

8.2 Example: Split-plot - Woodstock vegetation

The study design

An example of a split-plot design is provided by a study of the effect of fire disturbance and grazers (excluded using fences) on the composition of plant assemblages on the central western slopes of New South Wales in south-eastern Australia ([Prober *et al.* \(2007\)](#)). The design (shown schematically in Fig. 8.4) included the following:

- Blocks (random with $r = 4$ levels).
- Factor A: Fire frequency (fixed with $a = 4$ levels: 0 yrs, 2 yrs, 4 yrs, or 8 yrs).
- Whole plots (random and nested within Blocks and Fire frequency, unreplicated).
- Factor B: Fencing (fixed with $b = 2$ levels: fenced or unfenced).
- Sub-plots (random and nested within all of the above, unreplicated).

The two fencing treatments were randomly allocated to two sub-plots (measuring 5 m $\times$ 5 m) within each fire treatment (whole plots) and there is one of each fire treatment (4 whole plots) randomised within each block. Relative abundances (cover) of higher plant species within each sub-plot were estimated using a point-intercept technique (an 8 mm dowel placed vertically at each of 50 points on a grid across each plot). Although the design was set up at each of two locations (Woodstock and Monteagle) and data were obtained over a number of years (see [Prober *et al.* \(2007\)](#) for more details), we consider here only data from the Woodstock location collected in 2003.

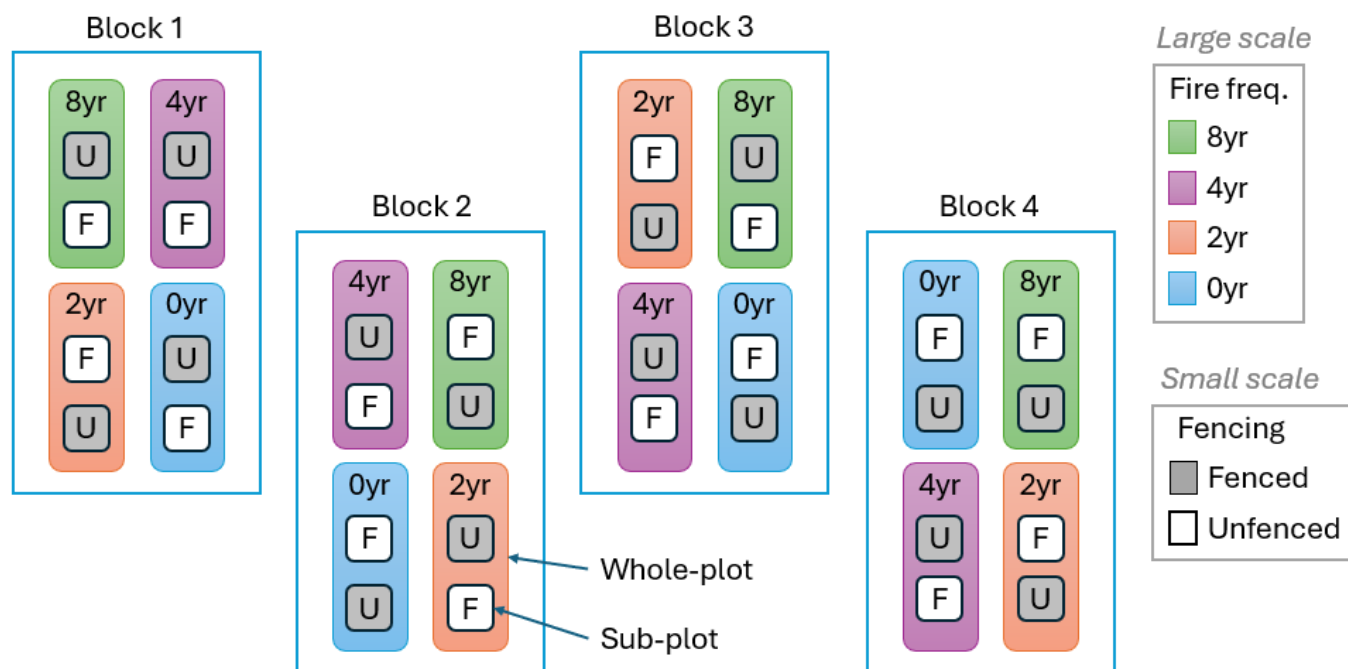


Fig. 8.4. Schematic diagram of the Woodstock split-plot design examining the potential effects of fire frequency and grazers (excluded using fences) on plant assemblages.

For the multivariate analysis, we also will exclude two species from the plant assemblage: *Poa sieberiana* (grey tussock grass) and *Themeda australis* (kangaroo grass), both dominant grasses, from the analysis. These were analysed separately in detail by [Prober et al. \(2007\)](#) ; our focus here instead will be on the more subtle potential responses of subsidiary forbs and exotic species.

Input data and select variables

1. Start running **PRIMER 8**, then click **File > Open...** to open the data file named 'Woodstock_grassland.pri' (found inside the 'Examples_P8 > Woodstock_grassland' folder).

PRIMER

File Edit Select View Wizards Pre-treatment Analyse Plots PERMANOVA+ Tools Window Help

