

## 3.2 Results don't match

When we compare the output obtained using the two different pieces of software, we can see that there are fundamental differences in the results (Fig. 1). They don't match!

### PERMANOVA in PRIMER

| Factors  |         |        |        |  |  |  |  |
|----------|---------|--------|--------|--|--|--|--|
| Name     | Abbrev. | Type   | Levels |  |  |  |  |
| Location | Lo      | Random | 4      |  |  |  |  |
| Site     | Si      | Random | 8      |  |  |  |  |
| Area     | Ar      | Random | 16     |  |  |  |  |

  

| PERMANOVA table of results |    |            |        |          |         |              |
|----------------------------|----|------------|--------|----------|---------|--------------|
| Source                     | df | SS         | MS     | Pseudo-F | P(perm) | Unique perms |
| Lo                         | 3  | 35564      | 11855  | 2.8086   | 0.0094  | 105          |
| Si(Lo)                     | 4  | 16883      | 4220.8 | 1.3564   | 0.0313  | 9815         |
| Ar(Si(Lo))                 | 8  | 24895      | 3111.8 | 1.232    | 0.0067  | 9674         |
| Res                        | 64 | 1.6165E+05 | 2525.7 |          |         |              |
| Total                      | 79 | 2.3899E+05 |        |          |         |              |

  

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

| Source     | EMS   |
|------------|---|
| Lo         | $1 \times V(\text{Res}) + 5 \times V(\text{Ar}(\text{Si}(\text{Lo}))) + 10 \times V(\text{Si}(\text{Lo})) + 20 \times V(\text{Lo})$ |
| Si(Lo)     | $1 \times V(\text{Res}) + 5 \times V(\text{Ar}(\text{Si}(\text{Lo}))) + 10 \times V(\text{Si}(\text{Lo}))$                          |
| Ar(Si(Lo)) | $1 \times V(\text{Res}) + 5 \times V(\text{Ar}(\text{Si}(\text{Lo})))$  |
| Res        | $1 \times V(\text{Res})$  |

  

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

| Source     | Numerator                                  | Denominator                                | Num. df | Den. df |
|------------|--|--|---------|---------|
| Lo         | $1 \times \text{Lo}$                       | $1 \times \text{Si}(\text{Lo})$            | 3       | 4       |
| Si(Lo)     | $1 \times \text{Si}(\text{Lo})$            | $1 \times \text{Ar}(\text{Si}(\text{Lo}))$ | 4       | 8       |
| Ar(Si(Lo)) | $1 \times \text{Ar}(\text{Si}(\text{Lo}))$ | $1 \times \text{Res}$                      | 8       | 64      |

  

Estimates of components of variation

| Source        | Estimate | Sq. root |
|---------------|----------|----------|
| V(Lo)         | 381.69   | 19.537   |
| V(Si(Lo))     | 110.9    | 10.531   |
| V(Ar(Si(Lo))) | 117.22   | 10.827   |
| V(Res)        | 2525.7   | 50.257   |

### adonis2 in R

```
> adonis2( D ~ Location/Site/Area, permutations = 9999 )
Permutation test for adonis under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 9999

adonis2(formula = D ~ Location/Site/Area, permutations = 9999)
      Df SumOfSqs      R2      F Pr(>F)
Location      3    35564 0.14881 4.6935 0.0001 ***
Location:Site  4    16883 0.07064 1.6711 0.0001 ***
Location:Site:Area  8    24895 0.10417 1.2320 0.0106 *
Residual      64   161648 0.67638
Total        79   238989 1.00000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Fig. 1. Comparison of results for the holdfast data using PERMANOVA in PRIMER and adonis2 in R.

More specifically, although the degrees of freedom and the sums of squares are effectively identical (in this particular case), the pseudo-F statistics and the p-values are not (Fig. 2).

### PERMANOVA in PRIMER

| Factors  |         |        |        |  |  |  |  |
|----------|---------|--------|--------|--|--|--|--|
| Name     | Abbrev. | Type   | Levels |  |  |  |  |
| Location | Lo      | Random | 4      |  |  |  |  |
| Site     | Si      | Random | 8      |  |  |  |  |
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| Res                        | 64 | 1.6165E+05 | 2525.7 |          |         |              |
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Details of the expected mean squares (EMS) for the model

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| Si(Lo)     | $1 \times V(\text{Res}) + 5 \times V(\text{Ar}(\text{Si}(\text{Lo}))) + 10 \times V(\text{Si}(\text{Lo}))$                          |
| Ar(Si(Lo)) | $1 \times V(\text{Res}) + 5 \times V(\text{Ar}(\text{Si}(\text{Lo})))$  |
| Res        | $1 \times V(\text{Res})$  |

  

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

| Source     | Numerator                                  | Denominator                                | Num. df | Den. df |
|------------|--|--|---------|---------|
| Lo         | $1 \times \text{Lo}$                       | $1 \times \text{Si}(\text{Lo})$            | 3       | 4       |
| Si(Lo)     | $1 \times \text{Si}(\text{Lo})$            | $1 \times \text{Ar}(\text{Si}(\text{Lo}))$ | 4       | 8       |
| Ar(Si(Lo)) | $1 \times \text{Ar}(\text{Si}(\text{Lo}))$ | $1 \times \text{Res}$                      | 8       | 64      |

  

Estimates of components of variation

| Source        | Estimate | Sq. root |
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```

The results for F-ratios and P-values do not match!

Fig. 2. Pseudo-F ratios and p-values do not match.

Why don't we get the same results as PERMANOVA in PRIMER when we run PERMANOVA using the adonis2 function in R? Unfortunately, the results obtained using adonis2 are incorrect. Basically,

adonis2 takes no notice of whether factors are fixed or random. The adonis2 function gives you no way of specifying the types of factors you are dealing with; **adonis2 treats all factors as if they are fixed**. In contrast, PERMANOVA does the analysis correctly by reference to the full study design specified by the end-user.

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Revision #16

Created 14 May 2024 01:45:51 by Marti

Updated 15 May 2024 02:04:05 by Marti