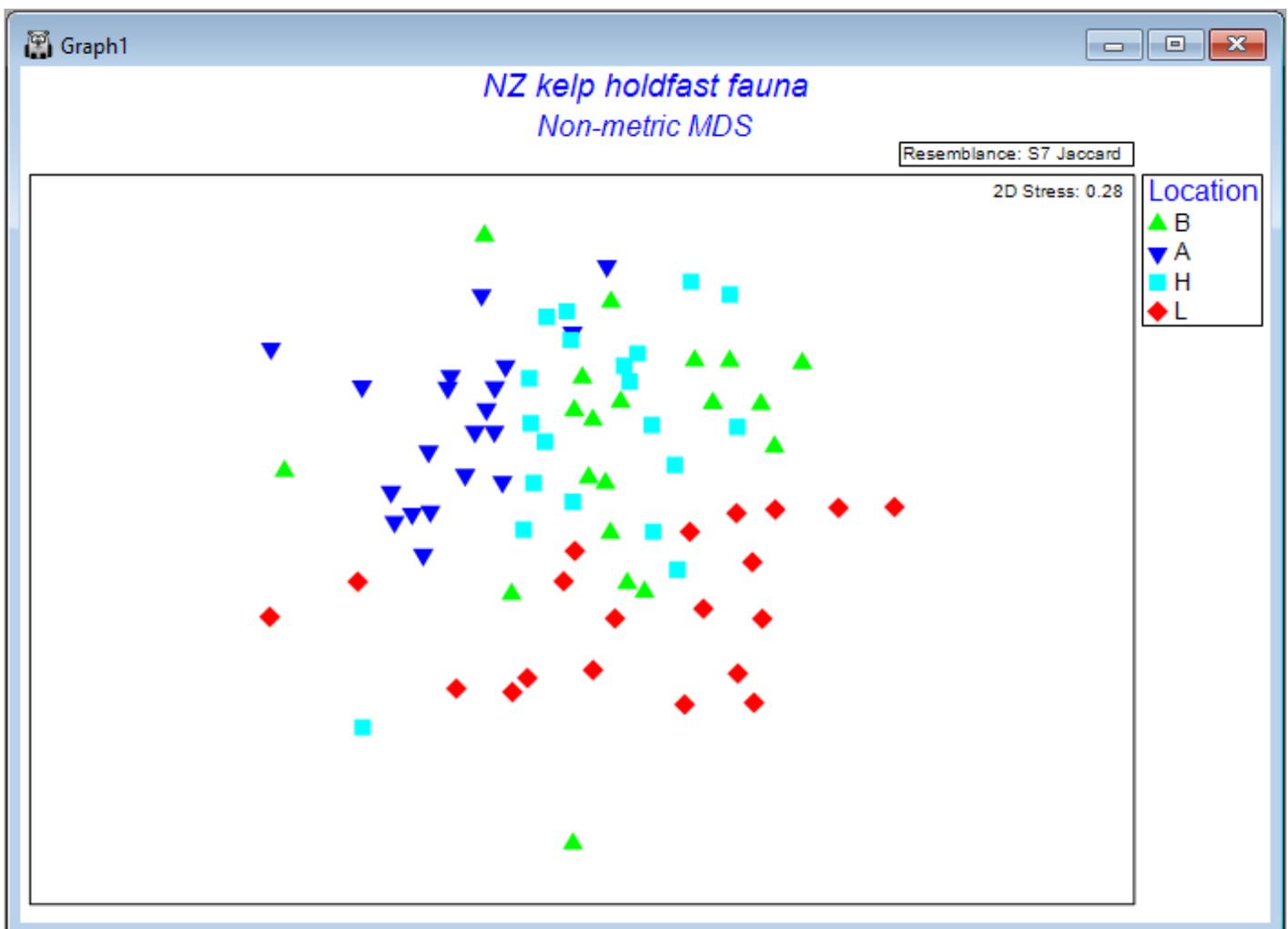


Step 5: Ordination of centroids

Having seen the results of a PERMANOVA analysis, it is natural to wish to see a **visualisation** of the patterns among centroids belonging to different groups or combinations of levels of different factors in the study design. In many cases, particularly if there is a large number of replicates in a complex study design, if one were simply to create an ordination of all individual sampling units, there would just be a lot of noise (the plot may look very messy), due to high residual variation. This tends to obscure salient patterns and important effects.

Ordination of sampling units (all replicates)

We can produce a non-metric MDS ordination of the holdfast data by starting from the Jaccard resemblance matrix and clicking on **Analyse > MDS > Non-metric MDS...**, taking all of the defaults, then clicking **OK**. The resulting configurations are very unsatisfactory with quite high stress, either in 2 dimensions (stress = 0.28, shown below), or 3 dimensions (stress = 0.20).



