

STAT183 - Biochar Study

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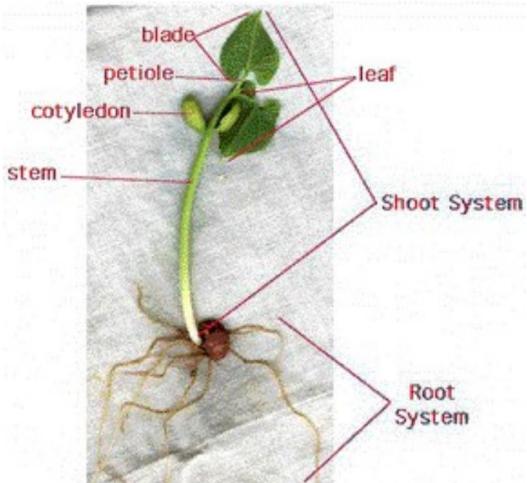
Introduction

Background and motivation



Background

- Biochar (charcoal) is a stable solid, rich in carbon. In agriculture, it can be used as soil amendment.
- Complete randomized design on 32 plots
 - Biochar treatment: present or absent
 - Wheat varieties: 76 or 1RS;
 - Soil types: soil or soil-sand;
- Randomly choose five plants in each plots and take average for each measurement.



Motivation

- **Variables of interest:**
 - shoot weight
 - shallow root weight
 - deep root weight
 - total root weight
 - root to shoot ratio
- **Factors:**
 - Wheat varieties: 76 or 1RS
 - Soil types: soil or soil-sand
 - Biochar treatment: present or absent
- **Objective:** To test the effects of the three main factors and their interactions on plant growth

02

Statistical Model

Three-way ANOVA



Statistical Model

Three-way ANOVA:

$$y_{ijkt} = \mu + a_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkt}$$

i – wheat variety (76, 1RS)

j – soil type (soil, soil – sand)

k – biochar treatment (yes, no)

t – replicate (1, ..., 4)

$$\epsilon_{ijkt} \sim N(0, \sigma^2)$$

Where:

- y_{ijk} : t measurement corresponding to the ith, jth, and kth factors
- μ : overall mean
- a_i : wheat affect
- β_j : soil effect
- γ_k : biochar treatment effect
- $(\alpha\beta)_{ij}$: interaction between wheat and soil
- $(\alpha\gamma)_{ik}$: interaction between wheat and biochar
- $(\beta\gamma)_{jk}$: interaction between soil and biochar
- $(\alpha\beta\gamma)_{ijk}$: interaction between wheat, soil, and biochar
- ϵ_{ijkt} : random error

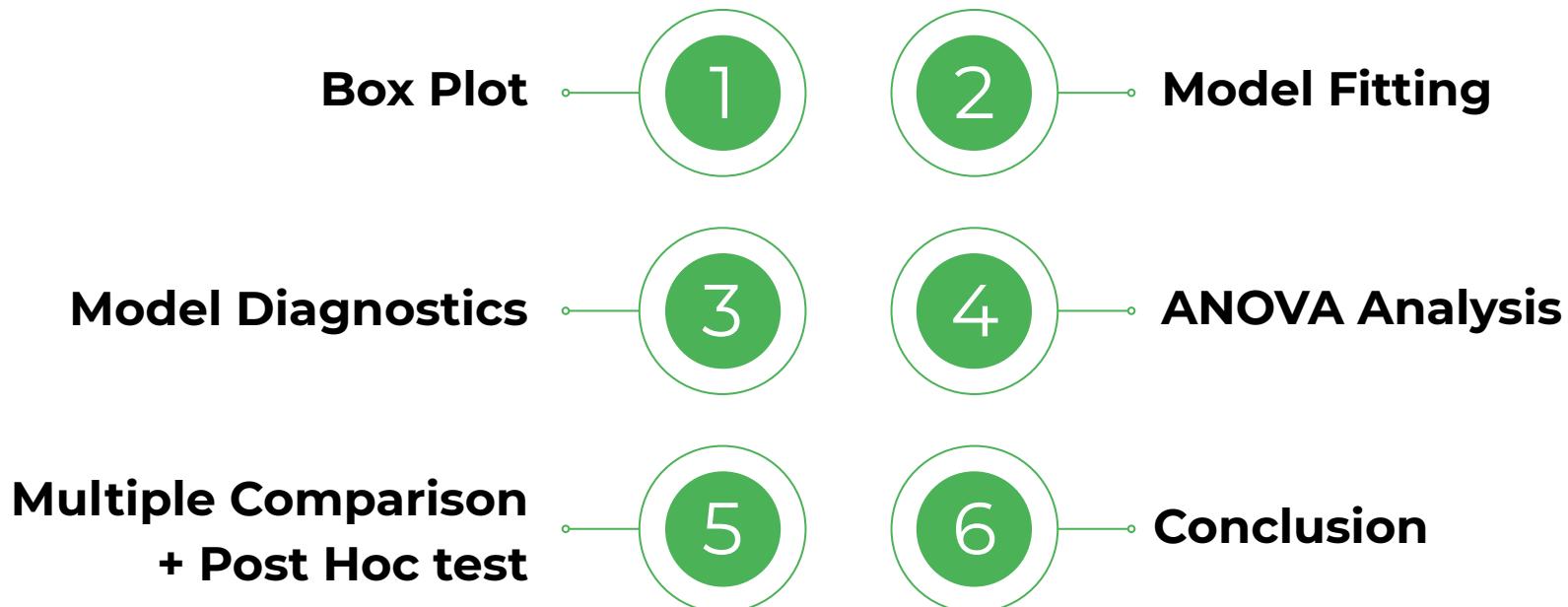
03

Methodology

Model diagnostics, ANOVA
analysis, multiple comparisons



Our Process

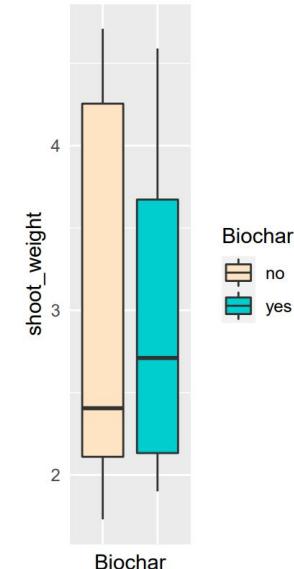
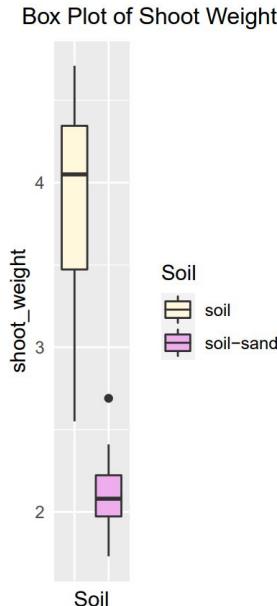
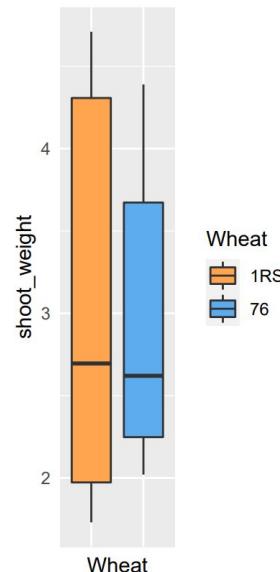




SHOOT WEIGHT

SHOOT WEIGHT: *BOX PLOT*

- Median for both wheat types is the most similar
- Soil and soil-sand are quite dissimilar
 - soil-sand has 1 outlier
- Presence or absence of biochar treatment somewhat similar



