Lecture 5: Ecological distance metrics; Principal Coordinates Analysis

Univariate testing vs. community analysis

- Univariate testing deals with hypotheses concerning individual taxa
  - Is this taxon differentially present/abundant in different samples?
  - Is this taxon correlated with a given continuous variable?
- What if we would like to draw conclusions about the community as a whole?

Useful ideas from modern statistics

- Distances between anything (abundances, presence-absence, graphs, trees);
- Direct hypotheses based on distances;
- Decompositions through iterative structuration;
- Projections;
- Randomization tests, probabilistic simulations.

Data → Distances → Statistics

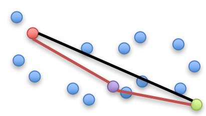
	S-1	S-2	...	S-16
OTU-1				
OTU-2				
OTU-3				
OTU-4				
OTU-5				
....				
OTU-10,000				

0.54															
0.54	0.34														
0.60	0.17	0.10													
0.01	0.75	0.17	0.72												
0.24	0.34	0.74	0.89	0.58											
0.08	0.43	0.82	0.91	0.69	0.64										
0.57	0.06	0.59	0.03	0.69	0.89	0.07									
0.88	0.68	0.59	0.49	0.19	0.64	0.54	0.26								
0.24	0.71	0.58	0.52	0.93	0.94	0.46	0.70	0.30							
0.92	0.46	0.17	0.21	0.17	0.02	0.81	0.22	0.45	0.59						
0.51	0.23	0.74	0.36	0.15	1.00	0.95	0.71	0.88	0.94	0.65					
0.29	0.30	0.75	0.28	0.04	0.45	0.15	0.44	0.59	0.89	0.07	0.19				
0.57	0.63	0.27	0.72	0.34	0.70	0.19	0.17	0.85	0.07	0.97	0.24	0.45			
0.95	0.53	0.86	0.01	0.10	0.63	0.40	0.10	0.51	1.00	0.20	0.41	0.11	0.14		
0.83	0.43	0.12	0.39	0.43	0.87	0.42	0.17	0.62	0.57	0.03	0.58	0.54	0.48	0.83	

Visualization (Principal coordinates analysis)
Statistical hypothesis testing (PERMANOVA)

What is a distance metric?

- Scalar function d(.,.) of two arguments
- d(x, y) >= 0, always nonnegative;
- d(x, x) = 0, distance to self is 0;
- d(x, y) = d(y, x), distance is symmetric;
- d(x, y) <= d(x, z) + d(z, y), triangle inequality.



WHAT ARE SOME GOOD DISTANCE METRICS?

Using distances to capture multidimensional heterogeneous information

- A “good” distance will enable us to analyze any type of data usefully
- We can build specialized distances that incorporate different types of information (abundance, trees, geographical locations, etc.)
- We can visualize complex data as long as we know the distances between objects (observations, variables)
- We can compute distances (correlations) between distances to compare them
- We can decompose the sources of variability contributing to distances in ANOVA-like fashion

Distance and similarity

- Sometimes it is conceptually easier to talk about similarities rather than distances
  - E.g. sequence similarity
- Any similarity measure can be converted into a distance metric, e.g.
  - S
  - If S is (0, 1), D=1-S
  - If S>0, D = 1/S or D = exp(-S)

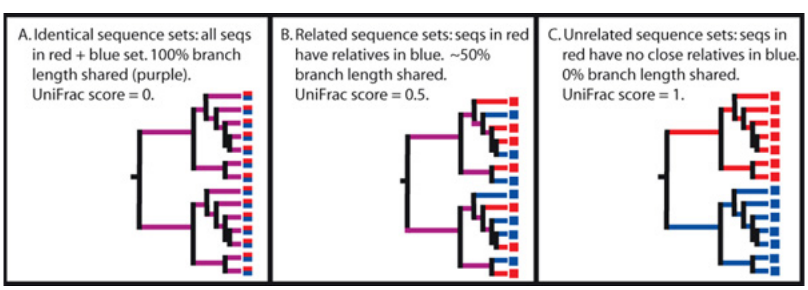
A few useful distances and similarity indices

