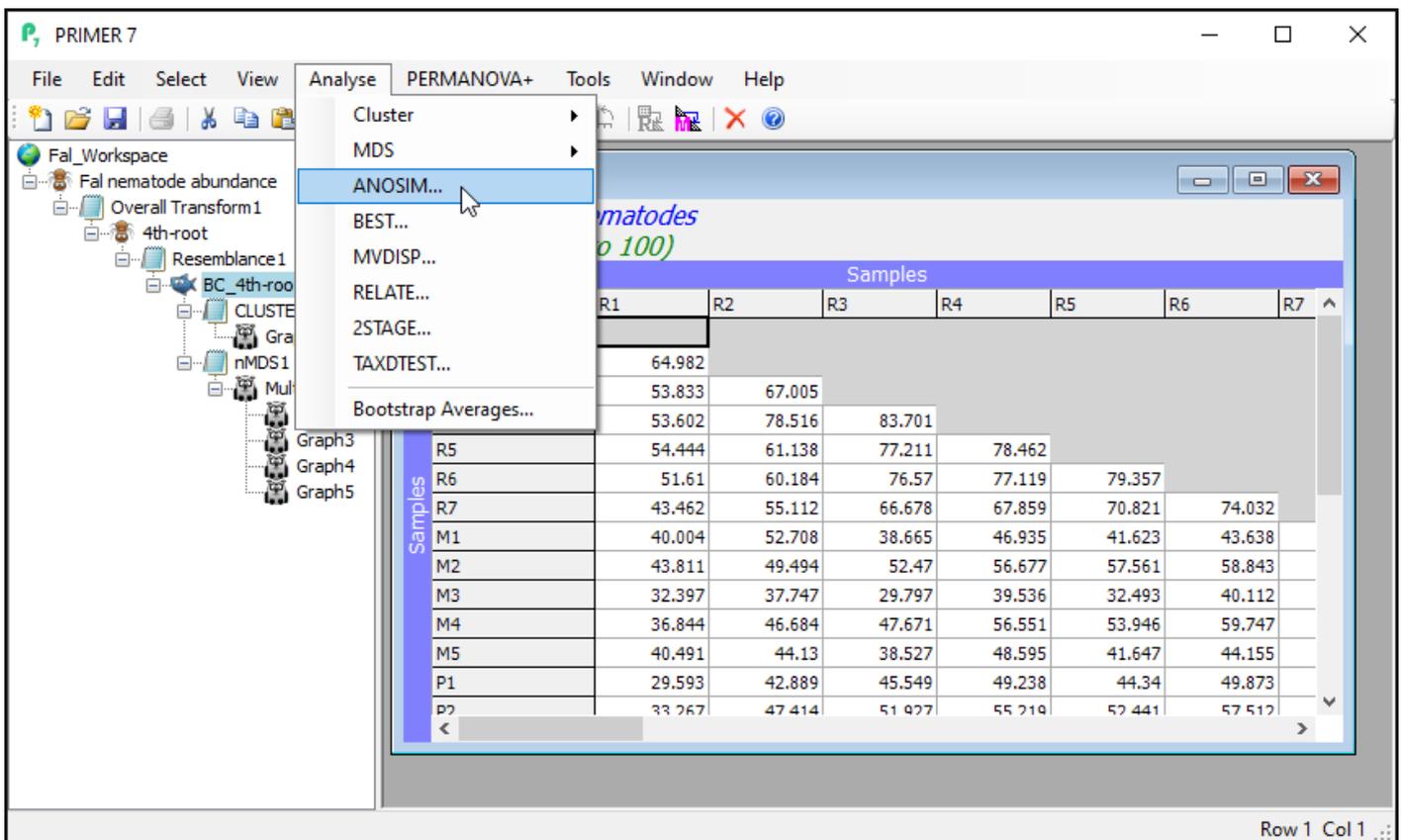


Step 5: ANOSIM test

The ordination plot gives us a visualisation of the rank-order relationships among the samples, based on the dissimilarity measure. Next, we may wish to test the null hypothesis that there are no differences among the five creeks. We can use a non-parametric multivariate permutation test called analysis of similarities (ANOSIM, Clarke (1993)) for this test. For more details on the ANOSIM test, see also Chapter 6 in 'Change in Marine Communities'. We will be doing a one-way ANOSIM test here (as there is a single factor in this example); for more information on multi-way designs with ordered and/or unordered factors, see Somerfield *et al.* (2021a) , Somerfield *et al.* (2021b) and Somerfield *et al.* (2021c) .