SHOOT WEIGHT: RESIDUALS AND Q-Q

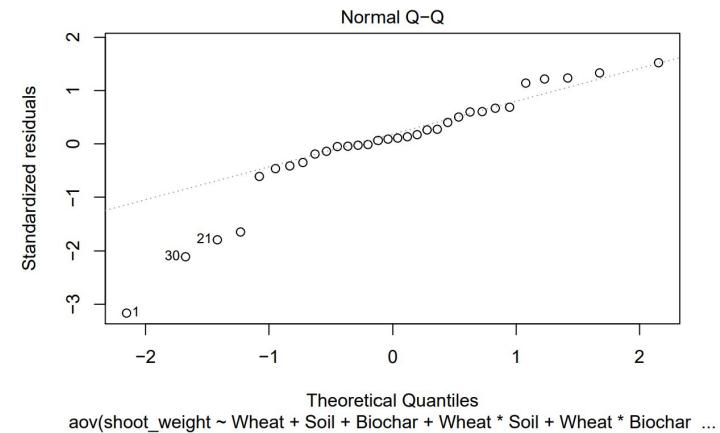
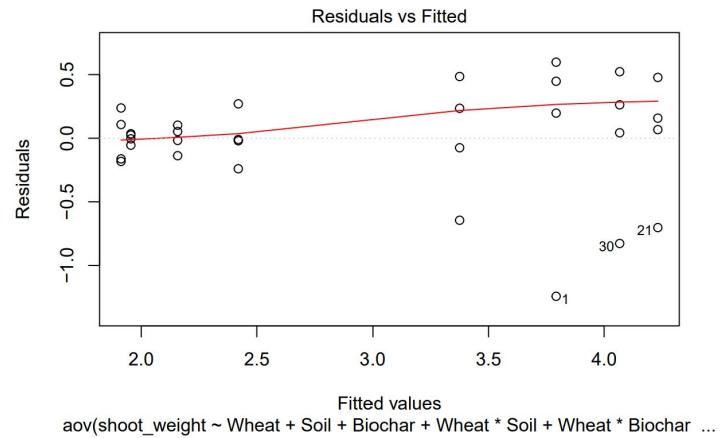
Normality Assumption

- dots don't seem to follow the line with some obvious outliers
- assumption is not met

Equal Variance Assumption

- seems that there may be a pattern
- assumption is not met

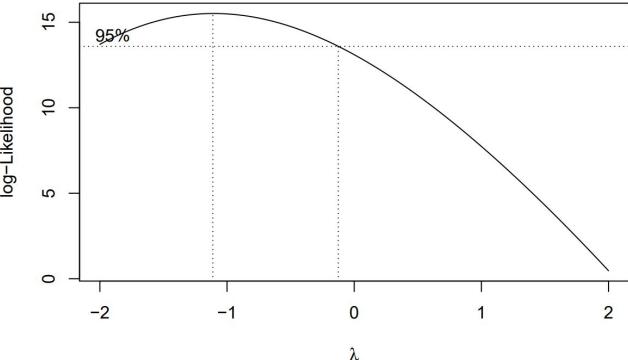
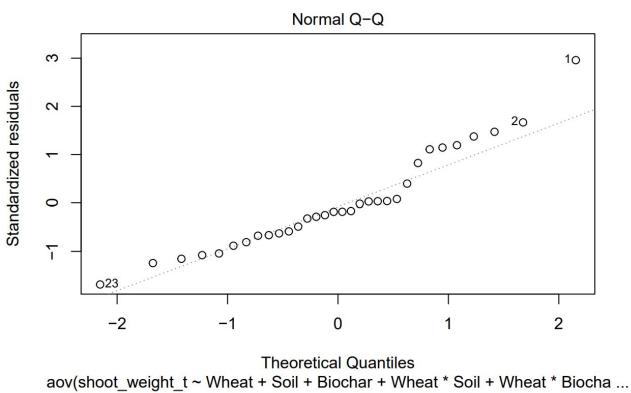
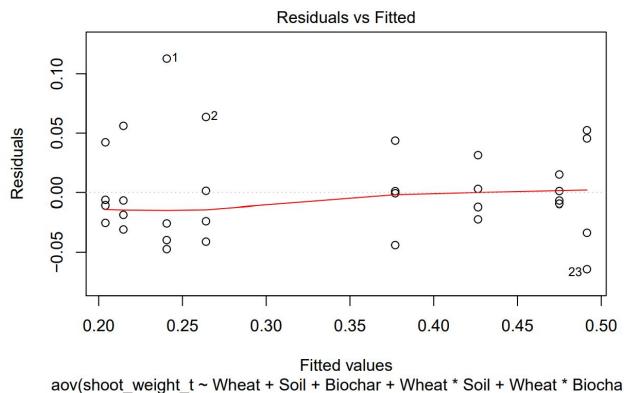
Transformation is needed!



SHOOT WEIGHT: BOX-COX TRANSFORMATION

After transformation:

- can see that QQ-plot is better with most points following the line
- can assume both assumptions met



SHOOT WEIGHT:

Fit Full Model

From Full Model:

- can conclude the interaction effect is not significant ($p\text{-value} = 0.47 > 0.05$)
- remove the 3-way interaction
 - Wheat:Soil:Biochar

```
## Analysis of Variance Table
##
## Response: shoot_weight_t
##                               Df  Sum Sq Mean Sq   F value    Pr(>F)
## Wheat                      1 0.00297 0.00297  1.5337  0.2275421
## Soil                       1 0.35828 0.35828 184.9350 8.996e-13 ***
## Biochar                     1 0.00050 0.00050  0.2603  0.6145718
## Wheat:Soil                  1 0.03098 0.03098 15.9906  0.0005285 ***
## Wheat:Biochar                1 0.00020 0.00020  0.1057  0.7479045
## Soil:Biochar                 1 0.00501 0.00501  2.5855  0.1209267
## Wheat:Soil:Biochar           1 0.00104 0.00104  0.5366  0.4709206
## Residuals                   24 0.04650 0.00194
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

SHOOT WEIGHT: *Fitting Order 2 Models*

Fitting Multiple Order 2 Models

- for every new model fit, assumptions are checked
- if there is no significant interaction effect ($p\text{-value} > 0.05$), remove the interaction
- refit model and check assumptions

```
anova(SW_model2)
```

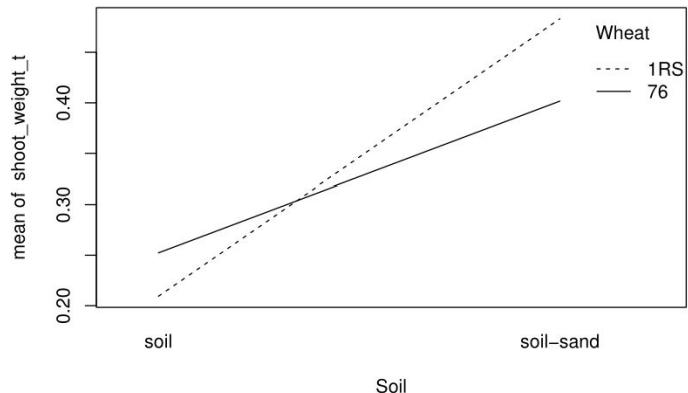
```
## Analysis of Variance Table
##
## Response: shoot_weight_t
##                               Df  Sum Sq Mean Sq   F value    Pr(>F)
## Wheat                  1 0.00297 0.00297   1.5626  0.2228583
## Soil                   1 0.35828 0.35828 188.4274 3.821e-13 ***
## Biochar                 1 0.00050 0.00050   0.2652  0.6110804
## Wheat:Soil              1 0.03098 0.03098 16.2926 0.0004512 ***
## Wheat:Biochar            1 0.00020 0.00020   0.1077  0.7455088
## Soil:Biochar             1 0.00501 0.00501   2.6343  0.1171235
```