- Distances:
  - Euclidean: (remember Pythagoras theorem) $\Sigma(x_i-y_i)^2$
  - Weighted Euclidean: $\chi^2 = \Sigma(e_i - o_i)^2/e_i$
  - Hamming/L1, Bray Curtis = $\Sigma\ \mathbf{1}_{\{xi=yi\}}$
  - Unifrac (later)
  - Jensen-Shannon: $(D(\mathbf{X}|\mathbf{M}) + D(\mathbf{Y}|\mathbf{M}))/2$, where
    - $\mathbf{M} = (\mathbf{X} + \mathbf{Y})/2$
    - Kullback-Leibler divergence: $D(\mathbf{X}|\mathbf{Y}) = \Sigma \ln[x_i/y_i]x_i$
- Similarity:
  - Correlation coefficient
  - Matching coefficient: $(f_{11}+f_{00})/(f_{11} + f_{10} + f_{01} + f_{00})$
  - Jaccard Similarity Index: $f_{11}/(f_{11} + f_{10} + f_{01})$

x\y	1	0
1	f_{11}	f_{10}
0	f_{01}	f_{00}

Unifrac distance (Lozupone and Knight, 2005)

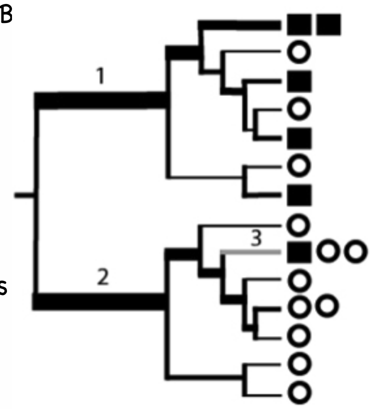
- Is a distance between groups of organisms related by a tree
- Definition: Ratio of the sum of the length of the branches leading to sample X or Y, but not both, to the sum of all branch lengths of the tree.



Weighted Unifrac (Lozupone et al., 2007)

$$\sum_{i=1}^{n} b_i \times |\frac{A_i}{A_T} - \frac{B_i}{B}$$

- n = number of branches in the
- b_i = length of the ith branch
- A_i = number of descendants of ith branch in group A
- A_T = total number of sequences in group A



Key warnings about the Unifrac family of distances.

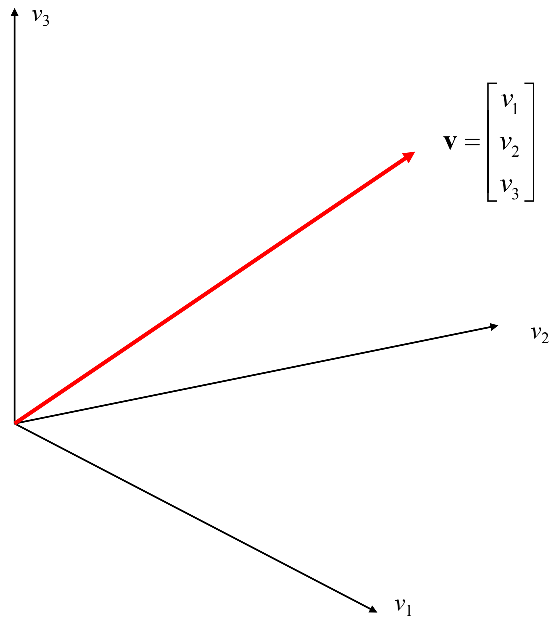
- The scaling of the tree is important.
- The rooting of the tree is important.

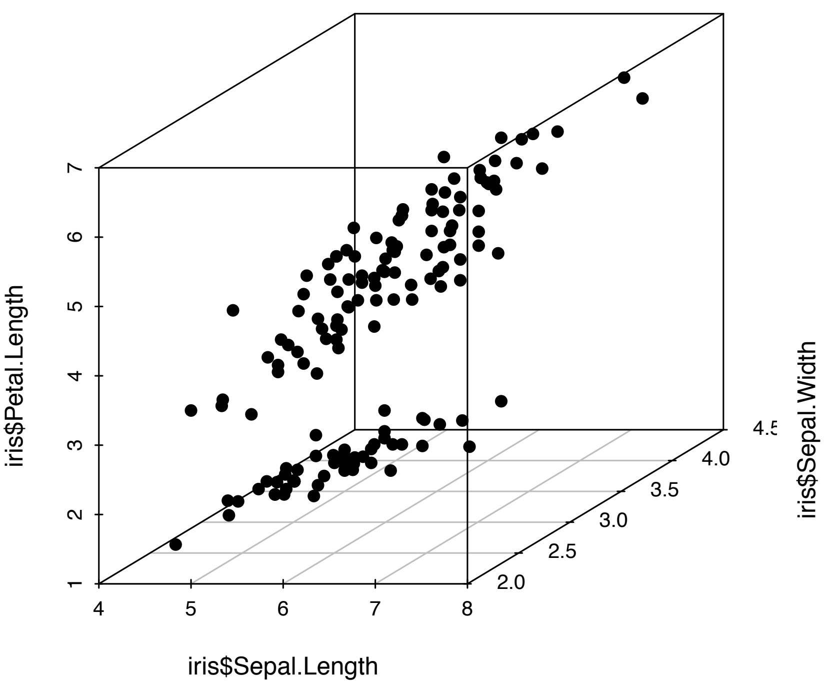
A note of warning!

