

## PRIMER/PERMANOVA Essentials

### Outline of Topics

1. Properties of multivariate data
  - a. Pre-treatment options, including transformation, standardization, normalization & dispersion weighting
  - b. Distance/dissimilarity/similarity measures for a variety of data types
2. Cluster analysis (**CLUSTER**), including tests for significant structure within clusters (**SIMPROF**) to permit non-arbitrary classifications of samples or variables (species)
3. Ordination *via* projection:
  - a. Principal component analysis (**PCA**); and
  - b. Principal coordinate analysis (**PCO**)
4. Ordination to preserve inter-sample relationships *via* non-metric, metric or threshold metric multi-dimensional scaling (**nMDS**, **mMDS**, **tmMDS**)
5. Relating biotic to abiotic data, including tests of association between resemblance matrices (**RELATE**); tests of seriation and cyclicity.
6. Finding optimal subsets of environmental (or other) variables that generate a ‘best’ match to patterns among samples based on species variables (**BEST – BIOENV**)
7. Non-parametric permutation tests for differences among *a priori* groups of samples:
  - a. Analysis of Similarities (**ANOSIM**);
  - b. Ordination of **Bootstrap averages**; and
  - c. Finding important variables (**SIMPER** and **BEST – BVSTEP**)
8. Diversity measures; Taxonomic distinctness; Taxonomic resemblance
9. Partitioning variation for high-dimensional data on the basis of a resemblance measure of choice and tests for differences in centroids (location) for *a priori* groups (**PERMANOVA**), including:
  - a. One-way and two-way cases & constructing *a priori* contrasts;
  - b. Tests for interactions and pairwise tests
10. Tests for homogeneity of multivariate dispersions and comparisons of beta diversity (**PERMDISP**); **PERMANOVA** tests for centroid differences in the presence of heterogeneity.
11. Complex multi-factor experimental designs (**PERMANOVA**), including:
  - a. Fixed/random factors; Crossed/nested relationships; Expectations of mean squares; Components of variation; Types of SS; Covariates; Pooling; Unbalanced designs
  - b. **Centroid plots** for Main Effects and Interactions; Residual plots.
12. Finite factors and asymmetrical designs (**PERMANOVA**)
13. Fitting multivariate response data (e.g., species) to continuous predictor variables (e.g., environmental), including:
  - a. model selection (**DISTLM**); and
  - b. dissimilarity-based redundancy analysis (**dbRDA**) to visualise fitted variation
14. Identifying when an observation is ‘unusual’, given previous observations (e.g., in a time-series / monitoring program) using multivariate dissimilarity-based **Control Charts**.
15. Wrap-up and Overview of the week / Open Q&A Session / Own-data Session.

