

multivariate models solutions

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```
# load code dependancies
library(vegan)

## Loading required package: permute
## Loading required package: lattice
## This is vegan 2.5-6
source('./scripts/utility_functions.R')
data(dune)
data(dune.env)
?dune
```

1. Conduct an indirect ordination on the dune plant community. Specifically, visually examine a NMDS plot using the bray-curtis distance metric. Below is some code to help you develop a potential plot that emphasizes the role of the environmental variable “Moisture”. Describe how you interpret the graphic. What is the goal of creating such a plot? Does this analysis suggest any interesting findings with respect to the dune vegetation?

```
dune_mds <- metaMDS(dune, trace = 0)
# fit enviornmental variables to ordination space
dune_fit <- envfit(dune_mds, dune.env)
dune_fit

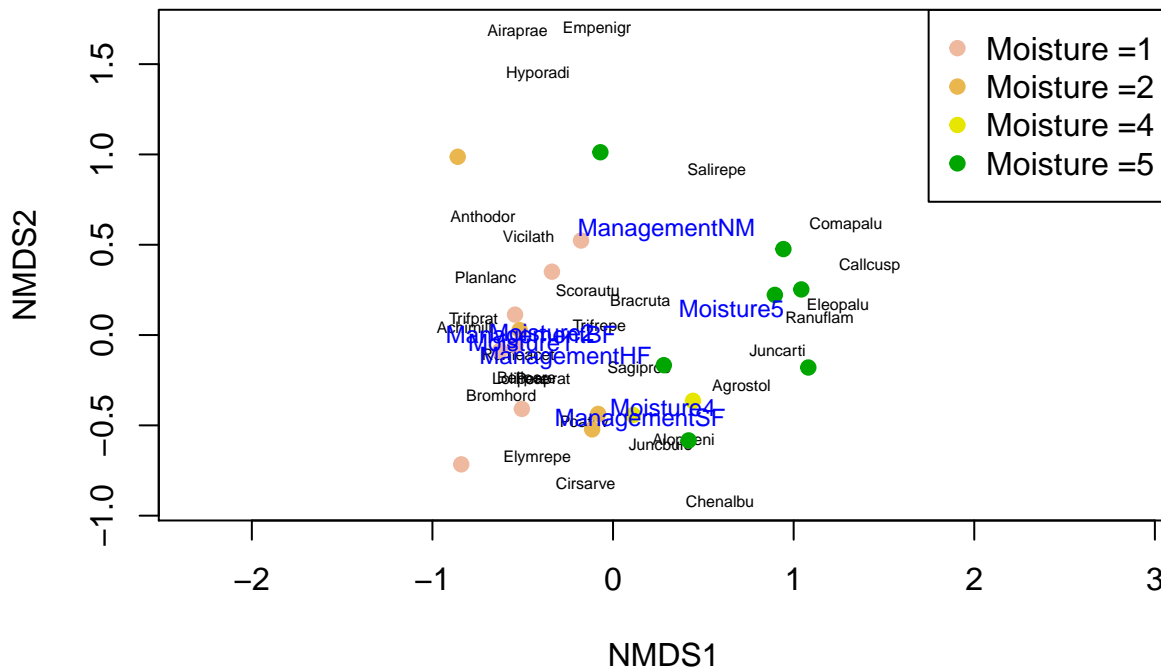
##
## ***VECTORS
##
##      NMDS1   NMDS2      r2 Pr(>r)
## A1 0.96474 0.26322 0.3649 0.017 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
##
## ***FACTORS:
##
## Centroids:
##      NMDS1   NMDS2
## Moisture1 -0.5101 -0.0403
## Moisture2 -0.3938 0.0139
## Moisture4 0.2765 -0.4033
## Moisture5 0.6561 0.1476
## ManagementBF -0.4534 -0.0102
## ManagementHF -0.2636 -0.1282
```

