Soil N and Fert N Analysis

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Necessary libraries

Data Organisation

Read from excel

```
all_data <- read_excel("15N_N_Uptake_MaturitySummed.xlsx", sheet = 1)
str(all_data)
## tibble [84 x 10] (S3: tbl_df/tbl/data.frame)
## $ Sample.ID : chr [1:84] "107 112 Pre" "207 212 Pre" "307 312 Pre" "101 106 Pre" ...
                : chr [1:84] "CR" "CR" "CR" "RF" ...
## $ Field
## $ Blk
                : num [1:84] 1 2 3 1 2 3 1 2 3 1 ...
## $ Topdress : chr [1:84] "N" "N" "N" "N" ...
## $ Stage : chr [1:84] "PrePlant" "PrePlant" "PrePlant" "PrePlant" ...
## $ fertiliser_N: num [1:84] 0 0 0 0 0 ...
## $ soil_N : num [1:84] 0 0 0 0 0 ...
## $ Days
                : num [1:84] 0 0 0 0 0 0 47 47 47 47 ...
## $ Year
                : num [1:84] 2021 2021 2021 2021 2021 ...
## $ Days graph : num [1:84] 0 0 0 0 0 46 46 46 46 ...
```

Clean up variables

```
all_data <- mutate_if(all_data, is.character, as.factor)</pre>
all_data$Blk <- as.factor(all_data$Blk)</pre>
all_data$Year <- as.factor(all_data$Year)</pre>
str(all_data)
## tibble [84 x 10] (S3: tbl df/tbl/data.frame)
## $ Sample.ID : Factor w/ 72 levels "101 106 minus H",..: 12 24 36 6 18 30 9 21 33 3 ...
                 : Factor w/ 2 levels "CR", "RF": 1 1 1 2 2 2 1 1 1 2 ...
## $ Field
## $ Blk
                 : Factor w/ 6 levels "1", "2", "3", "4", ...: 1 2 3 1 2 3 1 2 3 1 ...
## $ Topdress : Factor w/ 2 levels "N", "Y": 1 1 1 1 1 1 1 1 1 1 1 ...
            : Factor w/ 4 levels "Heading", "Maturity", ...: 4 4 4 4 4 4 3 3 3 3 ...
## $ Stage
## $ fertiliser_N: num [1:84] 0 0 0 0 0 ...
## $ soil_N : num [1:84] 0 0 0 0 0 ...
## $ Days
                 : num [1:84] 0 0 0 0 0 0 47 47 47 47 ...
                 : Factor w/ 2 levels "2021", "2022": 1 1 1 1 1 1 1 1 1 1 ...
## $ Year
## $ Days_graph : num [1:84] 0 0 0 0 0 46 46 46 46 ...
```