Perform the ANOSIM test

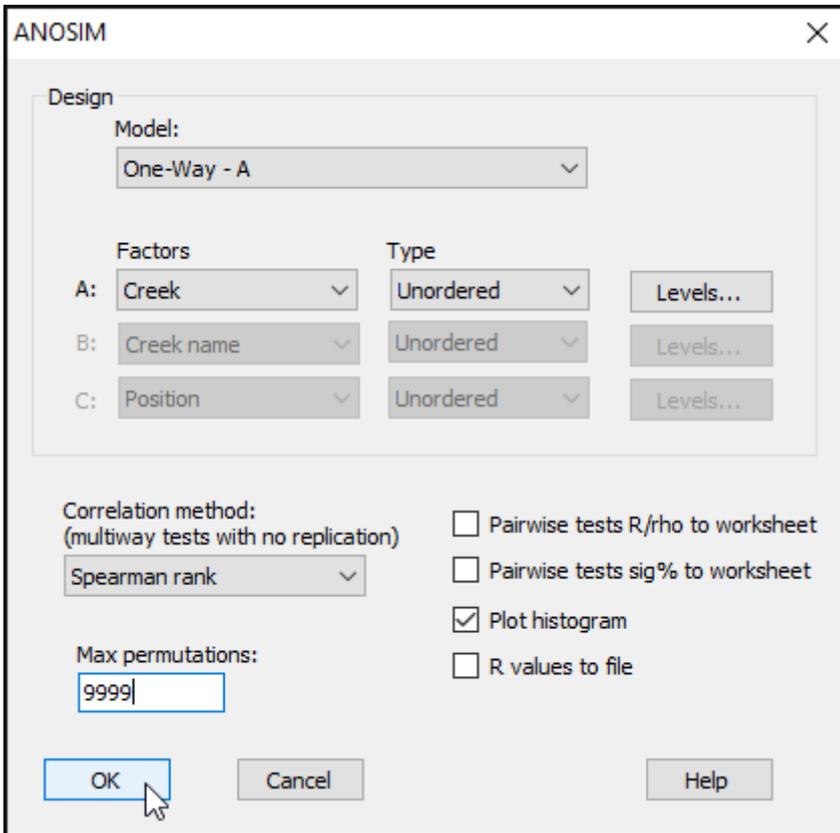
From the Bray-Curtis resemblance matrix (called 'BC_4th-root' in this example), click on **Analyse > ANOSIM...** and you will see the ANOSIM dialog.



