

N Uptake overtime

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Necessary libraries

Read and check data

```
#read data
master = read_excel('N_uptake_over_time.xlsx', sheet = 1)

master$Days <- as.numeric(master$Days)

master <- master %>% mutate_if(is.character, as.factor)
master$Nrate_kgha_F <- as.factor(master$Nrate_kgha)

table(master$Stage)
```

```
##
## Heading Maturity      PI
##      24      24      24
```

```
table(master$Treatment)
```

```
##
## CR FR
## 36 36
```

```
table(master$Blk)
```

```
##
## 1 2 3 4 5 6
## 12 12 12 12 12 12
```

```
table(master$Year)
```

```
##
## 2021 2022
## 36 36
```

```
#dataset looks good and complete so lets have some fun huehuehue
str(master)
```

```
## tibble [72 x 11] (S3: tbl_df/tbl/data.frame)
## $ SampleID      : Factor w/ 72 levels "403-minus-H",...: 37 49 64 46 58 70 40 52 61 43 ...
## $ Plot          : Factor w/ 24 levels "104","105","109",...: 1 5 10 4 8 12 2 6 9 3 ...
## $ Stage         : Factor w/ 3 levels "Heading","Maturity",...: 3 3 3 3 3 3 3 3 3 3 ...
## $ Treatment     : Factor w/ 2 levels "CR","FR": 2 2 2 1 1 1 2 2 2 1 ...
## $ Year          : num [1:72] 2021 2021 2021 2021 2021 2021 ...
## $ Topdress      : Factor w/ 1 level "0": 1 1 1 1 1 1 1 1 1 1 ...
## $ N_total_kgha  : num [1:72] 45 54.1 51.7 35.8 38 ...
## $ Blk           : Factor w/ 6 levels "1","2","3","4",...: 1 2 3 1 2 3 1 2 3 1 ...
## $ Days          : num [1:72] 47 47 47 47 47 47 47 47 47 47 ...
## $ Nrate_kgha    : num [1:72] 0 0 0 0 0 0 185 185 185 185 ...
## $ Nrate_kgha_F  : Factor w/ 2 levels "0","185": 1 1 1 1 1 1 2 2 2 2 ...
```

Split by Nrate

```
N_zero <- master %>% filter(Nrate_kgha == 0)
N_zero_average <- N_zero
N_zero_2021 <- N_zero %>% filter(Year ==2021)
N_zero_2022 <- N_zero %>% filter(Year ==2022)

N_185 <-master %>% filter(Nrate_kgha == 185)
N_185_average <- N_185
N_185_2021 <- N_185 %>% filter(Year ==2021)
N_185_2022 <- N_185 %>% filter(Year ==2022)
```

0N

Pooled_zero (Figure 4)

Pairise Comparisons average

```
N_zero_model_average <- lmer(N_total_kgha~Treatment*Stage*Year+(1|Blk:Treatment)+(1|Blk), data = N_zero.
```

```
## Warning: Some predictor variables are on very different scales: consider  
## rescaling
```

```
## boundary (singular) fit: see help('isSingular')
```

```
## Warning: Some predictor variables are on very different scales: consider  
## rescaling
```

```
anova(N_zero_model_average)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method  
##  
##           Sum Sq Mean Sq NumDF   DenDF F value    Pr(>F)  
## Treatment      16.32   16.321     1   8.9804   0.4878 0.50257  
## Stage        384.98  192.491     2  11.7612   5.7539 0.01809 *  
## Year           4.85    4.845     1   7.9968   0.1448 0.71343  
## Treatment:Stage  1.62    0.810     2  14.8449   0.0242 0.97610  
## Treatment:Year   11.44   11.439     1   8.0245   0.3419 0.57478  
## Stage:Year      381.77  190.885     2  16.1494   5.7059 0.01335 *  
## Treatment:Stage:Year 1.63    0.814     2  14.8432   0.0243 0.97601  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(N_zero_model_average)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: N_total_kgha ~ Treatment * Stage * Year + (1 | Blk:Treatment) +  
##           (1 | Blk)  
## Data: N_zero_average  
##  
## REML criterion at convergence: 182.6  
##  
## Scaled residuals:  
##      Min       1Q   Median       3Q      Max   
## -2.1504 -0.3816  0.0217  0.4700  1.8066   
##  
## Random effects:  
## Groups          Name          Variance Std.Dev.  
## Blk:Treatment (Intercept) 83.16     9.119  
## Blk           (Intercept)  0.00     0.000  
## Residual                33.45     5.784  
## Number of obs: 36, groups: Blk:Treatment, 12; Blk, 6
```