Sub dataset for "preplant" and "topdress"

```
preplant <- all_data %>% filter(Topdress == "N")
str(preplant)
```

```
## tibble [48 x 10] (S3: tbl_df/tbl/data.frame)
## $ Sample.ID : Factor w/ 72 levels "101 106 minus H",..: 12 24 36 6 18 30 9 21 33 3 ...
                 : Factor w/ 2 levels "CR", "RF": 1 1 1 2 2 2 1 1 1 2 ...
## $ Field
                 : Factor w/ 6 levels "1","2","3","4",..: 1 2 3 1 2 3 1 2 3 1 ...
## $ Blk
                 : Factor w/ 2 levels "N", "Y": 1 1 1 1 1 1 1 1 1 1 ...
## $ Topdress
                : Factor w/ 4 levels "Heading", "Maturity", ...: 4 4 4 4 4 4 3 3 3 3 ...
## $ Stage
## $ fertiliser N: num [1:48] 0 0 0 0 0 ...
## $ soil N
                : num [1:48] 0 0 0 0 0 ...
## $ Days
                 : num [1:48] 0 0 0 0 0 0 47 47 47 47 ...
## $ Year
                 : Factor w/ 2 levels "2021", "2022": 1 1 1 1 1 1 1 1 1 1 ...
## $ Days_graph : num [1:48] 0 0 0 0 0 46 46 46 46 ...
topdress <- all_data %>% filter(Topdress == "Y")
str(topdress)
## tibble [36 x 10] (S3: tbl_df/tbl/data.frame)
## $ Sample.ID : Factor w/ 72 levels "101 106 minus H",..: 10 22 34 4 16 28 11 23 35 5 ...
## $ Field
                 : Factor w/ 2 levels "CR", "RF": 1 1 1 2 2 2 1 1 1 2 ...
                 : Factor w/ 6 levels "1","2","3","4",...: 1 2 3 1 2 3 1 2 3 1 ...
## $ Blk
## $ Topdress
                 : Factor w/ 2 levels "N", "Y": 2 2 2 2 2 2 2 2 2 2 ...
## $ Stage
                : Factor w/ 4 levels "Heading", "Maturity", ...: 1 1 1 1 1 1 2 2 2 2 ...
## $ fertiliser_N: num [1:36] 10.99 9.79 9.19 9.3 10.05 ...
                : num [1:36] 71.9 65.8 59.8 80.9 73.6 ...
## $ soil N
                 : num [1:36] 81 81 81 81 81 81 124 124 124 124 ...
## $ Days
## $ Year
                : Factor w/ 2 levels "2021", "2022": 1 1 1 1 1 1 1 1 1 1 ...
## $ Days_graph : num [1:36] 81 81 81 81 81 81 127 127 127 127 ...
```

Initial look

Year interaction

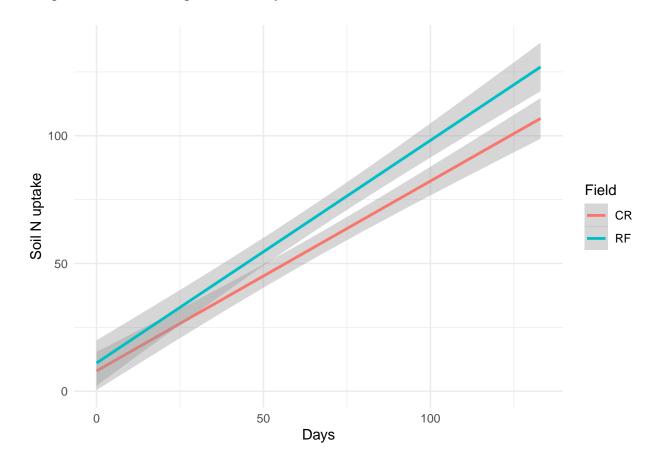
```
soil_N_try <- lm(soil_N~Field*Year+Stage, data = preplant)</pre>
anova(soil_N_try)
## Analysis of Variance Table
##
## Response: soil_N
              Df Sum Sq Mean Sq F value
                                            Pr(>F)
## Field
              1
                 1512 1511.8 20.8981 4.399e-05 ***
## Year
              1
                     1
                            0.9 0.0122
                                            0.9125
## Stage
              3 71063 23687.6 327.4475 < 2.2e-16 ***
## Field:Year 1
                  12
                          11.6
                                0.1608
                                            0.6905
## Residuals 41
                  2966
                           72.3
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' 1
fert_N_try <- lm(fertiliser_N~Field*Year+Stage, data = preplant)</pre>
anova(fert_N_try)
```

```
## Analysis of Variance Table
##
## Response: fertiliser_N
                 Sum Sq Mean Sq F value
##
             Df
                                            Pr(>F)
                   35.8
## Field
                           35.8
                                 1.1973 0.280249
## Year
              1
                  294.6
                          294.6
                                  9.8470 0.003147 **
## Stage
              3 15449.4 5149.8 172.1176 < 2.2e-16 ***
## Field:Year 1
                  119.9
                          119.9
                                  4.0060 0.051986 .
## Residuals 41
                 1226.7
                           29.9
## Signif. codes: 0 '*** 0.001 '** 0.01 '* 0.05 '.' 0.1 ' ' 1
```

Seems that only field has a very strong effect. Days expected to be very strong as N uptake will increase over time.

