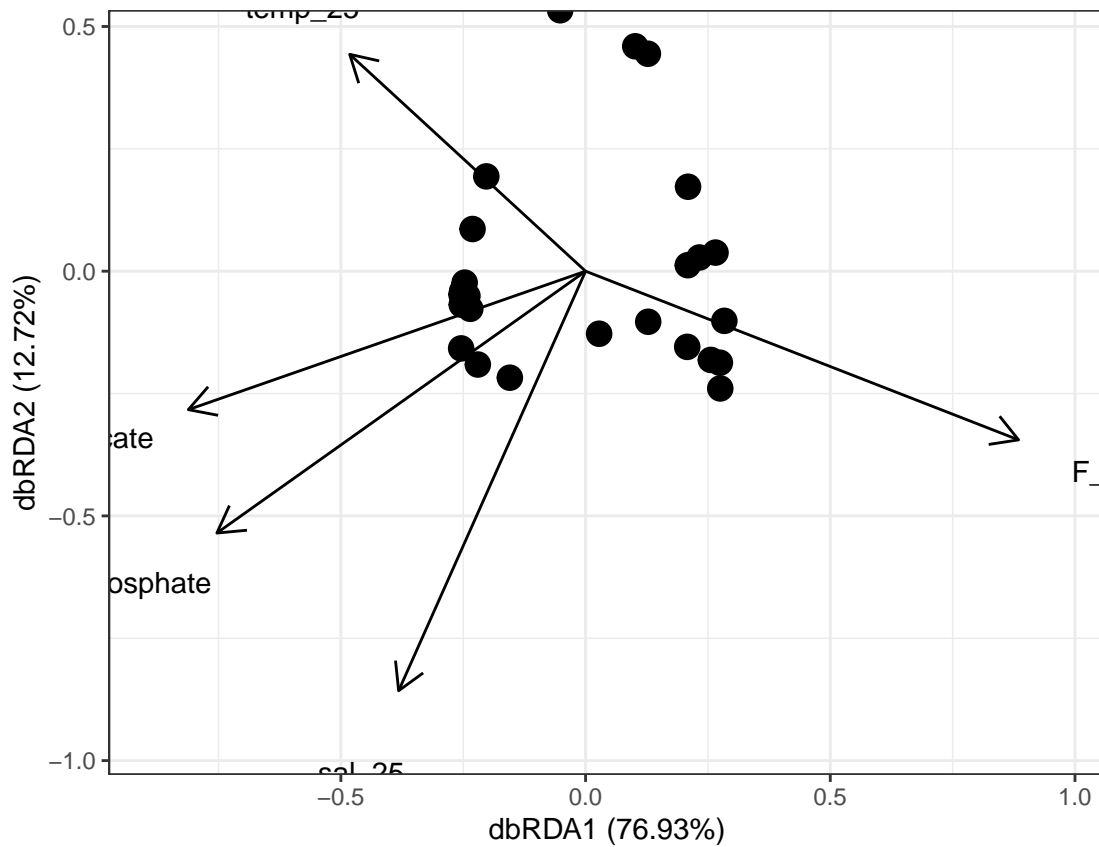
Plot the ordination

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)
```

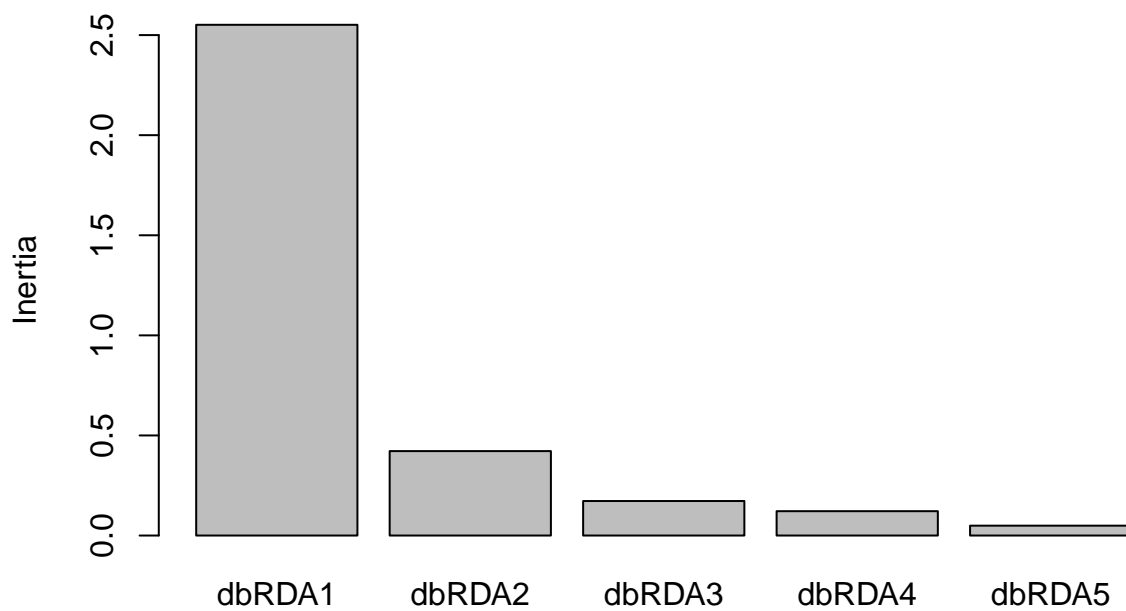


The plot

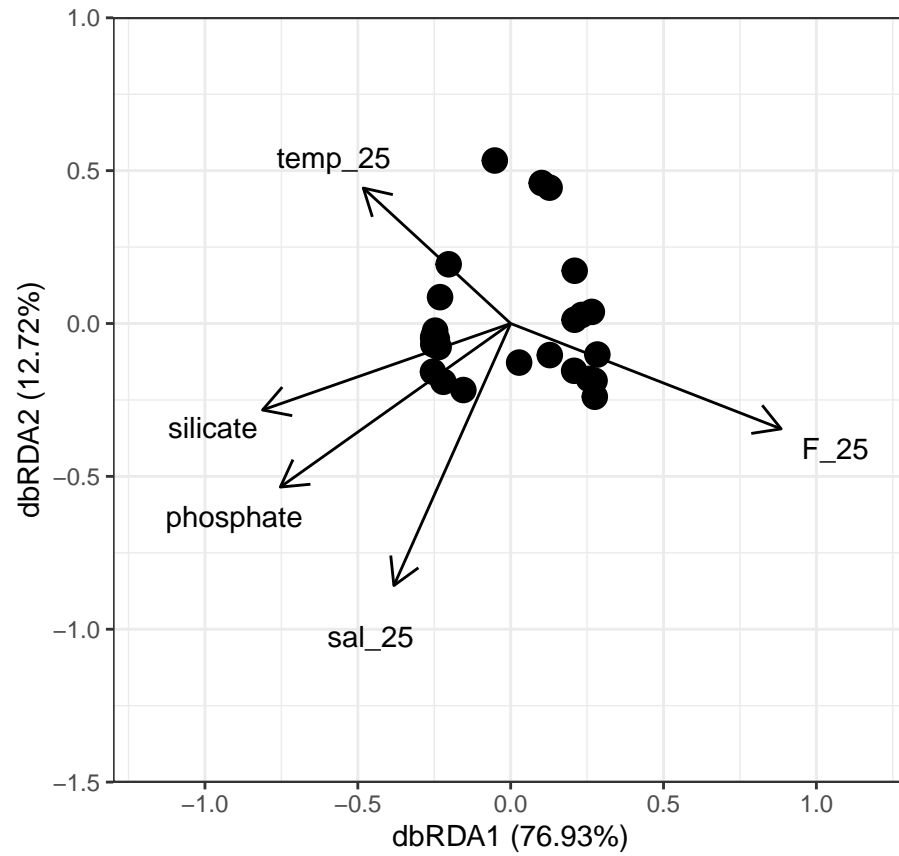