```
anova(SW_model3)
```

```
## Analysis of Variance Table
##
## Response: shoot_weight_t
##                               Df  Sum Sq Mean Sq   F value    Pr(>F)
## Wheat                  1 0.00297 0.00297   1.6181  0.214610
## Soil                   1 0.35828 0.35828 195.1239 1.348e-13 ***
## Biochar                 1 0.00050 0.00050   0.2746  0.604672
## Wheat:Soil              1 0.03098 0.03098 16.8716 0.000353 ***
## Soil:Biochar             1 0.00501 0.00501   2.7279  0.110637
```

SHOOT WEIGHT:

Final Model: Order 2 with interaction



```
anova(SW_model5)
```

```
## Analysis of Variance Table
##
## Response: shoot_weight_t
##              Df  Sum Sq Mean Sq   F value    Pr(>F)
## Wheat        1 0.00297 0.00297   1.5622 0.2216880
## Soil         1 0.35828 0.35828 188.3789 5.884e-14 ***
## Wheat:Soil   1 0.03098 0.03098 16.2884 0.0003816 ***
***
```

- Final Model: shoot weight = wheat +soil + wheat*soil
- Final Model has 1 significant interaction furthered shown in the interaction plot
 - Wheat: Soil

SHOOT WEIGHT:

Multiple Comparison-EMMeans

- since interaction between wheat and soil is significant, we will perform multiple comparison
- The effect of wheat is significant when soil type is “soil-sand” (p-value = 0.0012 < 0.05) in terms of shoot weight
- The effect of Soil is significant at each level of wheat type (both p-value < 0.0001) in terms of shoot weight.

```
emmeans(SW_model5, list(pairwise ~ Wheat|Soil))
```

```
## $`emmeans of Wheat | Soil`  
## Soil = soil:  
##   Wheat emmean      SE df lower.CL upper.CL  
##   1RS     0.209 0.0154 28    0.178    0.241  
##   76      0.252 0.0154 28    0.221    0.284  
##  
##  
## Soil = soil-sand:  
##   Wheat emmean      SE df lower.CL upper.CL  
##   1RS     0.483 0.0154 28    0.452    0.515  
##   76      0.402 0.0154 28    0.370    0.433  
##  
## Confidence level used: 0.95  
##  
## $`pairwise differences of Wheat | Soil`  
## Soil = soil:  
##   2       estimate      SE df t.ratio p.value  
##   1RS - 76   -0.0430 0.0218 28  -1.970  0.0588  
##  
##  
## Soil = soil-sand:  
##   2       estimate      SE df t.ratio p.value  
##   1RS - 76   0.0815 0.0218 28   3.738  0.0008
```

```
emmeans(SW_model5, list(pairwise ~ Soil|Wheat))
```

```
## $`emmeans of Soil | Wheat`  
## Wheat = 1RS:  
##   Soil      emmean      SE df lower.CL upper.CL  
##   soil     0.209 0.0154 28    0.178    0.241  
##   soil-sand 0.483 0.0154 28    0.452    0.515  
##  
## Wheat = 76:  
##   Soil      emmean      SE df lower.CL upper.CL  
##   soil     0.252 0.0154 28    0.221    0.284  
##   soil-sand 0.402 0.0154 28    0.370    0.433  
##  
## Confidence level used: 0.95  
##  
## $`pairwise differences of Soil | Wheat`  
## Wheat = 1RS:  
##   2           estimate      SE df t.ratio p.value
```

Conclusion

● **For shoot weight:**

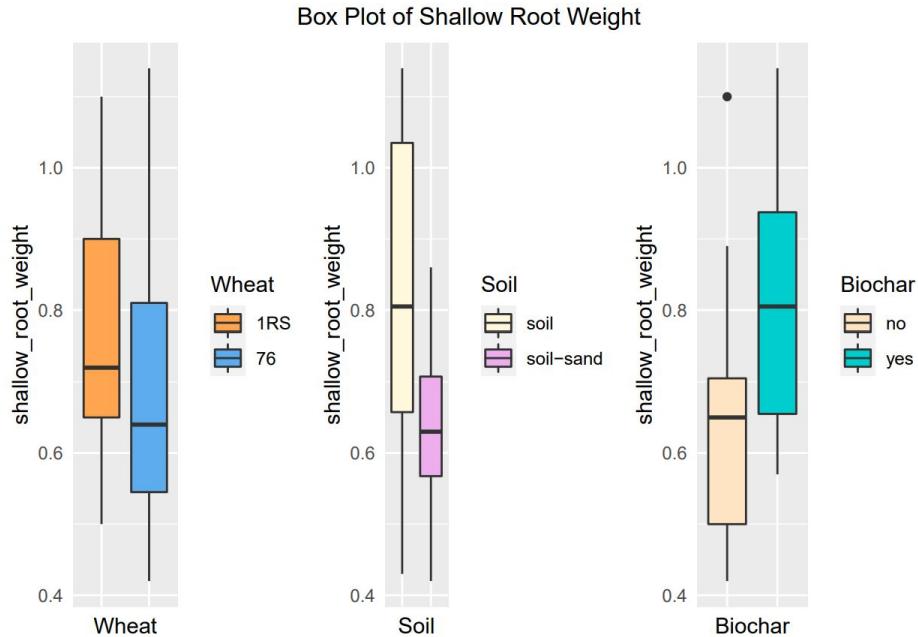
- use soil type: soil-sand
- use wheat type: 1RS



**SHALLOW
ROOT
WEIGHT**

SHALLOW ROOT WEIGHT: BOX PLOT

- Median for both wheat types is the most similar
- Soil and soil-sand are relatively dissimilar
- Presence or absence of biochar treatment relatively dissimilar
 - Absence of biochar has one outlier



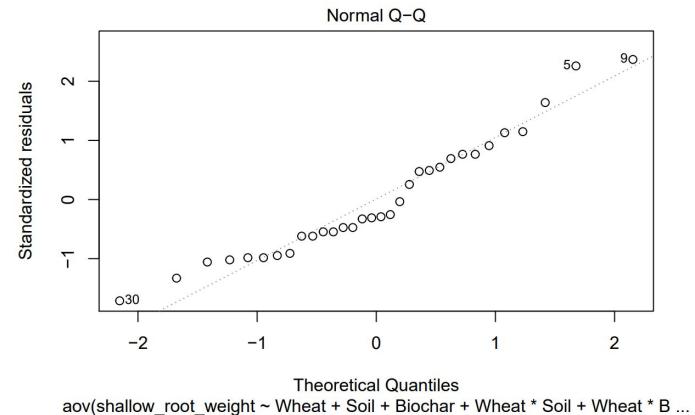
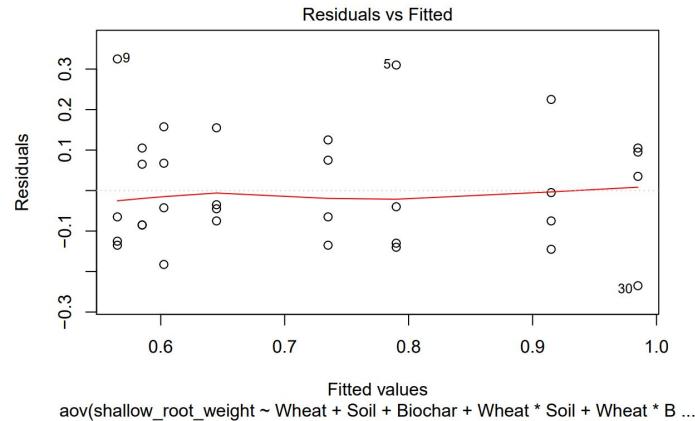
SHALLOW ROOT WEIGHT: *RESIDUALS AND Q-Q*

Normality Assumption

- dots seem to follow line with no obvious deviation
- assumption is met

Equal Variance Assumption

- no obvious pattern
- assumption is met



SHALLOW ROOT WEIGHT:

Fit Full Model

From Full Model:

- can conclude the interaction effect is not significant ($p\text{-value} = 0.25 > 0.05$)
- remove the 3-way interaction
 - Wheat:Soil:Biochar