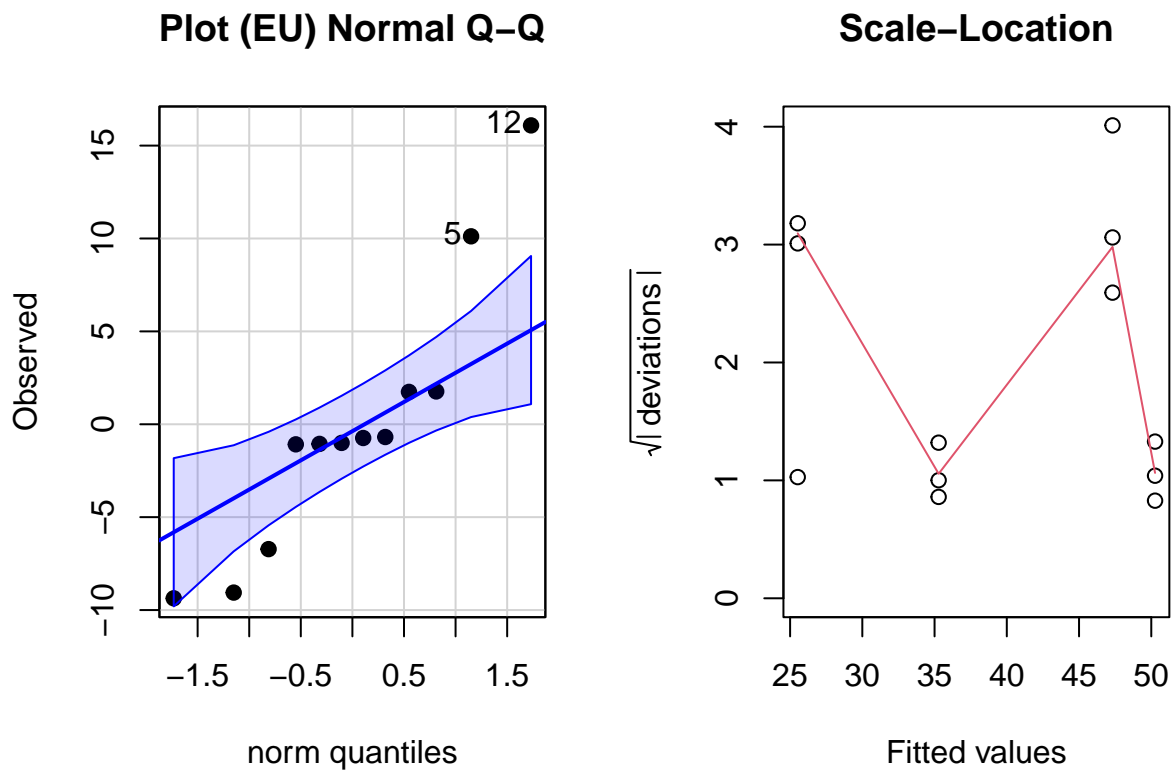
```

##
## Fixed effects:
##
##               Estimate Std. Error      df t value
## (Intercept)    4.054e+02  1.782e+04  1.192e+01  0.023
## TreatmentFR   -1.387e+04  2.521e+04  1.189e+01 -0.550
## StageMaturity -1.349e+04  1.350e+04  1.593e+01 -0.999
## StagePI        1.934e+04  1.350e+04  1.595e+01  1.432
## Year          -1.737e-01  8.817e+00  1.192e+01 -0.020
## TreatmentFR:StageMaturity  1.965e+03  1.909e+04  1.597e+01  0.103
## TreatmentFR:StagePI    1.252e+02  1.909e+04  1.598e+01  0.007
## TreatmentFR:Year      6.870e+00  1.247e+01  1.189e+01  0.551
## StageMaturity:Year    6.676e+00  6.679e+00  1.593e+01  1.000
## StagePI:Year        -9.579e+00  6.679e+00  1.595e+01 -1.434
## TreatmentFR:StageMaturity:Year -9.669e-01  9.445e+00  1.597e+01 -0.102
## TreatmentFR:StagePI:Year -6.178e-02  9.445e+00  1.598e+01 -0.007
##
##               Pr(>|t|)
## (Intercept)    0.982
## TreatmentFR    0.592
## StageMaturity  0.333
## StagePI        0.171
## Year           0.985
## TreatmentFR:StageMaturity  0.919
## TreatmentFR:StagePI    0.995
## TreatmentFR:Year      0.592
## StageMaturity:Year    0.332
## StagePI:Year        0.171
## TreatmentFR:StageMaturity:Year  0.920
## TreatmentFR:StagePI:Year  0.995
##
## Correlation of Fixed Effects:
##      (Intr) TrtmFR StgMtr StagPI Year   TrFR:SM TrFR:SPI TrFR:Y StgM:Y
## TreatmentFR -0.707
## StageMatrty -0.379  0.268
## StagePI     -0.379  0.268  0.500
## Year        -1.000  0.707  0.379  0.379
## TrtmntFR:SM  0.268 -0.379 -0.707 -0.354 -0.268
## TrtmnFR:SPI  0.268 -0.379 -0.354 -0.707 -0.268  0.500
## TrtmntFR:Yr  0.707 -1.000 -0.268 -0.268 -0.707  0.379  0.379
## StgMtrty:Yr  0.379 -0.268 -1.000 -0.500 -0.379  0.707  0.354  0.268
## StagePI:Yer  0.379 -0.268 -0.500 -1.000 -0.379  0.354  0.707  0.268  0.500
## TrtmFR:SM:Y -0.268  0.379  0.707  0.354  0.268 -1.000 -0.500 -0.379 -0.707
## TrtFR:SPI:Y -0.268  0.379  0.354  0.707  0.268 -0.500 -1.000 -0.379 -0.354
##
##      StPI:Y TFR:SM:
## TreatmentFR
## StageMatrty
## StagePI
## Year
## TrtmntFR:SM
## TrtmnFR:SPI
## TrtmntFR:Yr
## StgMtrty:Yr
## StagePI:Yer
## TrtmFR:SM:Y -0.354
## TrtFR:SPI:Y -0.707  0.500

