VEGETATION DESCRIPTION AND ANALYSIS

LABORATORY 5 AND 6

ORDINATIONS USING PC-ORD AND INSTRUCTIONS FOR LAB AND WRITTEN REPORT

LABORATORY 5 (OCT 4, 2017)
PC-ORD 1

BRAY & CURTIS ORDINATION AND THE SPECIES DATA

Introduction

We will use the ordination program PC-ORD to analyze the field data collected at the Colleen Site A research site, Prudhoe Bay, Alaska. PC-ORD provides most of the types of output that are useful for ordination analysis and numerical classification.

This handout outlines the steps for: (a) preparing the data (b) starting PC-ORD, (c) importing the data matrix, (d) performing Bray and Curtis, and (e) graphing a variety of output from the ordination. You should also spend time going through some of the other options and windows that are not discussed here and browse the online PC-ORD help manual to get a better idea of some of the functions.

Jump ahead to page 7 to read the instructions for the Lab 5 and 6 written report (100 points).

Logging in

If you are using the Biology Department's PC computers, log in with username "biolab", password "ygoloib"

Prepare your data

The data are provided on the class website and have been sent via email in the Excel Workbook:

The summary of all plot data are in the Excel Workbook:

- ***Colleen spp and env data, field and PCORD format20170929.xlsx***

- These data were used to create the PC-Ord-compatible files:
  - ***Colleen species dataPCORD.wk1***
  - ***Colleen envi data PCORD.wk1***

NOTES

The species matrix was constructed by

1. Converting the Br.-Bl. cover abundance scores to quantitative values using find/replace
   - + = 1
   - r = 2
   - 1 = 3
   - 2 = 16
   - 3 = 38

- 4 = 63
- 5 = 88
- blank = 0 (zero fill the whole matrix)

2. Transpose rows to columns
3. Make sure that relevé labels are in Text format: T1-005-T, T1-005-C, T1-010-T, T1-010-C, etc.
4. Insert 3 rows at top:
    - Row 1, col. 1: number of plots "29"; col. 2 "plots" (29, plots)
    - Row 2, col. 1: number of species "56"; col. "species" (56, species)
    - blank, Q, Q, Q, … (for quantitative). Note: all species cover values are quantitative variables, vs. catigorical variables for many of the environmental variables, which are labeled "C" in the environmental matrix.
    5. Export as Lotus123 file
    - Lotus 1-2-3 (*.wk1)
    - Because of the restrictions on the lab computers, you need to save it as a comma separated variable file (*.csv), then re-name it with a .wk1 extension (so that PC-ORD can find it). When you bring it into PC-ORD, it will ask you if you want to convert it.

Doing the ordination

Start PC-ORD

Double click on the **PC-ORD** icon on the desktop.

Load main and secondary matrices

Click on **File/Open/Main Matrix.** Load the file you've created as the **main matrix**. The main matrix is the species matrix. The **secondary matrix** is the matrix of environmental factors – we'll work with that next week. The main matrix will appear on the screen. You can minimize the window by clicking on the left-hand button in the upper right part of the main matrix window.

Bray and Curtis (Polar ordination)

Pull down the **Ordination** menu in the **PC-ORD** window and click on **Bray-Curtis**. You will get a "Bray-Curtis Setup" window with several options for distance measures, axis projection geometry, residual distances, endpoint selection method, etc. Click **OK** for the default parameters. You will then get a window requesting a title that will appear on all results and graph files for this ordination. Type in something like "Colleen Bray and Curtis Plot Ordination". Click **OK**. You will then get a "Process" window showing progress on the calculations. When the processing is complete, the "RESULTS.TXT" and the "GRAPHROW.GPH" files will appear briefly on the screen, followed by an ordination graph message. Click **OK**; and both the Results and Graph files should be on the screen.

Examine the results and graph files

Print the **RESULT.TXT** file by pulling down the File menu in the PC-ORD window; click **Print** and then click on **RESULT.TXT**. The results file contains a variety of information describing the details of the Bray and Curtis method. These include the options that were selected, endpoints of the axes, sum of squares of non-redundant distances, regression coefficients, the amount of information extracted by each axis, and the scores for each plot along three ordination axes. The GRAPHROW.GPH contains the coordinates for producing a graph of the ordination. You can hide the RESULT.TXT and GRAPHROW.GPH windows by clicking on the left-hand box in the upper right corner of the windows.

Click the "Graph Ordination" option under the Graph menu in the PC-ORD window. Choose 2D. This will show the "Graph" window with many option buttons. We will go through a few options to get a feel for the possibilities. Each button has a descriptive balloon explaining the function. The explanation will appear by touching the cursor arrow to the button.

Graph the B&C plot ordination

Graph of plots in ordination space. Click on the Simple Scatterplot button (second from left on top). An ordination of plots should appear on the screen. This may or may not have the points labeled depending on the previous setting in the "Preferences" menu.