Visualisation

```
ggplot(preplant, aes(x = Days, y = soil_N, color = Field)) +
  geom_smooth(method = gam) +
  labs(x = "Days", y = "Soil N uptake", color = "Field", linetype = "Year") +
  scale_linetype_manual(values = c("solid", "dashed")) +
  theme_minimal()
```



Preplant Soil N

Remove day 0

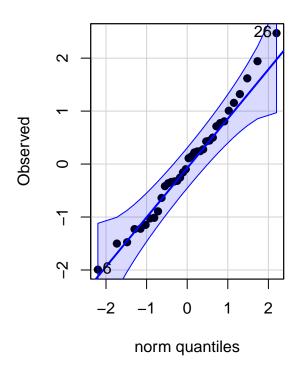
```
preplant_no0 <-subset(preplant, Days != 0)</pre>
str(preplant_no0)
## tibble [36 x 10] (S3: tbl_df/tbl/data.frame)
## $ Sample.ID : Factor w/ 72 levels "101 106 minus H",..: 9 21 33 3 15 27 7 19 31 1 ...
## $ Field
                : Factor w/ 2 levels "CR", "RF": 1 1 1 2 2 2 1 1 1 2 ...
                : Factor w/ 6 levels "1","2","3","4",..: 1 2 3 1 2 3 1 2 3 1 ...
## $ Blk
## $ Topdress : Factor w/ 2 levels "N", "Y": 1 1 1 1 1 1 1 1 1 1 1 ...
## $ Stage : Factor w/ 4 levels "Heading", "Maturity", ..: 3 3 3 3 3 3 1 1 1 1 ...
## $ fertiliser_N: num [1:36] 48.5 37 41.9 46.7 36.2 ...
## $ soil_N : num [1:36] 54 49.6 60 71.4 62.1 ...
                : num [1:36] 47 47 47 47 47 47 81 81 81 81 ...
## $ Days
## $ Year : Factor w/ 2 levels "2021", "2022": 1 1 1 1 1 1 1 1 1 1 ...
## $ Days_graph : num [1:36] 46 46 46 46 46 46 81 81 81 81 ...
```

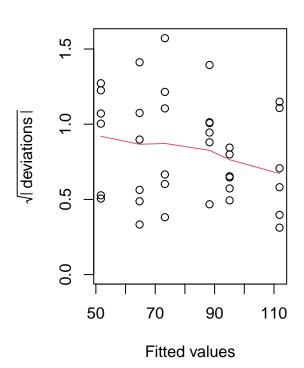
Model selection and testing

```
preplant_soil_N_model <- lm(soil_N~Field*Stage, data = preplant_no0)
pls205_diagnostics(preplant_soil_N_model)</pre>
```

Plot (EU) Normal Q-Q

Scale-Location





```
preplant_soil_N_means = emmeans(preplant_soil_N_model,spec = 'Field',by = 'Stage')
preplant_soil_N_effects = contrast(preplant_soil_N_means, method = 'pairwise', adjust = "tukey")
summary(preplant_soil_N_effects)
```

```
## Stage = Heading:
   contrast estimate
                       SE df t.ratio p.value
   CR - RF
               -15.0 5.22 30 -2.880 0.0073
##
## Stage = Maturity:
   contrast estimate
                       SE df t.ratio p.value
   CR - RF
               -16.8 5.22 30 -3.221 0.0031
##
## Stage = PI:
## contrast estimate
                       SE df t.ratio p.value
   CR - RF
               -13.0 5.22 30 -2.498 0.0182
```

cld(preplant_soil_N_means)