```

```
## fit warnings:
## Some predictor variables are on very different scales: consider rescaling
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
pls205_diagnostics(N_zero_model_average, EU="Blk:Treatment")
```



```
N_zero_means_average = emmeans(N_zero_model_average, spec = 'Treatment', by = 'Stage')
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
N_zero_effects_average = contrast(N_zero_means_average, method = 'pairwise', adjust = "tukey")
summary(N_zero_effects_average)
```

```
## Stage = Heading:
## contrast estimate SE df t.ratio p.value
## CR - FR -18.0 6.23 6.03 -2.892 0.0274
##
## Stage = Maturity:
## contrast estimate SE df t.ratio p.value
## CR - FR -28.6 6.23 6.03 -4.592 0.0037
##
## Stage = PI:
## contrast estimate SE df t.ratio p.value
```

```
## CR - FR      -18.4 6.23 6.03 -2.949 0.0255
##
## Results are averaged over the levels of: Year
## Degrees-of-freedom method: kenward-roger
```

```
N_zero_effects_average_summary <-
  as.data.frame(summary(N_zero_effects_average)) %>%
  mutate(
    p_value = case_when(
      p.value < 0.05 ~ "*",          # For p-values less than 0.05
      TRUE ~ sprintf("%.2f", p.value) # Force two decimal places for all other p-values
    )
  ) %>%
  mutate(Days = case_when(
    Stage %in% c("PI") ~ "47",
    Stage %in% c("Heading") ~ "82",
    Stage %in% c("Maturity") ~ "134",
    TRUE ~ "Other" # This line handles cases where plot is not listed
  )) %>%
  mutate(Days = as.numeric(Days))

cld(N_zero_means_average)
```

```
## Stage = Heading:
## Treatment emmean SE df lower.CL upper.CL .group
## CR          54.3 4.41 11.9 44.7 63.9 1
## FR          72.3 4.41 11.9 62.7 81.9 2
##
## Stage = Maturity:
## Treatment emmean SE df lower.CL upper.CL .group
## CR          63.2 4.41 11.9 53.6 72.8 1
## FR          91.8 4.41 11.9 82.2 101.4 2
##
## Stage = PI:
## Treatment emmean SE df lower.CL upper.CL .group
## CR          30.4 4.41 11.9 20.8 40.0 1
## FR          48.8 4.41 11.9 39.2 58.4 2
##
## Results are averaged over the levels of: Year
## Degrees-of-freedom method: kenward-roger
## 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.
```

