

1.18 Additional assumptions

Recall that we assume for the analysis of a one-way design by PERMANOVA that the multivariate observations are independent and identically distributed (i.i.d.) under a true null hypothesis. When more complex (multi-way) designs are analysed, a few more assumptions are added, due to the way partitioning is done and the method of permutation used (generally, the method used is permutation of residuals under a reduced model). More specifically, for multi-way designs, PERMANOVA fits an additive linear model to the multivariate samples in the space of the chosen resemblance measure. This assumes that effects of factors and their interactions can be modeled meaningfully in this additive fashion, as opposed to using, say, a non-linear, multiplicative or other approach (e.g., see [Millar, Anderson & Zunun \(2005\)](#)). It also assumes that the errors (which may be estimated using the residuals after fitting a given PERMANOVA model) are i.i.d. across the full design in the space of the chosen resemblance measure.

At present, it is unknown to what extent and in what circumstances departures from these assumptions might affect error rates or interpretations of results from PERMANOVA. Plots showing distributions of residuals vs fitted values (e.g., from either PERMANOVA or DISTLM models, see [chapter 4](#)) for each of a series of PCO dimensions (see [chapter 3](#)) in order of decreasing importance, for example, and also multivariate ordination plots of residuals could provide helpful diagnostic tools. Further study is warranted to develop these tools and to investigate the effects of violations of the assumptions, especially for mixed models, models with covariates or unbalanced designs (which are all discussed in more detail later in this chapter) and even for simple designs that include interactions. In addition, the good asymptotic properties of the methods of permutation of residuals used by PERMANOVA (and DISTLM) require reasonable sample sizes within the cells. Small numbers of observations within cells will result in inter-correlations among residuals. Although exchangeability of errors (implying independence and homogeneity) and additive effects in the space of the resemblance measure are fairly modest assumptions, we nevertheless look forward to future studies where the full implications of the use of these models in different situations and with different resemblance measures may become clearer. In the meantime, simulations done with univariate data having highly non-normal error structures indicate that the permutation methods implemented by PERMANOVA are quite stable for reasonable sample sizes (as indicated above) and when observations are i.i.d.

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