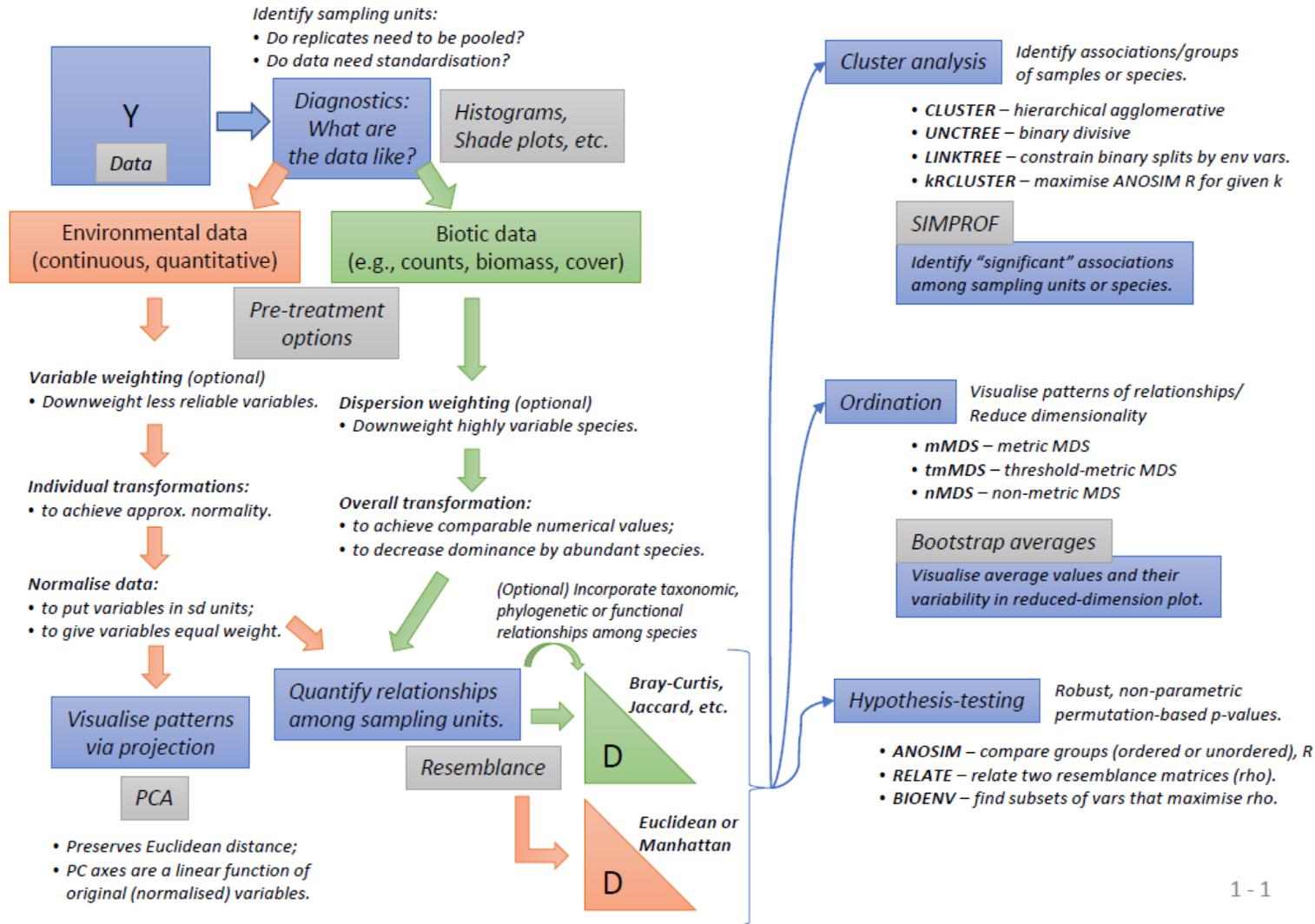
# PRIMER/PERMANOVA Essentials

## Provisional Time-Table

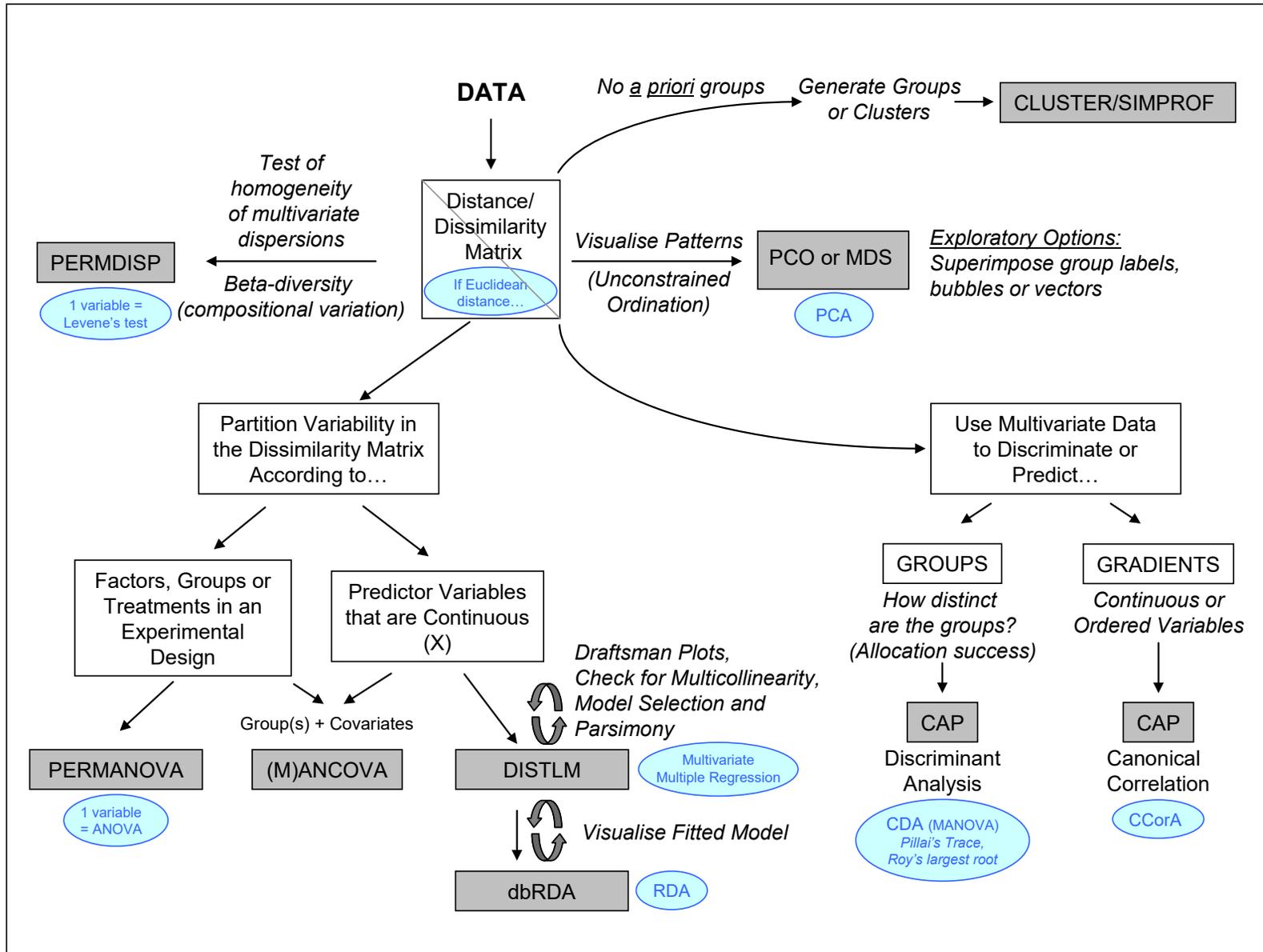
The time-table below should be taken as a rough guide. Breaks will be taken at the specified times each day, but individual topics may flow over or under allotted time-slots, depending on the depth of coverage, questions asked and ensuing discussions. Participants will be given real data sets to analyse during practical sessions. Participants are encouraged to ask questions and discuss their own data with the presenter throughout the week.

	Monday	Tuesday	Wednesday	Thursday	Friday
<b>Session 1</b> 8:30 – 10:30	1. Pre-treatment options; resemblance measures	4. Ordination with nMDS, mMDS, tmMDS (cont'd)	7. SIMPER; BVSTEP (continued)	10. Test centroids when dispersions differ (PERMANOVA)	13. DISTLM / dbRDA
<b>Coffee Break</b> 10:30 – 11:00					
<b>Session 2</b> 11:00 – 12:30	2. CLUSTER; SIMPROF	5. RELATE; seriation or cyclical models	8. Diversity	11. Complex designs (PERMANOVA)	14. Multivariate Control Charts
<b>Lunch</b> 12:30 – 13:30					
<b>Session 3</b> 13:30 – 15:30	3. Ordination with PCA/PCO	6. BEST; BIOENV; global test	9. PERMANOVA: (one-way; two-way; interactions; contrasts)	11. Centroid plots (PERMANOVA, cont'd)	15. Wrap up / Overview / Q&A
<b>Coffee Break</b> 15:30 – 16:00					
<b>Session 4</b> 16:00 – 17:30	4. Ordination with nMDS	7. ANOSIM & Bootstrap averages	10. PERMDISP; beta diversity	12. Finite factors; asymm. designs (PERMANOVA, cont'd)	15. Q&A / 'Own-data' Session

