27. *Practical.* ANOVA and associated tests

This lab focuses on applying the concepts from Chapter 23, Chapter 24, Chapter 25, and Chapter 26 in Jamovi (The Jamovi Project, 2022). The focus will be on ANOVA and associated tests, with 4 exercises in total. The data for this lab are inspired by the doctoral work of Dr Lidia De Sousa Teixeira at the University of Stirling. This work included a nutrient analysis of agricultural soil in different regions of Angola. Measuring soil nutrient concentrations is essential for assessing soil quality, and this data includes analysis of Nitrogen (N), Phosphorus (P), and Potassium (K) concentrations.

Figure 27.1.: Images from a project led by the Dr Lidia De Sousa Teixeira at the University of Stirling

Here we will focus on testing whether or not the concentrations of N, P, and K differ among 2 different sites and 3 soil profiles (upper, middle, and lower). To complete this lab, download the Angola_soils.csv dataset (right click and "Save Link As…", then save it with the extension '.csv'). All concentrations of Nitrogen, Phosphorus, and Potassium are given in parts per million (ppm).

27.1. One-way ANOVA (site)

Suppose that we first want to test whether or not mean Nitrogen concentration is the same in different sites. Notice that there are only 2 sites in the dataset, Funda and Bailundo. We could therefore also use an Independent samples t-test. We will do this first, then compare the t-test output to the ANOVA output. What are the null (H_0) and alternative (H_A) hypotheses for the t-test.

H_0 : _____

H_A : _____

Before running a t-test, remember that we need to check the assumptions of a t-test to see if any of them are violated (see Chapter 21.4). Use the **Assumption Checks** in Jamovi as we did in the previous practical (Chapter 22.4) to test for normality and homogeneity of variances in Nitrogen concentration. What can you conclude from these 2 tests?

Normality conclusion: _____

Homogeneity of variances conclusion: _____

Given the conclusions from the checks of normality and homogeneity of variances above, what kind of test should you use use to see if the mean Nitrogen concentration is significantly different in Funda versus Bailundo?

Test: _____

Run the test above in Jamovi. What is the p-value of the test, and what conclusion do you make about Nitrogen concentration at the two sites?

P: _____

Conclusion: _____

Now we will use an ANOVA to test if the mean Nitrogen concentration differs between sites. Remember from Chapter 23 that the ANOVA compares the variance among groups to the variance within groups, calculating an F statistic and finding where F is in the null F distribution. To run an ANOVA, navigate to the 'Analyses' tab in Jamovi, then select the 'ANOVA' button in the toolbar. From the ANOVA pulldown, select 'One-Way ANOVA' (Figure 27.2).

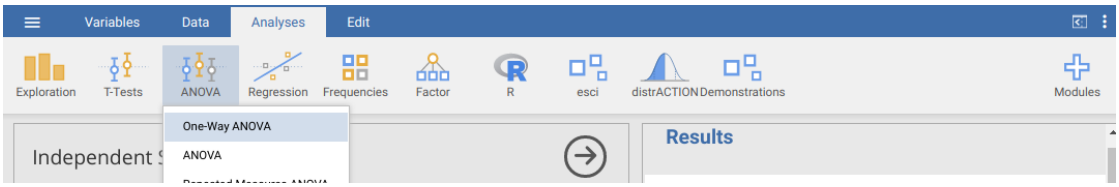

Figure 27.2.: Jamovi toolbar in the Analyses tab, which includes an option for running an ANOVA.

After selecting the one-way ANOVA, a familiar interface will open up. Place 'Nitrogen' in the Dependent Variables box and 'site' in the Grouping Variable box. Although we have already checked the assumptions of normality and homogeneity of variances when we ran the t-test, check these boxes under **Assumption Checks** too (Figure 27.3).