Graph_average

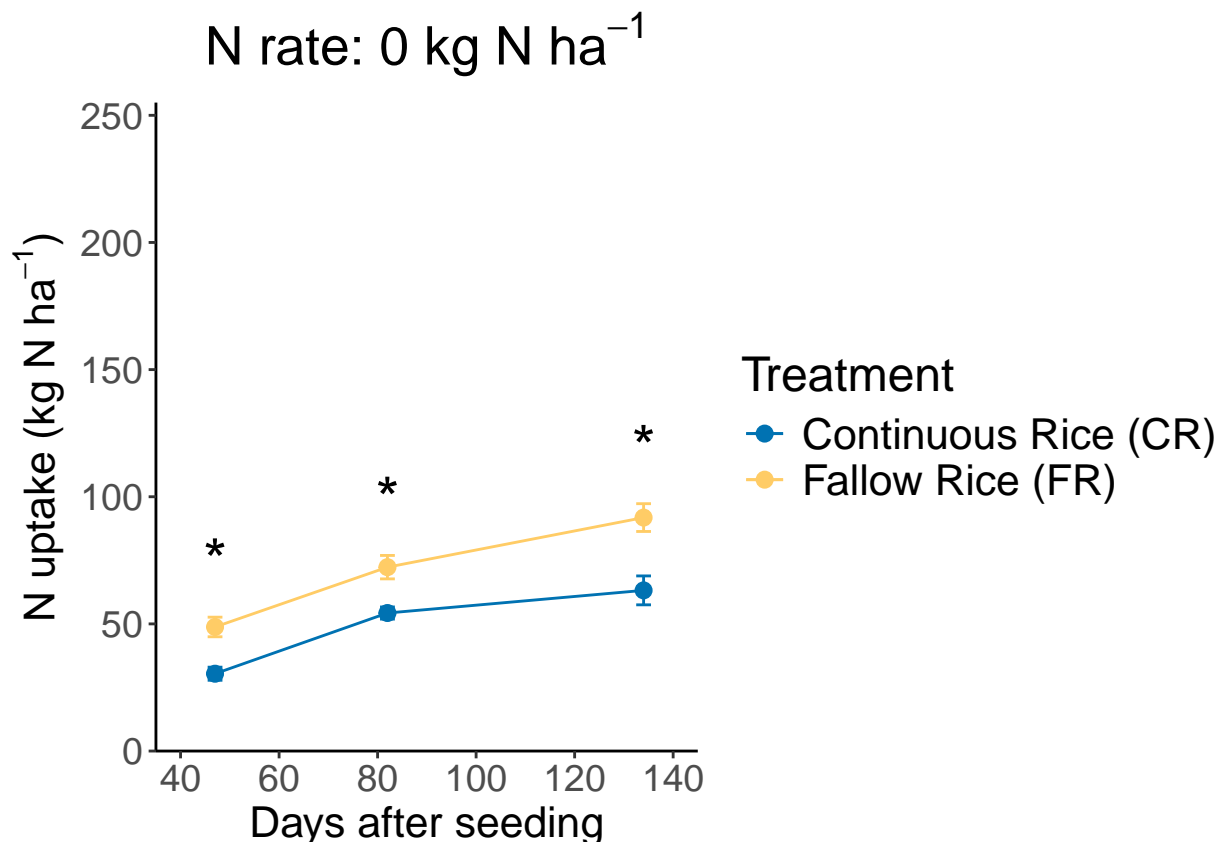
```
N_zero_graphing_average <- N_zero_average %>%
  group_by(Treatment, Days) %>%
  mutate(N_total_kgha_se = sd(N_total_kgha)/sqrt(6)) %>%
```

```
summarise(N_total_kgha = mean(N_total_kgha), N_total_kgha_se = mean(N_total_kgha_se)),
left_join(N_zero_effects_average_summary %>% select(Days, p_value), by = "Days")
```

'summarise()' has grouped output by 'Treatment'. You can override using the
'.groups' argument.

```
N_zero_graph_average <-
ggplot(N_zero_average, aes(x=Days, y=N_total_kgha, color=Treatment))+
  geom_point(data=N_zero_graphing_average, size=2.5)+
  geom_line(data=N_zero_graphing_average)+
  scale_color_manual(values=c("#0072B2", "#FFCC66"), labels = c("Continuous Rice (CR)", "Fallow Rice (FR)"),
  scale_x_continuous(name="Days after seeding", limits = c(35, 145), expand = c(0, 0), breaks = seq(0, 145, 20)),
  scale_y_continuous(name=expression("N uptake (kg N ha"^-1)*"), limits = c(0, 255), expand = c(0, 0)),
  geom_errorbar(data=N_zero_graphing_average, aes(ymin=N_total_kgha-N_total_kgha_se, ymax=N_total_kgha+N_total_kgha_se),
  #geom_vline(xintercept = c(41, 50, 78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme_classic()+
  theme(axis.text = element_text(size = 14), axis.title = element_text(size=16))+
  theme(legend.text = element_text(size = 16), legend.title = element_text(size = 18))+
  theme(plot.title = element_text(hjust = 0.5, size = 20, face = "bold"))+
  geom_text(data=N_zero_graphing_average %>% filter(Treatment == "FR"), aes(x=Days, y=N_total_kgha+N_total_kgha_se,
  label=sprintf(p_value)), size=8, vjust=-0.5, color="black")+
  ggtitle(expression("N rate: 0 kg N ha"^-1)*")
```

N_zero_graph_average



185N

Pooled_185 (Figure 4)