```
## ManagementNM  0.2958  0.5790
## ManagementSF  0.1506 -0.4670
## UseHayfield   -0.1568  0.3248
## UseHaypastu   -0.0412 -0.3370
## UsePasture     0.2854  0.0844
## Manure0        0.2958  0.5790
## Manure1       -0.2482 -0.0215
## Manure2       -0.3079 -0.1866
## Manure3        0.3101 -0.2470
## Manure4       -0.3463 -0.5583
##
## Goodness of fit:
##              r2 Pr(>r)
## Moisture    0.5014 0.001 ***
## Management  0.4134 0.007 **
## Use         0.1871 0.128
## Manure      0.4247 0.022 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

The output from the function `envfit` suggests that the categorical variables **Moisture** and **Management** are the two most important variables for explaining the site placements in ordination space. In other words, these variables coorelate the strongest to the primary axes of variation in species composition identified by the analysis.

Let's examine our ordination plot to see if this seems reasonable.

```
plot(dune_mds, type='n')
text(dune_mds, 'sp', cex=.5)
# generate vector of colors
mois_lvs <- sort(unique(dune.env$Moisture))
color_vect <- rev(terrain.colors(length(mois_lvs) + 1))[-1]
points(dune_mds, 'sites', pch=19,
       col=color_vect[dune.env$Moisture])
# add enviornmental variables for which their p value was less than 0.01
plot(dune_fit, p.max = 0.01, cex=.75)
legend('topright', paste("Moisture =", mois_lvs, sep=''),
      col=color_vect, pch=19)
```



In the above plot, the points are colored by their Moisture level. It appears that sites of different moisture levels are arrayed along the first ordination axis (i.e., x-axis) which by definition is the dominant gradient in species composition. The management levels are arrayed vertically along the second axis indicating that they are associated with an secondary orthogonal (i.e., independent) axis of variation in the plant community.

Notice too that the graphic is a bit cluttered and messy. Cleaning up these plots takes time and care. One simple fix would be to rename the variables **Moisture** and **Management** so that their labels are shorter. Alternatively, if the effect of **Moisture** is largely linear as it appears in the ordination then it could be treated as a single numeric vector rather than a factor with mutiple centroids.

2. Carry out a direct ordination using CCA in order to test any potential hypotheses that you developed after examining the MDS plot. Specifically, carry out a test of the entire model (i.e., including all constrained axes) and also carry out tests at the scale of individual explanatory variables you included in your model if you included more than one variable. Plot your results.

```
# a very terse way to specify to use all enviornmental variable is use the "."
# notation, but I don't recommend this because it is not the clearest way to
# indicate what variables are in the model for a human reader
dune_cca <- cca(dune ~ . , data=dune.env)
# alternatively I perfer this specification
dune_cca <- cca(dune ~ A1 + Moisture + Management + Use + Manure,
               data=dune.env)
# examine output
dune_cca
```

```
## Call: cca(formula = dune ~ A1 + Moisture + Management + Use + Manure,
## data = dune.env)
##
##              Inertia Proportion Rank
## Total          2.1153      1.0000
## Constrained    1.5032      0.7106   12
## Unconstrained  0.6121      0.2894    7
## Inertia is scaled Chi-square
## Some constraints were aliased because they were collinear (redundant)
```

```
##
## Eigenvalues for constrained axes:
##   CCA1   CCA2   CCA3   CCA4   CCA5   CCA6   CCA7   CCA8   CCA9   CCA10  CCA11
## 0.4671 0.3410 0.1761 0.1532 0.0953 0.0703 0.0589 0.0499 0.0318 0.0260 0.0228
##   CCA12
## 0.0108
##
## Eigenvalues for unconstrained axes:
##   CA1    CA2    CA3    CA4    CA5    CA6    CA7
## 0.27237 0.10876 0.08975 0.06305 0.03489 0.02529 0.01798
```

