



The resulting project (*Aggregated SplitPlot.c5p*) contains one analysis named *Age* that demonstrates the permutation test for the effect of stand age adjusted for soil type with averaged whole-plots. The age effect is not significant (pseudo- $F=2.30$ ,  $P=0.156$ ).

With restricted permutation types, Canoco does not always allow you to specify the design, particularly if you have deleted some cases, or if they were excluded in unimodal methods because they contained only zero values in the response data. In such cases, the project aggregation described above might help for testing whole-plot variables, while the testing of split-plot variables can often proceed with the help of permutation within blocks, as described in the following sub-section.

It might be instructive to see what happens if we attempt to test the *Age* variable using all data (analysis *Age error* in the *SplitPlot.c5p* project). For the permutation test, we again specify the *Hierarchical design* option, but what about the number of split-plot per whole plot? Note that there are 64 plots in the data file. We cannot choose 12 ( $4 \times 3$ ), because  $64/12$  is not a whole number. We can choose 8 ( $64/8$ ) because the result is a whole number. Canoco accepts 8 and executes the analysis, but from the case arrangement listed in the *Log* page we see that the permutation test used 8 whole-plots, whereas there are only 6 stands. The lesson is to always check the case arrangement in restricted permutation tests in the *Log* page.

### 6.5.6.3 Testing the effect of split-plot variables

Testing the effect of split-plot variables is best carried out using unrestricted permutations within blocks. For this, the whole-plots (*Stand*) must be specified as covariate(s) and as permutation blocks. The *LH* analysis gives an example in which the effect of the treatment of litter and humus is tested. Note it is not a problem that the number of plots differs between blocks: 12 in stands 1-4 and 8 in the stands 5 and 6. Similarly, in the *LH x Age* analysis, it is tested whether the effect of the treatment of litter and humus differs among stands of different age (with the adjustment for possible *LH x Soil type* interaction). The interaction is judged significant (pseudo- $F=3.3$ ,  $P=0.002$ ).

For illustration only, the *LH split-plot* analysis shows how the *LH* effect can be tested without using the *Stand* factor as a covariate: split-plots are permuted, whereas whole-plots are not.

We close this example with the *Figure 1* analysis used to reproduce Figure 1 of Baar & Ter Braak (1996). The number of species and the number of sporocarps per plot are defined as supplementary response variables, while soil acidity and nutrient concentrations in the soil are used as supplementary variables. To define supplementary response variables and/or the supplementary variable role, one must execute the Analysis Setup Wizard in the non-QuickWizard mode (see section 6.1.1). Also note that this analysis performs no permutation tests, as there is no permutation type that would be correct for all the explanatory variables used in the analysis.