Pairise Comparisons average

```
N_185_model_average <- lmer(N_total_kgha~Treatment*Stage*Year+(1|Blk:Treatment)+(1|Blk), data = N_185_a
```

```
## Warning: Some predictor variables are on very different scales: consider  
## rescaling
```

```
## Warning: Some predictor variables are on very different scales: consider  
## rescaling
```

```
anova(N_185_model_average)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method  
##               Sum Sq Mean Sq NumDF    DenDF F value    Pr(>F)  
## Treatment      1153.90  1153.90     1   7.1358   5.1355 0.05710 .  
## Stage          2192.99  1096.49     2  12.4098   4.8800 0.02731 *  
## Year            258.73   258.73     1   4.0014   1.1515 0.34364  
## Treatment:Stage    669.73   334.87     2  17.6678   1.4904 0.25228  
## Treatment:Year    1253.89  1253.89     1   4.0847   5.5805 0.07612 .  
## Stage:Year         847.05   423.53     2  15.9844   1.8849 0.18408  
## Treatment:Stage:Year 670.06   335.03     2  17.6676   1.4911 0.25213  
## ---  
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(N_185_model_average)
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: N_total_kgha ~ Treatment * Stage * Year + (1 | Blk:Treatment) +  
##      (1 | Blk)  
##      Data: N_185_average  
##  
## REML criterion at convergence: 219.4  
##  
## Scaled residuals:  
##      Min       1Q   Median       3Q      Max  
## -1.17772 -0.49840  0.02605  0.46417  2.14576  
##  
## Random effects:  
##      Groups      Name      Variance Std.Dev.  
## Blk:Treatment (Intercept)  46.42     6.813  
## Blk          (Intercept) 116.44    10.791  
## Residual                224.69    14.990  
## Number of obs: 36, groups: Blk:Treatment, 12; Blk, 6  
##  
## Fixed effects:
```



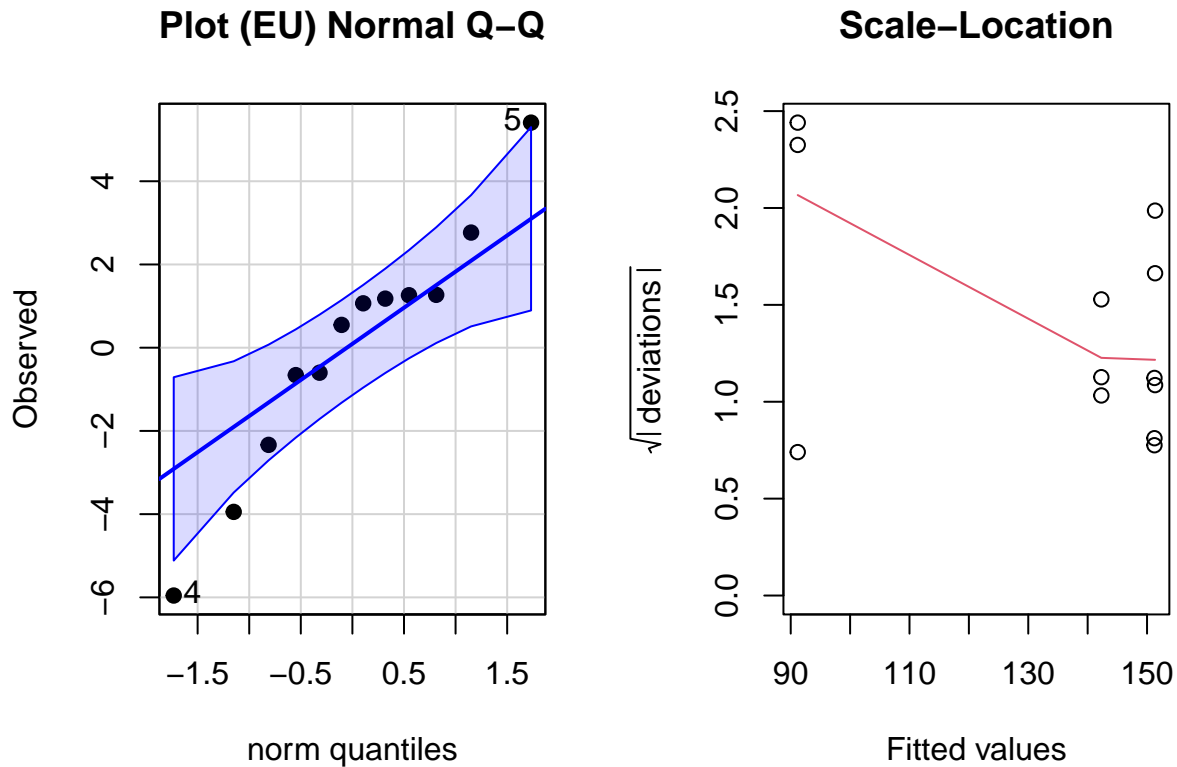
```

##               Estimate Std. Error      df t value
## (Intercept)      30043.353   32493.255    14.793    0.925
## TreatmentFR      -40406.266   38433.832    14.219   -1.051
## StageMaturity    -4416.434   34989.275    16.097   -0.126
## StagePI          73416.715   34989.275    16.077    2.098
## Year             -14.778     16.074    14.793   -0.919
## TreatmentFR:StageMaturity    7575.236   49482.306    16.120    0.153
## TreatmentFR:StagePI    -63111.188   49482.307    16.131   -1.275
## TreatmentFR:Year         20.002     19.013    14.219    1.052
## StageMaturity:Year         2.192     17.309    16.097    0.127
## StagePI:Year        -36.344     17.309    16.077   -2.100
## TreatmentFR:StageMaturity:Year    -3.744     24.478    16.120   -0.153
## TreatmentFR:StagePI:Year     31.223     24.478    16.131    1.276
##               Pr(>|t|)
## (Intercept)         0.3700
## TreatmentFR         0.3107
## StageMaturity       0.9011
## StagePI             0.0520 .
## Year                0.3726
## TreatmentFR:StageMaturity    0.8802
## TreatmentFR:StagePI    0.2202
## TreatmentFR:Year       0.3103
## StageMaturity:Year     0.9008
## StagePI:Year          0.0519 .
## TreatmentFR:StageMaturity:Year    0.8803
## TreatmentFR:StagePI:Year    0.2202
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) TrtmFR StgMtr StagPI Year   TrFR:SM TrFR:SPI TrFR:Y StgM:Y
## TreatmentFR -0.591
## StageMatrty -0.538  0.455
## StagePI     -0.538  0.455  0.500
## Year        -1.000  0.591  0.538  0.538
## TrtmntFR:SM  0.381 -0.644 -0.707 -0.354 -0.381
## TrtmnFR:SPI  0.381 -0.644 -0.354 -0.707 -0.381  0.500
## TrtmntFR:Yr  0.591 -1.000 -0.455 -0.455 -0.591  0.644  0.644
## StgMtrty:Yr  0.538 -0.455 -1.000 -0.500 -0.538  0.707  0.354  0.455
## StagePI:Yer  0.538 -0.455 -0.500 -1.000 -0.538  0.354  0.707  0.455  0.500
## TrtmFR:SM:Y -0.381  0.644  0.707  0.354  0.381 -1.000 -0.500 -0.644 -0.707
## TrtFR:SPI:Y -0.381  0.644  0.354  0.707  0.381 -0.500 -1.000 -0.644 -0.354
##      StPI:Y TFR:SM:
## TreatmentFR
## StageMatrty
## StagePI
## Year
## TrtmntFR:SM
## TrtmnFR:SPI
## TrtmntFR:Yr
## StgMtrty:Yr
## StagePI:Yer
## TrtmFR:SM:Y -0.354
## TrtFR:SPI:Y -0.707  0.500