Seeing a bit of a 'mess' when we plot replicates like this is actually not too surprising in many cases, and particularly in this case, considering the very high variation (high turnover) in the

identities of molluscs among holdfasts at small spatial scales (within areas), as seen on the previous page (recall that the residuals contributed by far the greatest source of variation to this system).

Ordination of distances among centroids

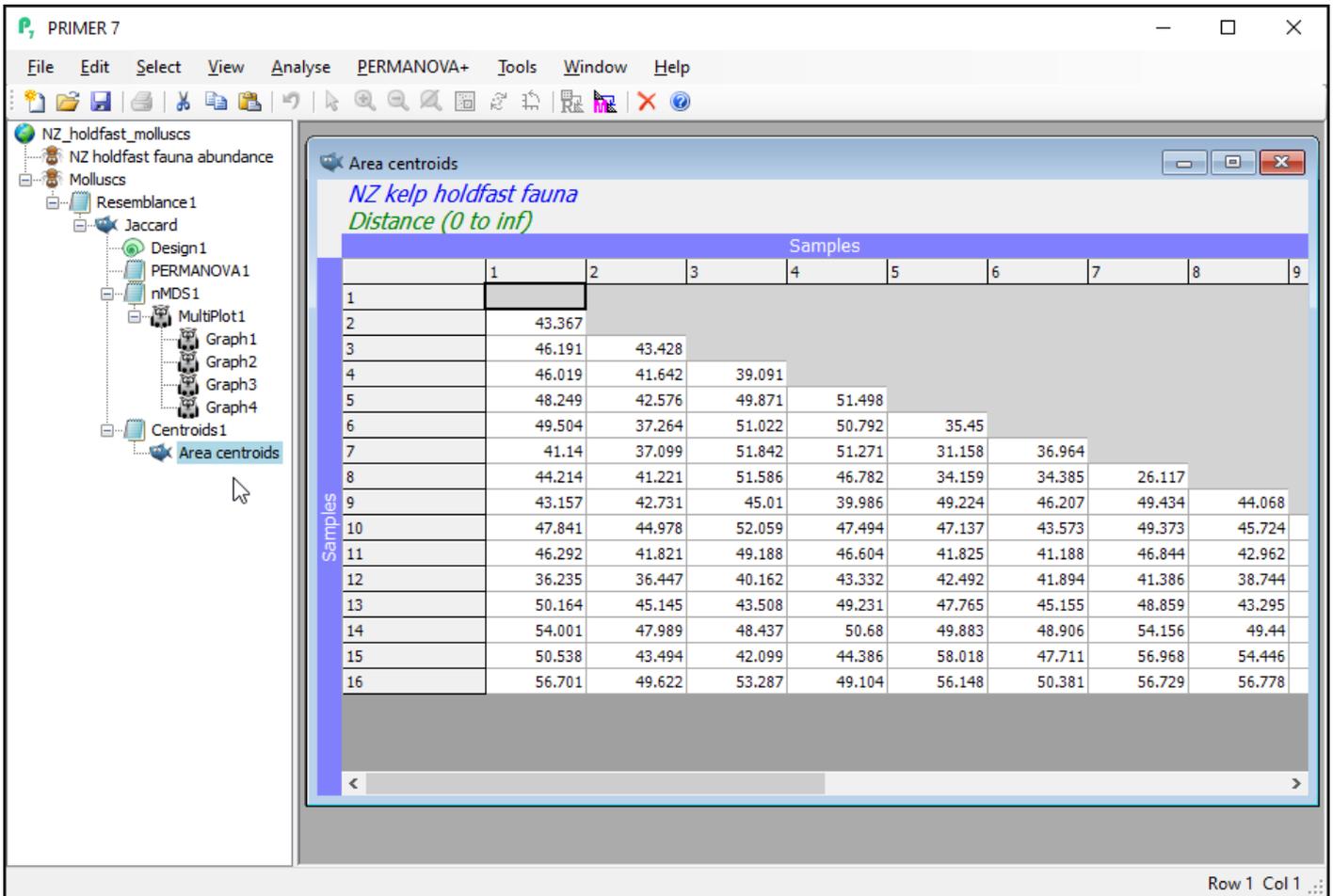
We can instead examine an ordination plot of the **centroids** in the space of the resemblance measure. In multi-factor designs when there is more than one factor and these factors are crossed with one another, we may need to create a single factor that consists of combinations of levels of the crossed factors (using **Edit > Factors... > Combine...**), but in the case of a fully nested design, and where nested factors all utilise unique labels (such as we have here), we can proceed without such a step.

1. From the 'Jaccard' resemblance matrix, click **PERMANOVA+ > Distances Among Centroids**, then in the 'Centroids' dialog, choose (Grouping factor: **Area**), and click **OK**.

