8.3 Example: Repeated measures - Victorian avifauna

The study design

An example of a repeated-measures sampling design (Fig. 8.5) is provided in a study of Victorian avifauna by Mac Nally & Timewell (2005) . The data consist of counts of p = 27 nectarivorous bird species at each of eight sites having different levels of flowering intensity within the Rushworth State Forest in Victoria, Australia. One pair of sites (S1, S2) had heavy flowering ('good' sites), another pair (S3, S4) had intermediate flowering ('medium' sites), and a third pair (S5, S6) had relatively little flowering ('poor' sites). Two sites (S7, S8) were near the good sites (called 'adjacent' sites) and these were also sampled to explore the potential for 'spill-over' effects. Sampling of the bird assemblages was done using a strip transect method and each of the 8 sites was sampled repeatedly at four different time points.

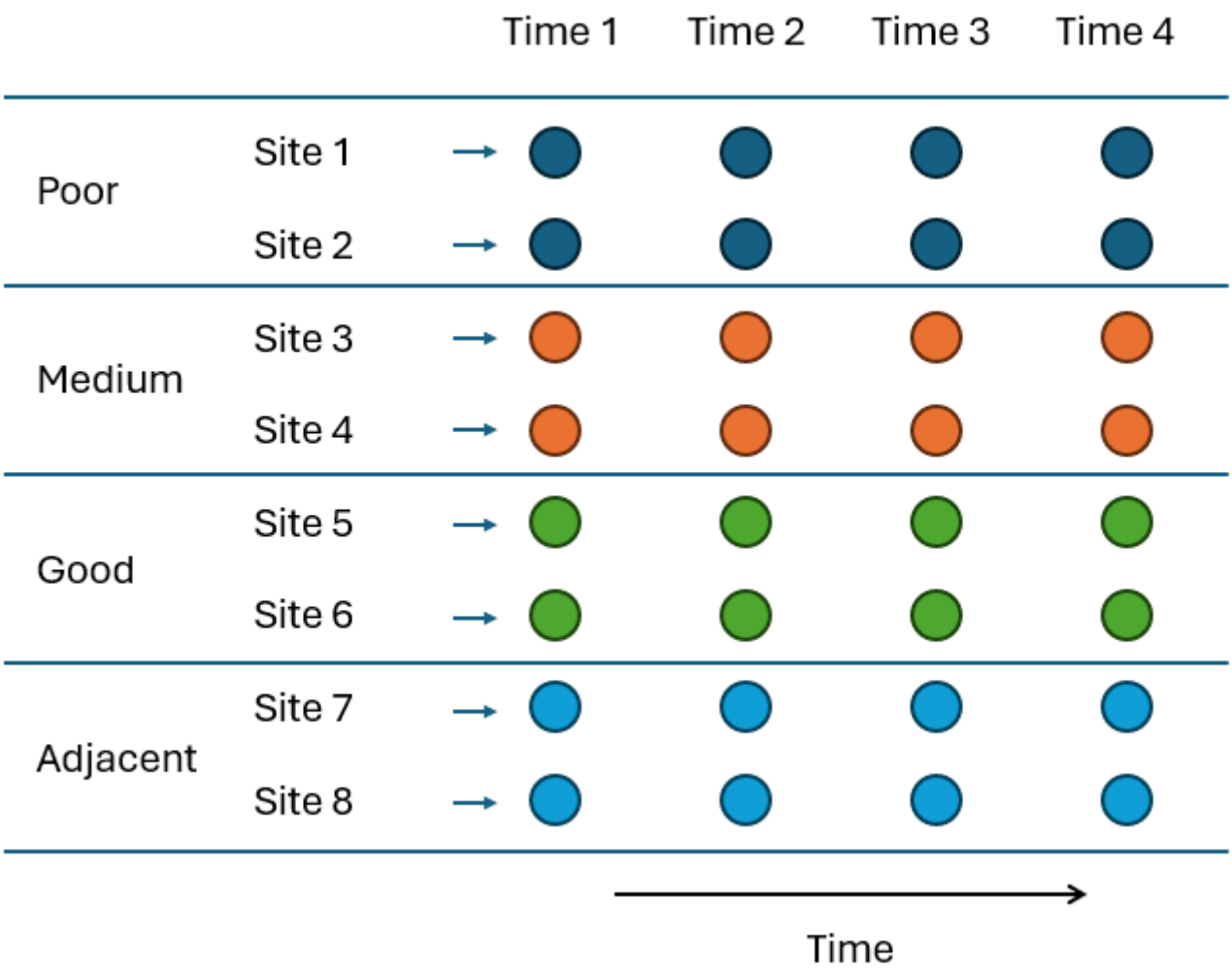
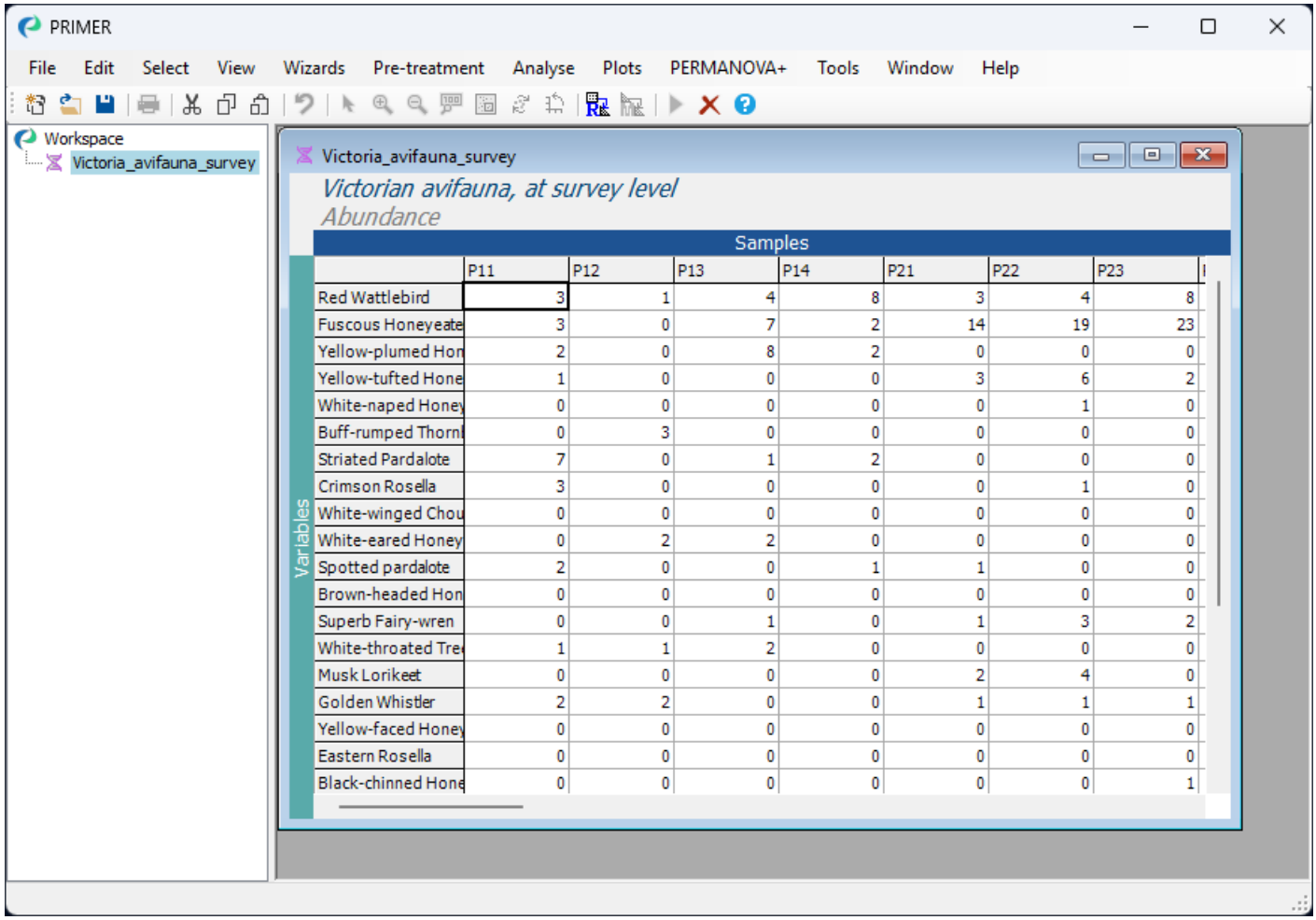


