

# 0.4 Changes from DOS to PERMANOVA+ for PRIMER

## The new Windows interface

1. All of the original DOS routines have been fully re-written, translated from their original FORTRAN into the new Microsoft .NET environment, as used by PRIMER v6. This gives the software a fully modern Windows user interface.
2. The integration of the add-on package with the current PRIMER v6 is seamless and complete, with true multi-tasking, providing a greatly enhanced ability to both visualise and formally analyse multivariate data from within a single package.
3. There are no fixed size constraints on data matrices, the number of factors, or group sizes for any analysis. The limitations are imposed only by the total available memory.
4. Other major advantages of PRIMER v6 shared by the PERMANOVA+ add-on include: workspaces and explorer tree navigation; multiple input formats (including from Excel, or 3-column format); label matching (avoiding the need to worry about the order of data in worksheets, provided the labels used are consistent); data handling operations (sorting, ranking, merging, transforming, summing, etc.); easy cutting and pasting of text results or graphics; multiple output formats (.txt, .rtf, .xls, .jpg, .tif, .emf, etc.); factors on samples (or indicators on variables) can be read in from (or saved to) Excel or text formats along with the data; data can be averaged or summed according to factor levels or combinations of them; levels of multiple factors can be identified with symbols and labels on enhanced 2-d and 3-d plots. Importantly, the package offers a great range of pre-treatment transformations or standardisations, as well as the additional choice of more than 50 resemblance measures.

## Development of the methods

5. The new PERMANOVA routine can now be used to analyse any balanced or unbalanced experimental design, either with or without covariables, for fixed, random or mixed models, either with or without hierarchical nesting. There is no limit on the number of factors that can be analysed. A new algorithm ensures correct derivation of expected mean squares (EMS) and correct construction of pseudo- $F$  ratios for each term in the model, with tests done using permutations.
6. A new 'Design' type of worksheet file (\*.ppd) is introduced into PRIMER with this add-on package. Its sole function is to outline the correct experimental design for subsequent analysis by PERMANOVA. It is linked to the resemblance matrix to be analysed, and so utilises important information regarding the available factors and their levels accordingly. When created, the user specifies whether factors are fixed or random and whether they

are nested within one or more other factors. A new feature is the ability to *test* the design file using dummy data. This will output EMS's and will identify the numerator and denominator mean squares and degrees of freedom for each term in the model. This allows the user to check a specified design and to consider the potential power for individual terms in the model, even before data are collected.

7. Pair-wise tests in PERMANOVA are now done taking into account the position of the factor of interest within the full experimental design. In the DOS version, the data corresponding only to the two levels of the factor being compared were extracted and considered in isolation for each test. The Windows version, however, retains the full experimental design and treats the pair-wise test effectively as a 1df contrast between those two levels. This ensures that the correct denominator is used for all tests and that the structure of the full design, including any necessary conditioning, is maintained (which is especially important for unbalanced designs or when there are covariates).
8. Terms which traditionally have had "no test" in PERMANOVA, due to the complexity of the design (usually caused by multiple random effects), are now tested using correct *linear combinations* of appropriate mean squares.
9. Pooling or the exclusion of one or more individual terms in the model is now possible in PERMANOVA, as is the analysis of models lacking replication, such as randomised blocks, split-plots and repeated measures. The order in which terms are fitted can also be specified explicitly by the user (important in either unbalanced designs or designs with covariates where Type I SS are used).
10. The user can now choose the type of sums of squares to use (Type I, II or III SS) in PERMANOVA. These types are equivalent for balanced designs, but not for unbalanced designs or designs with covariates. An enhanced algorithm even allows for imbalance in the actual cell structure of the model, which is especially useful in the context of asymmetrical experimental designs (e.g., [Underwood \(1994\)](#) , [Glasby \(1997\)](#) ).
11. The new PERMANOVA routine also allows the user to specify and test particular 1 df contrasts of interest (such as a treatment *versus* a number of different control groups) as well as the interaction of such contrasts with other factors.
12. The method used to fit covariables in the DOS version of PERMANOVA was a naïve approach that did not adjust the EMS's of the other terms in the ANOVA model accordingly<sup>3</sup>. Thus, the results obtained from the two versions will differ for a given (balanced) data set if there are covariables (the new Windows version is correct).
13. In PERMDISP, the user now has the option of whether distances are calculated to centroids or to spatial medians and also whether *P*-values are obtained using permutations or tabled values for the *F* ratio (the default is to use centroids and permutations, as recommended in [Anderson \(2006\)](#) ). This speeds up the routine considerably, as clearly it is not necessary to perform all four possible combinations of these approaches, as is done in the DOS version.
14. The PCO routine yields graphical output that has all of the facilities that similar graphical objects (like PCA plots) in PRIMER possess, including choices for symbols and labels, the ability to observe and spin 3-d plots, and to superimpose bubbles. A new graphical feature offered with the PERMANOVA+ add-on is the ability to overlay vectors that correspond to either individual or partial correlations between variables in a worksheet and ordination axes. These may be the original variables, transformed variables, or some other variables

