multivariate models solutions

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```
# load code dependancies
library(vegan)

## Loading required package: permute

## Loading required package: lattice

## This is vegan 2.6-4

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?

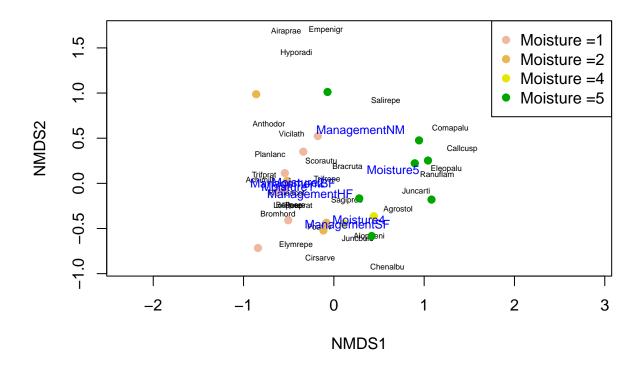
```
dune_mds <- metaMDS(dune, trace = 0)
# fit enviornmental variables to ordination space
dune_fit <- envfit(dune_mds, dune.env)
dune_fit</pre>
```

```
##
## ***VECTORS
##
       NMDS1
               NMDS2
                          r2 Pr(>r)
##
## A1 0.96473 0.26323 0.3649 0.012 *
## 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
                0.2957 0.5790
## ManagementSF
                0.1506 - 0.4670
## UseHayfield -0.1568 0.3248
## UseHaypastu
               -0.0412 -0.3370
## UsePasture
                0.2854 0.0844
## Manure0
                0.2957 0.5790
## Manure1
               -0.2482 -0.0215
## Manure2
               -0.3079 -0.1866
## Manure3
                0.3102 -0.2470
## Manure4
                -0.3463 -0.5583
##
## Goodness of fit:
##
                 r2 Pr(>r)
## Moisture
             0.5014 0.002 **
## Management 0.4134 0.009 **
             0.1871 0.129
## Manure
             0.4247 0.025 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 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 explaning 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 oridination plot to see if this seems reasonable.



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.

Call: cca(formula = dune ~ A1 + Moisture + Management + Use + Manure,

```
## data = dune.env)
##
                 Inertia Proportion Rank
##
                             1.0000
## Total
                  2.1153
## Constrained
                  1.5032
                             0.7106
                                       12
## Unconstrained 0.6121
                             0.2894
## Inertia is scaled Chi-square
## Some constraints or conditions were aliased because they were 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]</pre>
```

```
## $adj.r.squared
## [1] 0.2249886
```

After 2000 permutations you can see that the adjusted r-squared stablized 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)
                  1.5032 1.4325 0.036 *
## Model
            12
## 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')
```

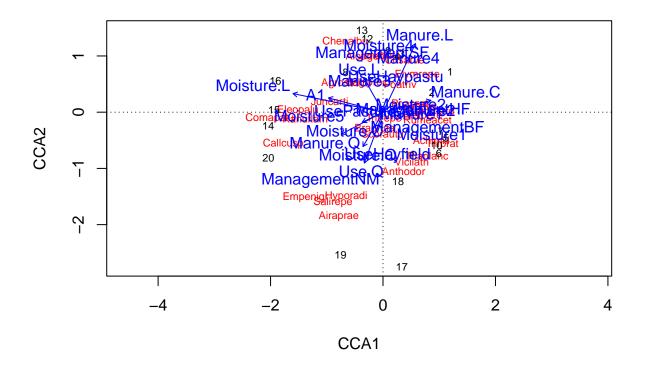
Permutation test for cca under reduced model