Fig. 8.5 Schematic diagram of the repeated-measures sampling design to study bird assemblages in response to flowering intensity by Mac Nally & Timewell (2005) .

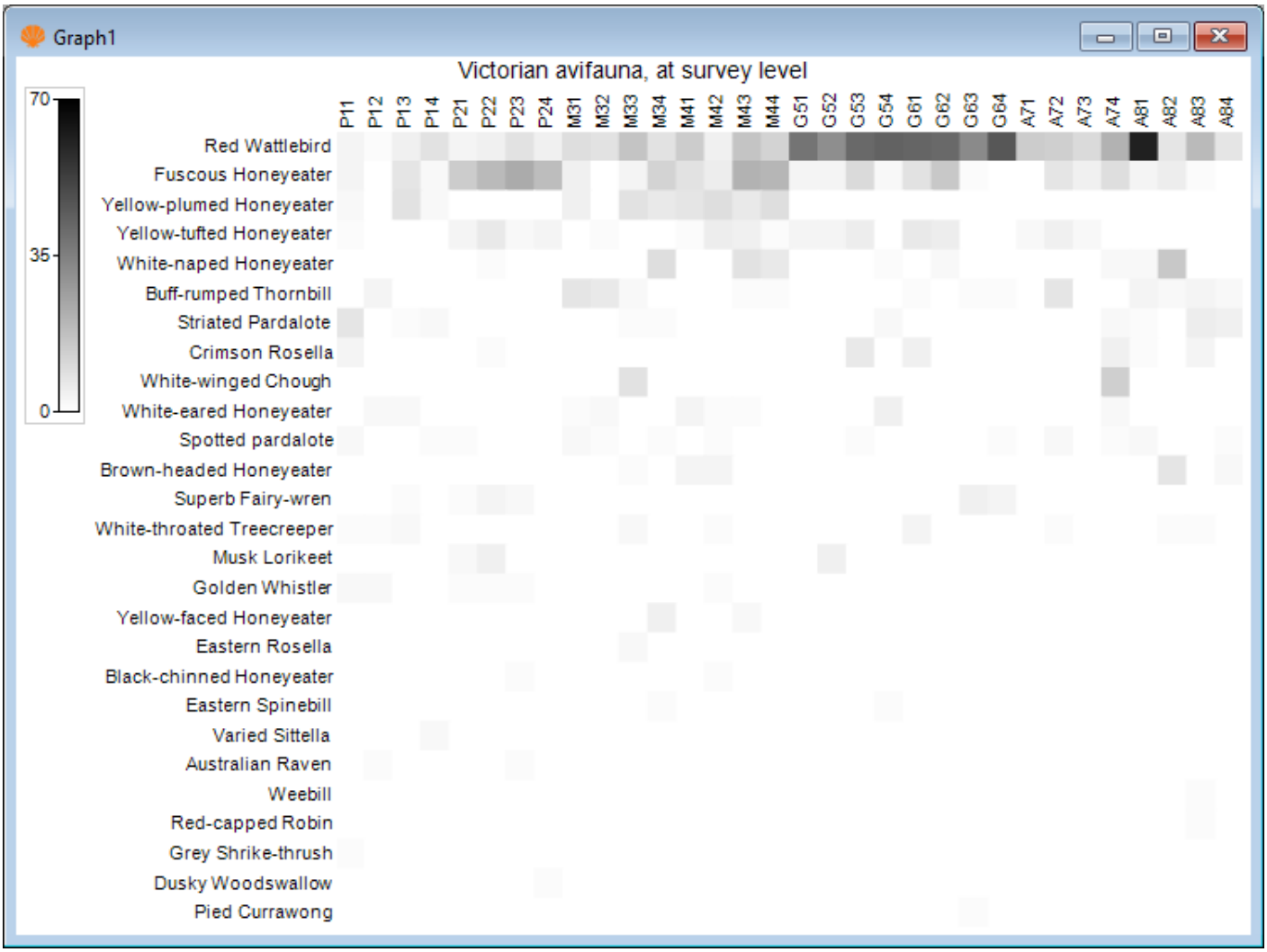
Input data and select a pre-treatment option

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

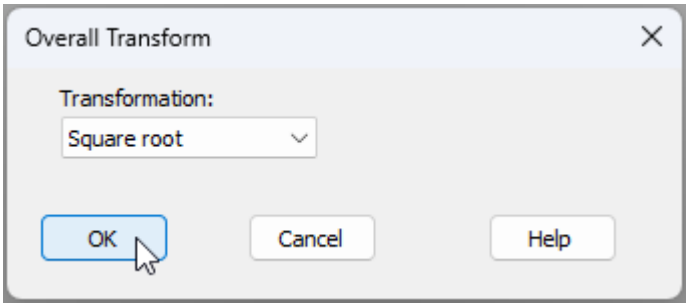


	P11	P12	P13	P14	P21	P22	P23
Red Wattlebird	3	1	4	8	3	4	8
Fuscous Honeyeate	3	0	7	2	14	19	23
Yellow-plumed Hon	2	0	8	2	0	0	0
Yellow-tufted Hone	1	0	0	0	3	6	2
White-naped Honey	0	0	0	0	0	1	0
Buff-rumped Thornb	0	3	0	0	0	0	0
Striated Pardalote	7	0	1	2	0	0	0
Crimson Rosella	3	0	0	0	0	1	0
White-winged Chou	0	0	0	0	0	0	0
White-eared Honey	0	2	2	0	0	0	0
Spotted pardalote	2	0	0	1	1	0	0
Brown-headed Hon	0	0	0	0	0	0	0
Superb Fairy-wren	0	0	1	0	1	3	2
White-throated Tre	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 Honey	0	0	0	0	0	0	0
Eastern Rosella	0	0	0	0	0	0	0
Black-chinned Hone	0	0	0	0	0	0	1