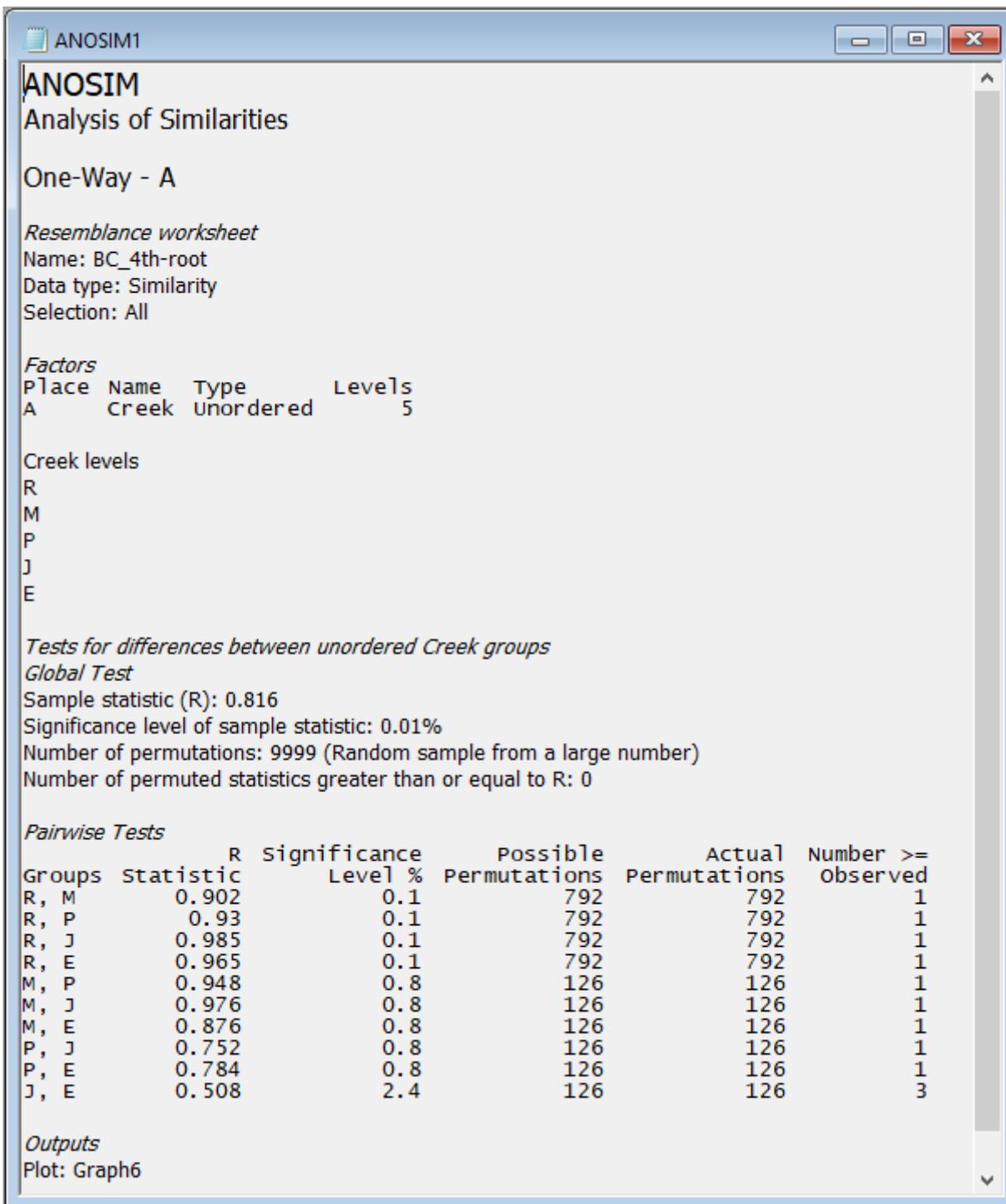
The screenshot shows the PRIMER 7 interface. The 'Analyse' menu is open, and 'ANOSIM...' is selected. The background displays a Bray-Curtis resemblance matrix for samples R1-R7 and M1-M5. The matrix values are as follows:

	R1	R2	R3	R4	R5	R6	R7
R1	64.982						
R2	53.833	67.005					
R3	53.602	78.516	83.701				
R4	54.444	61.138	77.211	78.462			
R5	51.61	60.184	76.57	77.119	79.357		
R6	43.462	55.112	66.678	67.859	70.821	74.032	
R7	40.004	52.708	38.665	46.935	41.623	43.638	
M1	43.811	49.494	52.47	56.677	57.561	58.843	
M2	32.397	37.747	29.797	39.536	32.493	40.112	
M3	36.844	46.684	47.671	56.551	53.946	59.747	
M4	40.491	44.13	38.527	48.595	41.647	44.155	
M5	29.593	42.889	45.549	49.238	44.34	49.873	
P1	33.267	47.414	51.927	55.219	52.441	57.512	

Here, we are performing a one-way ANOSIM (there is just one factor whose levels are un-ordered), so we can leave most of the defaults in the dialog as they are. We should, however, increase the permutations to a larger value, so change the 'Max permutations' from the default value of 999 to 9999, then click 'OK'.