- "Garbage in, garbage out"
- Wrong normalization => wrong distance => wrong answer
- However, given the many choices there isn't much beyond prior knowledge, experience and intuition to guide in selection of the distance.

PRINCIPAL COORDINATES ANALYSIS - MULTIDIMENSIONAL SCALING

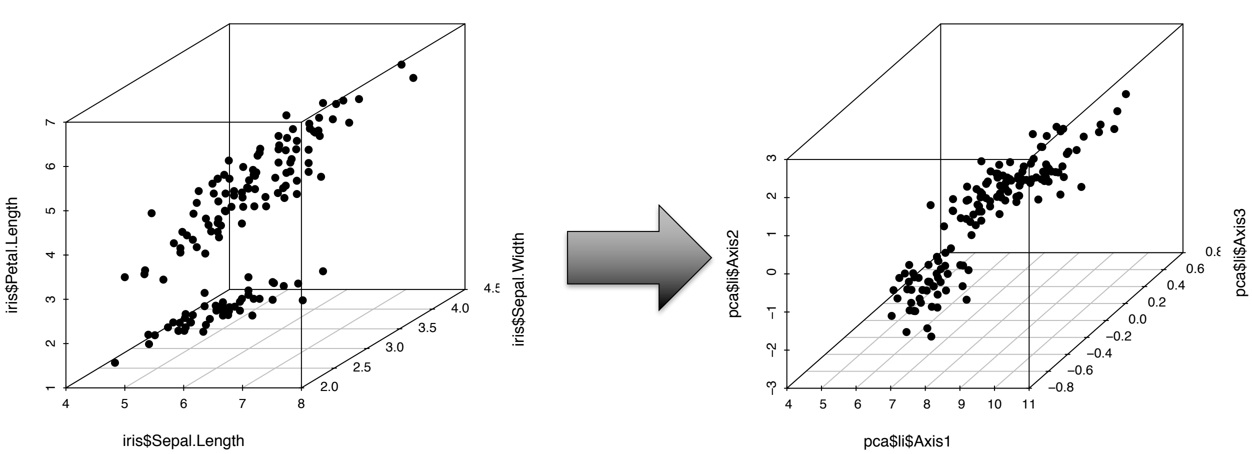
Every multivariate sample can be represented as a vector in some vector space





Vector Basis

- A basis is a set of linearly independent (dot product is zero) vectors that **span** the vector space.
- **Spanning** the vector space: Any vector in this vector space may be represented as a linear combination of the basis vectors.
- The vectors forming a basis are orthogonal to each other. If all the vectors are of length 1, then the basis is called orthonormal.



Basic idea for analysis of multidimensional data

- Compute distances
- Reduce dimensions
- Embed in Euclidean space
- The general framework behind this process is called **Duality diagram**: ($\mathbf{X}_{nxp}$, $\mathbf{Q}_{pxp}$, $\mathbf{D}_{nxn}$)
  - $\mathbf{X}_{nxp}$ (centered) data matrix
  - $\mathbf{Q}_{pxp}$ column weights (weights on variables)
  - $\mathbf{D}_{nxn}$ row weights (weights on observations)

Duality diagram defines the geometry of multivariate analysis

$$\begin{tikzcd}
\mathbb{R}^{p*} \arrow[r, "X"'] \arrow[d, shift left, "V"] & \mathbb{R}^{n} \arrow[d, shift right, "D"'] \\
\mathbb{R}^{p} \arrow[u, shift left, "Q"] & \mathbb{R}^{n*} \arrow[l, "X^t"] \arrow[u, shift right, "W"']
\end{tikzcd}$$

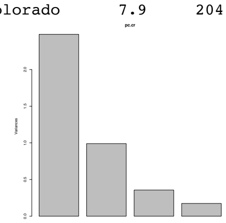
- $V = X^T DX$
- $W = XQX^T$
- Duality:
  - The eigen decomposition of VQ leads to eigen-decomposition of WD
- *Inertia* is equal to trace (sum of the diagonal elements) of VQ or WD.