The output from the CCA model including all the environmental variables indicates the model explains $100 * 1.50 / 2.12 = 71\%$ of the variance in species composition. However, we included a lot of variables in our model so it is probably a good idea to compute the adjusted R-squared statistic as well.

```
dune_cca_r2 <- RsquareAdj(dune_cca, nperm=2000)
dune_cca_r2[2]
```

```
## $adj.r.squared
## [1] 0.2167921
```

After 2000 permutations you can see that the adjusted r-squared stabilized around 0.22 which is quite a bit smaller than the raw R^2 . This indicates that the model was overfit to the data because it had many spurious explanatory variables. Let's examine if the model and particular variables that are statistically significant.

```
# test for model significance
anova(dune_cca)
```

```
## Permutation test for cca under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ A1 + Moisture + Management + Use + Manure, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## Model      12      1.5032 1.4325 0.034 *
## Residual    7       0.6121
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# test for marginal effects of each variable
anova(dune_cca, by='margin')
```

```
## Permutation test for cca under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ A1 + Moisture + Management + Use + Manure, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## A1           1   0.11070 1.2660 0.238
## Moisture      3   0.31587 1.2041 0.221
## Management    2   0.15882 0.9081 0.586
## Use           2   0.13010 0.7439 0.784
## Manure        3   0.25490 0.9717 0.493
## Residual      7   0.61210
```

The first permutation-based ANOVA indicates that the model does explain more variance than random, but the effect size is pretty weak as indicated by the small F-statistic. The second ANOVA which examined the

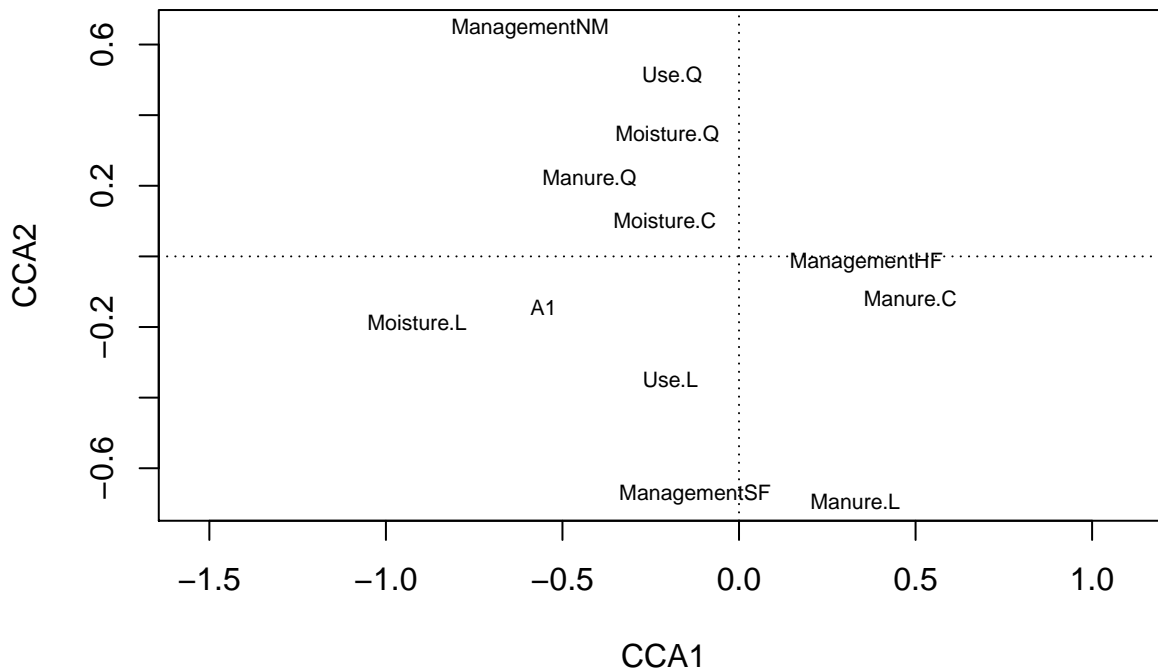
Let's plot our results now:

CCA2

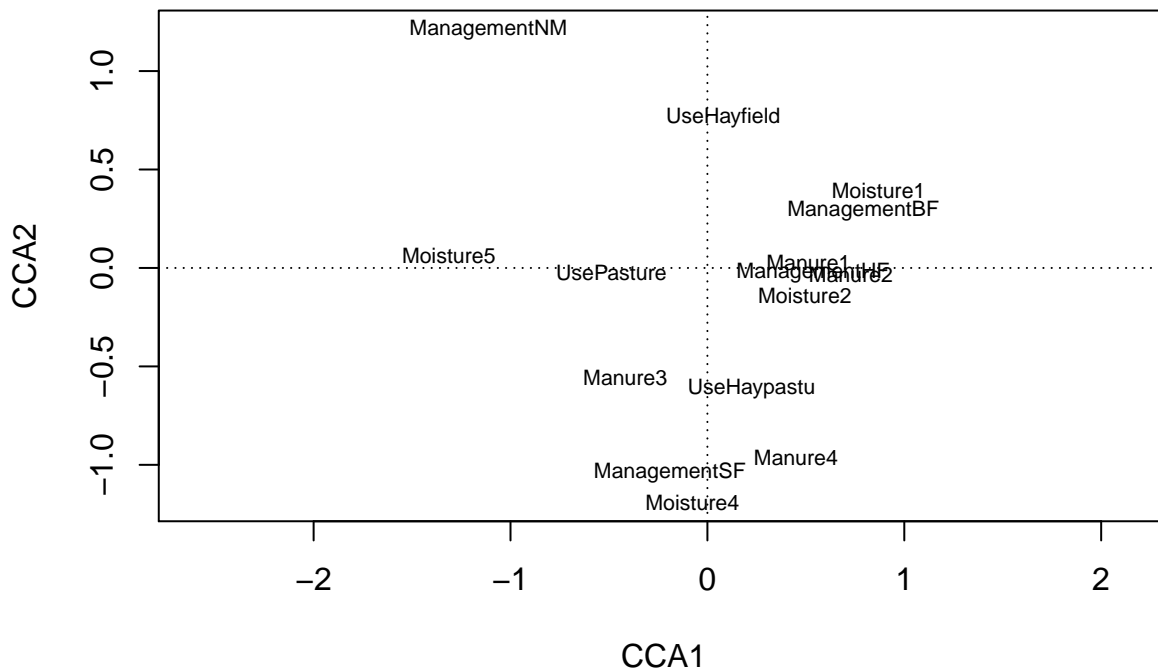
CCA1

A CCA plot with CCA1 on the x-axis (range -4 to 4) and CCA2 on the y-axis (range -1 to 2). The plot displays two types of sampling points: open circles and red plus signs. Environmental variables are represented by blue vectors originating from the center (0,0). The vectors are labeled: 'Use.Q' (pointing up and slightly left), 'Moisture.Q' (pointing up and left), 'Manure.Q' (pointing up and left), 'Moisture.C' (pointing left), 'AT' (pointing left), 'Moisture.L' (pointing left), 'Use.L' (pointing down and left), 'Manure.L' (pointing down and right), 'Manure.C' (pointing right), and 'Manure.Q' (pointing right). The red plus signs are clustered in the upper-left quadrant, while the open circles are more widely distributed, with some in the upper-right and lower-right quadrants.

```
# this is cleaner but the blue 'X' are enviornmentnal variables that need labels
# if we want to focus in on the enviornmentnal variables we can use
plot(dune_cca, display='bp')
```



```
# for categorical variables it typically makes more sense to display centroids
plot(dune_cca, display='cn')
```



The third plot indicates that the first axis is primarily being loaded on by the linear component of the moisture variable, the thickness of soil, and the cubic component of the Manure variable (interpreting exactly what a cubic component means is difficult). The second axis is more strongly related to differences in management as the three classes of management are spread broadly along this axis.