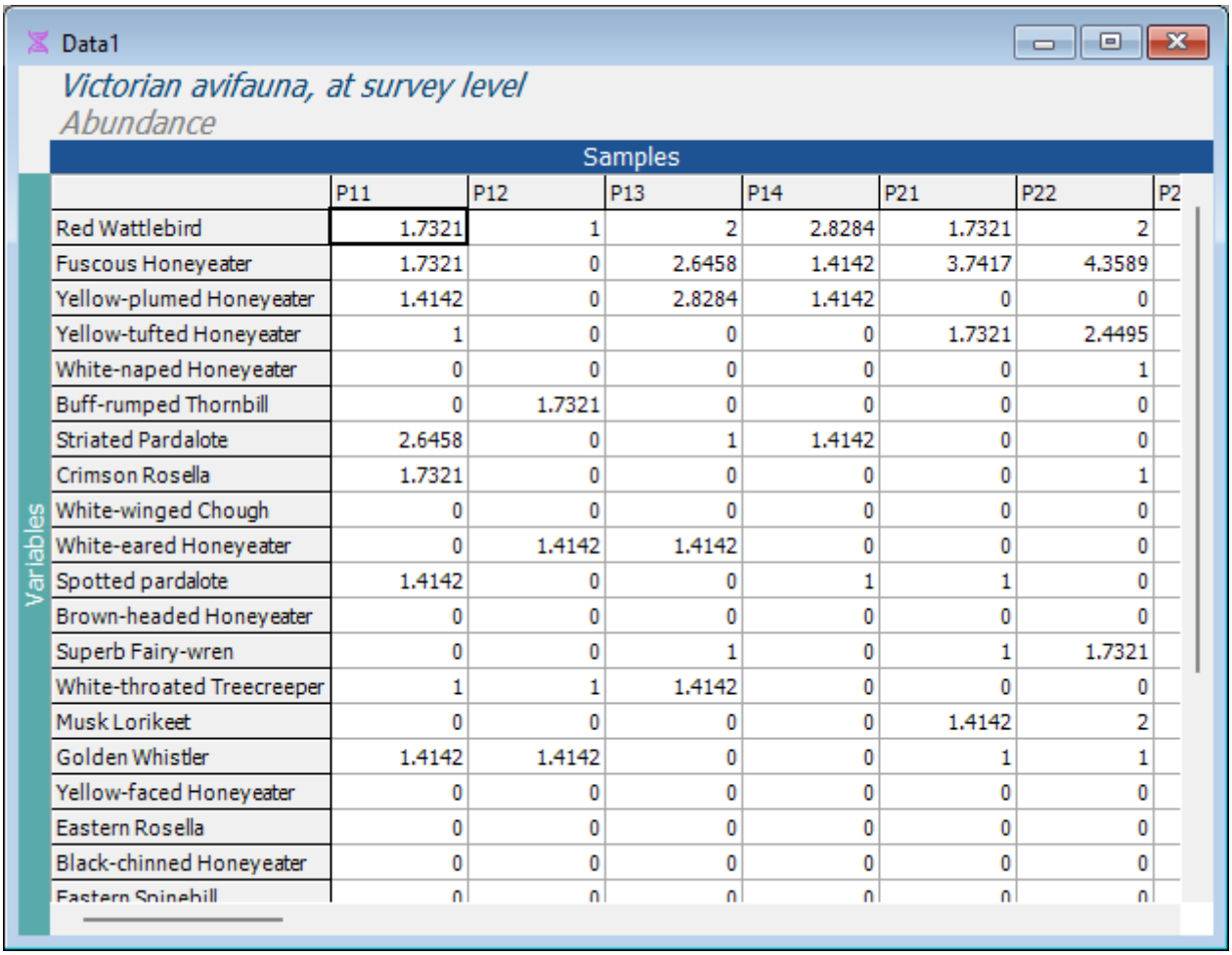
2. From the 'Victoria_avifauna_survey' data sheet in the Explorer tree inside PRIMER, click **Plots** > **Shade Plot**. It is evident from the resulting shade plot ('Graph1) that there is a lot of 'white space', and the range of abundance values is from 0 to 70. Thus a mild (e.g., square-root) transformation would be a sensible choice here as a pre-treatment option, to balance the relative importance of abundant *vs* rare taxa in the calculation of the resemblance measure.



3. From the 'Victoria_avifauna_survey' data sheet, click **Pre-treatment** > **Transform(overall)...**, and in the 'Overall Transform' dialog window, choose 'Transformation: Square root from the drop-down menu, then click '**OK**'.



The square-root transformed data are now provided in a data sheet called 'Data1':



Data1

Victorian avifauna, at survey level

Abundance

Samples

Variables

	P11	P12	P13	P14	P21	P22	P2
Red Wattlebird	1.7321	1	2	2.8284	1.7321	2	
Fuscous Honeyeater	1.7321	0	2.6458	1.4142	3.7417	4.3589	
Yellow-plumed Honeyeater	1.4142	0	2.8284	1.4142	0	0	
Yellow-tufted Honeyeater	1	0	0	0	1.7321	2.4495	
White-naped Honeyeater	0	0	0	0	0	1	
Buff-rumped Thornbill	0	1.7321	0	0	0	0	
Striated Pardalote	2.6458	0	1	1.4142	0	0	
Crimson Rosella	1.7321	0	0	0	0	1	
White-winged Chough	0	0	0	0	0	0	
White-eared Honeyeater	0	1.4142	1.4142	0	0	0	
Spotted pardalote	1.4142	0	0	1	1	0	
Brown-headed Honeyeater	0	0	0	0	0	0	
Superb Fairy-wren	0	0	1	0	1	1.7321	
White-throated Treecreeper	1	1	1.4142	0	0	0	
Musk Lorikeet	0	0	0	0	1.4142	2	
Golden Whistler	1.4142	1.4142	0	0	1	1	
Yellow-faced Honeyeater	0	0	0	0	0	0	
Eastern Rosella	0	0	0	0	0	0	
Black-chinned Honeyeater	0	0	0	0	0	0	
Eastern Spinebill	0	0	0	0	0	0	

4. From the square-root transformed data ('Data1'), click **Plots** > **Shade Plot**, and you will see in the resulting shade plot graphic (called 'Graph2' in the Explorer tree) a more even distribution of abundance information across all bird taxa (less white space), with transformed abundance values now ranging from 0-8.

Calculate dissimilarities and visualise patterns

Now we are ready to calculate dissimilarities (or similarities) based on the transformed data and use this to visualise patterns of relationships among the sampling units, based on the fauna they contain, using ordination methods.