Principal Component Analysis (PCA)

- Let Q = I and D = 1/n I and let X be centered.
- $VQ = X^TDXQ = 1/n\ X^TX$.
- The inertia Tr(VQ) = sum of the variances.
- PCA decomposes the variance of X into independent components.
- To decompose the inertia means to find the eigen-system of VQ or equivalently WD matrices.
- Eigenvalues give the amount of inertia explained in corresponding dimension.
- Eigenvectors give the dimensions of variability.

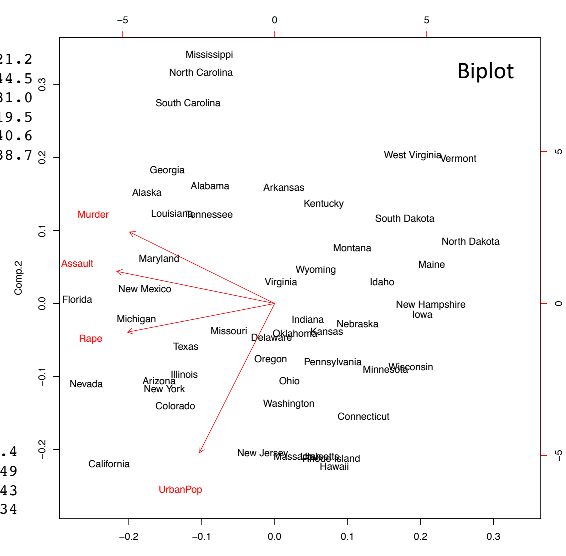
Example PCA

```
head(USArrests)
      Murder Assault UrbanPop Rape
Alabama       13.2     236       58 21.2
Alaska        10.0     263       48 44.5
Arizona        8.1     294       80 31.0
Arkansas       8.8     190       50 19.5
California     9.0     276       91 40.6
Colorado       7.9     204       78 38.7
```



Screeplot: plot of inertia

```
Loadings:
         Comp.1 Comp.2 Comp.3 Comp.4
Murder   -0.536  0.418 -0.341  0.649
Assault  -0.583  0.188 -0.268 -0.743
UrbanPop -0.278 -0.873 -0.378  0.134
Rape     -0.543 -0.167  0.818
```



Centering

- Let Y be not centered data matrix with n observations (rows) and p variables (columns)
- Let **H** = (**I** – 1/n **1**x**1')**
- Then X = HY is centered

From Euclidean distances to PCA to PCoA

- Note that if **D** is a Euclidean distance, then
- **X X**' = 1/n **H** $\mathbf{D}^{(2)}$ **H**.
- PCoA is a generalization of PCA in that knowledge of **X** is not required, all you need to represent the points is **D**, the inter-point distance matrix.

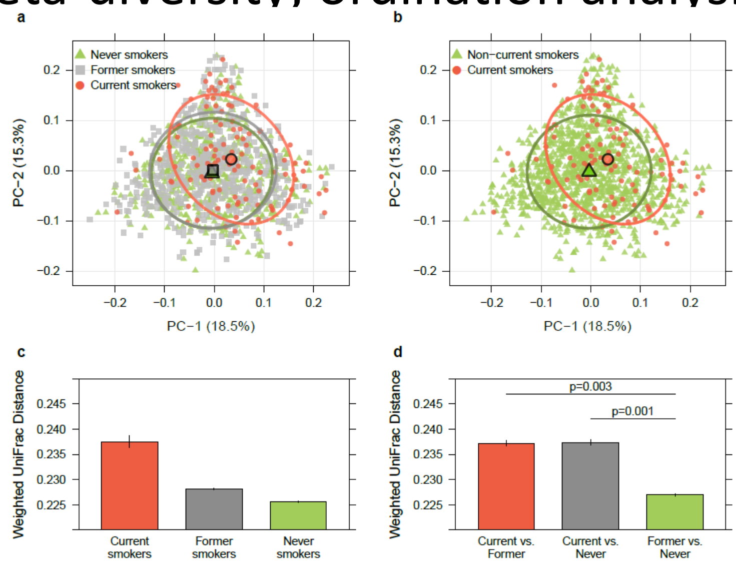
Representation of (arbitrary) distances in Euclidean space