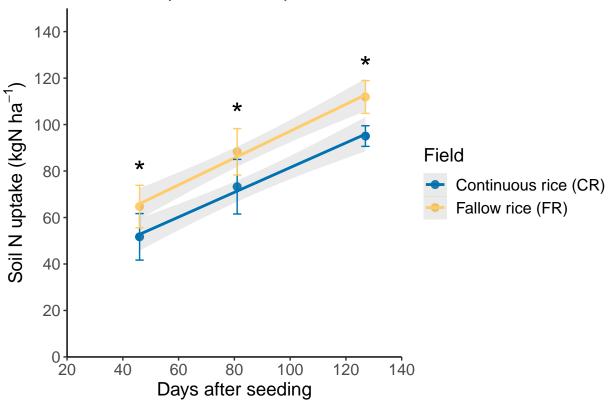
```
## Stage = Heading:
## Field emmean SE df lower.CL upper.CL .group
## CR 73.3 3.69 30 65.7 80.8 1
## RF 88.3 3.69 30 80.8 95.8 2
##
```

```
## Stage = Maturity:
## Field emmean
                 SE df lower.CL upper.CL .group
           95.0 3.69 30
                            87.5
                                    102.6 1
          111.9 3.69 30
                            104.3
                                     119.4
## RF
##
## Stage = PI:
                  SE df lower.CL upper.CL .group
## Field emmean
           51.7 3.69 30
## CR.
                            44.1
                                     59.2 1
## RF
           64.7 3.69 30
                             57.2
                                     72.3
##
## Confidence level used: 0.95
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
         then we cannot show them to be different.
##
##
         But we also did not show them to be the same.
```

Graphing preplant soil_N mean and SD

```
preplant_soilN_graphing <- preplant_no0 %>% group_by(Field, Days_graph) %>%
  mutate(soil_N_sd = sd(soil_N)) %>%
  summarise(soil_N = mean(soil_N),
            soil_N_sd = mean(soil_N_sd))
## 'summarise()' has grouped output by 'Field'. You can override using the
## '.groups' argument.
preplant_soil_N_graph <-</pre>
ggplot(preplant_no0, aes(x=Days_graph, y=soil_N, color=Field))+
  geom_point(data=preplant_soilN_graphing, size=2.5)+
  geom_smooth(method = lm, alpha=0.2)+
  scale color manual(values=c("#0072B2","#FFCC66"), name = "Field", labels = c("Continuous rice (CR)",
  scale_x_continuous(name="Days after seeding", limits = c(20, 140), expand = c(0, 0), breaks = seq(0,
  scale_y_continuous(name=expression("Soil N uptake (kgN ha"^{-1}*")"), limits = c(0, 150), expand = c(
  geom_errorbar(data=preplant_soilN_graphing, aes(ymin=soil_N-soil_N_soi, ymax=soil_N+soil_N_sd), width=
  #geom_vline(xintercept = c(41, 50, 78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme_classic()+theme(axis.text = element_text(size = 12), axis.title = element_text(size=14))+
  theme(legend.text = element text(size = 12), legend.title = element text(size = 14))+
  ggtitle("Preplant soil N uptake")+
  theme(plot.title = element_text(hjust = 0.5, size = 15))+
  annotate(
  "text",
  x = c(46,81, 127), # X-axis positions for annotations
  y = c(75,100, 120), # Y-axis positions for annotations
  label = "*",
  size = 8.
  vjust = 0 # Adjust vertical position of asterisks
preplant_soil_N_graph
```





```
ggsave(preplant_soil_N_graph, filename = "preplant_soil_N_graph.png", height = 15, width = 20, units =
## 'geom_smooth()' using formula = 'y ~ x'
```

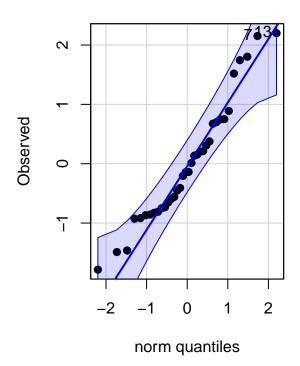
Preplant Fert N

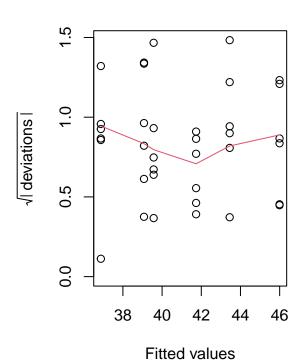
Model selection and testing

```
preplant_fert_N_model_huehue <- lm(fertiliser_N~Field*Stage+Year, data = preplant_no0)
preplant_fert_N_model <- lm(fertiliser_N~Field*Stage, data = preplant_no0)
pls205_diagnostics(preplant_fert_N_model)</pre>
```