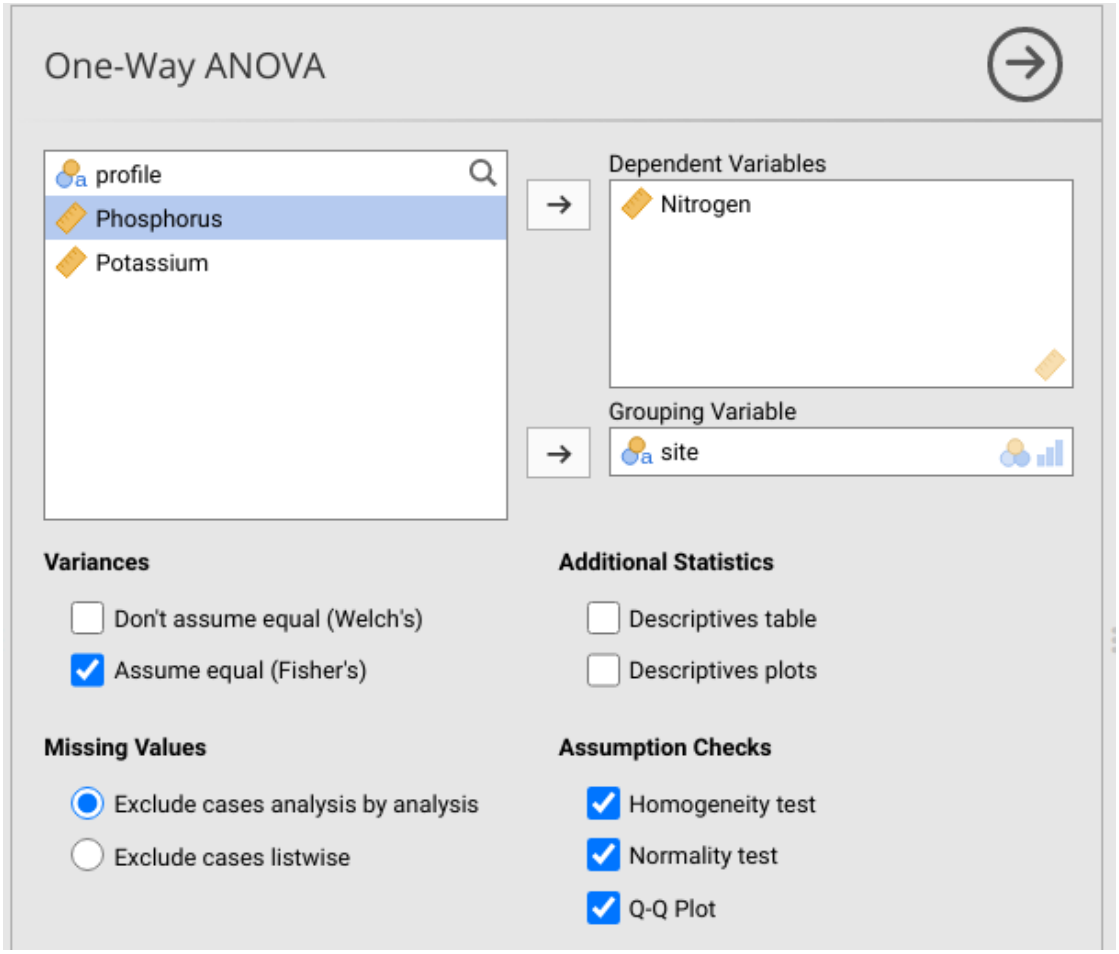

Figure 27.3.: Jamovi interface for running a one-way ANOVA to test if Nitrogen concentration (ppm) differs among sites in soils of Angola. Data for this test were inspired by the doctoral thesis of Dr Lidia De Sousa Teixeira.

Confirm that the Shapiro-Wilk test of normality and the Levene's test of homogeneity of variances are consistent with what you concluded when testing the assumptions of the t-test above. Since there is no reason to reject the null hypothesis that group variances are equal, we can use the Fisher's One-Way ANOVA by checking 'Assume equal (Fisher's)' under **Variances** (see Figure 27.3). A table called 'One-Way ANOVA' will appear in the panel on the right. Write down the test statistic (F), degrees of freedom, and p-values from this table below.

F: _____

df1: _____

df2: _____

P: _____

Remember from Chapter 23 that the ANOVA calculates an F statistic (mean variance among groups divided by mean variance withing groups). This F statistic is then compared to the null F distribution with the correct degrees of freedom to calculate the p-value. Use the interactive app to visualise this from the above Jamovi output. To do this, move the 'Variance 1' slider in the app until it is approximately equal to F, then change df1 and df2 to the values above. From the interactive app, what is the approximate area under the curve (i.e., the orange area) where the F value on the x-axis is greater than your calculated F?