Label the plot numbers. If the plot numbers are not labeled, this can be done by clicking on the Preferences button (right hand button in the "Graph" window). A "Preferences" window with many font and format options will appear. Click the box next to Label plots, and then on OK.

Change the font size of labels. You may want to change the location and size of the labels for easier reading. In the "Preferences" window, click on Font. Change the font size to 8 point. Click OK. Change the location of the labels by clicking on a label and moving it with the cursor.

Display axes 1, 3 and 2, 3. Click on the 1 vs 3 button to display axes 1 and 3. Click on the 2 vs 3 button to display axes 2 and 3. Try the 3D option to see all three axes at once (you have to close the 2D window, and click on Graph/Graph Ordination/3D).

Print the graph in a format that displays the results well. To delete the output windows after printing, click the X in the upper-right corner of the window.
Experiment with different ordination options. Close all the output files (you could save these if you wanted to), and start the ordination again, choosing different distance measures or endpoint method. Compare the results with the one you printed.

Bray and Curtis "species" ordination

To do a species ordination in Bray and Curtis, it is first necessary to transpose the Main Matrix so that the columns are plots and the rows are species. This can be done by pulling down the **Modify Data** menu in the PC-ORD window, and then clicking on **Transpose Main Matrix**. Follow the same procedures for graphing the species ordination as for the

plot ordination. The ordination space shows the locations of the central tendencies for all the species. What do the results tell us about the species we found at our sites?

Spend quite a bit of time becoming familiar with how to do ordinations and graphing the ordinations staying with the Bray and Curtis ordination approach. Once you are comfortable with how to make and interpret the plots, result files, and graph files, decide on a set of graphs that you would like to use for your paper.

First choose a set of preferences (colors, symbol shapes, font sizes, for the legend points, arrows in joint plot, etc.) and a consistent approach to title your ordination plots, graph files, and results files (e.g. Colleen Bray and Curtis plot ordination; Colleen Bray and Curtis species ordination; Colleen Bray and Curtis plot ordination graph file; Colleen Bray and Curtis results file)

Do a plot ordination and a species ordination. Save the plots, graphs, and results file for both in a folder titled "**your name_Output from PCORD Colleen Analysis**". If using a Biology department computer, make sure to save the folder and files on a USB drive, and not on the computer hard drive. Make an accompanying Word file with notes regarding your thoughts regarding each plot and key points of interpretation. Print and write down critical graphs/results. You will need these for the ordination lab report (instructions p. 7) in which you will compare the different ordination methods. You will be asked to select the "best" ordination method and to defend that choice.

LABORATORY 6 (OCT 18, 2017)
PC-ORD-2

MORE ORDINATION METHODS & ENVIRONMENTAL DATA

Bringing in the Second Matrix (environmental matrix)

Repeat the steps for making a Bray and Curtis ordination and graphing the results from last lesson (pp 2-3). Be sure to bring in both the **main matrix** (the species matrix, *Colleen species dataPCORD.wk1* and the **secondary matrix** (the environmental matrix, *Colleen envi data PCORD.wk1*).

Label by polygon centers and troughs (C-T). This is one of the environmental variables (1 = centers, 2 = troughs). Change the color and the symbol so the two sites look different. Under **Preferences/Form**at, click the **Symbol** code categories option. Click on the **Legend Symbol/Color** bar and choose some options.

Overlay the species data. Plots of individual species cover can be shown within the ordination space by clicking on the Overlay main matrix button. Keep the plots labeled according to C-T so you can better see the distribution of environmental variables for each type. This will display scaled symbols for each plot according to the relative abundance of the given species in each plot. By examining the scatter plots, trends of the species cover along the axes can be determined. Other species can be selected by using the menu underneath the displayed species name. Go through all the species (e.g., ERIANG, CARMEM, CARROT, etc.) and get a feel for how the patterns change with species you may know, dry species, wet species, shrubs, sedges, lichens, mosses.

Overlay the environmental data. Click on **the Overlay second matrix** button. Keep the plots labeled according to C-T so you can better see the distribution of environmental variables for each type. Look at the side plots and see how the environmental variable varies across both axes of the ordination. Also note the size of the symbols on the ordination increase with the magnitude of the variable. The scaling of the size of the symbols varies according to the range of the variable within the data set. So be careful in interpreting the meaning of these differences. Variables with small possibly meaningless ranges for a given variable will have the same range of symbol sizes as variables with large ranges for a variable.

The distribution of values for environmental variables can be explored in the same manner as the cover values for the species. Click on the **Overlay second matrix** button. To display groups of values for a given variable, go into the **Preferences** menu and code all the values of a given class with the same color. Explore the patterns for several site factors. Do the patterns make sense?

Go through all the environmental variables: Water cover, water depth, soil moisture, thaw, elevation, etc., to get a feel for how the environmental variables vary across the ordination space.