Plot (EU) Normal Q-Q

Scale-Location





```
preplant_fert_N_means = emmeans(preplant_fert_N_model,spec = 'Field',by = 'Stage')
preplant_fert_N_effects = contrast(preplant_fert_N_means, method = 'pairwise', adjust = "tukey")
summary(preplant_fert_N_effects)
```

```
## Stage = Heading:
## contrast estimate SE df t.ratio p.value
## CR - RF     0.492 4.2 30     0.117     0.9074
##
## Stage = Maturity:
## contrast estimate SE df t.ratio p.value
## CR - RF     -2.543 4.2 30     -0.606     0.5492
##
## Stage = PI:
## contrast estimate SE df t.ratio p.value
## CR - RF     -4.860 4.2 30     -1.158     0.2561
```

cld(preplant_fert_N_means)

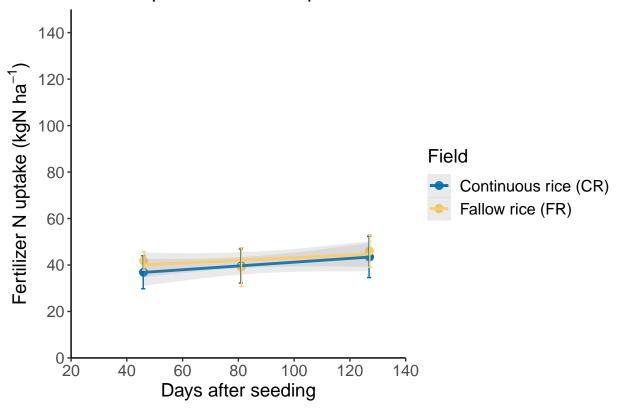
```
## Stage = Heading:
## Field emmean SE df lower.CL upper.CL .group
## RF 39.1 2.97 30 33.0 45.1 1
## CR 39.6 2.97 30 33.5 45.6 1
##
```

```
## Stage = Maturity:
                 SE df lower.CL upper.CL .group
## Field emmean
           43.5 2.97 30
                            37.4
                                     49.5 1
            46.0 2.97 30
                             39.9
                                      52.1 1
## RF
##
## Stage = PI:
## Field emmean
                  SE df lower.CL upper.CL .group
                                      42.9 1
                            30.8
## CR
           36.9 2.97 30
## R.F
           41.7 2.97 30
                             35.7
                                      47.8 1
##
## Confidence level used: 0.95
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
         then we cannot show them to be different.
##
##
         But we also did not show them to be the same.
```

Graphing preplant fert_N mean and SD

```
preplant_fertiliser_N_graphing <- preplant_no0 %>% group_by(Field, Days_graph) %>%
  mutate(fertiliser_N_sd = sd(fertiliser_N)) %>%
  summarise(fertiliser N = mean(fertiliser N),
            fertiliser_N_sd = mean(fertiliser_N_sd))
## 'summarise()' has grouped output by 'Field'. You can override using the
## '.groups' argument.
preplant_fertiliser_N_graph <-</pre>
ggplot(preplant_no0, aes(x=Days_graph, y=fertiliser_N, color=Field))+
  geom_point(data=preplant_fertiliser_N_graphing, size=2.5)+
  geom_smooth(method = lm, alpha=0.2)+
  scale color manual(values=c("#0072B2","#FFCC66"), name = "Field", labels = c("Continuous rice (CR)",
  scale_x_continuous(name="Days after seeding", limits = c(20, 140), expand = c(0, 0), breaks = seq(0,
  scale y continuous(name=expression("Fertilizer N uptake (kgN ha"^{-1}*")"), limits = c(0, 150), expan
  geom_errorbar(data=preplant_fertiliser_N_graphing, aes(ymin=fertiliser_N-fertiliser_N_sd, ymax=fertil
  #geom_vline(xintercept = c(41, 50, 78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme classic()+
  theme(axis.text = element_text(size = 12), axis.title = element_text(size=14))+
  theme(legend.text = element_text(size = 12),legend.title = element_text(size = 14))+
  ggtitle("Preplant fertilizer N uptake")+
  theme(plot.title = element_text(hjust = 0.5, size = 15))
preplant_fertiliser_N_graph
```