P: _____

Slide the 'Variance 1' to the left now until you find the F value where the probability density in the tail (orange) is about $P = 0.05$. Approximately, what is this threshold F value above which we will reject the null hypothesis?

Approximate threshold F: _____

What should you conclude regarding the null hypothesis that sites have the same mean?

Conclusion: _____

Look again at the p-value from the one-way ANOVA output and the Student's t-test output. Are the two values the same, or different? Why might this be?

Next, we will run a one-way ANOVA to test the null hypothesis that all profiles have the same mean Nitrogen concentration.

27.2. One-way ANOVA (profile)

We will now run an ANOVA to see if Nitrogen concentration differs among profiles. In this dataset, there are lower, middle, and upper profiles, which refer to the location on along a slope from which soil samples were obtained. Using the same approach as the previous Exercise 27.1, we will run a one-way ANOVA with profile as the independent variable instead of site. Again, navigate to the 'Analyses' tab in Jamovi, then select the 'ANOVA' button in the toolbar. From the ANOVA pulldown, select 'One-Way ANOVA' (Figure 27.2). First check the assumptions of normality and homogeneity of variances. What can you conclude?

Normality conclusion: _____

Homogeneity of variances conclusion: _____

It appears that the assumptions of normality and homogeneity of variances are met. We can therefore proceed with the one-way ANOVA. Run the one-way ANOVA with the assumption of equal variances (i.e., Fisher's test). What are the output statistics in the One-Way ANOVA table?

F: _____

df1: _____

df2: _____

P: _____

From these statistics, what do you conclude about the difference in Nitrogen concentration among sites?

Conclusion: _____

In the previous Exercise 27.1, we used an interactive app to visualise the relationship between the F statistic and the p-value. We can do the same thing with the distrACTION module in Jamovi. To do this, go to the distrACTION option in the Jamovi toolbar and select 'F-Distribution' from the pulldown menu. Place the df1 and df2 from the One-Way ANOVA table into the df1 and df2 boxes under **Parameters** (ignore λ). Under **Function**, select 'Compute probability', then place the F value from the One-Way ANOVA table in the box for x1. Write down the 'Probability' value from the Results table in the panel to the right.

Probability: _____

Note that this is the same value (perhaps with a rounding error) as the p-value from the One-Way ANOVA table above. We can also find the threshold value of F, above which we will reject the null hypothesis. To do this, check the 'Compute quantile' box and set p = 0.95 in the box below. From the Results table, what is the critical F value

('Quantile'), above which we would reject the null hypothesis that all groups have the same mean?

Critical F value: _____

Note that the objective of working this out in the distrACTION module (and with the interactive app) is to help explain what these different values in the One-Way ANOVA table actually mean. To actually test the null hypothesis, the One-Way ANOVA output table is all that we really need.

Finally, note that in the ANOVA pulldown from the Jamovi toolbar, the option 'ANOVA' is just below the 'One-way ANOVA' that we used in this exercise and Exercise 27.1. This is just a more general tool for running an ANOVA, which includes the two-way ANOVA that we will use in Exercise 27.5 below. For now, give this a try by selecting 'ANOVA' from the pull down menu. In the ANOVA interface, place 'Nitrogen' into the 'Dependent Variable' box and 'Profile' in the 'Fixed Factors' box (Figure 27.4).