Based upon the large difference between the R^2 and R^2 adjusted values, the output of the marginal variables tests, and the graphical results of the CCA indicate that many of the variables included in the model do not have strong explanatory power. Additionally, interpretation of the nomial variables with their polynomial constrats (the L-linear, Q-quadratic, and C-cubic terms) is not straightforward.

In an effort to improve model interpretation and to decrease the chance of overfitting the community data, let's examine a simpler model with only the moisture and management variables. A1 appeared to have a similar effect as the linear moisture component (notice both arrows share a similar angle) so we'll ignore it here.

```
dune_cca_MM <- cca(dune ~ Moisture + Management, data=dune.env)
dune_cca_MM_r2 <- RsquareAdj(dune_cca_MM, 2000)
dune_cca_MM_r2

## $r.squared
## [1] 0.4738772
##
## $adj.r.squared
## [1] 0.2319815

anova(dune_cca_MM)

## Permutation test for cca under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ Moisture + Management, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## Model      6    1.0024 1.9515 0.003 **
## Residual  13    1.1129
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(dune_cca_MM, by='margin')

## Permutation test for cca under reduced model
## Marginal effects of terms
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ Moisture + Management, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## Moisture    3    0.39854 1.5518 0.044 *
## Management  3    0.37407 1.4565 0.054 .
## Residual   13    1.11289
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

anova(dune_cca_MM, dune_cca)

## Permutation tests for cca under reduced model
## Permutation: free
## Number of permutations: 999
##
## Model 1: dune ~ Moisture + Management
## Model 2: dune ~ A1 + Moisture + Management + Use + Manure
##   ResDf ResChiSquare Df ChiSquare      F Pr(>F)
```

```
## 1    13      1.1129
## 2     7      0.6121  6    0.50079 0.9545  0.559
```

The above analysis indicates that this reduced model is less overfit (note it actually has a higher adjusted R^2 because the spurious variables were dropped). Both moisture and management are relevant variables (i.e., statistically significant), but their effects are not mind blowing.

Note that it is difficult to visually compare the strength of a continuous variable such as A1 with the categorical variables because the relative distance of the label seems to be scaled differently. The ANOVA analysis is useful for parsing out the relative importances when different classes of variables are involved.

One solution here is to maintain the ranking of these nominal categorical variables but treat their effects as purely linear by re-encoding them as numeric vectors. An alternative approach is to treat them like unranked categorical variables in which case a separate mean (i.e., centroid) is estimated for each level.

Let's examine the utility of both approaches here.

```
dune.env$Moist_cat <- as.factor(as.numeric(dune.env$Moisture))
dune.env$Moist_lin <- as.numeric(dune.env$Moisture)

dune_cca_cat <- cca(dune ~ Moist_cat + Management, data = dune.env)
anova(dune_cca_cat, by = 'terms')
```

```
## Permutation test for cca under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ Moist_cat + Management, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## Moist_cat  3    0.62831 2.4465 0.001 ***
## Management 3    0.37407 1.4565 0.050 *
## Residual   13    1.11289
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dune_cca_cat)
```

```
## $r.squared
## [1] 0.4738772
##
## $adj.r.squared
## [1] 0.2291386
```

```
dune_cca_lin <- cca(dune ~ Moist_lin + Management, data = dune.env)
anova(dune_cca_lin, by = 'terms')
```

```
## Permutation test for cca under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## Model: cca(formula = dune ~ Moist_lin + Management, data = dune.env)
##           Df ChiSquare      F Pr(>F)
## Moist_lin  1    0.41081 4.8952 0.001 ***
## Management 3    0.44563 1.7700 0.007 **
## Residual   15    1.25883
## ---
```