Workspace

Victoria_avifauna_survey

Victoria_avifauna_survey

Victorian avifauna, at survey level

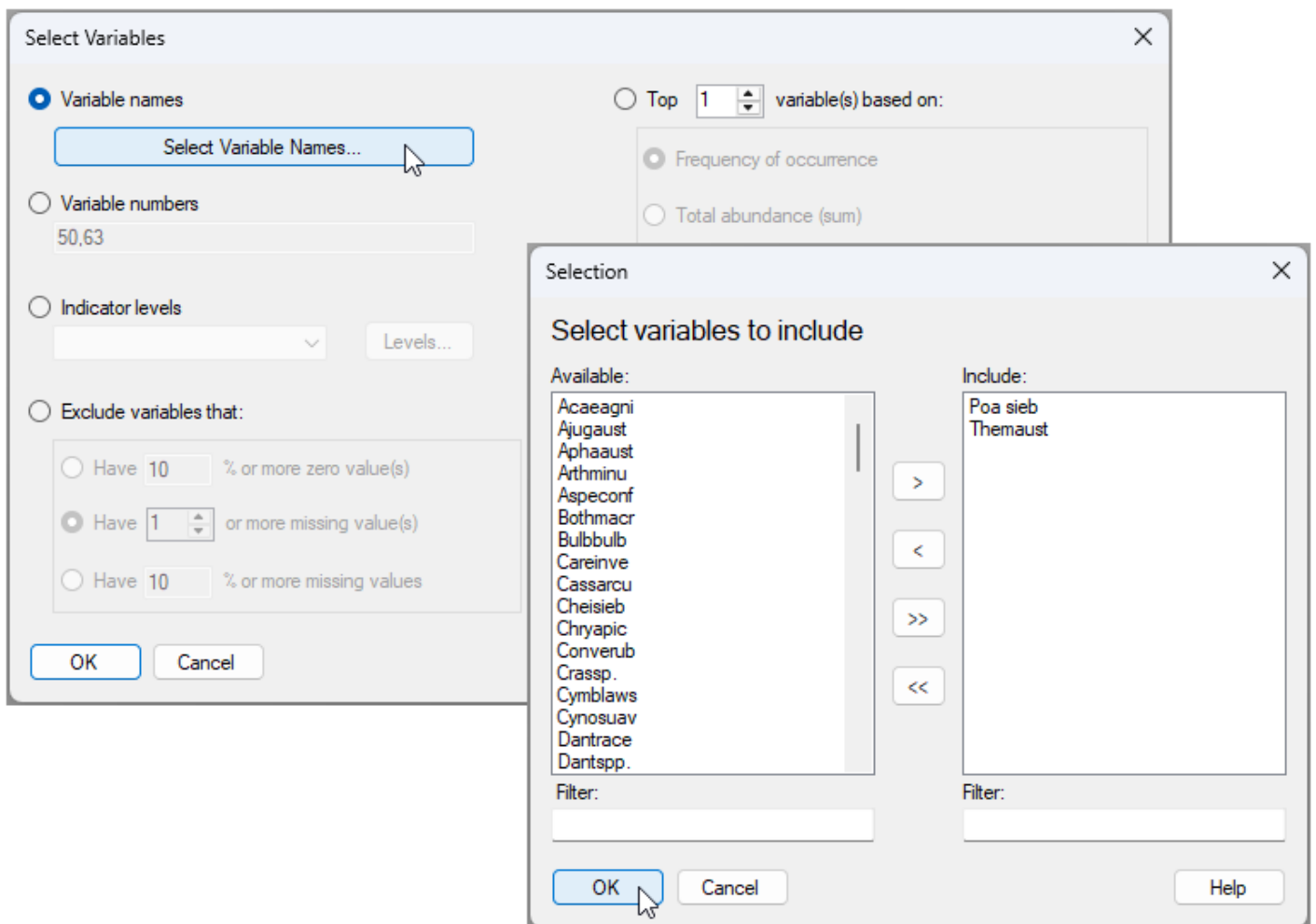
Abundance

	P11	P12	P13	P14	P21	P22	P23
Red Wattlebird	3	1	4	8	3	4	8
Fuscous Honeyeater	3	0	7	2	14	19	23
Yellow-plumed Honeyeater	2	0	8	2	0	0	0
Yellow-tufted Honeyeater	1	0	0	0	3	6	2
White-naped Honeyeater	0	0	0	0	0	1	0
Buff-rumped Thornbill	0	3	0	0	0	0	0
Striated Pardalote	7	0	1	2	0	0	0
Crimson Rosella	3	0	0	0	0	1	0
White-winged Chough	0	0	0	0	0	0	0
White-eared Honeyeater	0	2	2	0	0	0	0
Spotted pardalote	2	0	0	1	1	0	0
Brown-headed Honeyeater	0	0	0	0	0	0	0
Superb Fairy-wren	0	0	1	0	1	3	2
White-throated Treecreeper	1	1	2	0	0	0	0
Musk Lorikeet	0	0	0	0	2	4	0
Golden Whistler	2	2	0	0	1	1	1
Yellow-faced Honeyeater	0	0	0	0	0	0	0
Eastern Rosella	0	0	0	0	0	0	0
Black-chinned Honeyeater	0	0	0	0	0	0	1
Eastern Spinehill	0	0	0	0	0	0	0

Row 1 Col 1

We want to select all variables *except* the two dominant grasses. We will first find these two variables in the data file, highlight them and then *invert* that highlighting in order to select the *remaining* species. The two dominant grasses *Poa sieberiana* and *Themeda australis* are named '**Poa sieb**' and '**Themaus**' in the data file, respectively.

- Click **Select > Variables...** > (\$\bullet\$ Variable names), then click on the button 'Select Variable Names...'. In the resulting dialog, start typing '**Poa**' in the 'Filter:' box under the 'Available' box on the left. When you find '**Poa sieb**', you can click on its name, then click the right arrow  to move this over to the 'Include' box on the right. Repeat the same operation to find and include '**Themaus**'. Once both variables you want to select are in the 'Include' box, click '**OK**', then click '**OK**' in the 'Select Variables' dialog window.



The data sheet with only these 2 species selected will look blue in colour, like this:

Woodstock_grassland

Woodstock, abundance (cover) of higher plant species

Abundance

	Variables	
	Poa sieb	Themaust
W03Tu1	7	15
W03Fu1	21	1
W03Eu1	22	2
W03Cu1	27	0.5
W03Tu2	3	23
W03Fu2	12	12
W03Eu2	2	14
W03Cu2	18	17
W03Tu3	17	13
W03Fu3	1	8
W03Eu3	13	9
W03Cu3	31	5
W03Tu4	9	30
W03Fu4	17	2
W03Eu4	14	8
W03Cu4	27	3
W03Tf1	10	23
W03Ff1	14	2
W03Ef1	15	3
W03Cf1	26	1
W03Tf2	3	23
W03Ff2	31	7
W03Ef2	13	13
W03Cf2	15	11

3. From the data sheet having only these 2 species selected, perform the following steps:

- click **Select > All**. This will show the full spreadsheet of all species once again, but now those two species will just be *highlighted* (appearing in orange / pale yellow) within the sheet.
- click **Edit > Invert Highlight**. Now all of the species except 'Poa' and 'Themaust' (and all of the rows) will be highlighted.
- click **Select > Highlighted**. Now all of the species except 'Poa' and 'Themaust' will be selected (hence blue).
- click **Tools > Duplicate**, to produce a new separate data sheet (which now omits those two grass species) called 'Data1'.