5. From the square-root transformed data ('Data1'), 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

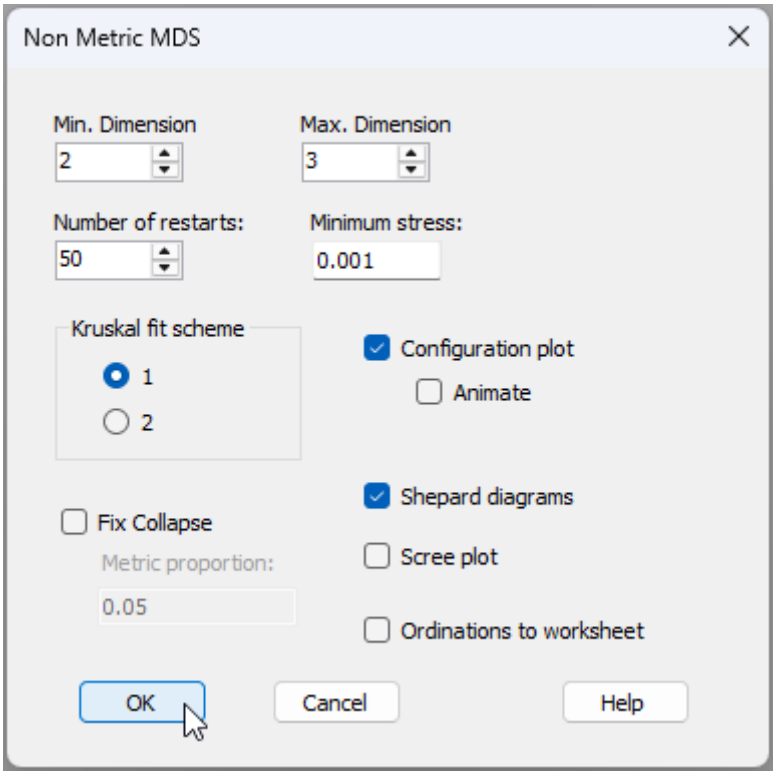
Victorian avifauna, at survey level

Similarity (0 to 100)

Samples

Samples	P11	P12	P13	P14	P21	P22	P23	P24	M31
P11									
P12	30.154								
P13	50.23	34.377							
P14	56.774	11.733	53.502						
P21	48.412	20.855	44.96	39.291					
P22	42.215	17.315	40.554	27.286	78.202				
P23	38.295	28.554	43.841	36.992	70.897	70.274			
P24	37.109	12.095	43.669	36.99	69.977	65.059	68.281		
M31	46.363	38.042	57.465	61.794	39.967	28.985	37.851	38.032	
M32	31.393	51.023	32.524	42.123	36.747	24.76	34.576	33.962	70.1
M33	42.049	27.106	59.963	49.098	23.685	22.501	29.343	28.054	54.8
M34	43.224	8.2296	55.743	58.388	43.693	40.047	41.68	42.495	54.
M41	40.872	22.732	67.012	48.839	43.957	39.893	49.069	51.455	62.
M42	56.751	33.663	63.595	45.376	55.349	47.077	51.592	47.406	56.6
M43	34.217	22.359	51.273	39.338	46.647	53.763	53.932	56.459	57.4
M44	38.584	17.432	53.988	45.49	47.943	54.057	57.566	59.462	58.2
G51	36.127	11.635	34.034	44.393	48.908	43.419	51.771	58.744	43.6

6. From the Bray-Curtis resemblance matrix ('Resem1'), click **Analyse** > **MDS** > **Non-metric MDS (nMDS)...**. Leaving all of the defaults in the 'Non Metric MDS' dialog window, click '**OK**'.



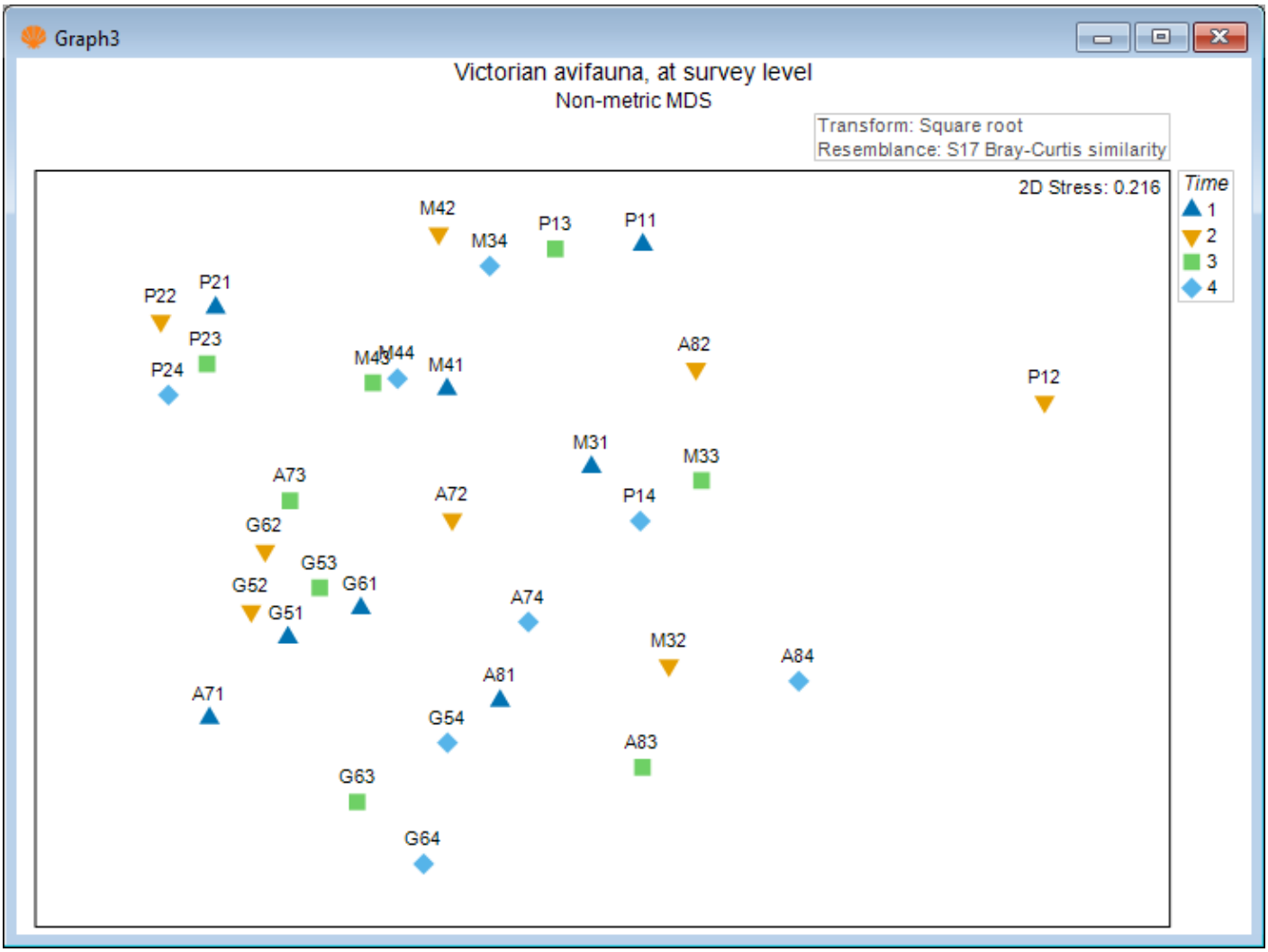
You will see a new item called 'MultiPlot1' in the Explorer tree, which contains 4 different graphics. Click on the '+' symbol next to the word 'MultiPlot1' in the Explorer tree, like so:

and this will "unfurl" (in the Explorer tree) to show all of the graphics in the MultiPlot:

- 'Graph3' (the 2D MDS plot),
- 'Graph4' (the Shepard diagram for the 2D MDS plot),
- 'Graph5' (the 3D MDS plot), and
- 'Graph6' (the Shepard diagram for the 3D MDS plot).