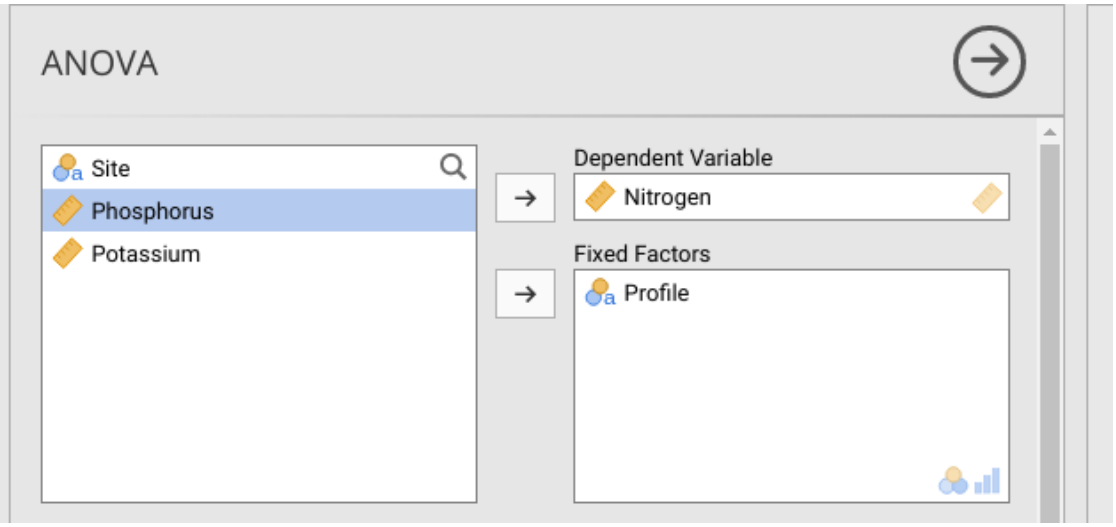

Figure 27.4.: Jamovi interface for running an ANOVA to test if Nitrogen concentration (ppm) differs among soil profiles in Angola. Data for this test were inspired by the doctoral thesis of Dr Lidia De Sousa Teixeira.

The output in the right panel shows an ANOVA table. It includes the sum of squares of the among-group (Profile) and within-group (Residuals) sum of squares and mean square. This is often how ANOVA results are presented in the literature. Fill in the table below with the information for degrees of freedom, F, and P.

Table 27.1.: ANOVA output testing the null hypothesis that mean Nitrogen concentration is the same across 3 different soil profiles in Angola. Data for this test were inspired by the doctoral thesis of Dr Lidia De Sousa Teixeira.

	Sum of Squares	df	Mean Square	F	p
Profile	16888.18606		8444.09303		
Residuals	118092.02927		2460.25061		

Now that we have established from the one-way ANOVA that mean Nitrogen concentration is not the same across all soil profiles, we can use a test of multiple comparisons to test which profile(s) are significantly different from one another.

27.3. Multiple comparisons

In this exercise, we will pick up where we left of in the ANOVA of Exercise 27.2. We have established that not all soil profiles have the same mean. Next, we will run a post hoc multiple comparisons test to evaluate which, if any, soil profiles have different means. In the ANOVA input panel, scroll down to the pulldown option called 'Post Hoc Tests' (Figure 27.5).

Move 'Profile' to the box to the right, then select they 'Tukey' checkbox under **Correction**, as shown in Figure 27.5. Doing this will run the Tukey's honestly significant difference (HSD) test introduced in Chapter 24. The output will appear in the panel on the right in a table called 'Post Hoc Tests'. Note that these post-hoc tests use the t-distribution to test for significance. Find the p-values associated with the Tukey's HSD (P_{tukey}) for each profile pairing. Report these below.

Tukey's HSD Lower - Middle: P = _____

Tukey's HSD Lower - Upper: P = _____

Tukey's HSD Middle - Upper: P = _____

From this output, what can we conclude about the difference among soil profiles?

Next, instead of running a Tukey's HSD test, we will use a series of t-tests with a Bonferonni correction. Check the box for 'Bonferonni' in the ANOVA Post Hoc Tests input panel, then find the p-values for the Bonferonni correction ($p_{bonferonni}$). Note that we do not need to change the α threshold ourselves (i.e., we do not need to see if P is less than $\alpha = 0.05/3 = 0.016667$ instead of $\alpha = 0.05$). Jamovi modifies the p-values