# Analysis Pathways in PRIMER



# Overview of Tools and Methods in PERMANOVA+



## Some key and well-cited papers on PRIMER / PERMANOVA+ methods

### PRIMER

- Clarke KR (1990) Comparisons of dominance curves. *J Exp Mar Biol Ecol* 138: 143-157
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18: 117-143
- Clarke KR (1999) Non-metric multivariate analysis in community-level ecotoxicology. *Environ Toxicol Chem* 18: 118-127
- Clarke KR, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. *Mar Ecol Prog Ser* 92: 205-219
- Clarke KR, Chapman MG, Somerfield PJ, Needham HR (2006) Dispersion-based weighting of species counts in assemblage analyses. *Mar Ecol Prog Ser* 320: 11-27
- Clarke KR, Gorley RN (2001, 2006, 2015) *PRIMER v5, v6, v7: User manual/tutorial*. PRIMER-E, Plymouth, UK, 91pp, 192pp, 296pp
- Clarke KR, Green RH (1988) Statistical design and analysis for a 'biological effects' study. *Mar Ecol Prog Ser* 46: 213-226
- Clarke KR, Somerfield PJ, Airoidi L, Warwick RM (2006) Exploring interactions by second-stage community analyses. *J Exp Mar Biol Ecol* 338: 179-192
- Clarke KR, Somerfield PJ, Chapman MG (2006) On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *J Exp Mar Biol Ecol* 330: 55-80
- Clarke KR, Somerfield PJ, Gorley RN (2008). Exploratory null hypothesis testing for community data: similarity profiles and biota-environment linkage. *J Exp Mar Biol Ecol* 366: 56-69
- Clarke KR, Somerfield PJ, Gorley RN (2016) Clustering in non-parametric multivariate analyses. *J Exp Mar Biol Ecol* 483: 147-155.
- Clarke KR, Tweedley JR, Valesini FJ (2014) Simple shade plots aid better long-term choices of data pre-treatment in multivariate assemblage studies. *J Mar Biol Assoc UK* 94: 1-16
- Clarke KR, Warwick RM (1994, 2001, 2014) *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. PRIMER-E, Plymouth, UK. 1st ed: 144pp; 2nd ed: 172pp. 3rd ed: (authors: Clarke KR, Gorley RN, Somerfield PJ, Warwick RM) 260pp
- Clarke KR, Warwick RM (1998) Quantifying structural redundancy in ecological communities. *Oecologia* 113: 278-289
- Clarke KR, Warwick RM (1998) A taxonomic distinctness index and its statistical properties. *J Appl Ecol* 35: 523-531
- Clarke KR, Warwick RM (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Mar Ecol Prog Ser* 216: 265-278
- Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. *Mar Ecol Prog Ser* 8: 37-52
- Somerfield PJ, Clarke KR (1995) Taxonomic levels, in marine community studies, revisited. *Mar Ecol Prog Ser* 127: 113-119
- Somerfield PJ, Clarke KR (2013) Inverse analysis in non-parametric multivariate analyses: distinguishing groups of associated species which covary coherently across samples. *J Exp Mar Biol Ecol* 449: 261-273
- Somerfield PJ, Clarke KR, Gorley RN (2021a) A generalized analysis of similarities (ANOSIM) statistic for designs with ordered factors. *Austral Ecology* 46: 901-910.
- Somerfield PJ, Clarke KR, Gorley RN (2021b) Analysis of similarities (ANOSIM) for 2-way layouts using a generalized ANOSIM statistic, with comparative notes on Permutational Multivariate Analysis of Variance (PERMANOVA). *Austral Ecology* 46: 911-926.
- Somerfield PJ, Clarke KR, Gorley RN (2021c) Analysis of similarities (ANOSIM) for 3-way designs. *Austral Ecology* 46: 927-941.

- Somerfield PJ, Clarke KR, Olsford F (2002) A comparison of the power of categorical and correlational tests applied to community ecology data from gradient studies. *J Anim Ecol* 71: 581-593
- Warwick RM, Clarke KR (1991) A comparison of some methods for analysing changes in benthic community structure. *J Mar Biol Ass UK* 71: 225-244
- Warwick RM, Clarke KR (1993) Increased variability as a symptom of stress in marine communities. *J Exp Mar Biol Ecol* 172: 215-226
- Warwick RM, Clarke KR (1995) New 'biodiversity' measures reveal a decrease in taxonomic distinctness with increasing stress. *Mar Ecol Prog Ser* 129: 301-305
- Warwick RM, Clarke KR (1998) Taxonomic distinctness and environmental assessment. *J appl Ecol* 35: 532-543
- Warwick RM, Clarke KR (2001) Practical measures of marine biodiversity based on relatedness of species. *Oceanog Mar Biol Ann Rev* 39: 207-231