wasn't very pretty. Specifying limits to the plot might help:

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

isa.rarified.db

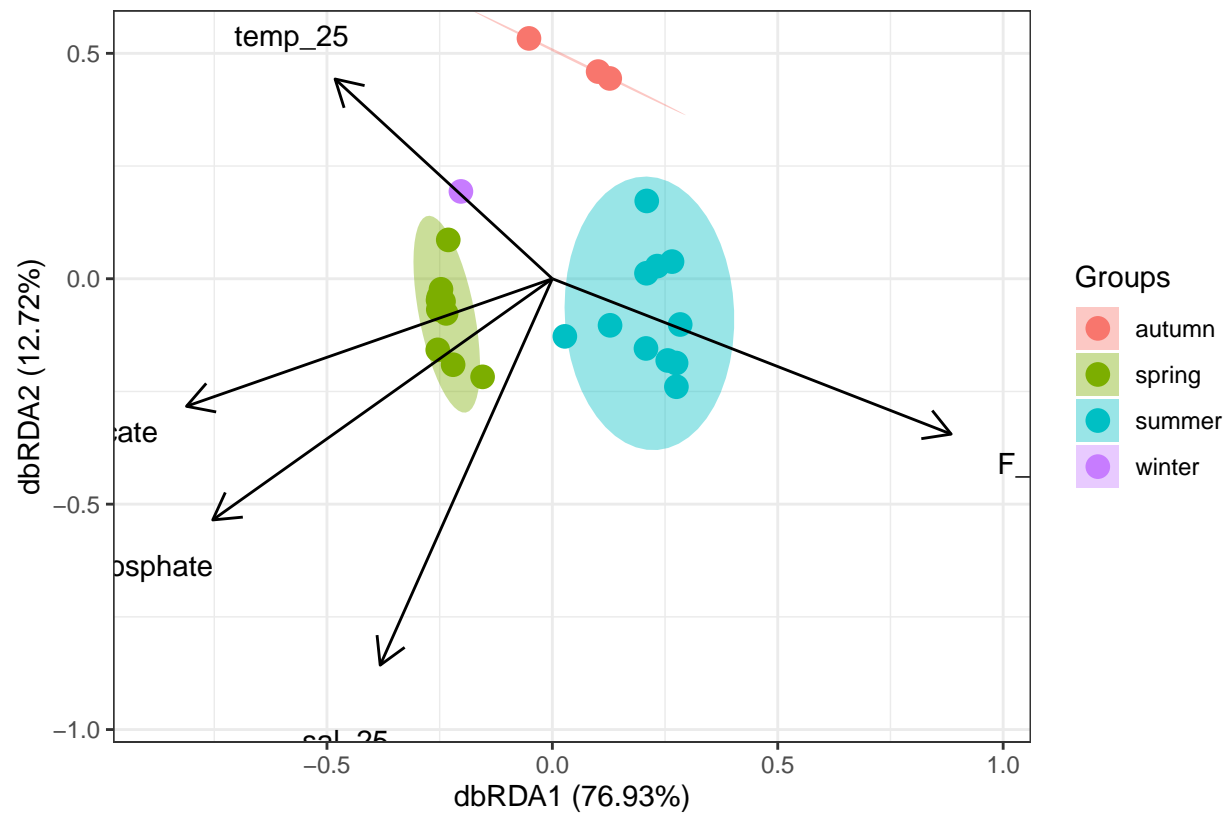


```