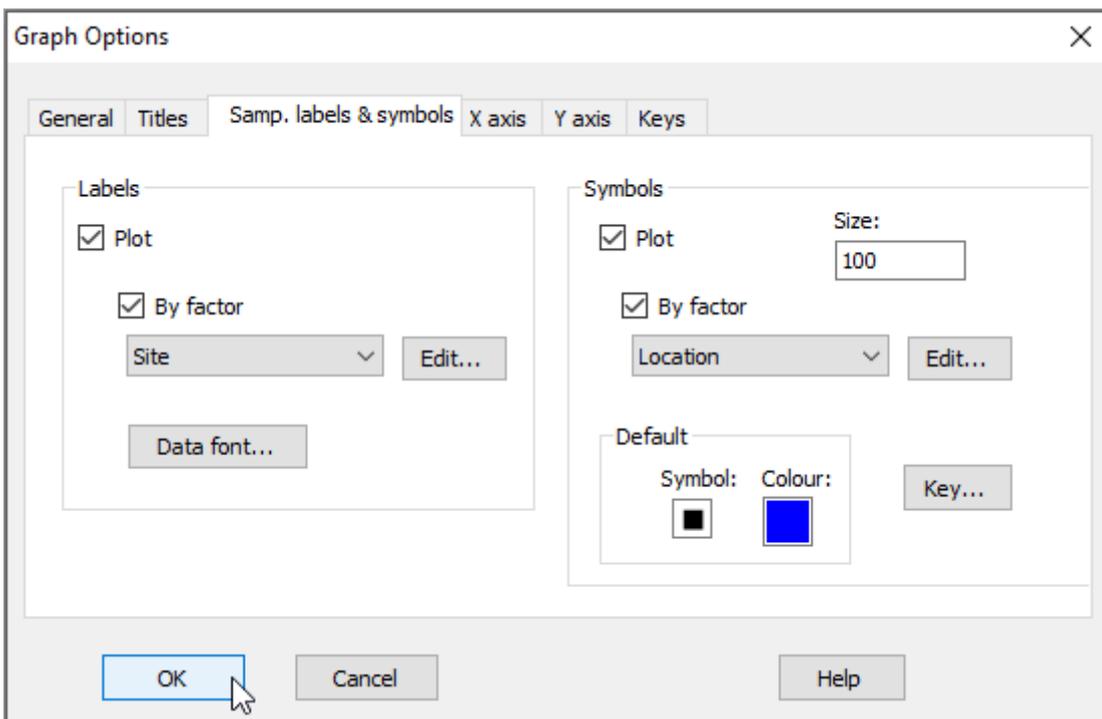
The screenshot shows the PRIMER 7 interface. The 'PERMANOVA+' menu is open, with 'Distance Among Centroids...' selected. The 'Centroids' dialog box is open, showing 'Area' as the grouping factor. The background shows a 'Samples' table with Jaccard dissimilarity values for 16 areas (Berghan11c to Berghan14c).

	Berghan11c	Berghan11d	Berghan11e	Berghan14a	Berghan14b	Berghan14c
Berghan11d		50	33.333			40
Berghan11e		44.444	50			33.333
Berghan14a		26.667	26.667	50		26.667
Berghan14b		30.769	30.769	30.769	50	
Berghan14c		14.286	14.286	14.286	14.286	50
Berghan14d		30.769	30.769	30.769	30.769	30.769
Berghan14e		31.111	31.111	31.111	31.111	31.111
Berghan33a		8.333	8.333	8.333	8.333	8.333
Berghan33b		8.333	8.333	8.333	8.333	8.333
Berghan33c		31.111	31.111	31.111	31.111	31.111
Berghan33d		20.833	20.833	20.833	20.833	20.833
Berghan33e		42.308	29.167	46.667	32.258	36.667
Berghan34a		32.143	24	37.5	41.379	28.125
Berghan34b		28.571	15.385	26.471	33.333	25
Berghan34c		30.769	21.739	28.125	22.581	31.034
Berghan34d		23.81	33.333	26.923	15.385	20