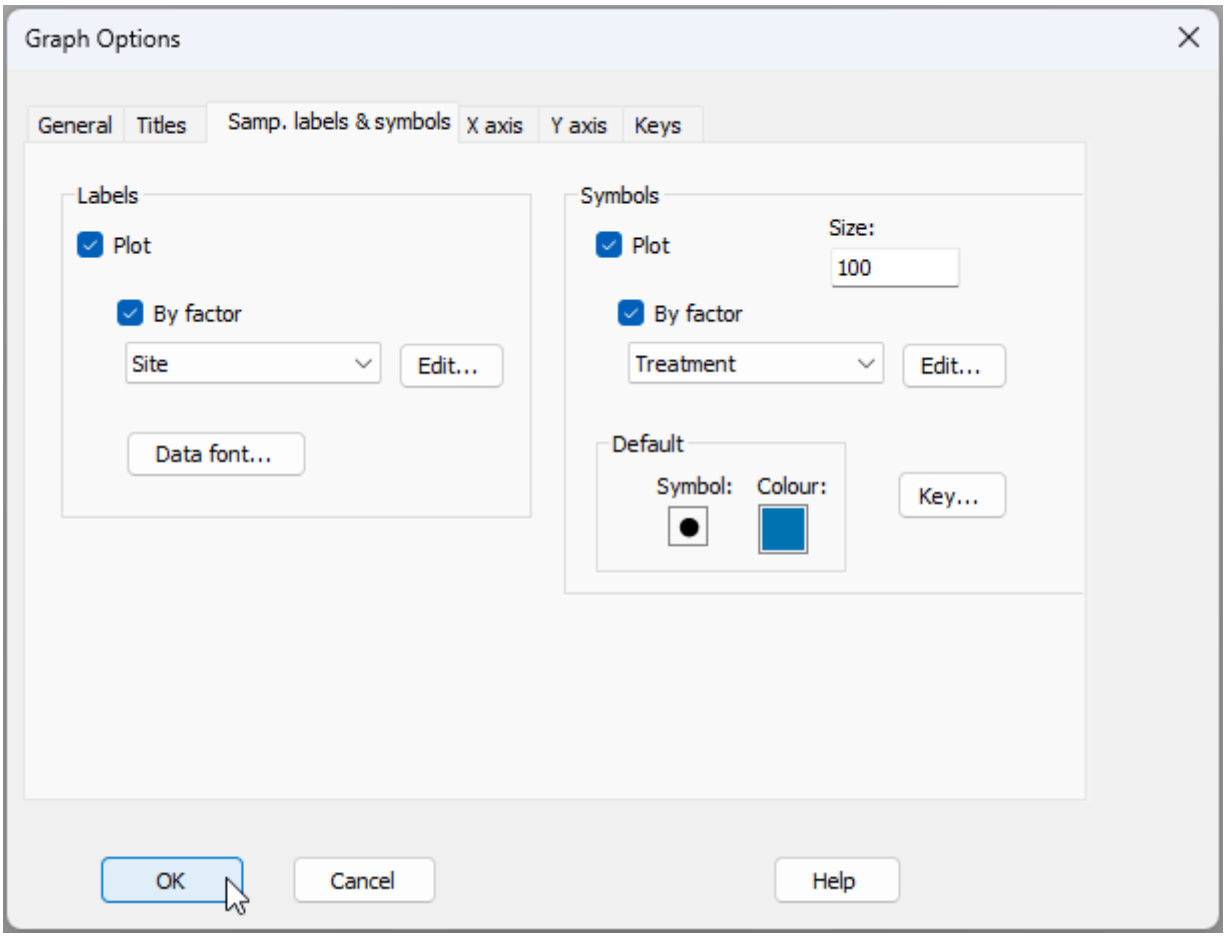
You can also simply click any of the individual graphics within the 'MultiPlot1' graphic itself to see it on its own.