Calculate the resemblance matrix

For analysis of composition of the assemblage, we will apply a square-root transformation, followed by the Bray-Curtis resemblance measure.

4. From the 'Data1' data sheet, click **Pre-treatment > Transform(overall)...** and choose 'Square root' from the drop-down menu, then click **OK**. This will generate a new data sheet item containing the transformed data, called 'Data2'.

- From the square-root transformed data ('Data2'), click **Analyse > Resemblance...** and in the 'Resemblance' dialog window, choose (Measure: $\bullet$ Bray-Curtis similarity) and (Analyse between: $\bullet$ Samples), then click **OK**. This will yield a resemblance matrix item in the explorer tree, called 'Resem1'.

Resem1
Woodstock, abundance (cover) of higher plant species
 Similarity (0 to 100)

	W03Tu1	W03Fu1	W03Eu1	W03Cu1	W03Tu2	W03Fu2	W03Eu2
W03Tu1							
W03Fu1	55.751						
W03Eu1	55.012	54.026					
W03Cu1	58.238	56.898	57.742				
W03Tu2	46.89	46.547	56.111	35.585			
W03Fu2	51.523	48.84	53.626	40.607	58.474		
W03Eu2	37.565	43.807	54.944	32.2	63.153	63.643	
W03Cu2	51.397	38.044	52.228	36.466	47.935	52.565	45.999
W03Tu3	45.015	50.131	55.895	36.31	58.923	64.605	59.686
W03Fu3	55.34	42.608	50.169	43.272	53.898	55.342	60.688
W03Eu3	51.927	51.101	52.971	53.553	57.833	67.094	53.257
W03Cu3	56.696	61.102	60.067	62.706	44.747	53.538	43.302
W03Tu4	34.413	32.106	46.116	34.81	49.548	57.5	59.685
W03Fu4	47.259	43.133	54.81	48.449	48.019	44.255	48.036
W03Eu4	47.536	45.123	52.482	45.576	64.274	65.363	70.959
W03Cu4	42.349	40.592	45.059	54.493	30.855	33.854	33.703
W03Tf1	65.794	54.665	54.463	46.659	55.559	63.186	51.22
W03Ff1	55.24	66.402	53.065	62.63	39.582	46.221	36.988
W03Ef1	59.572	63.579	77.049	63.338	52.108	53.902	46.839
W03Cf1	44.376	56.447	56.553	65.916	34.98	39.306	25.178

Create the design file

Recall that running a PERMANOVA will require us first to set up a design file in accordance with our study's experimental/sampling design.

- From the 'Resem1' matrix, click **PERMANOVA+ > Create PERMANOVA Design...**, then, in the resulting design file (called 'Design1'), click three times on the button to add a row () so that there are four rows in total, as shown below.

Design1

Woodstock, abundance (cover) of higher plant species

Add row Remove row

Factor	Nested in	Type	Contrasts
		Fixed	
		Fixed	
		Fixed	
		Fixed	

Sources of variation

Terms... Pool...

☐ Use short names

Sums of Squares

☒ Type I (sequential)

☐ Type II (conditional)

☐ Type III (partial)

Covariables

Worksheet:

☐ Group covariables (indicator)

Make interaction terms available:

☒ Factor-by-covariable

☐ Covariable-by-covariable

Dispersions

☐ Allow for heterogeneity

Groups...

Term identifying groups with different dispersions:

7. **Choose the name of the factor you want in each row** - In this design file (called 'Design1'), double-click inside each cell of the first column (headed 'Factor') in order to choose the following factors so that they occur sequentially in rows 1, 2, 3 and 4, respectively: row 1 = 'Block', row 2 = 'Fire frequency', row 3 = 'WholePlot', and row 4 = 'Fencing', as shown below:

Design1

Woodstock, abundance (cover) of higher plant species

Add row Remove row

Factor	Nested in	Type	Contrasts
Block		Fixed	
Fire frequency		Fixed	
WholePlot		Fixed	
Fencing		Fixed	

Sources of variation

Terms... Pool...

☐ Use short names

Sums of Squares

☒ Type I (sequential)

☐ Type II (conditional)

☐ Type III (partial)

Covariables

Worksheet:

☐ Group covariables (indicator)

Make interaction terms available:

☒ Factor-by-covariable

☐ Covariable-by-covariable

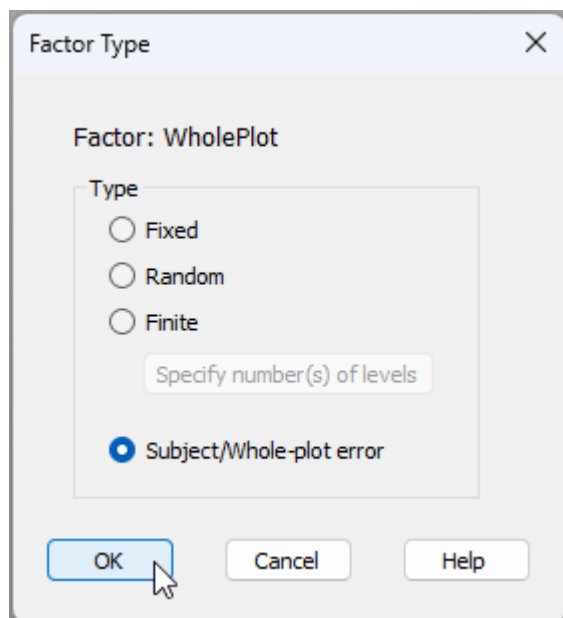
Dispersions

☐ Allow for heterogeneity

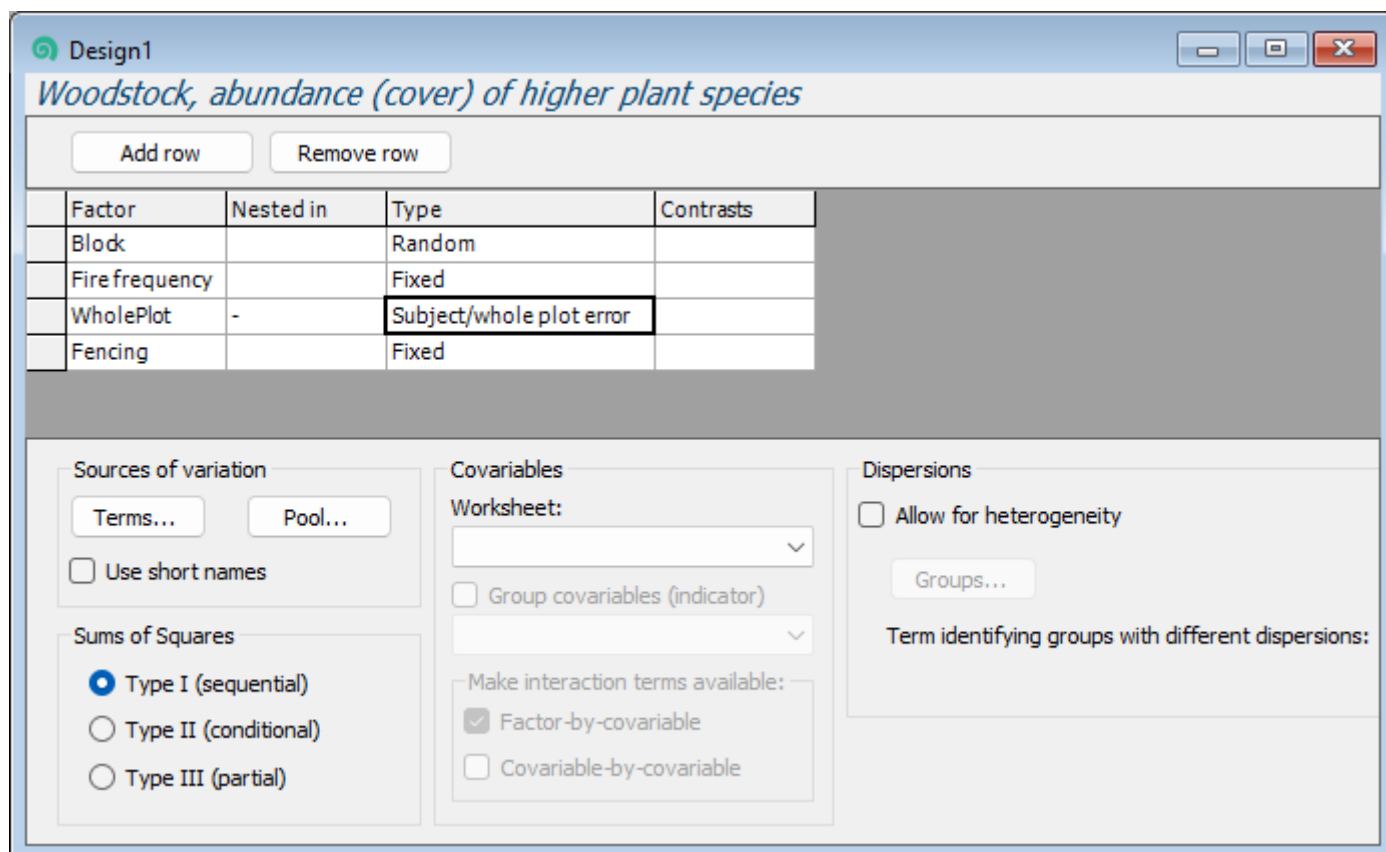
Groups...

Term identifying groups with different dispersions:

8. **Input the factor types** - Next, in column 3, we need to specify the 'Type' of each factor. By default, everything begins by being listed as 'Fixed'. To change this for any given factor, we double-click on the word 'Fixed' in its respective row and the 'Factor Type' dialog will pop up. For the present design, we are happy to treat the factors of 'Fire frequency' and 'Fencing' as 'Fixed', but we need to specify carefully that:
- 'Block' is of Type 'Random', and
 - 'WholePlot' is of Type 'Subject/Whole-plot error', like so:



Having done that, the final correct design file (called 'Design1') for this split-plot study will look like this:

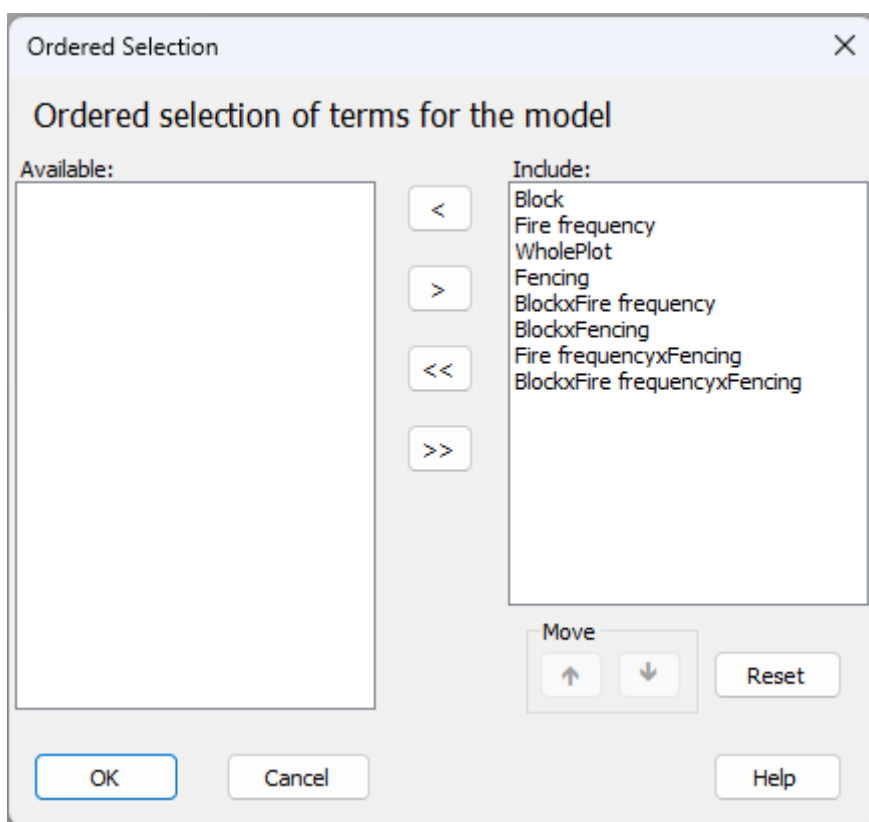


Further design considerations

Before we run the analysis, it is worth pointing out a couple of things about this design file.