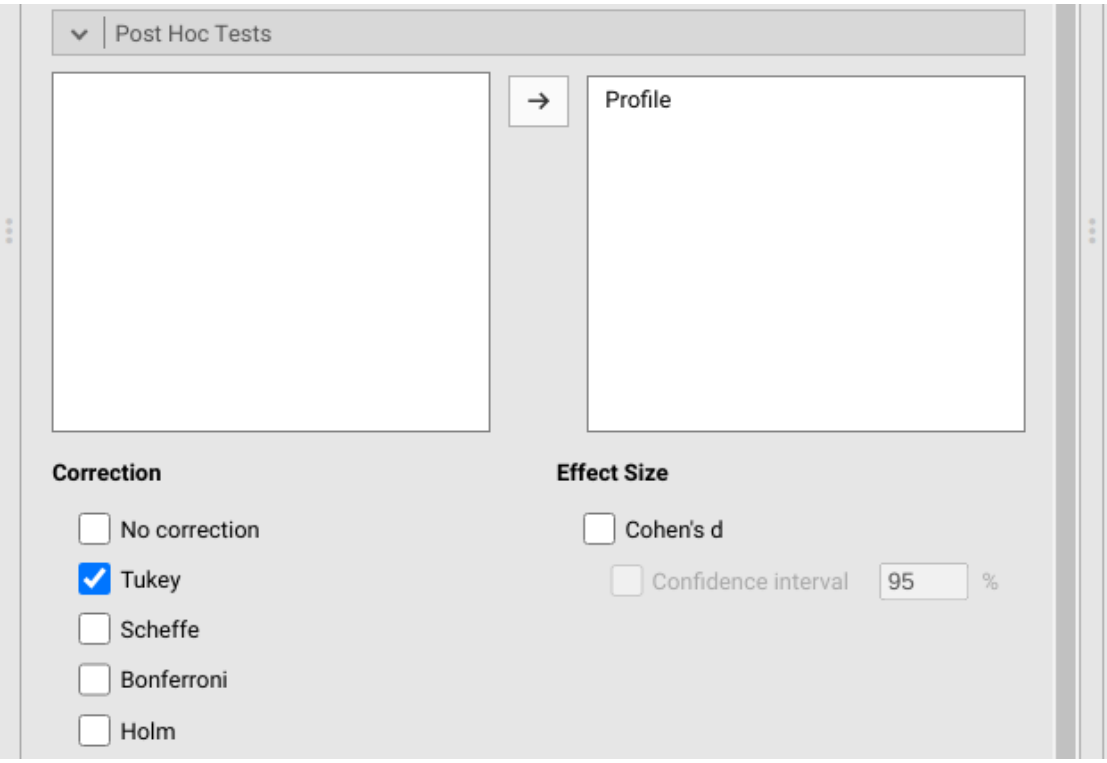

Figure 27.5.: Jamovi interface for running post hoc tests following an ANOVA.

appropriately for the Bonferonni correction (we can see the difference by clicking the checkbox for 'No correction' in the Post Hocs Tests input panel). Report the p-values for the Bonferonni correction below.

Bonferonni Lower - Middle: P = _____

Bonferonni Lower - Upper: P = _____

Bonferonni Middle - Upper: P = _____

In general, how are the p-values different between the Tukey's HSD and the Boneferroni correction? Are they about the same, higher, or lower?

What does this difference mean in terms of making a Type I error? In other words, based on this output, are we more likely to make a Type I error with the Tukey's HSD test or the Bonferroni test?

Note that we ran the Tukey's HSD test and the Bonferroni test separately. This is because, when doing a post-hoc test, we should choose which test to use in advance. This will avoid biasing our results to get the conclusion that we *want* rather than the conclusion that is *accurate*. If, for example, we first decided to use a Bonferroni correction, but then found that none of our p-values were below 0.05, it would not be okay to try a Tukey's HSD test instead in hopes of changing this result. This kind of practice is colloquially called 'p-hacking' (or 'data dredging'), and it causes an elevated risk of Type I error and a potential for bias in scientific results. Put more simply, trying to game the system to get results in which $P < 0.05$ can lead to mistakes in science (Head et al., 2015). Specifically, p-hacking can lead us to believe that there are patterns in nature where none really exist, which is definitely something that we want to avoid!

27.4. Kruskall-Wallis H test

In this exercise, we will apply the nonparametric equivalent of the one-way ANOVA, the Kruskall-Wallis H test. Suppose that we now want to know if Potassium concentration differs among soil profiles. We therefore want to test the null hypothesis that the mean Potassium concentration is the same for all soil profiles. Before opening the ANOVA input panel, have a look at a histogram of Potassium concentration. How would you describe the distribution? Do the data appear to be normally distributed?