7. Click on 'Graph3' to see the 2D MDS plot. The default plot in the output here looks a bit messy at first:

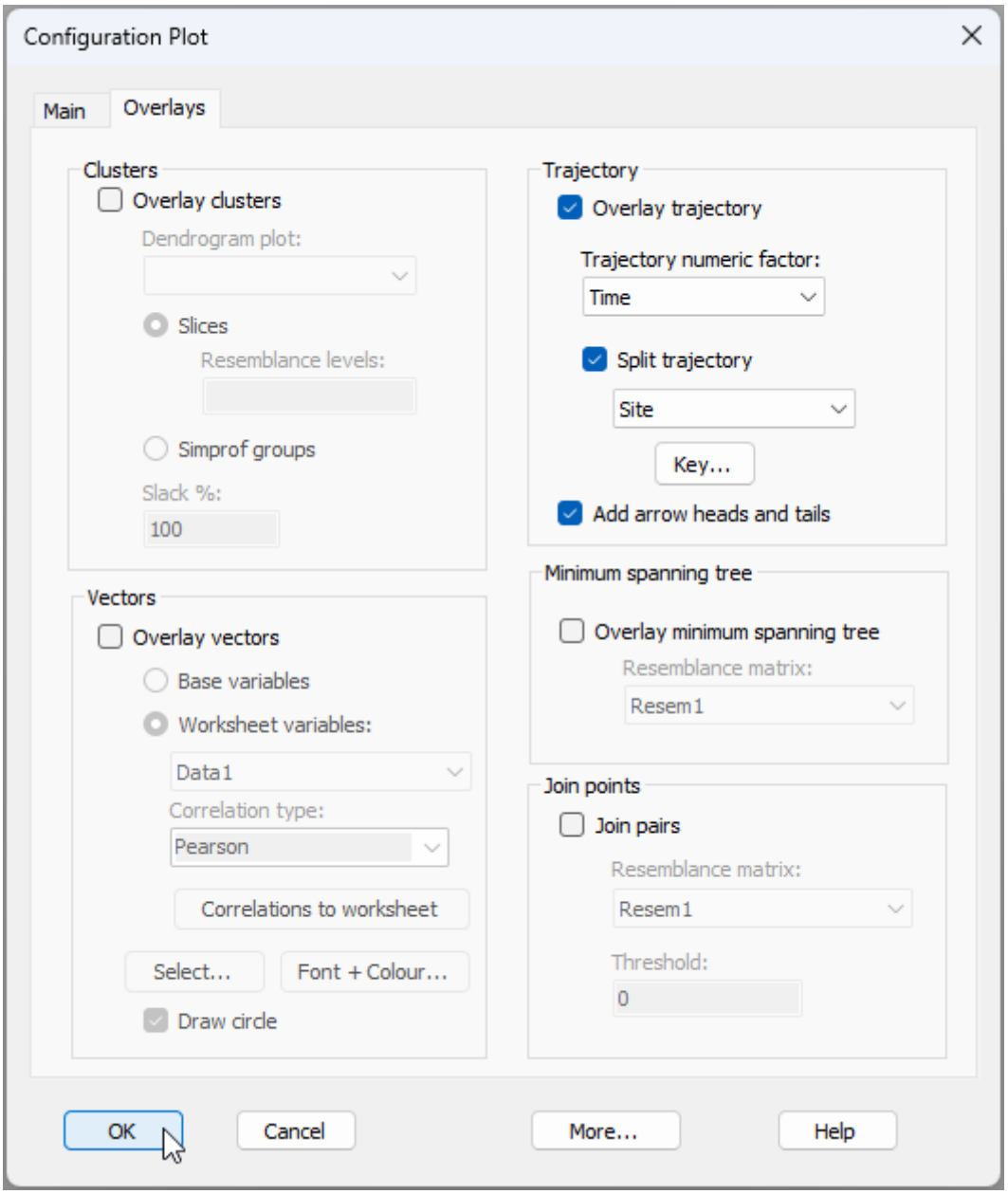


We will do two things (visually) to this graphic to make it easier to see the potential effects of important factors in this study.

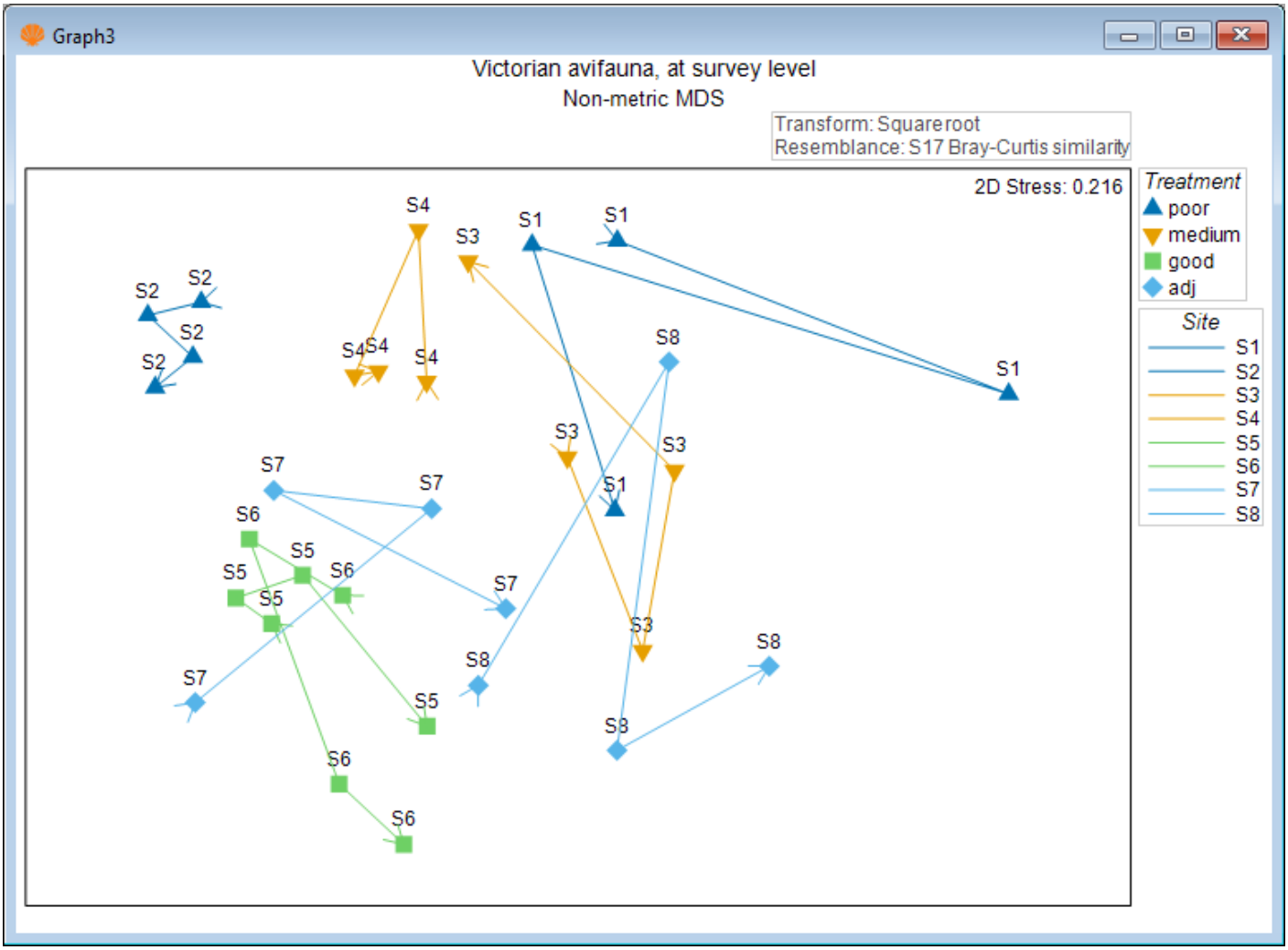
First, we will *change the labels and symbols* so that they correspond to spatial factors of interest. Click **Graph** > **Sample Labels & Symbols...** and change the **Labels** to reflect the factor of 'Site', and change the **Symbols** to reflect the factor of **Treatment**, as shown below, then click **OK**.



Second, we will *superimpose a trajectory to connect observations from the same site through time*. Click **Graph** > **Special**, then click on the 'Overlays' tab in the 'Configuration Plot' dialog, and under the word 'Trajectory', tick the box to $\checkmark$Overlay trajectory > Trajectory numeric factor: Time and $\checkmark$Split trajectory by Site, as shown below. Note that you can (optionally) click on the 'Key' button (Key...) here to make the colours of individual Site trajectory lines match their Treatment colour, then click **OK**.