First, you will notice that there is a dash ('-') in the 'Nested in' column for the factor of 'WholePlot'. This is because whole-plots, having been identified as such, are deemed effectively to contribute an 'error' into the study design, which means they are necessarily nested within all of the broad-scale factors (in the present case, the broad-scale factors are 'Block' and 'Fire frequency'). Having specified that 'WholePlots' are of Type 'Subject/Whole-plot error', it is not necessary to also articulate this nested structure as well. The dash ('-') merely indicates this column has been 'taken care of' internally, so-to-speak, for the whole-plot factor.

Second, if you click on the 'Terms...' button (Terms...), you will see (on the right-hand side, in a box under the word 'Include:') a list of all of the terms included (by default) in the full PERMANOVA model implied by the design that you have specified.[¶] In the present case, it looks like this:



What do we know about this design already?

- (i). The 'Block $\times$ Fire frequency' interaction term is not actually estimable, because there is no replication of the Fire-frequency treatments in each block.
- (ii). The 'Block $\times$ Fire frequency $\times$ Fencing' interaction term is also inestimable, because there is no replication of the two fencing treatments in each whole-plot.
- (iii). The 'Block $\times$ Fencing' interaction term would not typically be included in a classical analysis of a split-plot design like this. This is primarily because blocks are set up at large scales and the fencing treatment is applied at small scales.

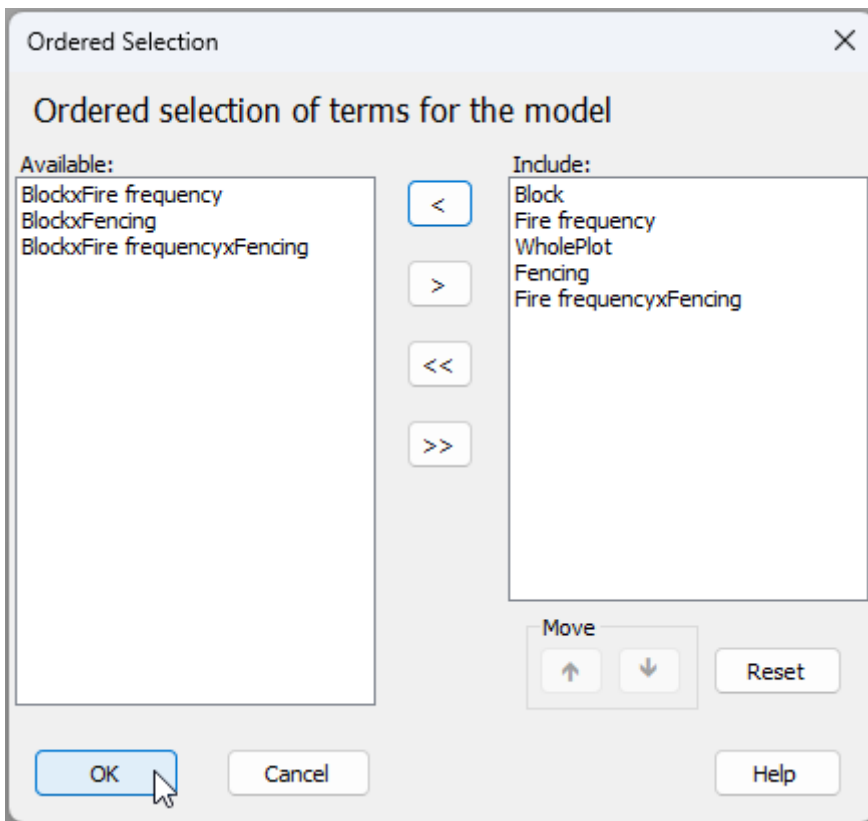
It turns out that, classically, *none* of the interactions involving the factor of 'Block' would be included in the ANOVA partitioning for this split-plot design. The sources of variation and degrees of freedom for this example, according to a traditional split-plot ANOVA partitioning, are shown in Table 8.1

Table 8.1. Sources of variation and degrees of freedom ($\textit{df}$) for a classical split-plot design, where factor A = 'Fire frequency' and factor B = 'Fencing', as per the Woodstock example. Note that 'Whole-plot total' is not an additional source of variation, but rather corresponds to the total variation at the broad scale, i.e., among all of the whole plots, which may be relevant for terms in the top half of the table only. (The line corresponding to 'Whole-plot total' can simply be omitted).

Source	$\textit{df}$
Block	$(r-1) = 3$
Fire frequency	$(a-1) = 3$
Whole-plot error	$(r-1)(a-1) = 9$
Whole-plot total	$(ra-1) = 15$
-----	-----
Fencing	$(b-1) = 1$
Fire frequency $\times$ Fencing	$(a-1)(b-1) = 3$
Sub-plot error	$a(b-1)(r-1) = 12$
Total	$abr - 1 = 31$

If we run the PERMANOVA directly, without manually removing any of the interactions involving the factor of 'Block', then both (i) 'Block $\times$ Fire frequency' and (ii) 'Block $\times$ Fire frequency $\times$ Fencing' interaction terms will be removed automatically in the PERMANOVA run anyway, as these two terms are inestimable. However (in this example), the (iii) 'Block $\times$ Fencing' interaction term will remain in the model, simply because it is (technically) estimable.