We can test the assumption of normality using a Shaprio-Wilk test. This can be done in the Descriptives panel of Jamovi, or we can do it in the One-Way ANOVA panel. To do it in the one-way ANOVA panel, first select 'ANOVA' from the pull down menu as we did at the end of Exercise 27.2. In the ANOVA interface, place 'Potassium' into the 'Dependent Variable' box and 'Profile' in the 'Fixed Factors' box. Next, scroll down to the 'Assumption Checks' pulldown menu and select all 3 options. From the Levene's test, the Shapiro-Wilk test, and the Q-Q plot, what assumptions of ANOVA might be violated?

Given the violation of ANOVA assumptions, we should consider a non-parametric option. As introduced in Chapter 25, the Kruskall-Wallis H test is a non-parametric alternative to a one-way ANOVA. Like other non-parametric tests introduced in this book, the Kruskall-Wallis H test uses the ranks of a dataset instead of the actual values. To run a Kruskall-Wallis H test, select the Analyses tab, then the 'ANOVA' button from the Jamovi toolbar. In the pulldown ANOVA menu, choose 'One-Way ANOVA: Kruskall-Wallis' (second to last one down the list; Figure 27.6).

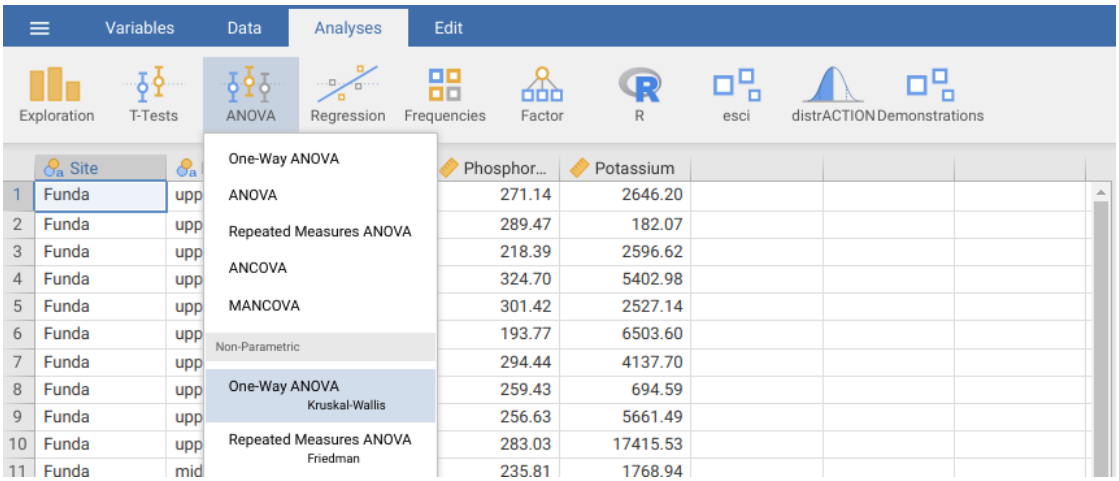

Figure 27.6.: Jamovi toolbar for selecting a Kruskall-Wallis test.

The Kruskall-Wallis input is basically the same as the one-way ANOVA input. We just need to put 'Potassium' in the dependent variable list and 'Profile' in the Grouping Variable box. The output table includes the test statistic (Jaomvi uses a χ^2 value as a test statistic, which we will introduce in Week 9, degrees of freedom, and p-value). Report these values below.

χ^2 : _____

df: _____

P: _____

From the above output, should we reject or not reject our null hypothesis?

H_0 : _____

Note that the Kruskall-Wallis test in Jamovi also includes a type of multiple comparisons tests (DSCF pairwise comparisons checkbox). We will not use the Dwass-Steel-Critchlow-Fligner pairwise comparisons, but the general idea is the same as the Tukey's HSD test for post hoc multiple comparisons in the ANOVA.

27.5. Two-way ANOVA

Since we have two types of categorical variables (site and profile), we might want to know if either has a significant effect on the concentration of an element, and if there is any interaction between site and profile. The two-way ANOVA was introduced in Chapter 26 with an example of fig wasp wing lengths. Here we will test the effects of site, profile, and their interaction on Nitrogen concentration. Recall from Chapter 26 that a two-way ANOVA actually tests 3 separate null hypotheses. Write these null hypotheses down below (the order does not matter).