The resulting 2D nMDS graphic will look like this:



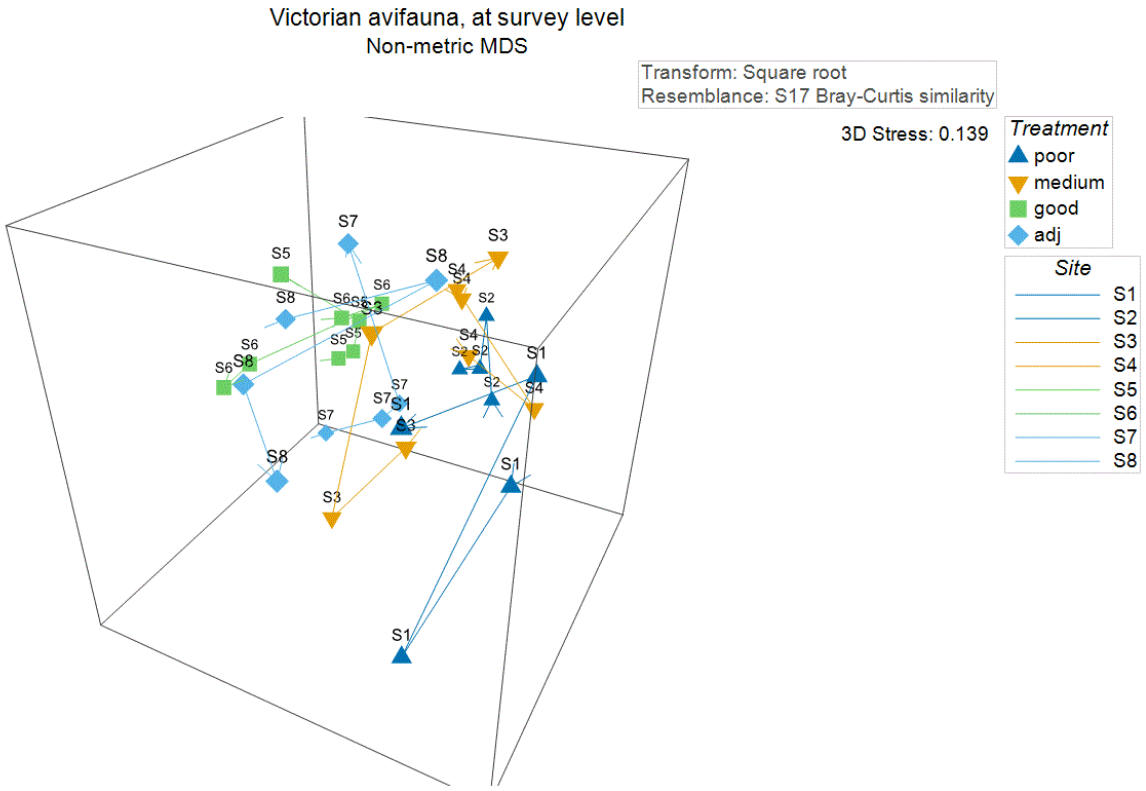
We can see here that there is a general pattern of gradual change in bird assemblages as you go from the 'good' (high flowering intensity) sites (in green) through the 'adjacent' (light blue) and 'medium' (orange) sites, towards the 'poor' sites (in dark blue); i.e., a sort of gradient from the lower left of the nMDS plot to the upper right. However, there is also quite a substantial difference in the bird assemblages seen at the two 'poor' sites (S1 *vs* S2), with S2 not really sitting in the appropriate place along the observed gradient. The variation through time (the overall length of each trajectory) also seems to differ somewhat across the sites.

An important thing to note here also is the relatively high stress of 0.216. This suggests that we should not read too much into any of the patterns we see on this 2D plot. Indeed, as the stress is higher than the usual 'rule-of-thumb' cut-off for interpretability of nMDS plots generally (stress = 0.20), we should really consider looking at the 3-dimensional nMDS ordination here instead.

8. To look at the 3D MDS plot, click on 'Graph5' and start by doing the same two operations we did on the 2D plot, i.e., change the labels and symbols to correspond to 'Site' and ' Treatment', respectively, then superimpose trajectories through time for each site (and adjust the Key for the 'Site' factor so that the colour of the line for each site corresponds to the colour of the treatment to which it belongs).

It is difficult to get a real sense of the patterns being shown in a 3D plot unless you see it in motion. From 'Graph5', click **Graph** > **Spin**, and you will see a series of buttons at the top of the graphic that look like this:

Hit the 'play' button ( ) and the graphic will start to spin. The blue slider permits you to increase or decrease the speed of the spin and you can change the angle of your view of the 3D image by clicking and dragging your mouse on it. You can also hit the red dot to record the spinning action, as shown in the animated $*$.gif image below (click on the image below to enlarge your view and see this as it would appear within PRIMER).

