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*V(Res) + 5*V(Ar(Si(Lo))) + 10*V(Si(Lo)) + 20*V(Lo)
Si(Lo)	1*V(Res) + 5*V(Ar(Si(Lo))) + 10*V(Si(Lo))
Ar(Si(Lo))	1*V(Res) + 5*V(Ar(Si(Lo)))
Res	1*V(Res)

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

Source	Numerator	Denominator	Num.df	Den.df
Lo	1*Lo	1*Si(Lo)	3	4
Si(Lo)	1*Si(Lo)	1*Ar(Si(Lo))	4	8
Ar(Si(Lo))	1*Ar(Si(Lo))	1*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
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*V(Res) + 5*V(Ar(Si(Lo))) + 10*V(Si(Lo)) + 20*V(Lo)
Si(Lo)	1*V(Res) + 5*V(Ar(Si(Lo))) + 10*V(Si(Lo))
Ar(Si(Lo))	1*V(Res) + 5*V(Ar(Si(Lo)))
Res	1*V(Res)

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

Source	Numerator	Denominator	Num.df	Den.df
Lo	1*Lo	1*Si(Lo)	3	4
Si(Lo)	1*Si(Lo)	1*Ar(Si(Lo))	4	8
Ar(Si(Lo))	1*Ar(Si(Lo))	1*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

```
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Number of permutations: 9999

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                  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
```

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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