of interest.

15. A new tool provided in PERMANOVA+ allows the user to calculate distance matrices among centroids identified by factors (or cells consisting of combinations of factors) *in the space of a chosen resemblance measure*. From this, centroids can then be further analysed or viewed in ordinations (handy for complex designs), also enabling the visualisation of relative effect sizes.
16. The DOS version of DISTLM and its cousin, DISTLM\_forward, have been dramatically enhanced in the DISTLM routine of PERMANOVA+ for PRIMER. The DOS version only performed forward selection on the basis of the simple  $R^2$  criterion, whereas the new DISTLM is a true multivariate regression modelling tool on the basis of a chosen resemblance measure. The user may now choose among four model selection procedures (forward selection, backwards elimination, step-wise selection, or the “best” of all possible combinations) on the basis of any of 4 model selection criteria ( $R^2$ , adjusted  $R^2$ , and multivariate analogues of AIC or BIC). In addition, if one specifies “best” as the model selection procedure, then one may also choose how many of the top solutions to view and also whether to view relatively brief or more detailed results.
17. The new DISTLM routine in PERMANOVA+ uses the existing utility in PRIMER of specifying sets of variables using indicators, so that these may be kept together during model selection (e.g., spatial, temporal and environmental variables, [Anderson & Gribble \(1998\)](#)). Identifying sets of variables is also useful for binary indicators or matrices that code for a categorical variable or factor in model selection or fitting.
18. DISTLM also allows the user to explicitly identify particular predictor variables (or sets of them) to be included or excluded in the model (or in the model selection activity), and optionally can also specify the order in which the variables (or sets) are to be fit.
19. The relationship between patterns in a resemblance matrix and a set of predictor variables (such as environmental variables) can be observed directly using a constrained ordination: distance-based redundancy analysis (dbRDA). This is a completely new routine. Vector overlays of the predictor variables are superimposed automatically. Like PCO, the dbRDA ordination routine also takes full advantage of all of the tools offered in other PRIMER Windows graphics (e.g., choice of which axes to view, in two or in three dimensions, ample choice of labels and symbols, etc.). DISTLM is also directly linked to dbRDA, providing an instant constrained ordination, if desired, for any given fitted model.
20. The CAP routine in PERMANOVA+ for PRIMER includes graphical output as well as the usual text output and diagnostics that would be familiar to users of the former CAP program in DOS. This graphical output (as for PCO or dbRDA) has all of the usual advantages of other PRIMER ordination graphics, including the new general vector overlay tool available in PERMANOVA+.
21. A brand new feature of the CAP routine is the ability to add new samples into the CAP model and “see where they fall”. This is highly useful for model validation and for classifying new samples whose group identity is unknown. The positions of the new samples are given in the canonical space in the text output file and visually in the CAP ordination graphic. If the CAP analysis focused on discriminating groups, then the new samples are also allocated to a new group based on their positions in the canonical space.

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<sup>3</sup> Unfortunately, when a covariable is added to an ANOVA model, then the terms in the model are no longer orthogonal (independent) of one another, even if the design is balanced.

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