```
## Analysis of Variance Table
##
## Response: shallow_root_weight
##                               Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat                      1 0.06753 0.067528 2.6900 0.114021
## Soil                       1 0.23633 0.236328 9.4143 0.005274 ***
## Biochar                     1 0.27195 0.271953 10.8334 0.003076 ***
## Wheat:Soil                  1 0.02475 0.024753 0.9861 0.330615
## Wheat:Biochar                1 0.00113 0.001128 0.0449 0.833905
## Soil:Biochar                 1 0.06213 0.062128 2.4749 0.128767
## Wheat:Soil:Biochar           1 0.03445 0.034453 1.3725 0.252889
## Residuals                   24 0.60248 0.025103
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

SHALLOW ROOT WEIGHT:

Fitting Order 2 Models

Fitting Multiple Order 2 Models

- for every new model fit, assumptions are checked
- if there is no significant interaction effect ($p\text{-value} > 0.05$), remove the interaction
- refit model and check assumptions

```
anova(SRW_model2)
```

```
## Analysis of Variance Table
##
## Response: shallow_root_weight
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.06753 0.067528  2.6505 0.116054
## Soil         1 0.23633 0.236328  9.2761 0.005409 **
## Biochar      1 0.27195 0.271953 10.6744 0.003150 **
## Wheat:Soil   1 0.02475 0.024753  0.9716 0.333730
## Wheat:Biochar 1 0.00113 0.001128  0.0443 0.835039
## Soil:Biochar  1 0.06213 0.062128  2.4386 0.130954
```

```
anova(SRW_model3)
```

```
## Analysis of Variance Table
##
## Response: shallow_root_weight
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.06753 0.067528  2.7517 0.109169
## Soil         1 0.23633 0.236328  9.6301 0.004574 **
## Biochar      1 0.27195 0.271953 11.0818 0.002612 **
## Wheat:Soil   1 0.02475 0.024753  1.0087 0.324480
## Soil:Biochar  1 0.06213 0.062128  2.5316 0.123671
```

SHALLOW ROOT WEIGHT:

Final Model: Order 2

- Final Model
 - srw = soil + biochar
- Final Model has no significant interaction

```
anova(SRW_model6)

## Analysis of Variance Table
##
## Response: shallow_root_weight
##           Df  Sum Sq Mean Sq F value    Pr(>F)
## Soil       1 0.23633 0.236328  8.6483 0.006371 ***
## Biochar    1 0.27195 0.271953  9.9520 0.003725 ***
```

SHALLOW ROOT WEIGHT: *Multiple Comparison-EMMeans*

- no significant interaction so will test for main effects
- Tukeys
 - soil types are significantly different
 - presence or absence of biochar is significantly different
- soil is significantly greater than soil-sand
- presence of biochar is significantly greater than no absence

```
emmeans(SRW_model6, list(pairwise ~ Soil))

## $`emmeans of Soil`
##   Soil      emmean     SE df lower.CL upper.CL
##   soil      0.814 0.0413 29    0.729    0.898
##   soil-sand 0.642 0.0413 29    0.557    0.726
##
## Results are averaged over the levels of: Biochar
## Confidence level used: 0.95
##
## $`pairwise differences of Soil`
##   1           estimate     SE df t.ratio p.value
##   soil - (soil-sand) 0.172 0.0584 29 2.941  0.0064
##
## Results are averaged over the levels of: Biochar

emmeans(SRW_model6, list(pairwise ~ Biochar))

## $`emmeans of Biochar`
##   Biochar emmean     SE df lower.CL upper.CL
##   no      0.636 0.0413 29    0.551    0.720
##   yes     0.820 0.0413 29    0.735    0.905
##
## Results are averaged over the levels of: Soil
## Confidence level used: 0.95
##
## $`pairwise differences of Biochar`
##   1       estimate     SE df t.ratio p.value
##   no - yes -0.184 0.0584 29 -3.155  0.0037
##
## Results are averaged over the levels of: Soil
```

Conclusion

● **For shallow root weight:**

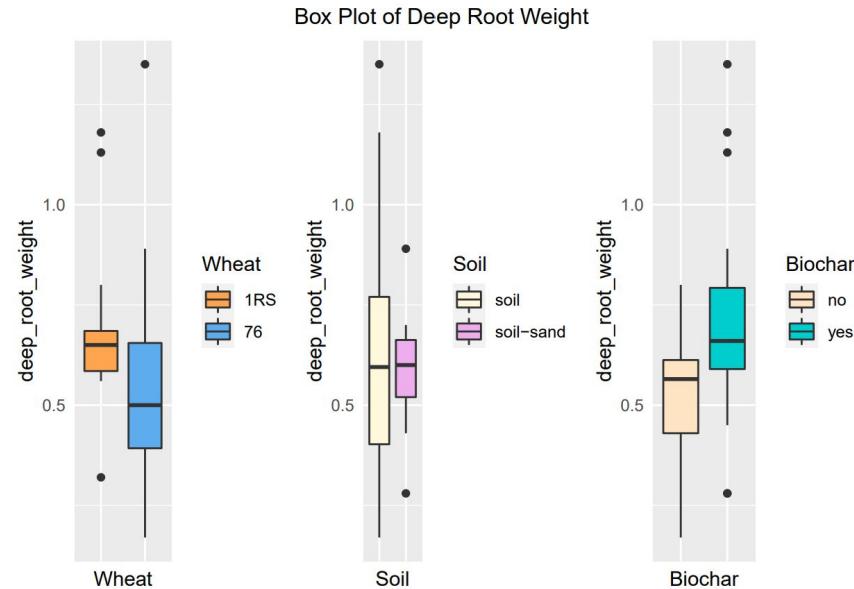
- use soil type: soil-sand
- biochar treatment: should be present



**DEEP
ROOT
WEIGHT**

DEEP ROOT WEIGHT: BOX PLOT

- Median for both wheat types is the most dissimilar
 - 1RS has 3 outliers, 76 has 1 outlier
- Median for soil and soil-sand is the most similar
 - Soil has 1 outlier
 - Soil-sand has 2 outliers
- Presence or absence of biochar treatment dissimilar
 - Presence of biochar has 3 outliers



DEEP ROOT WEIGHT: *RESIDUALS AND Q-Q*

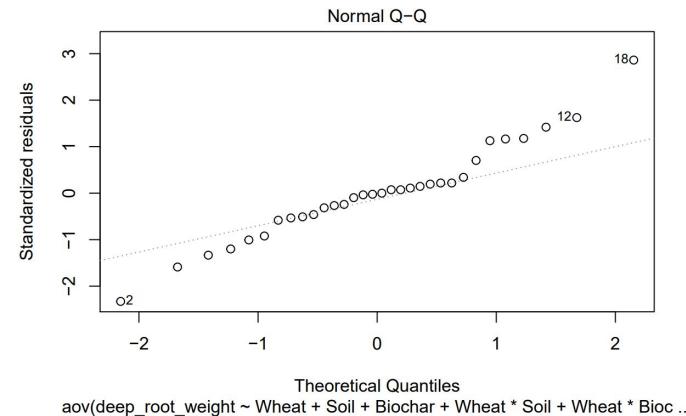
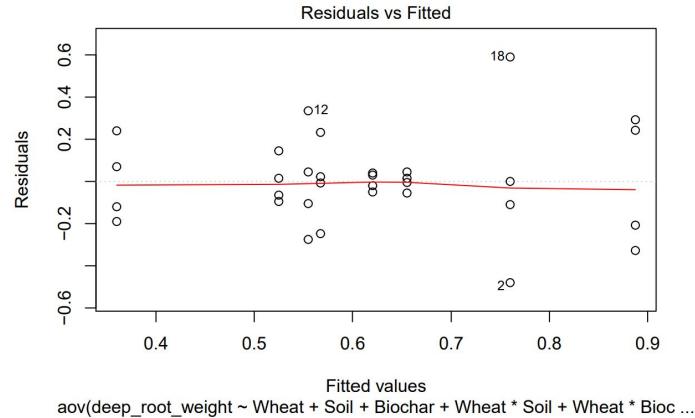
Normality Assumption