### **PERMANOVA+**

- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecol* 26: 32-46
- Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. *Can J Fish Aquat Sci* 58: 626-639
- Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62: 245-253
- Anderson MJ (2008) Animal-sediment relationships revisited: characterising species' distributions along an environmental gradient using canonical analysis and quantile regression splines. *J Exp Mar Biol Ecol* 366: 16-27
- Anderson MJ (2017) [Permutational Multivariate Analysis of Variance \(PERMANOVA\)](#). *Wiley StatsRef: Statistics Reference Online*. 1 – 15. Article ID: stat07841.
- Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell HV, Comita LS, Davies KF, Harrison SP, Kraft NJB, Stegen JC, Swenson NG (2011) Navigating the multiple meanings of  $\beta$  diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14: 19-28
- Anderson MJ, Connell SD, Gillanders BM, Diebel CE, Blom WM, Landers TJ, Saunders JE (2005) Relationships between taxonomic resolution and spatial scales of multivariate variation in kelp holdfast assemblages. *J Anim Ecol* 74: 636-646
- Anderson MJ, de Valpine P, Punnett A, Miller AE (2019) A pathway for multivariate analysis of ecological communities using copulas. *Ecol Evol* 9: 3276-3294
- Anderson MJ, Diebel CE, Blom WM, Landers TJ (2005) Consistency and variation in kelp holdfast assemblages: spatial patterns of biodiversity for the major phyla at different taxonomic resolutions. *J Exp Mar Biol Ecol* 320: 35-56
- Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. *Ecol Lett* 9: 683-693
- Anderson MJ, Gorley RN, Clarke KR (2008) *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*. PRIMER-E: Plymouth, UK, 214pp
- Anderson MJ, Gorley RN, Terlizzi A (2025) The incremental progression from fixed to random factors in the analysis of variance: a new synthesis. *Aust N Z J Stat* 67: 3-30.
- Anderson MJ, Gribble NA (1998) Partitioning the variation among spatial, temporal and environmental components in a multivariate data set. *Aust J Ecol* 23: 158-167
- Anderson MJ, Legendre P (1999) An empirical comparison of permutation methods for tests of partial regression coefficients in a linear model. *J Statist Comput Sim* 62: 271-303
- Anderson MJ, Millar RB (2004) Spatial variation and effects of habitat on temperate reef fish assemblages in northeastern New Zealand. *J Exp Mar Biol Ecol* 305(2): 191-221

- Anderson MJ, Robinson J (2003) Generalized discriminant analysis based on distances. *Aust NZ J Stat* 45: 301-318
- Anderson MJ, Robinson J (2001) Permutation tests for linear models. *Aust NZ J Stat* 43: 75-88
- Anderson MJ, Santana-Garcon J (2015) Measures of precision for dissimilarity-based multivariate analysis of ecological communities. *Ecol Lett* 18: 66-73.
- Anderson MJ, ter Braak CJF (2003) Permutation tests for multi-factorial analysis of variance. *J Statist Comput Sim* 73: 85-113
- Anderson MJ, Walsh DCI (2013) What null hypothesis are you testing? PERMANOVA, ANOSIM and the Mantel test in the face of heterogeneous dispersions. *Ecol Monogr* 83: 557-574
- Anderson MJ, Walsh DCI, Clarke KR, Gorley RN, Guerra-Castro E (2017) Some solutions to the multivariate Behrens-Fisher problem for dissimilarity-based analyses. *Aust NZ J Stat.* 59: 57-79
- Anderson MJ, Walsh DCI, Sweatman WL, Punnett AJ (2022) Non-linear models of species' responses to environmental and spatial gradients. *Ecology Letters* 25: 2739-2752
- Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* 84: 511-525
- Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol Monogr* 69: 1-24
- McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82: 290-297
- Rolls RJ, Deane DC, Johnson SE, Heino J, Anderson MJ, Ellingsen KE (2023) Biotic homogenization and differentiation as directional change in beta diversity: synthesizing driver-response relationships to develop conceptual models across ecosystems. *Biological Reviews* 98: 1388-1423.