Preplant fertilizer N uptake



```
ggsave(preplant_fertiliser_N_graph, filename = "preplant_fertiliser_N_graph.png", height = 15, width = "
## 'geom_smooth()' using formula = 'y ~ x'
```

Topdress Soil N

Remove day 0

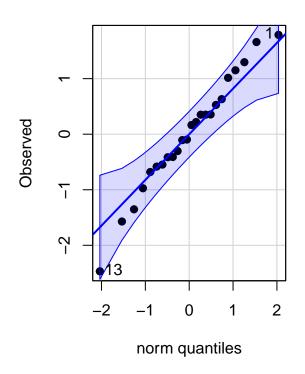
```
topdress_no0 <-subset(topdress, Days != 0)</pre>
str(topdress_no0)
## tibble [24 x 10] (S3: tbl_df/tbl/data.frame)
    $ Sample.ID
                  : Factor w/ 72 levels "101 106 minus H",..: 10 22 34 4 16 28 11 23 35 5 ...
                  : Factor w/ 2 levels "CR", "RF": 1 1 1 2 2 2 1 1 1 2 ...
    $ Field
##
                  : Factor w/ 6 levels "1","2","3","4",...: 1 2 3 1 2 3 1 2 3 1 ...
                  : Factor w/ 2 levels "N", "Y": 2 2 2 2 2 2 2 2 2 2 ...
##
    $ Topdress
                  : Factor w/ 4 levels "Heading", "Maturity", ...: 1 1 1 1 1 1 2 2 2 2 ...
    $ fertiliser_N: num [1:24] 10.99 9.79 9.19 9.3 10.05 ...
##
    $ soil N
                  : num [1:24] 71.9 65.8 59.8 80.9 73.6 ...
                  : num [1:24] 81 81 81 81 81 81 124 124 124 124 ...
##
   $ Days
    $ Year
                  : Factor w/ 2 levels "2021", "2022": 1 1 1 1 1 1 1 1 1 1 ...
    $ Days_graph : num [1:24] 81 81 81 81 81 81 127 127 127 127 ...
```

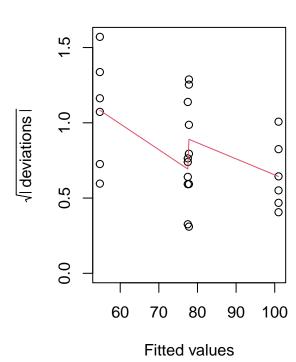
Model selection and testing

```
topdress_soil_N_model <- lm(soil_N~Field*Stage, data = topdress_no0)
pls205_diagnostics(topdress_soil_N_model)</pre>
```

Plot (EU) Normal Q-Q

Scale-Location





```
topdress_soil_N_means = emmeans(topdress_soil_N_model,spec = 'Field',by = 'Stage')
topdress_soil_N_effects = contrast(topdress_soil_N_means, method = 'pairwise', adjust = "tukey")
summary(topdress_soil_N_effects)
```

```
cld(topdress_soil_N_means)
```