- dots don't seem to follow the line with some obvious outliers
- assumption is not met

Equal Variance Assumption

- seems that there may be a pattern
- assumption is not met

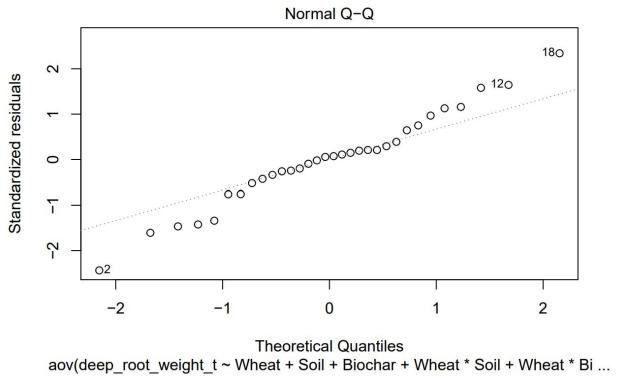
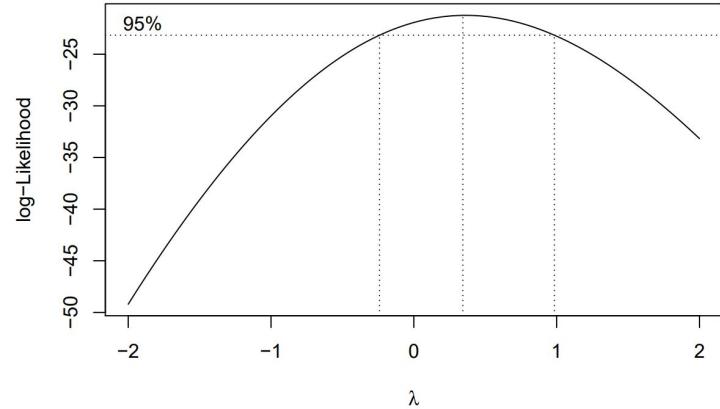
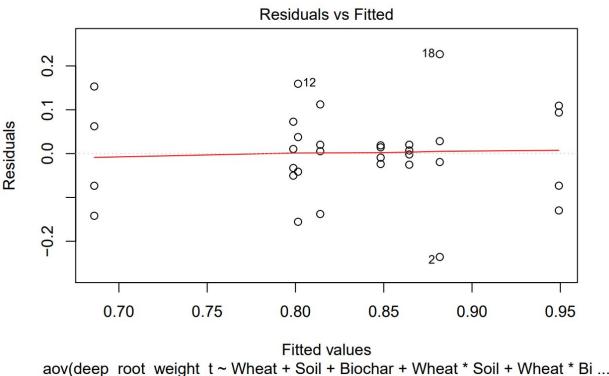
Transformation is needed!



DEEP ROOT WEIGHT: BOX-COX TRANSFORMATION

After transformation:

- can see that QQ-plot is better with most points following the line
- can assume both assumptions met



DEEP ROOT WEIGHT: *Fit Full Model*

From Full Model:

- can conclude the interaction effect is not significant ($p\text{-value} = 0.64 > 0.05$)
- remove the 3-way interaction
 - Wheat:Soil:Biochar

```
anova(DRW_model_new)

## Analysis of Variance Table
##
## Response: deep_root_weight_t
##                               Df  Sum Sq Mean Sq F value Pr(>F)
## Wheat                      1 0.047401 0.047401  3.7907 0.06333 .
## Soil                       1 0.000163 0.000163  0.0130 0.91005
## Biochar                     1 0.061163 0.061163  4.8912 0.03676 *
## Wheat:Soil                  1 0.003442 0.003442  0.2752 0.60466
## Wheat:Biochar                1 0.001107 0.001107  0.0885 0.76865
## Soil:Biochar                 1 0.048662 0.048662  3.8915 0.06016 .
## Wheat:Soil:Biochar           1 0.002740 0.002740  0.2191 0.64395
```

DEEP ROOT WEIGHT: *Fitting Order 2 Models*

Fitting Multiple Order 2 Models

- for every new model fit, assumptions are checked
- if there is no significant interaction effect (p-value > 0.05), remove the interaction
- refit model and check assumptions

```
anova(DRW_model12)
```

```
## Analysis of Variance Table
##
## Response: deep_root_weight_t
##              Df  Sum Sq Mean Sq F value Pr(>F)
## Wheat          1 0.047401 0.047401  3.9129 0.05904 .
## Soil           1 0.000163 0.000163  0.0135 0.90858
## Biochar        1 0.061163 0.061163  5.0489 0.03371 *
## Wheat:Soil     1 0.003442 0.003442  0.2841 0.59873
## Wheat:Biochar   1 0.001107 0.001107  0.0914 0.76496
## Soil:Biochar    1 0.048662 0.048662  4.0170 0.05599 .
```

```
anova(DRW_model13)
```

```
## Analysis of Variance Table
##
## Response: deep_root_weight_t
##              Df  Sum Sq Mean Sq F value Pr(>F)
## Wheat          1 0.047401 0.047401  4.0546 0.05451 .
## Soil           1 0.000163 0.000163  0.0139 0.90692
## Biochar        1 0.061163 0.061163  5.2318 0.03056 *
## Wheat:Soil     1 0.003442 0.003442  0.2944 0.59204
## Soil:Biochar    1 0.048662 0.048662  4.1625 0.05161 .
```

DEEP ROOT WEIGHT:

Final Model: Order 1

- Final Model
 - drw = biochar
- Final Model has no significant interaction

```
anova(DRW_model7)
```

```
## Analysis of Variance Table
##
## Response: deep_root_weight_t
##           Df  Sum Sq Mean Sq F value Pr(>F)
## Biochar     1 0.06116 0.061163   4.546 0.0413 *
## Residuals 30 0.40363 0.013454
```

DEEP ROOT WEIGHT: *Multiple Comparison-EMMeans*

- no significant interaction so will test for main effects
- EMMeans/Tukeys
 - presence or absence of biochar is significantly different
- presence of biochar is significantly greater than no absence

```
emmeans(DRW_model7, list(pairwise ~ Biochar))  
  
## $`emmeans of Biochar'  
##   Biochar emmean    SE df lower.CL upper.CL  
##   no        0.787 0.029 30     0.728    0.846  
##   yes       0.874 0.029 30     0.815    0.933  
##  
## Confidence level used: 0.95  
##  
## $`pairwise differences of Biochar'  
##   1      estimate    SE df t.ratio p.value  
##   no - yes -0.0874 0.041 30 -2.132  0.0413
```

Conclusion

● For deep root weight:

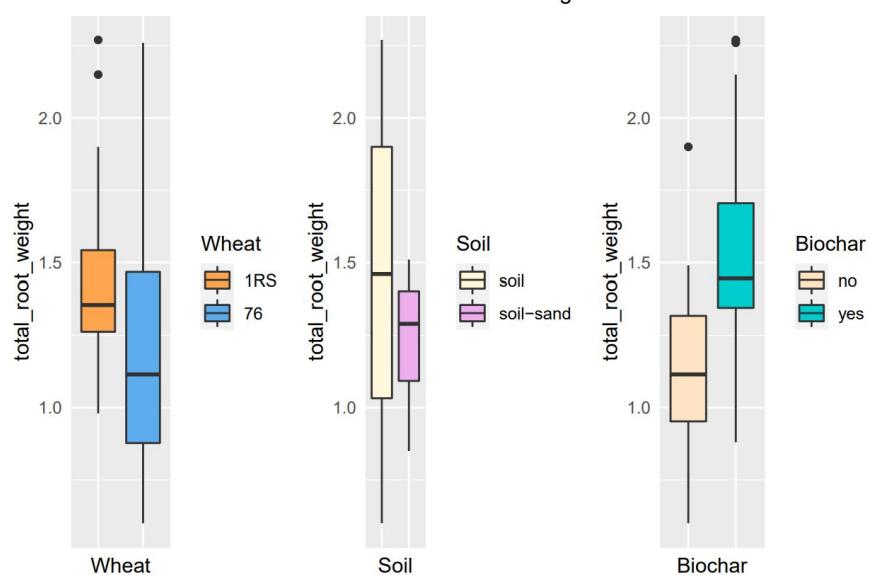
- biochar treatment: should be present



TOTAL ROOT WEIGHT

TOTAL ROOT WEIGHT: BOX PLOT

- Median for both wheat types is relatively dissimilar
 - 1RS has two outliers
- Median for soil and soil-sand are the most similar
- Presence or absence of biochar treatment is most dissimilar
 - Absence of biochar has 1 outlier, presence has 2 outliers



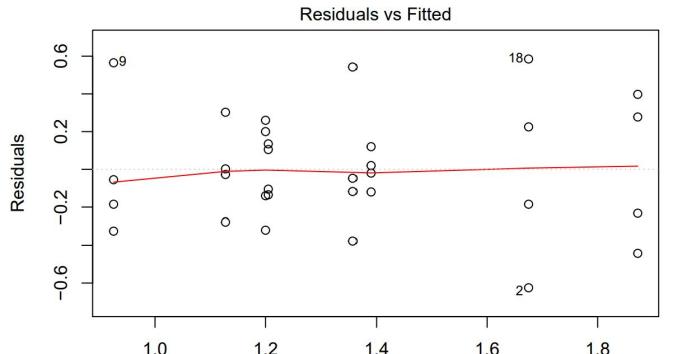
TOTAL ROOT WEIGHT: *RESIDUALS AND Q-Q*

Normality Assumption

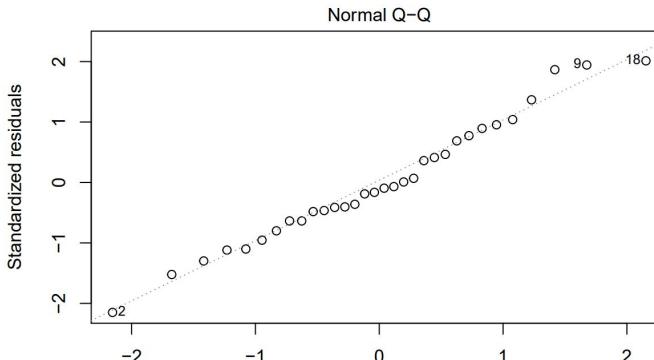
- dots seem to follow line with no obvious deviation
- assumption is met

Equal Variance Assumption

- no obvious pattern
- assumption is met



Normal Q-Q



TOTAL ROOT WEIGHT:

Fit Full Model

From Full Model:

- can conclude the interaction effect is not significant ($p\text{-value} = 0.47 > 0.05$)
- remove the 3-way interaction
 - Wheat:Soil:Biochar

```
anova(TRW_model)

## Analysis of Variance Table
##
## Response: total_root_weight
##                               Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat                      1  0.40275 0.40275  3.5740  0.070824 .
## Soil                       1  0.41178 0.41178  3.6541  0.067943 .
## Biochar                     1  1.15900 1.15900 10.2850  0.003776 **
## Wheat:Soil                  1  0.06570 0.06570  0.5831  0.452561
## Wheat:Biochar                1  0.00750 0.00750  0.0666  0.798579
## Soil:Biochar                 1  0.50753 0.50753  4.5038  0.044335 *
## Wheat:Soil:Biochar           1  0.06038 0.06038  0.5358  0.471268
```

TOTAL ROOT WEIGHT: *Fitting Order 2 Models*

Fitting Multiple Order 2 Models

- for every new model fit, assumptions are checked
- if there is no significant interaction effect (p-value > 0.05), remove the interaction
- refit model and check assumptions

```
anova(TRW_model_2)
```

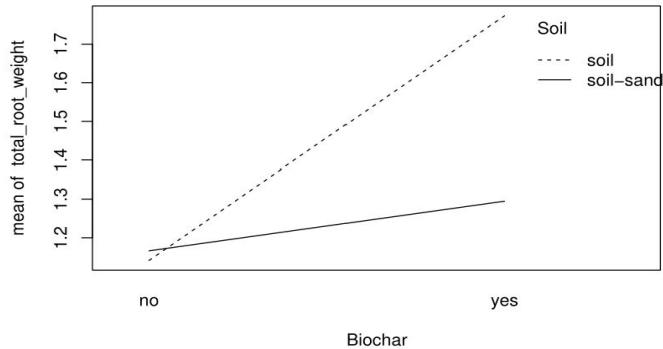
```
## Analysis of Variance Table
##
## Response: total_root_weight
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.40275 0.40275  3.6417 0.067898 .
## Soil         1 0.41178 0.41178  3.7233 0.065083 .
## Biochar      1 1.15900 1.15900 10.4796 0.003392 **
## Wheat:Soil   1 0.06570 0.06570  0.5941 0.448069
## Wheat:Biochar 1 0.00750 0.00750  0.0678 0.796638
## Soil:Biochar  1 0.50753 0.50753  4.5890 0.042105 *
```

```
anova(TRW_model_3)
```

```
## Analysis of Variance Table
##
## Response: total_root_weight
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.40275 0.40275  3.7771 0.062859 .
## Soil         1 0.41178 0.41178  3.8617 0.060169 .
## Biochar      1 1.15900 1.15900 10.8693 0.002831 **
## Wheat:Soil   1 0.06570 0.06570  0.6162 0.439568
## Soil:Biochar  1 0.50753 0.50753  4.7597 0.038365 *
```

TOTAL ROOT WEIGHT:

Final Model: Order 2 with interaction



```
anova(TRW_model_5)
```

```
## Analysis of Variance Table
##
## Response: total_root_weight
##                         Df Sum Sq Mean Sq F value    Pr(>F)
## Soil                      1 0.4118 0.41178  3.5576 0.069684 .
## Biochar                   1 1.1590 1.15900 10.0134 0.003726 **
## Soil:Biochar               1 0.5075 0.50753  4.3849 0.045435 *
```

- Final Model
 - $\text{trw} = \text{soil} + \text{biochar} + \text{soil} * \text{biochar}$
- Final Model has a significant interaction between soil and biochar further shown in interaction plot