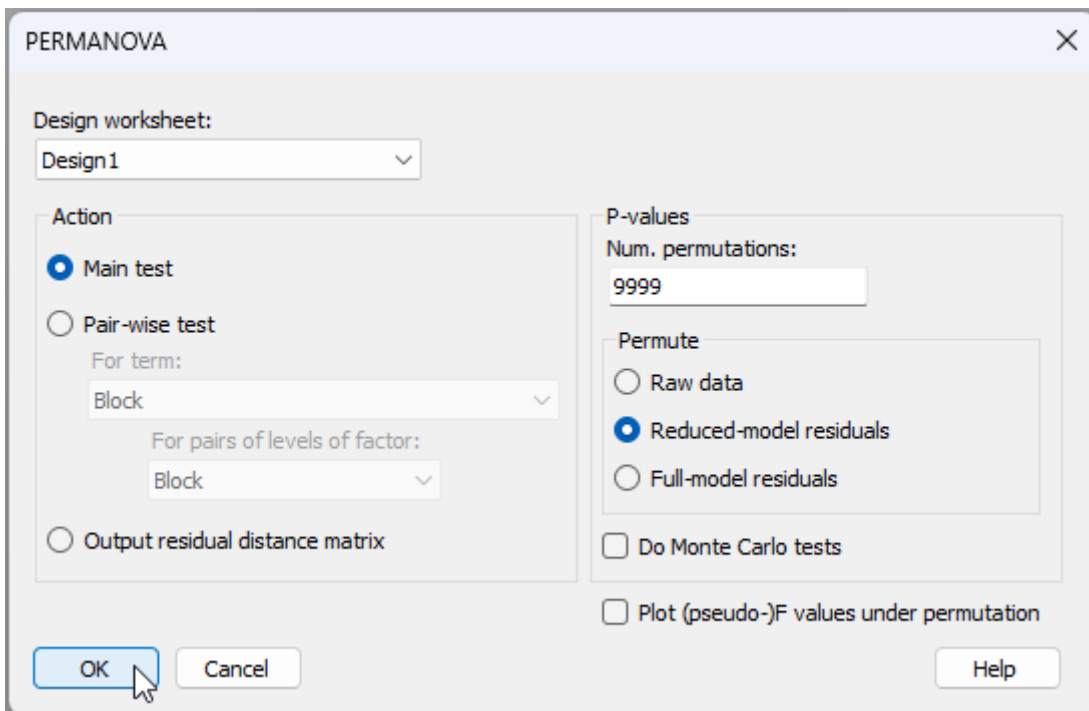
- 9. Remove unwanted interaction terms (optional)** - If you desire the classical split-plot design, you may wish to manually remove all of the interaction terms involving the factor of 'Block'.[‡] To do this, click on the 'Terms' button in the design file and (sequentially) click on each of the terms that you wish to omit from the model, then click on the left arrow () to move it over to the 'Available' column. The result (if you do choose to remove all of the interactions involving the factor of 'Block') will look like this:



Click '**OK**' and now the design file is all ready for the split-plot analysis.

Run the PERMANOVA

- From the original resemblance matrix '**Resem1**', click **PERMANOVA+ > PERMANOVA....** Ensure that the design worksheet is '**Design1**', leave all other defaults, and click '**OK**'.



The resulting output file (called '**PERMANOVA1**') looks like this:

PERMANOVA1

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Block	3	9891.5	3297.2	2.8394	0.0005	9905
Fire frequency	3	7244.2	2414.7	2.0794	0.0071	9914
wholePlot	9	10451	1161.2	2.0014	0.0001	9835
Fencing	1	840.46	840.46	1.4485	0.1349	9931
Fire frequencyxFencing	3	2252.1	750.69	1.2938	0.1136	9872
Res	12	6962.6	580.22			
Total	31	37642				

Estimates of components of variation

Source	Estimate	Sq.root
V(Block)	266.99	16.34
S(Fire frequency)	156.69	12.517
V(wholePlot)	290.51	17.044
S(Fencing)	16.265	4.033
S(Fire frequencyxFencing)	42.617	6.5282
V(Res)	580.22	24.088

Details of the expected mean squares (EMS) for the model

Source	EMS
Block	$1 \times V(\text{Res}) + 2 \times V(\text{wholePlot}) + 8 \times V(\text{Block})$
Fire frequency	$1 \times V(\text{Res}) + 2 \times V(\text{wholePlot}) + 8 \times S(\text{Fire frequency})$
wholePlot	$1 \times V(\text{Res}) + 2 \times V(\text{wholePlot})$
Fencing	$1 \times V(\text{Res}) + 16 \times S(\text{Fencing})$
Fire frequencyxFencing	$1 \times V(\text{Res}) + 4 \times S(\text{Fire frequencyxFencing})$

Construction of Pseudo-F ratio(s) from mean squares

Source	Numerator	Denominator	Num. df	Den. df
Block	1*Block	1*wholePlot	3	9
Fire frequency	1*Fire frequency	1*wholePlot	3	9
wholePlot	1*wholePlot	1*Res	9	12
Fencing	1*Fencing	1*Res	1	12
Fire frequencyxFencing	1*Fire frequencyxFencing	1*Res	3	12

There is a significant effect of fire frequency on the composition of these plant assemblages ($F_{3,9} = 2.08$, $P < 0.01$). There was not, however, a significant effect of fencing to exclude grazers ($F_{1,12} = 1.45$, $P > 0.10$), nor did fencing treatments change the overall effect of fire frequency (the Fire frequency $\times$ Fencing interaction term was not significant; $F_{3,12} = 1.29$, $P > 0.10$).

Run pair-wise comparisons

A natural next step might be to examine pairwise comparisons among the whole-plots having different fire frequencies.

- To run pairwise tests, start from the original resemblance matrix ('Resem1') and click **PERMANOVA+ > PERMANOVA....** Ensuring, once again, that the design worksheet is 'Design1', under 'Action', choose: (• Pair-wise test > (For term: Fire frequency > (For pairs of levels of factor: Fire frequency))). You might also (optionally) tick the option to '• Do Monte Carlo tests', simply because there are not very many whole plots to permute at the large spatial scale. Leaving everything else as the defaults, click 'OK', viz:

PERMANOVA

Design worksheet:
Design1

Action

☐ Main test

☒ Pair-wise test

For term:
Fire frequency

For pairs of levels of factor:
Fire frequency

☐ Output residual distance matrix

P-values

Num. permutations:
9999

Permute

☐ Raw data

☒ Reduced-model residuals

☐ Full-model residuals

☒ Do Monte Carlo tests

☐ Plot (pseudo-)F values under permutation

OK Cancel Help

Results of the pairwise comparisons are shown below ('PERMANOVA2'):

PERMANOVA2

PAIR-WISE TESTS

Term 'Fire frequency'

Groups	t	P(perm)	Unique perms	P(MC)
2yr, 4yr	1.511	0.0951	425	0.0776
2yr, 8yr	0.87175	0.6859	425	0.6186
2yr, nil	1.7834	0.0666	425	0.0393
4yr, 8yr	1.2337	0.2234	425	0.2292
4yr, nil	1.4125	0.1385	425	0.103
8yr, nil	1.5732	0.081	425	0.0766

Denominators

Groups	Denominator	Den. df
2yr, 4yr	1*wholePlot	3
2yr, 8yr	1*wholePlot	3
2yr, nil	1*wholePlot	3
4yr, 8yr	1*wholePlot	3
4yr, nil	1*wholePlot	3
8yr, nil	1*wholePlot	3