```

```
## fit warnings:
## Some predictor variables are on very different scales: consider rescaling
```

```
pls205_diagnostics(N_185_model_average, EU="Blk:Treatment")
```



```
N_185_means_average = emmeans(N_185_model_average, spec = 'Treatment', by = 'Stage')
```

```
## NOTE: Results may be misleading due to involvement in interactions
```

```
N_185_effects_average = contrast(N_185_means_average, method = 'pairwise', adjust = "tukey")
summary(N_185_effects_average)
```

```
## Stage = Heading:
## contrast estimate SE df t.ratio p.value
## CR - FR -28.0 9.51 14.5 -2.947 0.0103
##
## Stage = Maturity:
## contrast estimate SE df t.ratio p.value
## CR - FR -35.3 9.51 14.5 -3.711 0.0022
##
## Stage = PI:
## contrast estimate SE df t.ratio p.value
## CR - FR -34.6 9.51 14.5 -3.635 0.0026
##
```

```
## Results are averaged over the levels of: Year
## Degrees-of-freedom method: kenward-roger
```

```
N_185_effects_average_summary <-
  as.data.frame(summary(N_185_effects_average)) %>%
  mutate(
    p_value = case_when(
      p.value < 0.05 ~ "*",          # For p-values less than 0.05
      TRUE ~ sprintf("%.2f", p.value) # Force two decimal places for all other p-values
    )
  ) %>%
  mutate(Days = case_when(
    Stage %in% c("PI") ~ "47",
    Stage %in% c("Heading") ~ "82",
    Stage %in% c("Maturity") ~ "134",
    TRUE ~ "Other" # This line handles cases where plot is not listed
  )) %>%
  mutate(Days = as.numeric(Days))

cld(N_185_means_average)
```

```
## Stage = Heading:
## Treatment emmean SE df lower.CL upper.CL .group
## CR 169 8.04 14.8 151.5 186 1
## FR 197 8.04 14.8 179.5 214 2
##
## Stage = Maturity:
## Treatment emmean SE df lower.CL upper.CL .group
## CR 183 8.04 14.8 165.9 200 1
## FR 218 8.04 14.8 201.2 235 2
##
## Stage = PI:
## Treatment emmean SE df lower.CL upper.CL .group
## CR 117 8.04 14.8 99.6 134 1
## FR 151 8.04 14.8 134.1 168 2
##
## Results are averaged over the levels of: Year
## Degrees-of-freedom method: kenward-roger
## 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.
```