Joint plots

Joint-plot diagrams can be generated by clicking the **Joint plot (biplot)** button. Make a joint plot diagram for the Bray and Curtis ordination for axes 1 and 2. Play with the labeling so that the vectors are clearly labeled. You may want to reduce the number of vectors displayed by increasing r^2 (0.3, 0.4 etc.). Select the **Joint Plot Cutoff** from the **Graph** submenu or click on the toolbar button the displays the current cutoff value. Which environmental variable accounts for most of the variance in the ordination data? What is the strength of these correlations and are they positive or negative? What other environmental variables have high correlations with the ordination space.

Change the font size and locations of the biplot arrow labels. You may want to change the location and size of the biplot arrow labels for easier reading. In the **Preferences** window, click on the **Font** sheet and then click on **Joint plot labels**. Change the font size to 8 point. Click **OK**. Change the location of the labels by clicking on a label and moving it with the cursor.

Display axes 1, 3 and 2, 3. Click on the 1 vs 3 button to display axes 1 and 3. Click on the 2 vs 3 button to display axes 2 and 3. Try rotating your plot. Examine the trends in environmental variables along each axis. Print the joint plot diagram with axes 1 and 2 displayed.

Environmental correlations

Correlation between environmental variables and 1st and 2nd axis scores: Use the Kendall's Tau (rank correlation coefficient) to calculate the correlation of each environmental variable with 1st and 2nd Bray and Curtis axis. Click on the **Correlations with second matrix (∑2)** button. The results of the correlation analyses will appear with the list of r, r-sq, and Kendall's tau for axes 1, 2, and 3. Kendall's tau is a rank correlation coefficient similar to Spearman's rank correlation coefficient. The values closer to 1 or -1 are the strongest correlations. Values above about ± 0.4 are probably significant. Unfortunately, the significance of the correlations are not given in the output. Print or save this file for future reference.

Print out your best Bray and Curtis ordination. By hand, label the axes 1 and 2 with arrows showing the direction and strength of some of the highest correlations. These values can be found in the results files of the Bray and Curtis output. What does this information provide you? Use this as one of your graphs for the lab report. (You can also save this file, and add labels in a word-processing document)

Correspondence analysis

Run a Correspondence Analysis (**RA option** in the **Ordination** menu, RA for Reciprocal Averaging). Compare the ordination results with the polar ordination. Do you see the way that the DCA has changed the RA results, flattening the arch and spreading the ends of the axes?

If you have time, try two other ordination techniques that I will cover in class on Wednesday:

CCA ordination

Note: for CCA you will first have to modify the environmental matrix to contain fewer than 29 quantitative variables (the number of plots). Choose ones that you think are most important. If you switch the main and second matrix, you can do this editing with PC-ORD, using Modify Data/Delete Columns/You Specify. Use shift to select the environmental variables to delete. Save your new matrix, switch main and second, and run the ordination.

DCA, NMS and other ordination approaches

Run with default settings and examine results. DCA and NMS are both very popular methods. See what you get. Also try the other methods. See if you can understand the differences in the ordination approaches.

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Instructions for Labs 5 and 6 Written Report: 100 points (DUE Oct 18)

Present your favorite ordination result that you got from the class data, along with some other ordination plots. There is not one "right" answer to this, but demonstrate that you understand what the ordinations show, and how to evaluate between different ordination methods, and different settings within a method. Use the species and the environmental information to explore the meaning of the method that you select as "best".

Materials & Methods

Describe the software used for the ordination. The complete citation for the PC-ORD software is:

McCune, B., and M. J. Mefford. 2005. PC-ORD, Multivariate analysis of ecological data, Version 5 for Windows edition. MjM Software Design, Gleneden Beach, Oregon USA.

For the data, cite the file:

Env-Spp_Workbook20121109(1).xlsx

and the PC-Ord files:

Colleen species dataPCORD.wk1
Colleen envi data PCORD.wk1

Results

Present several (at least 5) different ordination from Labs 05 and 06. You can print out ordinations in the lab, or you can save them as .jpg files and import them into the text of your report. This will allow you to reduce the size of the graphics and get several on a page for better comparison. Describe all the settings for each ordination. If you import the graphics in PowerPoint you can also alter the graphics and legends to make them more comprehensible.

At least one of your graphs should include B&C **species ordination**, that you create by first transposing the main matrix.

At least one of your graphs should include **environmental information**.

Include a table of the **Kendall correlation coefficient** of the most important environmental variables for each axis of your "best" ordination.

Discussion

Compare the ordination methods, describing the assumptions and the type of analysis that the ordinations use and how well these apply to our data. Why does the ordination method that you chose work best for our data set?

Compare the ordination results. What they tell us about the sites that we sampled? Which method tells us more?

Explain what the species and environmental information tells us about the ordination space.