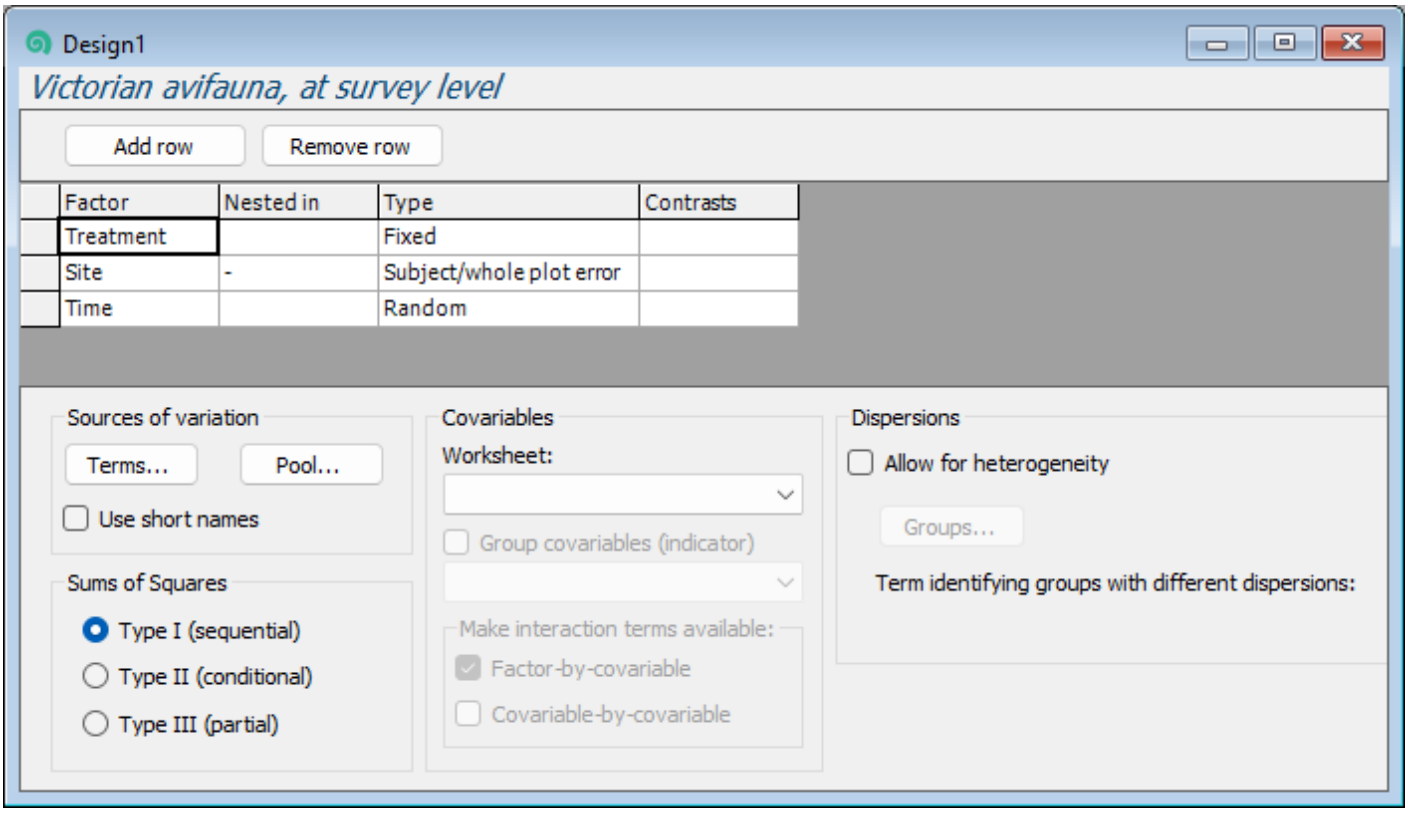


The patterns we see here are similar to what we saw in the 2D plot. There is a suggestion of a gradual shift in the bird assemblages with changes in flowering intensity, but there are also apparently substantial differences between sites of a similar flowering intensity, particularly between the two 'poor' sites.

Run the PERMANOVA

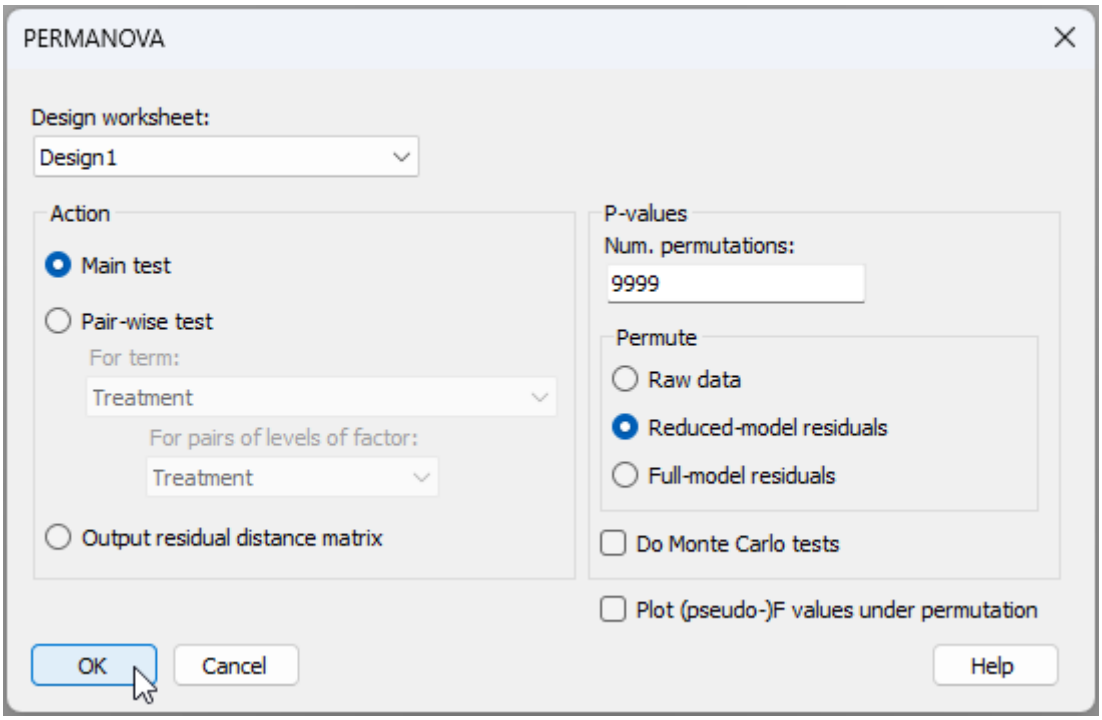
We need to create a design file, then run the PERMANOVA model on these data to formally test hypotheses regarding the potential effects of these factors.

10. From the Bray-Curtis resemblance matrix ('Resem1'), click **PERMANOVA+** > **Create PERMANOVA Design...**. Click the 'Add row' button (Add row) until there are (in this case) three rows (one for each factor): 'Treatment', 'Site' and then 'Time'. Next, we shall nominate 'Treatment' as a 'Fixed' factor, and 'Time' as a 'Random' factor. Note that individual *sites* are the specific items that are *repeatedly sampled*. Therefore, we need to specify 'Site' as a factor of type 'Subject/whole plot error'. The resulting Design file (called 'Design1') will look like this:



Now we are ready to run the analysis.

11. From the Bray-Curtis resemblance matrix ('Resem1'), click **PERMANOVA+** > **PERMANOVA**, take all of the defaults in the dialog and click '**OK**'.



The resulting PERMANOVA output file ('PERMANOVA1') will look like this:

PERMANOVA1

PERMANOVA
Permutational MANOVA

Resemblance worksheet
Name: Resem1
Data type: Similarity
Selection: All
Transform: Square root
Resemblance: S17 Bray-Curtis similarity

PERMANOVA design worksheet
Name: Design1
Title: Victorian avifauna, at survey level

Sums of squares type: Type I (sequential)
Permutation method: Permutation of residuals under a reduced model
Number of permutations: 9999

Factors

Name	Type	Levels
Treatment	Fixed	4
Site	Subject/whole plot error	8
Time	Random	4

Excluded terms
No terms excluded

Inestimable terms
No inestimable terms.

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Treatment	3	14959	4986.4	2.0206	0.068	105
Site	4	9871	2467.8	2.3715	0.0071	9931
Time	3	4512.1	1504	1.4453	0.1417	9919
TreatmentxTime	9	7793.9	865.99	0.83221	0.7386	9886
Res	12	12487	1040.6			
Total	31	49623				

There is significant variability from site to site *within* each of the different flowering intensity treatments ($F_{(4,12)}$ = 2.37, P = 0.0071). Over and above this site-to-site variation, however, there was only weak evidence of any treatment effects ($F_{(3,4)}$ = 2.02, P = 0.068).

These results make perfect sense by reference to the patterns seen in the nMDS plot(s), where we could discern a pattern of change in bird community structure with differences in flowering intensity, but also saw substantial site-to-site variation.

We might consider analysing the data again but without any pre-treatment transformation, in order to emphasise changes in the more abundant birds rather more (*try it!*). Another idea would be to analyse some other aspect or variable associated with these bird assemblages in response to the study design, such as species richness, or the univariate abundances of one or more dominant species (such as the *Red wattlebird*).

Ultimately, we might consider sampling a larger number of sites to increase the power of the test for Treatment effects (4 degrees of freedom in our denominator was not really very many).

Choosing sites to be as similar as possible to one another in other respects (environmentally) in order to reduce site-to-site variation would also be wise.

Revision #77
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