ggord(isa.rarified.db,xlims=c(-1.3,1.3), ylims=c(-1.5,1))
```



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

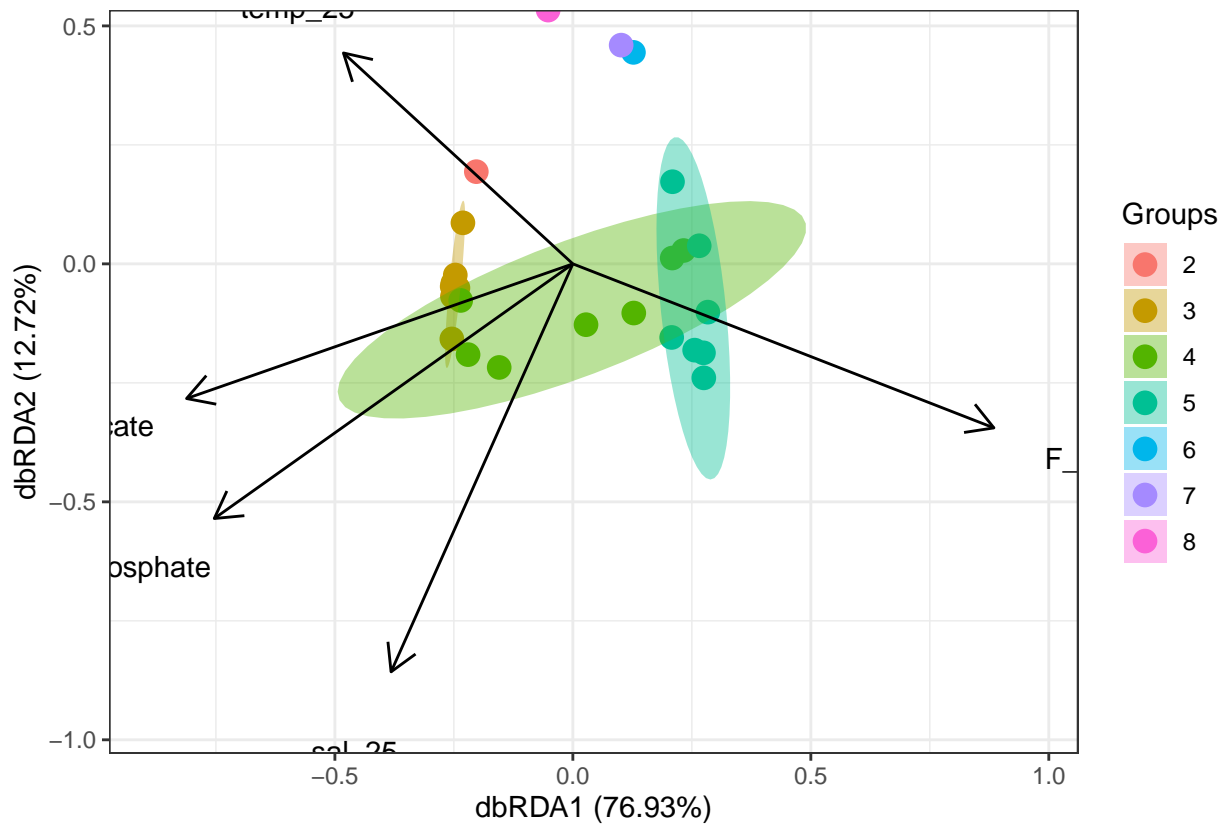
```
ggord(isa.rarified.db, isa.metadata.simp$seasons)