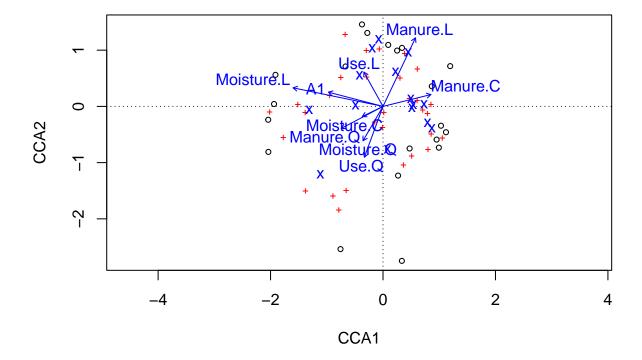
```
## Marginal effects of terms
## Permutation: free
## Number of permutations: 999
##
##
  Model: cca(formula = dune ~ A1 + Moisture + Management + Use + Manure, data = dune.env)
##
              Df ChiSquare
                                 F Pr(>F)
## A1
                    0.11070 1.2660
                                    0.214
               1
## Moisture
               3
                    0.31587 1.2041
                                    0.235
## Management
               2
                    0.15882 0.9081
                                    0.590
## Use
               2
                    0.13010 0.7439
                                    0.776
## Manure
               3
                    0.25490 0.9717
                                    0.518
               7
## Residual
                    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 marginal effects of each model term indicates that when the variables are forced to compete against one another that no single variable is statistically significant. The variable "A1" which is the thickness of the soil A1 horizon is the most important variable followed by the Moisture and Manure variables as indicated by their F-statistics.

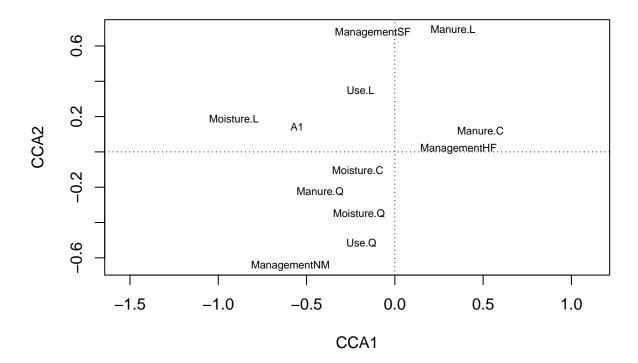
Let's plot our results now:

plot(dune_cca)





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



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 (intpreting 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. At 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</pre>
```

[1] 0.2331328

```
## 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 0.001 ***
## Residual 13
                  1.1129
## Signif. codes: 0 '***' 0.001 '**' 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 0.042 *
## Management 3
                  0.37407 1.4565 0.054 .
## Residual
             13
                  1.11289
## Signif. codes: 0 '***' 0.001 '**' 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
## 1
        13
                 1.1129
        7
## 2
                 0.6121 6
                             0.50079 0.9545
                                             0.57
```

The above analysis indicates that this reduced model 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.

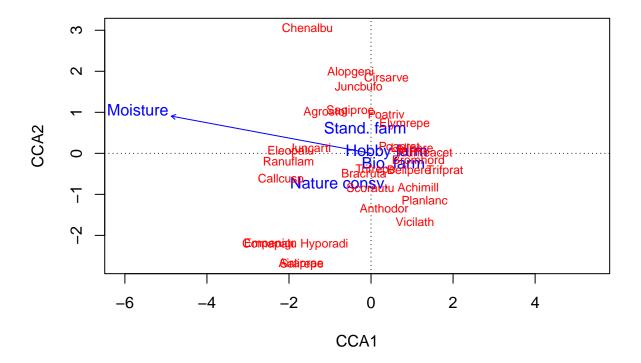
One solution here is to maintain the ranking of these nomial categorical variables but treat their effects as purely linear by re-encoding them as numeric vectors. An alternative approach is to treat them like unranked categorical variables in which case a separate mean (i.e., centroid) is estimated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

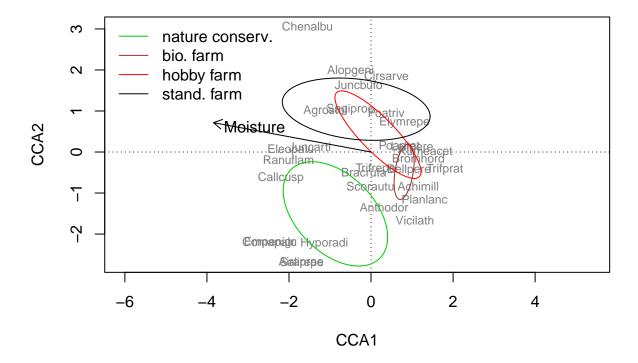
Let's examine the utility of both approaches here.

anova(dune_cca_MM)

```
dune.env$Moist_cat <- as.factor(as.numeric(dune.env$Moisture))</pre>
dune.env$Moist_lin <- as.numeric(dune.env$Moisture)</pre>
dune_cca_cat <- cca(dune ~ Moist_cat + Management, data = dune.env)</pre>
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 0.057 .
## Residual 13 1.11289
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
RsquareAdj(dune_cca_cat)
## $r.squared
## [1] 0.4738772
## $adj.r.squared
## [1] 0.2321424
dune_cca_lin <- cca(dune ~ Moist_lin + Management, data = dune.env)</pre>
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 0.016 *
## 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] 0.24654
```

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.





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 enviornment can explain (as in CCA). However, the direct ordination provides us a clear measures of variable importance and variance explained which are intuitive an easier to communicate. With the NMDS bringing in the enviornment 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 enviornmental gradient and decrease in prevalence away from the optima. In other words species are expected to have unimodal responses to the enviornment 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 vauge.