TOTAL ROOT WEIGHT: *Multiple Comparison-EMMeans*

- Interaction is significant so will perform multiple comparison
- Effect of Soil is significant when Biochar treatment is “yes” (p-value = 0.0088 < 0.05) in terms of total root weight
- Effect of Biochar is significant when Soil type is “soil” (p-value = 0.0009 < 0.05) in terms of total root weight

```
# compare the effect of Wheat at each level of Soil
emmmeans(TRW_model_5, list(pairwise ~ Soil|Biochar))

## $`emmeans of Soil | Biochar`
## Biochar = no:
##   Soil      emmean    SE df lower.CL upper.CL
##   soil      1.14 0.12 28    0.895   1.39
##   soil-sand 1.17 0.12 28    0.920   1.41
##
## Biochar = yes:
##   Soil      emmean    SE df lower.CL upper.CL
##   soil      1.77 0.12 28    1.527   2.02
##   soil-sand 1.29 0.12 28    1.049   1.54
##
## Confidence level used: 0.95
##
## $`pairwise differences of Soil | Biochar`
## Biochar = no:
##   2           estimate    SE df t.ratio p.value
##   soil - (soil-sand) -0.025 0.17 28 -0.147  0.8842
##
## Biochar = yes:
##   2           estimate    SE df t.ratio p.value
##   soil - (soil-sand)  0.479 0.17 28  2.814  0.0088

## $`emmeans of Biochar | Soil`
## Soil = soil:
##   Biochar emmean    SE df lower.CL upper.CL
##   no      1.14 0.12 28    0.895   1.39
##   yes     1.77 0.12 28    1.527   2.02
##
## Soil = soil-sand:
##   Biochar emmean    SE df lower.CL upper.CL
##   no      1.17 0.12 28    0.920   1.41
##   yes     1.29 0.12 28    1.049   1.54
##
## Confidence level used: 0.95
##
## $`pairwise differences of Biochar | Soil`
## Soil = soil:
##   2           estimate    SE df t.ratio p.value
##   no - yes   -0.632 0.17 28 -3.718  0.0009
##
## Soil = soil-sand:
##   2           estimate    SE df t.ratio p.value
##   no - yes   -0.129 0.17 28 -0.757  0.4554
```

Conclusion

● For total root weight:

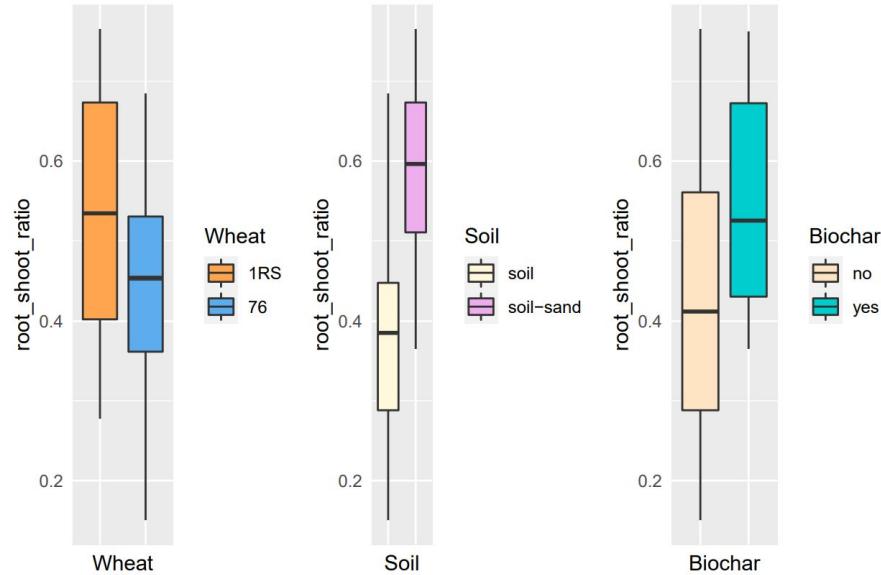
- use soil type: soil
- biochar treatment: should be present



ROOT SHOOT RATIO

ROOT-SHOOT RATIO: BOX PLOT

- 1RS and 76 wheat types are somewhat similar
- Soil and soil-sand are very dissimilar
- Presence or absence of biochar treatment somewhat dissimilar



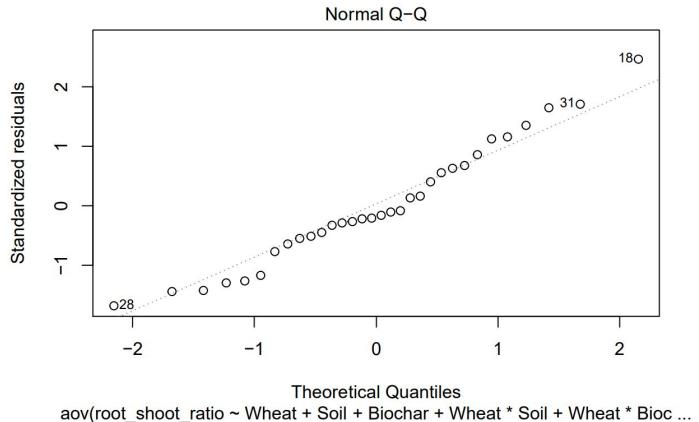
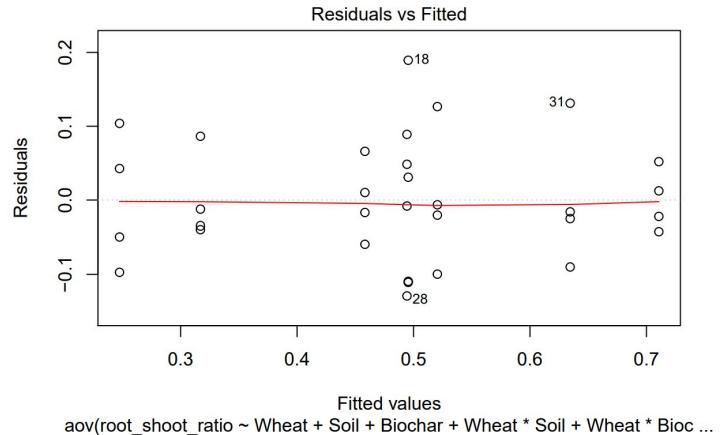
ROOT-SHOOT RATIO: *RESIDUALS AND Q-Q*

Normality Assumption

- dots seem to follow line with no obvious deviation
- assumption is met

Equal Variance Assumption

- no obvious pattern
- assumption is met



ROOT-SHOOT RATIO:

Fit Full Model

From Full Model:

- can conclude the interaction effect is not significant ($p\text{-value} = 0.10 > 0.05$)
- remove the 3-way interaction
 - Wheat:Soil:Biochar

```
anova(RSR_model)

## Analysis of Variance Table
##
## Response: root_shoot_ratio
##                               Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat                      1 0.06575 0.06575  8.3510  0.008050 **
## Soil                       1 0.35424 0.35424 44.9928 6.145e-07 ***
## Biochar                     1 0.09643 0.09643 12.2479  0.001844 **
## Wheat:Soil                  1 0.04441 0.04441  5.6404  0.025889 *
## Wheat:Biochar                1 0.00001 0.00001  0.0013  0.971988
## Soil:Biochar                 1 0.05750 0.05750  7.3030  0.012438 *
## Wheat:Soil:Biochar           1 0.02182 0.02182  2.7714  0.108963
```

ROOT-SHOOT RATIO:

Fitting Order 2 Models

Fitting Multiple Order 2 Models

- for every new model fit, assumptions are checked
- if there is no significant interaction effect ($p\text{-value} > 0.05$), remove the interaction
- refit model and check assumptions

```
anova(RSR_model_2)
```

```
## Analysis of Variance Table
##
## Response: root_shoot_ratio
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.06575 0.06575  7.7985 0.009880 **
## Soil         1 0.35424 0.35424 42.0157 8.668e-07 ***
## Biochar      1 0.09643 0.09643 11.4374 0.002371 **
## Wheat:Soil   1 0.04441 0.04441  5.2671 0.030399 *
## Wheat:Biochar 1 0.00001 0.00001  0.0012 0.972919
## Soil:Biochar  1 0.05750 0.05750  6.8198 0.015027 *
```

```
anova(RSR_model_3)
```