```



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

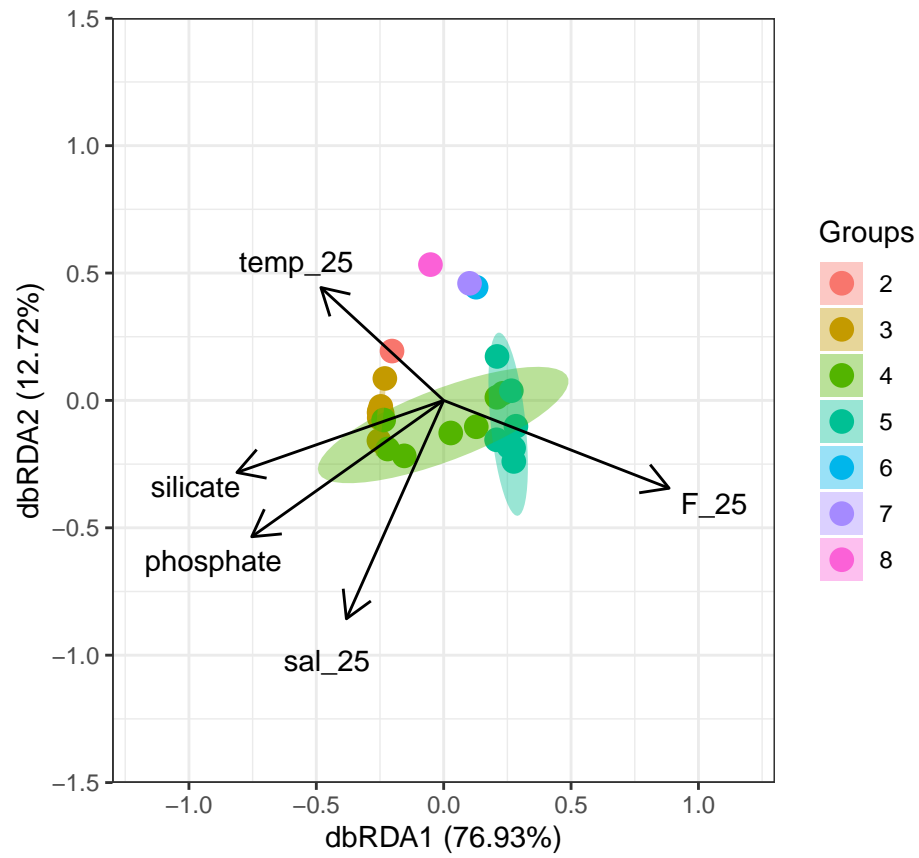
Or the months:

```
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")
```