Average Similarity between/within groups

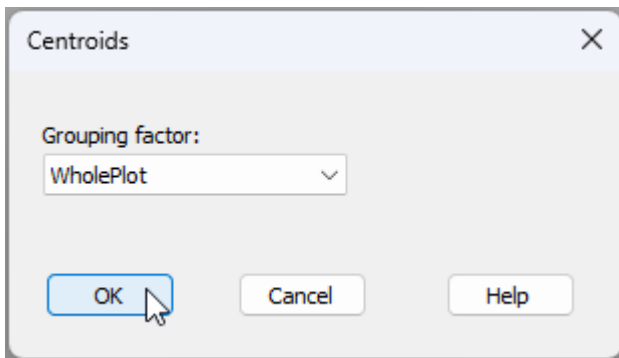
	2yr	4yr	8yr	nil
2yr	54.969			
4yr	50.554	52.879		
8yr	56.802	53.429	58.372	
nil	45.174	49.279	49.563	51.08

Despite the significant overall test, individual treatment levels are not detected as being significantly different from one another (at the 0.05 significance level) in the pair-wise tests.[†] This sort of thing can happen, as the overall (omnibus) F -test can have greater power, given its larger denominator degrees of freedom, compared to the individual pair-wise tests.

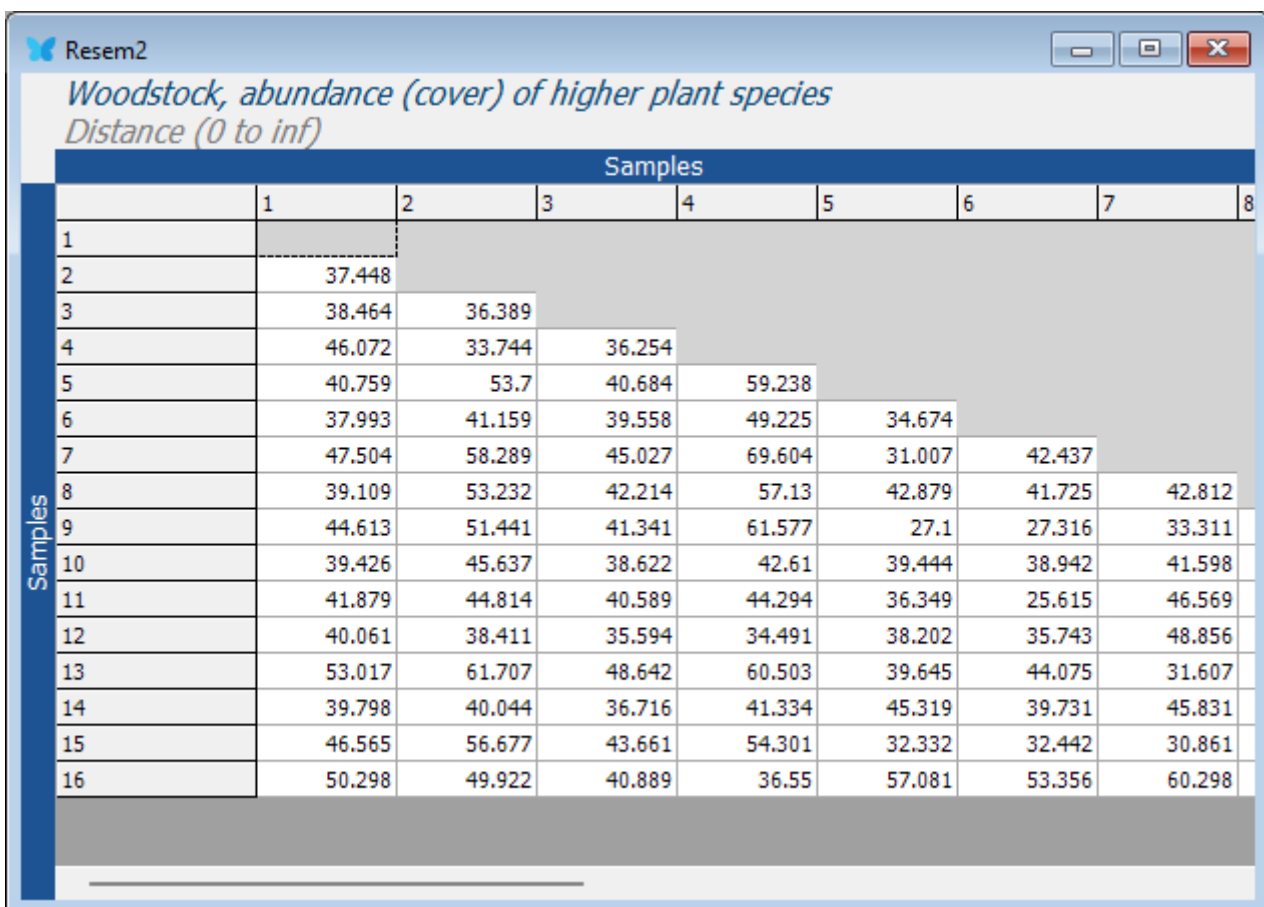
Ordination of whole-plot centroids

Given that fire frequency is assessed at the spatial scale of entire whole-plots (and fencing had no detectable effects), we might consider creating an ordination plot of (say) the whole-plot centroids, as a way to visualise the results.

- From the original resemblance matrix ('Resem1'), we can calculate distances among whole-plot centroids by clicking **PERMANOVA+ > Distance Among Centroids...**, and choosing the 'Grouping factor' as 'WholePlot', as shown below:



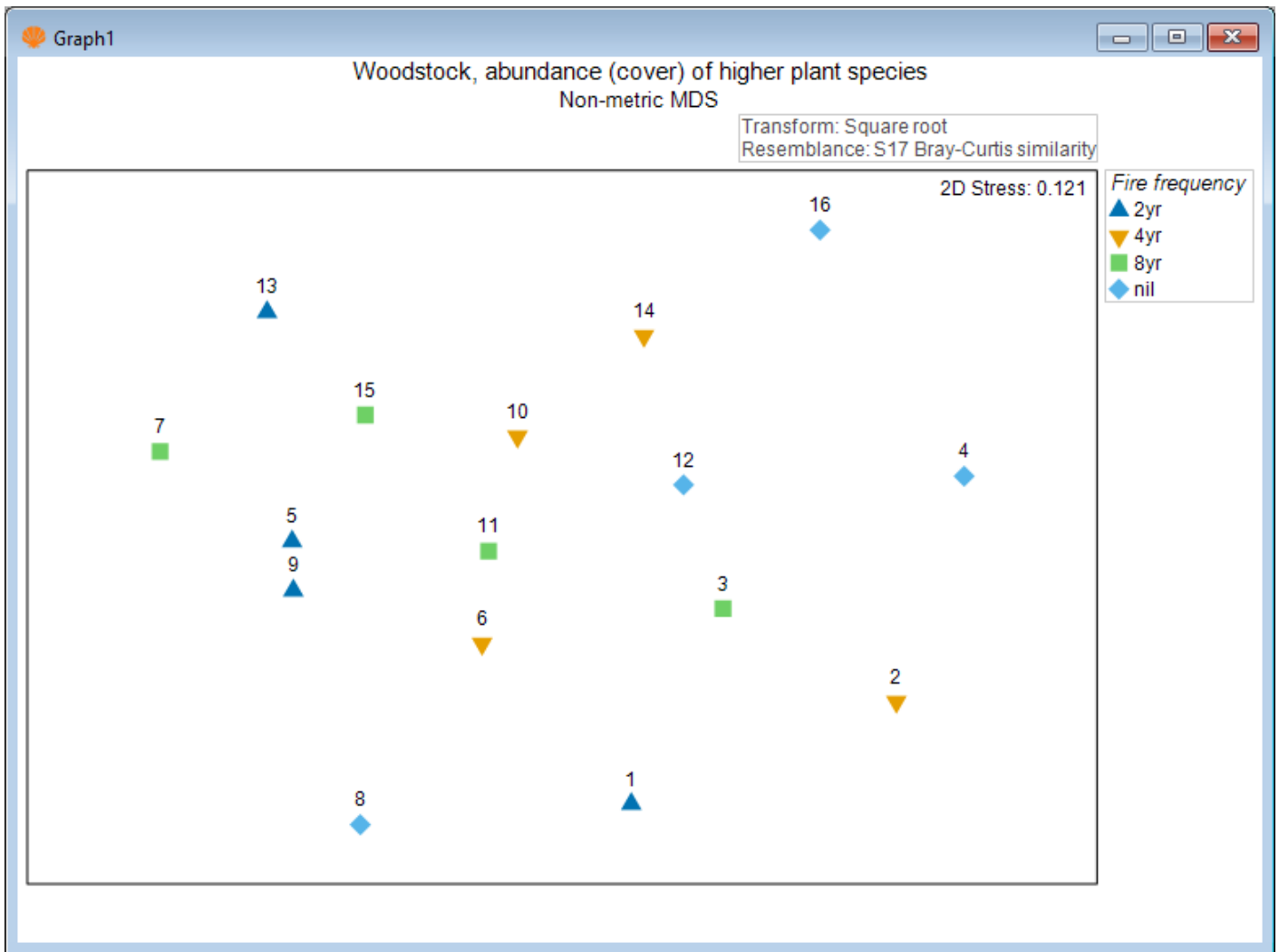
This will produce a new resemblance matrix of Bray-Curtis resemblances among the centroids for the 16 whole-plots, called 'Resem2':



		Samples							
		1	2	3	4	5	6	7	8
Samples	1								
	2	37.448							
	3	38.464	36.389						
	4	46.072	33.744	36.254					
	5	40.759	53.7	40.684	59.238				
	6	37.993	41.159	39.558	49.225	34.674			
	7	47.504	58.289	45.027	69.604	31.007	42.437		
	8	39.109	53.232	42.214	57.13	42.879	41.725	42.812	
	9	44.613	51.441	41.341	61.577	27.1	27.316	33.311	
	10	39.426	45.637	38.622	42.61	39.444	38.942	41.598	
	11	41.879	44.814	40.589	44.294	36.349	25.615	46.569	
	12	40.061	38.411	35.594	34.491	38.202	35.743	48.856	
	13	53.017	61.707	48.642	60.503	39.645	44.075	31.607	
	14	39.798	40.044	36.716	41.334	45.319	39.731	45.831	
	15	46.565	56.677	43.661	54.301	32.332	32.442	30.861	
	16	50.298	49.922	40.889	36.55	57.081	53.356	60.298	

- From the 'Resem2' matrix, produce a non-metric MDS plot by clicking **Analyse > MDS > Non-metric MDS (nMDS)...** Take all the defaults in the 'Non Metric MDS' dialog and click 'OK'. The resulting 2-dimensional nMDS plot (an item called 'Graph1' in the Explorer

tree) is shown below.



As indicated by the pair-wise tests, we do see that most of the assemblages corresponding to unburned whole-plots (light blue diamond-shaped symbols) tend to occur towards the left-hand side of the ordination plot, whereas most of the assemblages corresponding to whole-plots burned at the highest frequency of every 2 years (the dark blue triangle-shaped symbols) tend to occur towards the right-hand side. However, this is not a super clear split, and there is rather high variation among whole plots within any of these treatments. The patterns on this ordination are quite consistent with the rather marginal pairwise test results that we obtained using PERMANOVA. In other words, from a practical point of view (omitting the two dominant grasses) there may only be somewhat minor differences in these plant assemblages caused by fire frequency, if any, at least for the time-scales examined in this study.

Interaction terms are implied between any factors that are crossed with one another. By 'implied', we mean that they are conceivable, although they may not be estimable in all cases. That will depend on there being adequate replication. An interaction is not implied, however, between a given factor and another factor within which it is nested. For example, If we have Factor A and Factor B nested within Factor A, then the only two terms in this model are A and B(A). Factor B clearly cannot interact with Factor A; therefore no $A \times B$ interaction term is implied.

†The closest we come to statistical significance at the 0.05 level is for the comparison of unburned plots (0 years) with plots burned most frequently, i.e., every 2 years. In that case, we have $t = 1.78$ and the Monte Carlo p-value is 0.038, although the permutation p-value is 0.063, so this is a pretty marginal result.

‡An alternative method to achieve the same result here would be to specify the 'Block' factor also as being of Type 'Subject/Whole-plot error'. This will automatically remove all interactions of the 'Block' factor with any other term(s) in the model.

Revision #95

Created 5 May 2025 05:55:01 by Marti

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