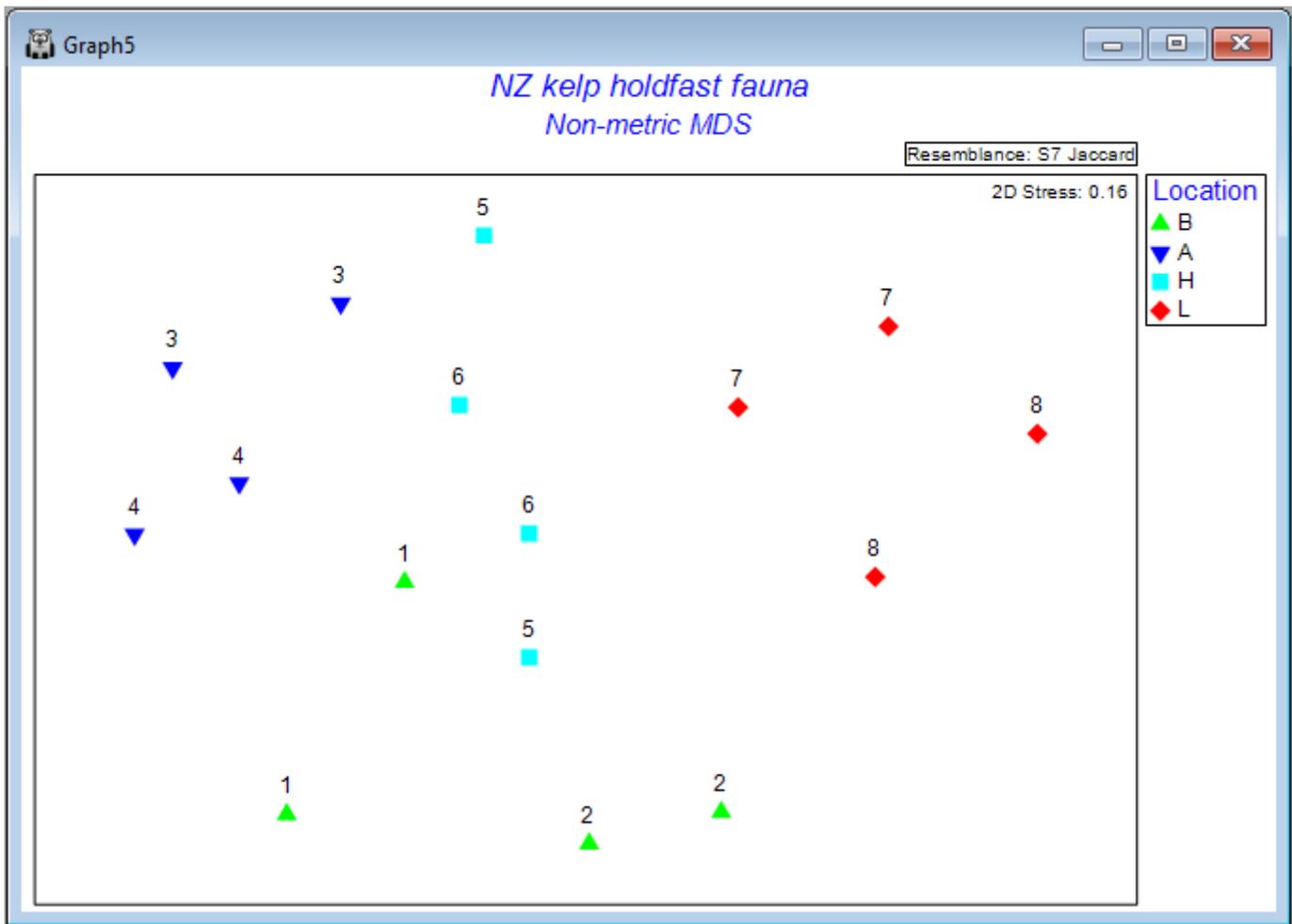
This will give you a matrix of Jaccard dissimilarities among the 16 Area centroids; each centroid being comprised of $n = 5$ replicate holdfasts within a given area. These are constructed in the space of the dissimilarity measure, which is not (quite) the same thing as calculating the arithmetic averages in the space of the original variables (i.e., that corresponds to a centroid in Euclidean space). In other words, these are the centroids just 'as PERMANOVA sees them' in *Jaccard* space, when doing the partitioning. You can re-name this matrix (from 'Resem1') to 'Area centroids'; i.e.,



- From the 'Area centroids' resemblance matrix, click **Analyse > MDS > Non-metric MDS...**, take all the defaults and click **OK**.
- From the 2D nMDS (probably called 'Graph5'), click on **Graph > Sample Labels & Symbols...**, then choose (Labels > Plot > By factor > Site) & (Symbols > Plot > By factor > Location), then click **OK**.



The result is a much more interpretable ordination plot, with far lower stress, viz:



Each point now represents the centroid (in Jaccard space) for $n = 5$ holdfasts in a given area. The numbers identify the 8 different sites, and the colours correspond to the 4 different locations. The patterns we see here are consistent with what was learned from the PERMANOVA analysis. More specifically, we can see that, within any particular location, the variation from one area to the next (any 2 centroids having the same symbol and number) is fairly similar to the variation between the two sites (any 2 centroids having the same colour, but a different number), and also that variation among locations (different colours) exceeds this - all four locations are clearly distinguishable from one another on the plot.

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