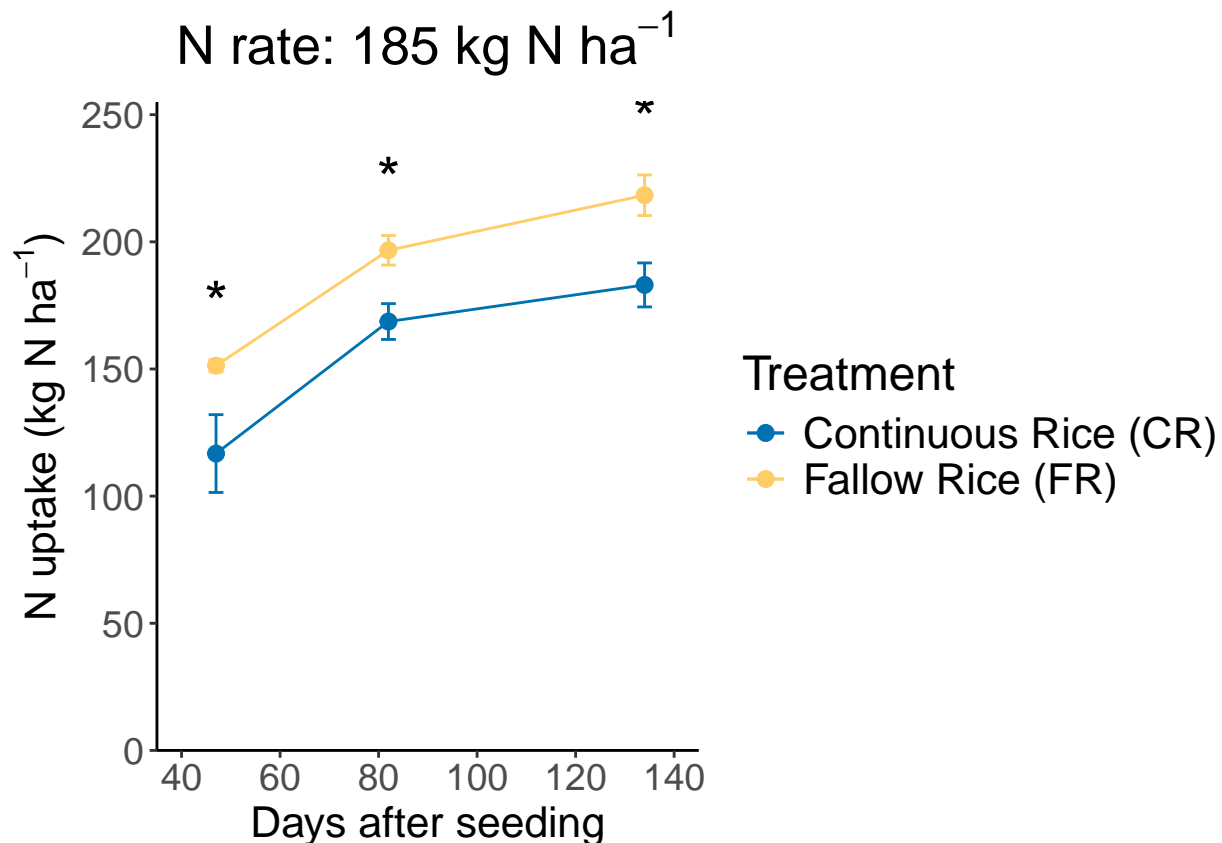
Graph_average

```
N_185_graphing_average <- N_185_average %>%
  group_by(Treatment, Days) %>%
  mutate(N_total_kgha_se = sd(N_total_kgha)/sqrt(6)) %>%
  summarise(N_total_kgha = mean(N_total_kgha), N_total_kgha_se = mean(N_total_kgha_se))
  left_join(N_185_effects_average_summary %>% select(Days, p_value), by = "Days")
```

'summarise()' has grouped output by 'Treatment'. You can override using the
'.groups' argument.

```
N_185_graph_average <-
ggplot(N_185_average, aes(x=Days, y=N_total_kgha, color=Treatment))+
  geom_point(data=N_185_graphing_average, size=2.5)+
  geom_line(data=N_185_graphing_average)+
  scale_color_manual(values=c("#0072B2", "#FFCC66"), labels = c("Continuous Rice (CR)", "Fallow Rice (FR)"))+
  scale_x_continuous(name="Days after seeding", limits = c(35, 145), expand = c(0, 0), breaks = seq(0, 145, 20))+
  scale_y_continuous(name=expression("N uptake (kg N ha"^-1)*"), limits = c(0, 255), expand = c(0, 0))+
  geom_errorbar(data=N_185_graphing_average, aes(ymin=N_total_kgha-N_total_kgha_se, ymax=N_total_kgha+N_total_kgha_se),
  #geom_vline(xintercept = c(41, 50, 78, 84, 121, 136), linetype = "dashed", color = "black") +
  theme_classic()+
  theme(axis.text = element_text(size = 14), axis.title = element_text(size=16))+
  theme(legend.text = element_text(size = 16), legend.title = element_text(size = 18))+
  theme(plot.title = element_text(hjust = 0.5, size = 20, face = "bold"))+
  geom_text(data=N_185_graphing_average %>% filter(Treatment == "FR"), aes(x=Days, y=N_total_kgha+N_total_kgha_se,
  label=sprintf(p_value)), size=8, vjust=-0.5, color="black")+
  ggtitle(expression("N rate: 185 kg N ha"^-1)*")
```