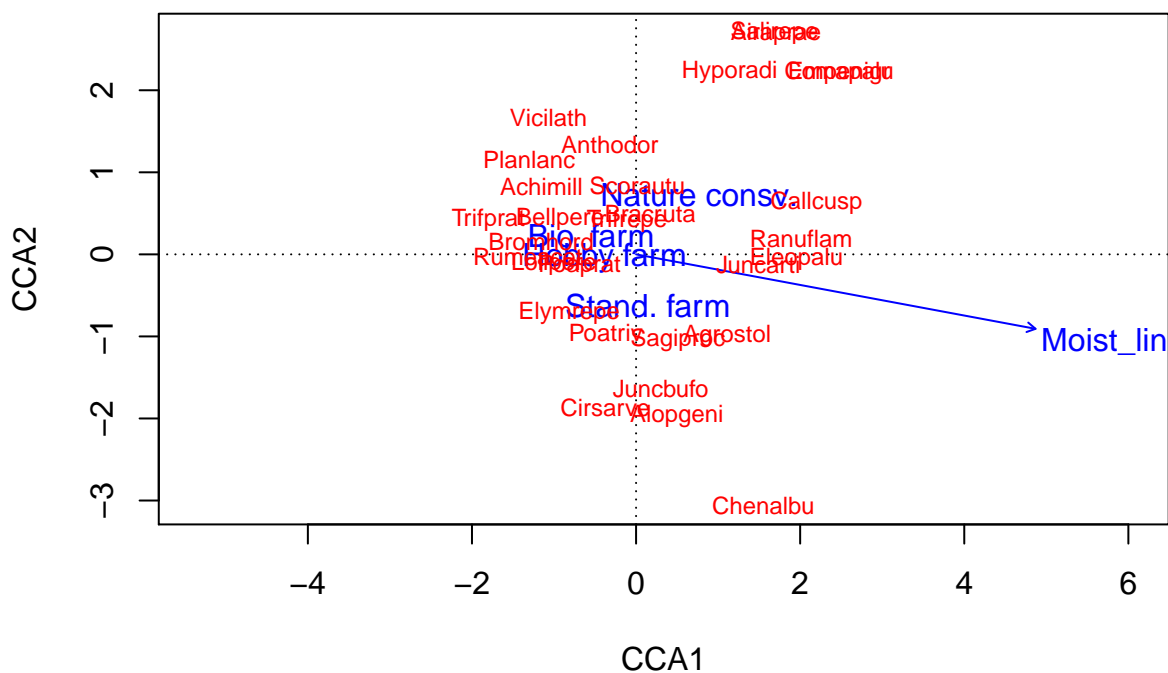
```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
RsquareAdj(dune_cca_lin)
```

```
## $r.squared
## [1] 0.4048847
##
## $adj.r.squared
## [1] 0.2466916
```

The anova tables above indicate that recoding Moisture as a pure categorical or linear variable does not greatly change the explanatory power of the model. Given that the linear effect of **Moisture** is easier to display and interpret, it has a slightly larger adjusted R^2 value, and it has fewer parameters this should be the way we use this variable in making our final figure and interpretations.

```
plot(dune_cca_lin, type = 'n', scaling = 'sites')
text(dune_cca_lin, 'cn', col = 'blue', scaling = 'sites',
     labels = c('Bio. farm', 'Hobby farm', 'Nature consv.', 'Stand. farm'))
text(dune_cca_lin, 'species', cex = 0.75, col = 'red', scaling = 'sites')
```