- The idea is to use singular value decomposition (SVD) on the centered interpoint distance matrix to extract Euclidean dimensions
- SVD: X = U S V, where S is diagonal matrix with diagonal elements s_1, s_2, …, s_n, and U and V are unit matrices (i.e. their determinant is 1 and they span their corresponding spaces)

PCoA details

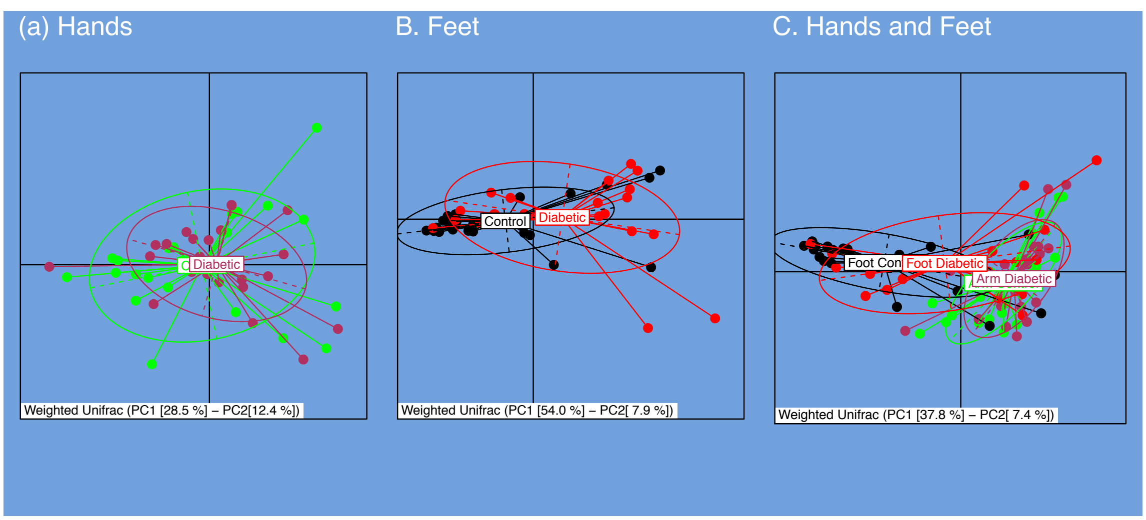
- Algorithm starting from **D** inter-point distances:
  - Center the rows and columns of the matrix of square (element-by-element) distances: $\mathbf{S} = -1/2\mathbf{H}\ \mathbf{D}^{(2)}\mathbf{H}$
  - Compute SVD by diagonalizing **S**, $\mathbf{S} = \mathbf{U}\ \mathbf{\Lambda}\ \mathbf{U}^T$
  - Extract Euclidean representations: $\underline{X} = \mathbf{U}\ \mathbf{\Lambda}^{1/2}$
- The relative values of diagonal elements of **Λ** gives the proportion of variability explained by each of the axes.
- The values of **Λ** should always be looked at in deciding how many dimensions to retain.

Beta-diversity; ordination analysis



ISME J. 2016 Mar 25. doi: 10.1038/ismej.2016.37

Differentiation of microbiota between diabetic and non-diabetic subjects and across body sites