N_185_graph_average

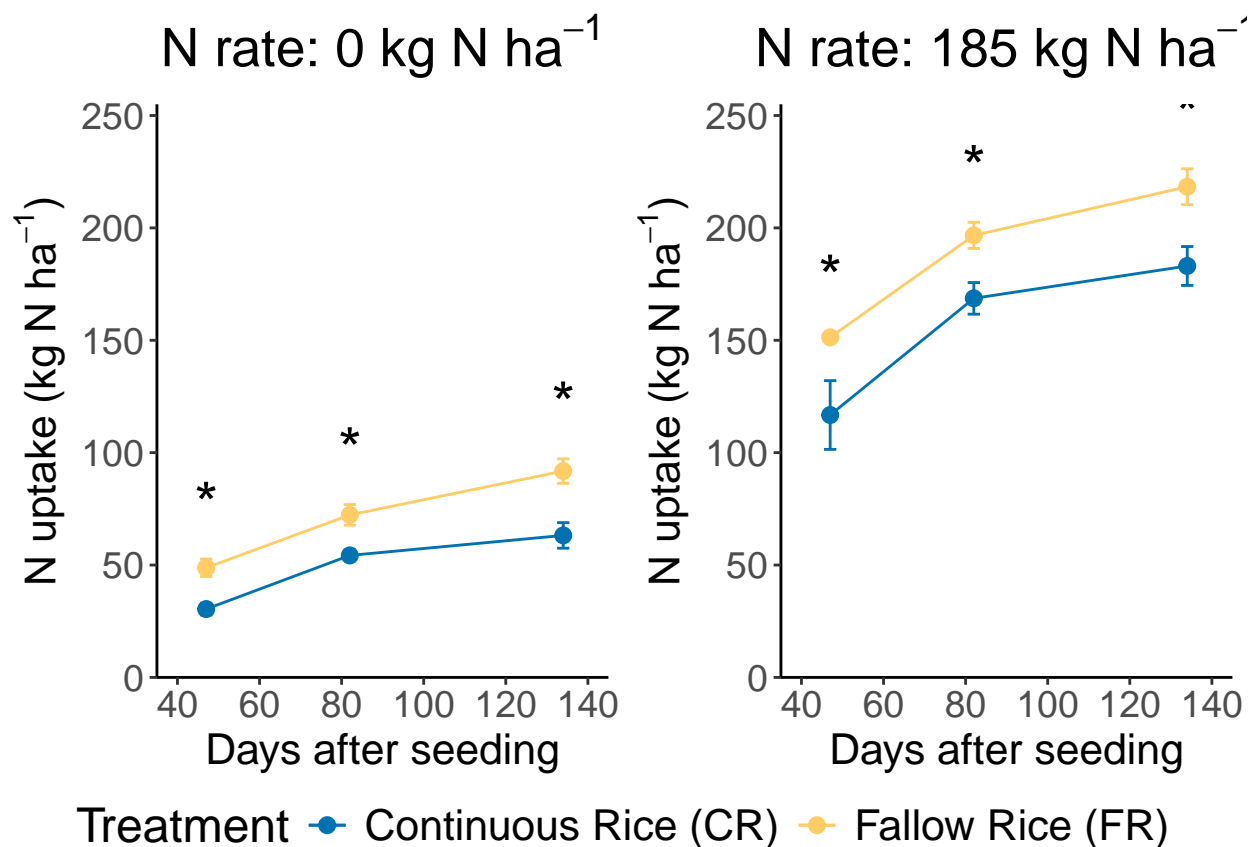


Combine graphs

```
all_N_uptake_overtime <- ggarrange(#N_zero_graph_2021,
                                   #N_zero_graph_2022,
                                   N_zero_graph_average,
                                   #N_185_graph_2021,
                                   #N_185_graph_2022,
                                   N_185_graph_average,

                                   nrow = 1,
                                   ncol = 2,
                                   common.legend = TRUE,
                                   legend= "bottom")
```

```
all_N_uptake_overtime
```



```
ggsave(all_N_uptake_overtime,
        filename = "all_N_uptake_overtime.jpg",
        path = "C:/Users/zhang/Documents/GitHub/FallowRice_ContinuousRice_AgronomicPerformance/Figures",
        height = 20, width = 30,
        units = "cm", dpi=400)
```