First H_0: _____

Second H_0: _____

Third H_0: _____

To test these null hypotheses again select 'ANOVA' from the pull down menu as we did at the end of Exercise 27.2. In the ANOVA interface, place 'Nitrogen' into the 'Dependent Variable' box and both 'Site' and 'Profile' in the 'Fixed Factors' box. Next, scroll down to the 'Assumption Checks' pulldown menu and select all 3 options. From the assumption checks output tables, is there any reason to be concerned about using the two-way ANOVA?

In the two-way ANOVA output, we see the same ANOVA table as in Exercise 27.2 (Table 27.1). This time, however, there are 4 rows in total. The first 2 rows correspond with tests of the main effects of Site and Profile, and the third row tests the interaction between these two variables. Fill in Table 27.2 with the relevant information from the two-way ANOVA output.

Table 27.2.: Two-way ANOVA output testing the effects of 2 sites and 3 different soil profiles on soil Nitrogen concentration in Angola. Data for this test were inspired by the doctoral thesis of Dr Lidia De Sousa Teixeira.

	Sum of Squares	df	Mean Square	F	p
Site	21522.18384		21522.18384		
Profile	22811.1368		11405.5684		
Site * Profile	16209.13035		8104.56517		
Residuals	80497.68348		1788.83741		

From this output table, should you reject or not reject your null hypotheses?

Reject First H_0?: _____

Reject Second H_0?: _____

Reject Third H_0?: _____

In non-technical language, what should you conclude from this two-way ANOVA?

Lastly, we can look at the interaction effect between Site and Profile visually. To do this, scroll down to the 'Estimated Marginal Means' pulldown option. Move 'Site' and 'Profile' from the box on the left to the 'Marginal Means' box on the right (Figure 27.7).

In the panel on the right hand side, a plot will appear under the heaading 'Estimated Marginal Means'. Based on what you learned in Chapter 26 about interaction effects, what can you say about the interaction between Site and Profile? Does one Profile, in particular, appear to be causing the interaction to be significant? How can you infer this from the Estimated Marginal Means plot?

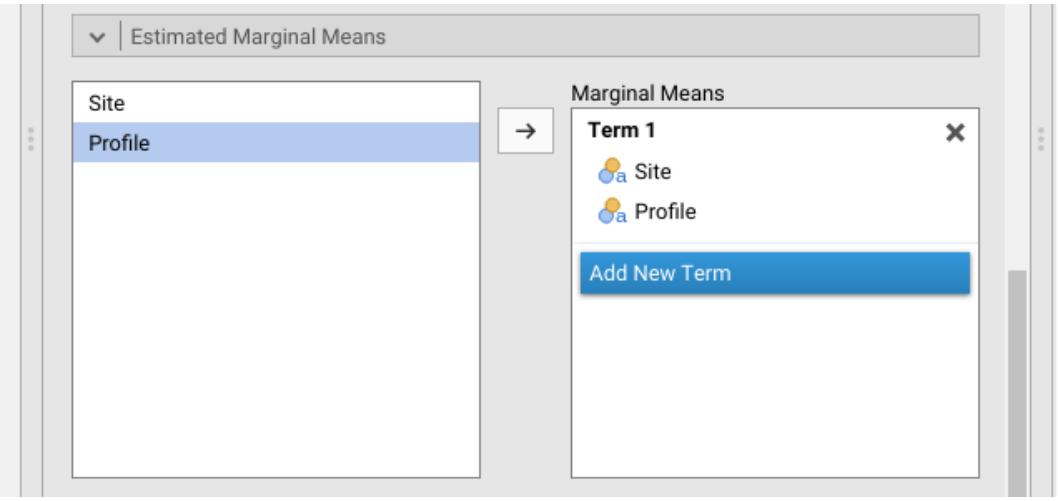

Figure 27.7.: Jamovi two-way ANOVA test with the pulldown menu for Estimated Marginal Means, which will produce a plot showing the interaction effect of the two-way ANOVA.

If you have time, try running a two-way ANOVA to test the effects of Site and Profile on Phosphorus concentration. Based on the ANOVA output, what can you conclude?