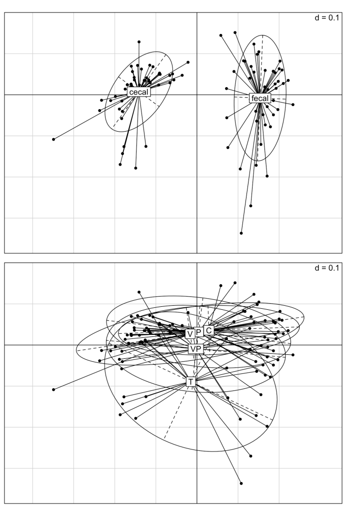


Redel et al. J Infect Dis. 2013 207(7):1105-14

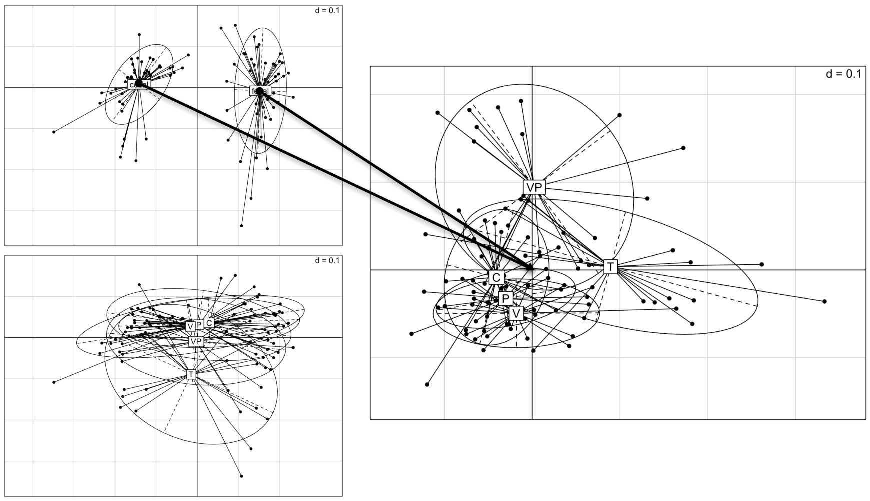
Correspondence analysis

- Is obtained by analyzing the eigen values of the chi-square transformed counts Y.
- $Y = \begin{pmatrix} y_{11} & y_{12} & y_{13} \\ y_{21} & y_{22} & y_{23} \\ y_{31} & y_{32} & y_{33} \end{pmatrix}, y_{i+} = \begin{pmatrix} y_{11} + y_{12} + y_{13} \\ y_{21} + y_{22} + y_{23} \\ y_{31} + y_{32} + y_{33} \end{pmatrix},$
- $y_{+j} = (y_{11} + y_{21} + y_{31}, \dots, \dots)$
- $Q = [q_{ij}] = \left[\frac{y_{ij}y_{++} - y_{i+}y_{+j}}{y_{++}\sqrt{y_{i+}y_{+j}}}\right]$
- SVD analysis of Q results in principal components for correspondence analysis
- Correspondence analysis preserves the chi-square distance.

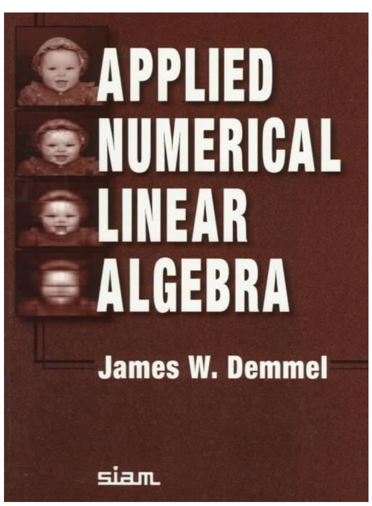
Within class analysis

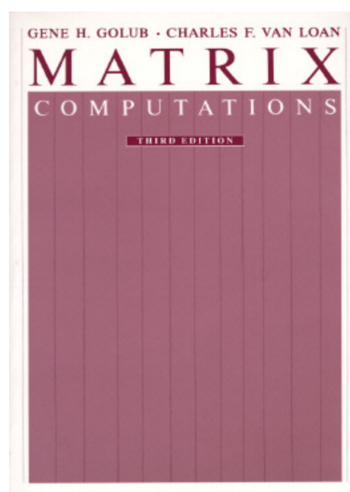


Within class analysis



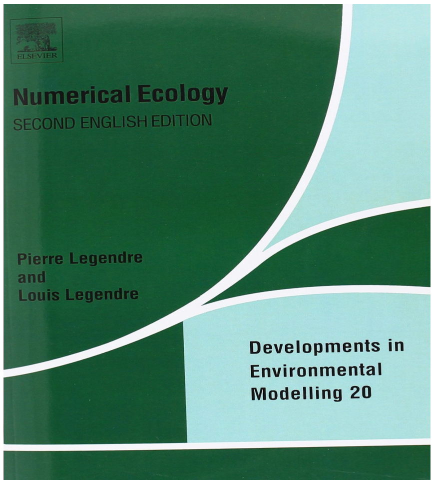
Suggested reading/references





+ any proof-based linear algebra text book.

Suggested reading



- Susan Holmes "Multivariate Data Analysis: The French Way", IMS Lecture Notes–Monograph Series, 2006.