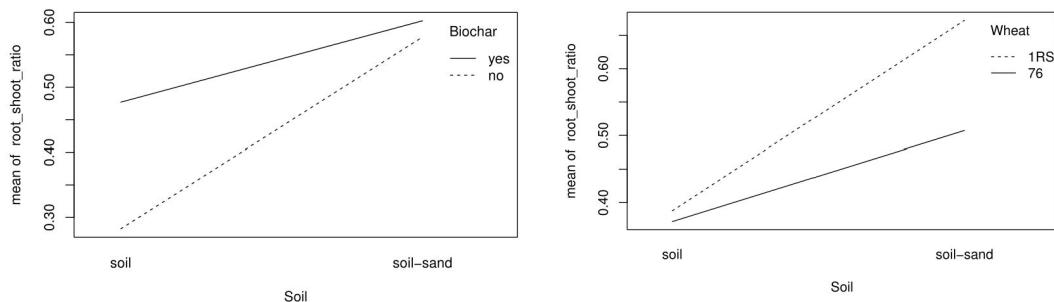
```
## Analysis of Variance Table
##
## Response: root_shoot_ratio
##              Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.06575 0.06575  8.1100 0.008488 **
## Soil         1 0.35424 0.35424 43.6942 5.199e-07 ***
## Biochar      1 0.09643 0.09643 11.8944 0.001931 **
## Wheat:Soil   1 0.04441 0.04441  5.4776 0.027209 *
## Soil:Biochar  1 0.05750 0.05750  7.0922 0.013110 *
```

ROOT-SHOOT RATIO:

Final Model: Order 2 with interactions

```
anova(RSR_model_3)
```

```
## Analysis of Variance Table
##
## Response: root_shoot_ratio
##             Df  Sum Sq Mean Sq F value    Pr(>F)
## Wheat        1 0.06575 0.06575  8.1100  0.008488 **
## Soil         1 0.35424 0.35424 43.6942 5.199e-07 ***
## Biochar      1 0.09643 0.09643 11.8944  0.001931 **
## Wheat:Soil   1 0.04441 0.04441  5.4776  0.027209 *
## Soil:Biochar 1 0.05750 0.05750  7.0922  0.013110 *
```



- Final Model
 - $rsr = wheat + soil + biochar + wheat*soil + soil*biochar$
- Final Model has 2 significant interaction further shown through interaction plots
 - wheat and soil
 - soil and biochar

ROOT-SHOOT RATIO: Multiple Comparison EMMeans

- Interaction is significant so we perform multiple comparison
- Effect of Wheat is significant when Soil type is “soil-sand” (p-value = 0.0011 < 0.05) in terms of root shoot ratio.
- Effect of Soil is significant at each level of Wheat type (both p-value < 0.05) in terms of root shoot ratio.
- Effect of Soil is significant when at each level of Biochar treatment (both p-value < 0.05) in terms of root shoot ratio.
- Effect of Biochar is significant when Soil type is “soil” (p-value = 0.0002 < 0.05) in terms of root shoot ratio

```
# compare the effect of Soil at each level of Wheat
emmeans(RSR_model_3, list(pairwise ~ Soil|Wheat))
```

```
## $'emmmeans of Soil | Wheat'
## Wheat = IRS:
##   Soil    emmean     SE df lower.CL upper.CL
##   soil     0.388 0.0318 26    0.322    0.453
##   soil-sand 0.673 0.0318 26    0.607    0.738
##
## Wheat = 76:
##   Soil    emmean     SE df lower.CL upper.CL
##   soil     0.371 0.0318 26    0.306    0.437
##   soil-sand 0.507 0.0318 26    0.442    0.573
##
## Results are averaged over the levels of: Biochar
## Confidence level used: 0.95
## $'pairwise differences of Soil | Wheat'
## Wheat = IRS:
##   2      estimate     SE df t.ratio p.value
##   soil - (soil-sand) -0.285 0.045 26 -6.329 <.0001
##
## Wheat = 76:
##   2      estimate     SE df t.ratio p.value
##   soil - (soil-sand) -0.136 0.045 26 -3.019 0.0056
##
## Results are averaged over the levels of: Biochar
```

```
# compare the effect of Soil at each level of Wheat
emmeans(RSR_model_3, list(pairwise ~ Biochar|Soil))
```

```
## $'emmmeans of Biochar | Soil'
## Soil = soil:
##   Biochar emmean     SE df lower.CL upper.CL
##   no      0.282 0.0318 26    0.217    0.348
##   yes     0.477 0.0318 26    0.411    0.542
##
## Soil = soil-sand:
##   Biochar emmean     SE df lower.CL upper.CL
##   no      0.577 0.0318 26    0.512    0.643
##   yes     0.603 0.0318 26    0.537    0.668
##
## Results are averaged over the levels of: Wheat
## Confidence level used: 0.95
##
## $'pairwise differences of Biochar | Soil'
## Soil = soil:
##   2      estimate     SE df t.ratio p.value
##   no - yes -0.195 0.045 26 -4.322 0.0002
##
## Soil = soil-sand:
##   2      estimate     SE df t.ratio p.value
##   no - yes -0.025 0.045 26 -0.556 0.5832
##
## Results are averaged over the levels of: Wheat
```

```
# compare the effect of Wheat at each level of Soil
emmeans(RSR_model_3, list(pairwise ~ Wheat|Soil))
```

```
## $'emmmeans of Wheat | Soil'
## Soil = soil:
##   Wheat emmean     SE df lower.CL upper.CL
##   IRS    0.388 0.0318 26    0.322    0.453
##   76     0.371 0.0318 26    0.306    0.437
##
## Soil = soil-sand:
##   Wheat emmean     SE df lower.CL upper.CL
##   IRS    0.673 0.0318 26    0.607    0.738
##   76     0.507 0.0318 26    0.442    0.573
##
## Results are averaged over the levels of: Biochar
## Confidence level used: 0.95
##
## $'pairwise differences of Wheat | Soil'
## Soil = soil:
##   2      estimate     SE df t.ratio p.value
##   IRS - 76  0.0162 0.045 26 0.359 0.7227
##
## Soil = soil-sand:
##   2      estimate     SE df t.ratio p.value
##   IRS - 76  0.1652 0.045 26 3.669 0.0011
##
## Results are averaged over the levels of: Biochar
```

```
# compare the effect of Wheat at each level of Soil
emmeans(RSR_model_3, list(pairwise ~ Soil|Biochar))
```

```
## $'emmmeans of Soil | Biochar'
## Biochar = no:
##   Soil    emmean     SE df lower.CL upper.CL
##   soil     0.282 0.0318 26    0.217    0.348
##   soil-sand 0.577 0.0318 26    0.512    0.643
##
## Biochar = yes:
##   Soil    emmean     SE df lower.CL upper.CL
##   soil     0.477 0.0318 26    0.411    0.542
##   soil-sand 0.603 0.0318 26    0.537    0.668
##
## Results are averaged over the levels of: Wheat
## Confidence level used: 0.95
##
## $'pairwise differences of Soil | Biochar'
## Biochar = no:
##   2      estimate     SE df t.ratio p.value
##   soil - (soil-sand) -0.295 0.045 26 -6.557 <.0001
##
## Biochar = yes:
##   2      estimate     SE df t.ratio p.value
##   soil - (soil-sand) -0.126 0.045 26 -2.791 0.0097
##
## Results are averaged over the levels of: Wheat
```

Conclusion

● For root shoot ratio:

- use soil type: soil-sand
- use wheat type: 1RS
- biochar treatment: should be present

THANK YOU