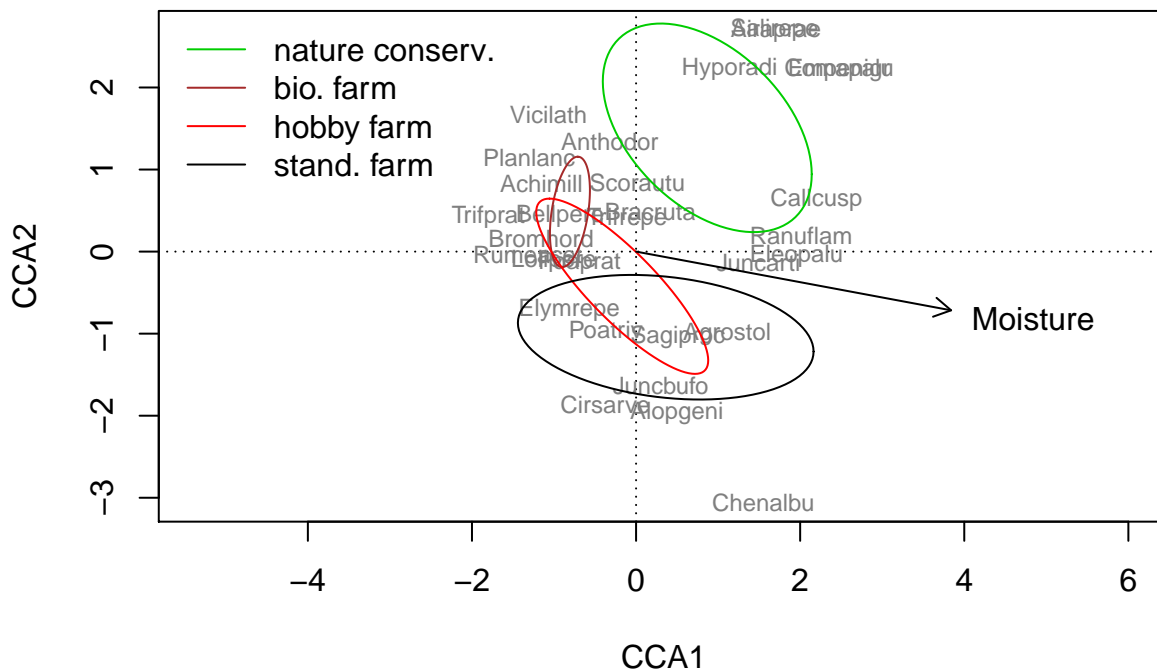


```
#points(dune_cca_lin, 'sites')
#ordiellipse(dune_cca_lin, Management, col=1:4, lwd=3)

# trying to get it prettier

plot(dune_cca_lin, type = 'n', scaling = 'sites')
text(dune_cca_lin, 'species', cex = 0.75, col = 'grey50', scaling = 'sites')
ordiellipse(dune_cca_lin, dune.env$Management,
             col=c('brown', 'red', 'green3', 'black'), label = FALSE, kind="ehull")
# still need to add arrow for just Moisture variable
bip <- scores(dune_cca_lin, display = 'bp', scaling = 'sites')
bip_scl <- (bip * ordiArrowMul(bip, fill = .8))[1, ]
arrows(0, 0, bip_scl[1], bip_scl[2], length = 0.1)
text(bip_scl[1] + 1, bip_scl[2] - 0.1, labels = 'Moisture')
```

```
#points(dune_cca_lin, 'sites', pch = 1, cex=0.75)
legend("topleft",
      c('nature conserv.', 'bio. farm', 'hobby farm', 'stand. farm'),
      col = c('green3', 'brown', 'red', 'black'), lty = 1, bty='n')
```



3. Do your two analyses agree with one another or complement one another or do these two analyses seem to be suggesting different take home messages? Which analysis do you find to be more useful?

This is a more subjective question but my personal take in this case is that the analyses are somewhat complementary. The NMDS is nice because you are ensuring that you are examining the dominant axes of variation not just the axes that the environment can explain (as in CCA). However, the direct ordination provides us a clear measures of variable importance and variance explained which are intuitive and easier to communicate. With the NMDS bringing in the environment is always a posthoc approach. One point to note is that the distance measures used in these two analyses are different. In the NMDS analysis the distance measure was the bray-curtis distance measure but in the CCA analysis chi-squared distances are computed. In the case of CCA the chi-squared distances have a clear ecological meaning, species are assumed to have an optima along an environmental gradient and decrease in prevalence away from the optima. In other words species are expected to have unimodal responses to the environment which is consistent with traditional ecological niche theory. The bray-curtis distance measure has no such theoretical foundation and its interpretation is thus much more vague.