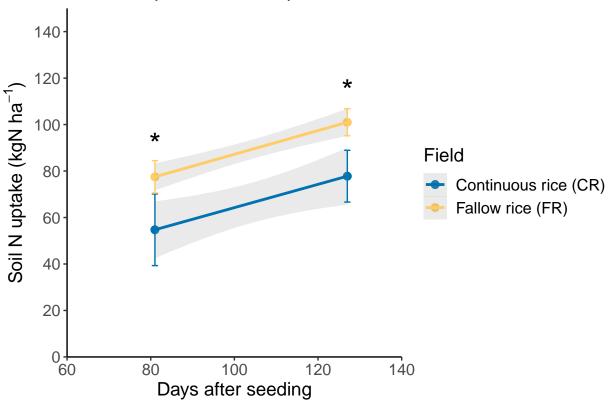
```
## Stage = Heading:
## Field emmean SE df lower.CL upper.CL .group
```

```
## CR
           54.7 4.3 20
                           45.8
                                    63.7 1
## R.F
           77.5 4.3 20
                           68.5
                                    86.5
##
## Stage = Maturity:
## Field emmean SE df lower.CL upper.CL .group
          77.8 4.3 20
                           68.8
## CR
                                   86.8 1
## RF
          101.0 4.3 20
                           92.0
                                   110.0
##
## Confidence level used: 0.95
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
        then we cannot show them to be different.
##
##
        But we also did not show them to be the same.
```

Graphing topdress soil_N mean and SD

```
topdress_soil_N_graphing <- topdress_no0 %>% group_by(Field, Days_graph) %>%
 mutate(soil_N_sd = sd(soil_N)) %>%
  summarise(soil_N = mean(soil_N),
            soil_N_sd = mean(soil_N_sd))
## 'summarise()' has grouped output by 'Field'. You can override using the
## '.groups' argument.
topdress_soil_N_graph <-</pre>
ggplot(topdress_no0, aes(x=Days_graph, y=soil_N, color=Field))+
  geom_point(data=topdress_soil_N_graphing, size=2.5)+
  geom_smooth(method = lm, alpha=0.2)+
  scale_color_manual(values=c("#0072B2","#FFCC66"), name = "Field", labels = c("Continuous rice (CR)",
  scale_x_continuous(name="Days after seeding", limits = c(60, 140), expand = c(0, 0), breaks = seq(0,
  scale_y_continuous(name=expression("Soil N uptake (kgN ha"^{-1}*")"), limits = c(0, 150), expand = c(
  geom_errorbar(data=topdress_soil_N_graphing, aes(ymin=soil_N-soil_N_sd, ymax=soil_N+soil_N_sd), width
  \#geom\_vline(xintercept = c(78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme classic()+
  theme(axis.text = element_text(size = 12), axis.title = element_text(size=14))+
  theme(legend.text = element_text(size = 12),legend.title = element_text(size = 14))+
  ggtitle("Topdress soil N uptake")+
  theme(plot.title = element_text(hjust = 0.5, size = 15))+
  annotate(
  "text",
  x = c(81, 127), \# X-axis positions for annotations
  y = c(87, 110), # Y-axis positions for annotations
  label = "*",
  size = 8,
  vjust = 0 # Adjust vertical position of asterisks
topdress_soil_N_graph
```





```
ggsave(topdress_soil_N_graph, filename = "topdress_soil_N_graph.png", height = 15, width = 20, units =
## 'geom_smooth()' using formula = 'y ~ x'
```

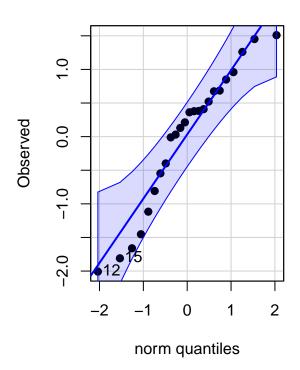
Topdress Fert N

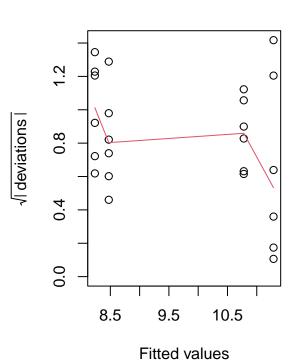
Model selection and testing

```
topdress_fert_N_model <- lm(fertiliser_N~Field*Stage, data = topdress_no0)
pls205_diagnostics(topdress_fert_N_model)</pre>
```