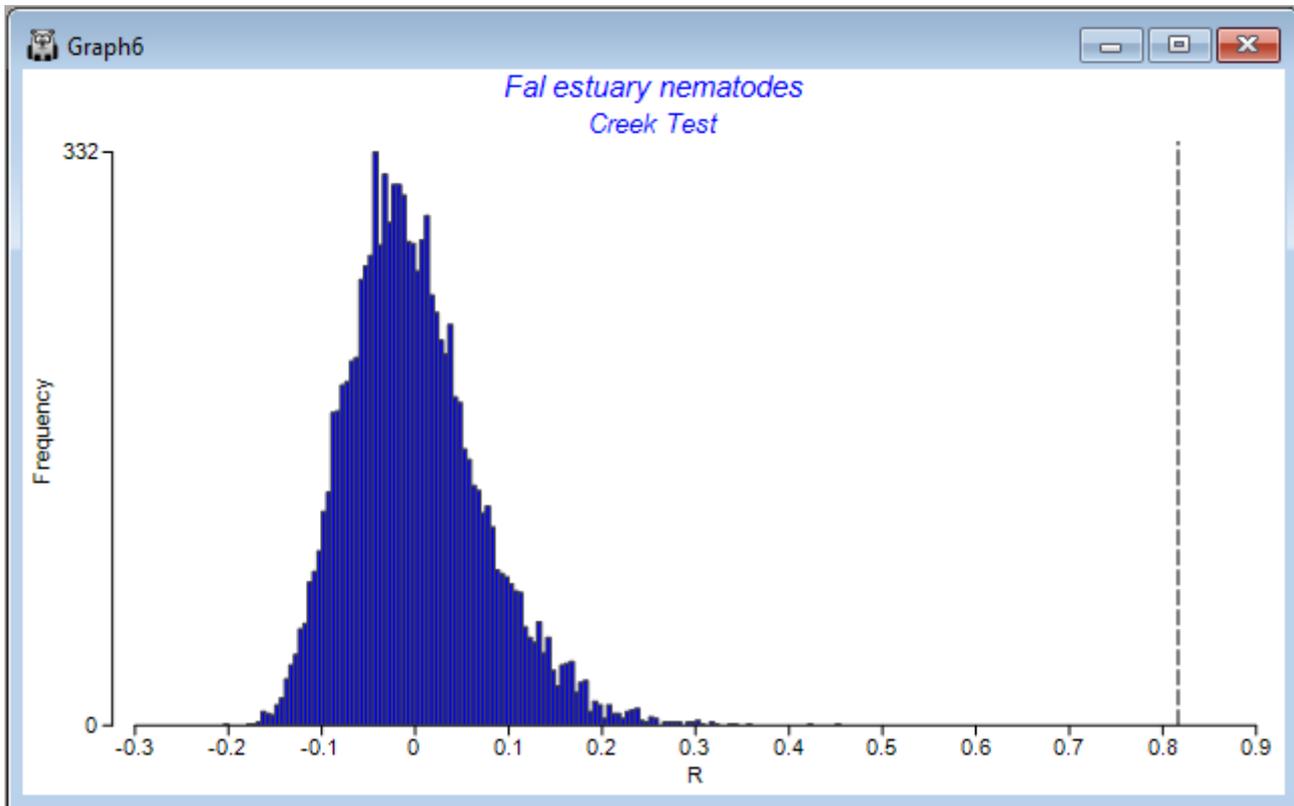


The results, including the overall ANOSIM test for differences among all groups, as well as the ANOSIM tests of all pair-wise comparisons, are provided in the output file called 'ANOSIM1'. The overall test is clearly highly significant, with $R = 0.816$ and a significance level of 0.01%. This corresponds to a p-value of $P = 0.0001$ (the smallest possible value attainable for 9999 permutations). Note that, in PRIMER, all p-values are reported for ANOSIM tests as 'significance levels', expressed as a percentage. For example, a p-value of $P = 0.0619$ would be given in the output file as a significance level of 6.19%.



The full set of pairwise tests is performed, and the strength of the differences between any pair of groups is well captured by the relative sizes of the R statistic - larger values indicate groups that are more different (more distinct or more easily distinguishable) from one another. The comparison of assemblages from St Just Creek (J) vs Percuil Creek (E) has the lowest R statistic ($R = 0.508$) of any tests done in this example, although this difference is still a statistically significant one ($P = 0.024$). Note that no 'correction' is being made here for multiple tests. The end-user is able to consider applying such a correction if desired (or may perhaps simply consider the frequency of 'significant' results obtained, given the number of tests performed), bearing in mind that each test is done using an exact permutation algorithm in order to generate the individual significance levels produced in the output, ignoring all other tests.

Also provided as output ('Graph6') is a histogram showing the distribution of values of the ANOSIM R statistic under permutation for the overall test. The observed value of the ANOSIM R statistic (0.816) is shown on the plot as a vertical dotted line. The purpose of the histogram is to show visually the departure of the observed value of R (or not) from the distribution of values of R expected under a true null hypothesis of 'no difference' among the groups.



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