6.18 Recommendations

1. For typical species abundance matrices, it is much preferable to use a non-parametric ANOSIM-type permutation test rather than classical MANOVA; the latter will almost always be totally invalid. A realistic alternative is the semi-parametric PERMANOVA tests of Anderson, Gorley & Clarke (2008) . These do make more assumptions, fitting additive linear models in a (complex) high-dimensional space defined by the (metric) resemblance matrix but, crucially, do not make unacceptable normality assumptions in carrying out their tests, which use (approximate) null distributions from permutation procedures. In simple designs, ANOSIM's greater robustness might be preferred; in more complex designs some questions can only be answered by PERMANOVA. This is a familiar balance from univariate statistics: non-parametric methods are more robust but give shallower inference, model estimation of parameters inevitably involves more assumptions but allows a deeper level of inference.

2. Choice of the level and type of replication should be carefully considered. Though it is difficult to define power for any of the ANOSIM (or PERMANOVA) tests, it is important to ensure sufficient samples are taken at the right level to generate enough permutations for meaningful significance levels. Equally important is that replicates which are crucial for the tests being undertaken should *genuinely* represent the condition being sampled: pseudo-replication is commonplace, e.g. analyses of sub-cores of single cores, or sets of spatially contiguous or temporally coincident samples which are unrepresentative of the extent of the sites or times about which inference is desired. Pseudo-replicates may still have an important role, when pooled, in providing enough material for sensible definition of a single replicate of that time or place, but the balance of collection or analysis effort at different levels of a design is often context dependent, and pilot experimentation will usually reap dividends for efficiency of the main study. As a general rule, design to provide fully representative replication at the level immediately below the effect of main interest, and use balanced crossed designs to eliminate non-negligible factors which are not the main focus of the study.

3. A point that cannot be over-stressed is that ANOSIM tests only apply to groups of samples specified *prior* to seeing (or collecting) the data. A dangerous misconception is that one can use a cluster analysis of the species abundance data to define sample groupings whose statistical validity can be established by performing an ANOSIM test for differences among those groups. This is *entirely wrong*, the reasoning being completely circular. Sometimes, independent data exists (e.g. environmental) which can permit the definition of groups to test with the biotic data. Another safe course here can be to use a first set of (biotic) data to define the groups of interest, i.e. to erect the hypothesis, and then to collect a further set of the same assemblage data to test that hypothesis. Alternatively, the SIMPROF procedure of Chapter 3 may allow you to make some (weaker) statements about structure in the data that is worth exploring in future studies. If prior structure exists, use it: where ANOSIM (or PERMANOVA) tests are valid, they are your most useful

testing tools.

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