Plot (EU) Normal Q-Q

Scale-Location





```
topdress_fert_N_means = emmeans(topdress_fert_N_model,spec = 'Field',by = 'Stage')
topdress_fert_N_effects = contrast(topdress_fert_N_means, method = 'pairwise', adjust = "tukey")
summary(topdress_fert_N_effects)
```

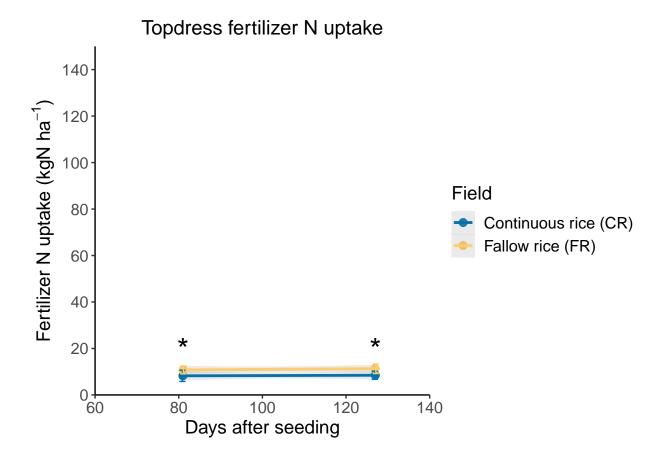
cld(topdress_fert_N_means)

```
## Stage = Heading:
##
   Field emmean
                    SE df lower.CL upper.CL .group
##
            8.24 0.817 20
                              6.53
                                       9.94 1
##
   RF
           10.78 0.817 20
                              9.07
                                      12.48
##
## Stage = Maturity:
  Field emmean
                    SE df lower.CL upper.CL .group
   CR
            8.47 0.817 20
                              6.77
                                      10.18 1
##
           11.29 0.817 20
## RF
                              9.58
                                      12.99
```

```
##
## Confidence level used: 0.95
## significance level used: alpha = 0.05
## NOTE: If two or more means share the same grouping symbol,
         then we cannot show them to be different.
##
         But we also did not show them to be the same.
```

Graphing topdress fert N mean and SD

```
topdress_fertiliser_N_graphing <- topdress_no0 %>% group_by(Field, Days_graph) %>%
  mutate(fertiliser_N_sd = sd(fertiliser_N)) %>%
  summarise(fertiliser_N = mean(fertiliser_N),
            fertiliser_N_sd = mean(fertiliser_N_sd))
## 'summarise()' has grouped output by 'Field'. You can override using the
## '.groups' argument.
topdress_fertiliser_N_graph <-</pre>
ggplot(topdress_no0, aes(x=Days_graph, y=fertiliser_N, color=Field))+
  geom_point(data=topdress_fertiliser_N_graphing, size=2.5)+
  geom_smooth(method = lm, alpha=0.2)+
  scale_color_manual(values=c("#0072B2","#FFCC66"), name = "Field", labels = c("Continuous rice (CR)",
  scale_x_continuous(name="Days after seeding", limits = c(60, 140), expand = c(0, 0), breaks = seq(0,
  scale_y_continuous(name=expression("Fertilizer N uptake (kgN ha"^{-1}*")"), limits = c(0, 150), expan
  geom_errorbar(data=topdress_fertiliser_N_graphing, aes(ymin=fertiliser_N-fertiliser_N_sd, ymax=fertil
  \#geom\_vline(xintercept = c(78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme_classic()+
  theme(axis.text = element_text(size = 12), axis.title = element_text(size=14))+
  theme(legend.text = element_text(size = 12),legend.title = element_text(size = 14))+
  ggtitle("Topdress fertilizer N uptake")+
  theme(plot.title = element_text(hjust = 0.5, size = 15))+
  annotate(
  "text",
  x = c(81, 127), \# X-axis positions for annotations
  y = c(15, 15), # Y-axis positions for annotations
  label = "*",
  size = 8.
  vjust = 0 # Adjust vertical position of asterisks
topdress_fertiliser_N_graph
```



ggsave(topdress_fertiliser_N_graph, filename = "topdress_fertiliser_N_graph.png", height = 15, width = 1