

# Plant Growth Indices

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## Load required R libraries

```
library(ggplot2)
library(car)
```

```
## Loading required package: carData
```

```
library(agricolae)
library(ggpubr)
library(emmeans)
library(multcompView)
library(plotrix) # for bar plots
library(lme4)
```

```
## Loading required package: Matrix
```

```
library(tidyverse)
```

```
## -- Attaching packages ----- tidyverse 1.3.2 --
```

```
## v tibble  3.1.8      v dplyr   1.0.10
## v tidyr   1.2.1      v stringr 1.5.0
## v readr   2.1.3      v forcats 0.5.2
## v purrr   1.0.1
```

```
## -- Conflicts ----- tidyverse_conflicts() --
```

```
## x tidyr::expand() masks Matrix::expand()
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()
## x tidyr::pack()    masks Matrix::pack()
## x dplyr::recode()  masks car::recode()
## x purrr::some()    masks car::some()
## x tidyr::unpack() masks Matrix::unpack()
```

```
library(RColorBrewer)
library(GGally)
```

```
## Registered S3 method overwritten by 'GGally':  
##   method from  
##   +.gg   ggplot2  
##  
## Attaching package: 'GGally'  
##  
## The following object is masked from 'package:emmeans':  
##  
##   pigs
```

```
library(lattice)  
library(ggthemes)  
library(DHARMA)
```

```
## This is DHARMA 0.4.6. For overview type '?DHARMA'. For recent changes, type news(package = 'DHARMA')
```

```
library(rmarkdown)  
library(dgof)
```

```
##  
## Attaching package: 'dgof'  
##  
## The following object is masked from 'package:stats':  
##  
##   ks.test
```

```
library(moments)
```

```
##  
## Attaching package: 'moments'  
##  
## The following objects are masked from 'package:agricolae':  
##  
##   kurtosis, skewness
```

```
library(glmmTMB)
```

## Set working directory

```
setwd("C:/ATP_PROJECT/Data")
```

## Load and prepare the data

Read in LEAF AREA data - trial 1

Read in LEAF AREA data - trial 2

Read in LEAF NUMBER data - trial 1

Read in LEAF NUMBER data - trial 2

```
LA1<-read.table("Leafarea_trial_1.txt",header=TRUE)
LA2<-read.table("Leafarea_trial_2.txt",header=TRUE)
LN1<-read.table("Number_of_leaves_trial_1.txt",header=TRUE)
LN2<-read.table("Number_of_leaves_trial_2.txt",header=TRUE)
```

## Save data in appropriate format:

Set the variable characteristics correctly for LA1, LA2, LN1, LN2

specify factor and numeric variables

```
LA1$amendment1 <- as.factor(LA1$amendment1)
LA1$time1 <- as.factor(LA1$time1)
LA1$plant_id1 <- as.factor(LA1$plant_id1)
LA1$trial1 <- as.factor(LA1$trial1)
LA1$leafarea1 <- as.numeric(LA1$leafarea1)
LA1$leaflength1 <- as.numeric(LA1$leaflength1)
LA1$leafwidth1 <- as.numeric(LA1$leafwidth1)

LA2$amendment2 <- as.factor(LA2$amendment2)
LA2$time2 <- as.factor(LA2$time2)
LA2$plant_id2 <- as.factor(LA2$plant_id2)
LA2$trial2 <- as.factor(LA2$trial2)
LA2$leafarea2 <- as.numeric(LA2$leafarea2)
LA2$leaflength2 <- as.numeric(LA2$leaflength2)
LA2$leafwidth2 <- as.numeric(LA2$leafwidth2)

LN1$amendment1 <- as.factor(LN1$amendment1)
LN1$time1 <- as.factor(LN1$time1)
LN1$trial1 <- as.factor(LN1$trial1)
LN1$plant_id1 <- as.factor(LN1$plant_id1)
LN1$no_leaves1 <- as.numeric(LN1$no_leaves1)

LN2$amendment2 <- as.factor(LN2$amendment2)
LN2$time2 <- as.factor(LN2$time2)
LN2$trial2 <- as.factor(LN2$trial2)
LN2$plant_id2 <- as.factor(LN2$plant_id2)
LN2$no_leaves2 <- as.numeric(LN2$no_leaves2)
```

## View data structure and summary stats

```
str(LA1)
```

```
## 'data.frame': 319 obs. of 7 variables:
## $ amendment1 : Factor w/ 3 levels "BSFF","MWF","NoFrass": 1 1 1 1 1 1 1 1 1 ...
## $ time1       : Factor w/ 4 levels "21","28","35",...: 1 1 1 1 1 1 1 1 1 ...
## $ trial1      : Factor w/ 1 level "1": 1 1 1 1 1 1 1 1 1 ...
## $ plant_id1   : Factor w/ 30 levels "1","2","3","4",...: 1 2 3 4 5 6 7 8 9 10 ...
## $ leaflength1: num 6.3 2.2 3.3 2.1 3 3 3.9 3 2.5 2.5 ...
## $ leafwidth1 : num 4.9 1.6 2 1.6 1.9 1.9 2.6 2.2 2.2 1.8 ...
## $ leafarea1 : num 25.89 3.77 5.42 3.77 4.98 ...
```

```
summary(LA1)
```

```
##      amendment1  time1    trial1    plant_id1    leaflength1    leafwidth1
## BSFF      :101   21:84    1:319    1           : 12    Min.      : 1.600    Min.      : 1.200
## MWF       :108   28:87           2           : 12    1st Qu.: 3.800    1st Qu.: 2.600
## NoFrass:110   35:88           3           : 12    Median : 7.000    Median : 4.500
##           42:60           4           : 12    Mean     : 7.852    Mean     : 4.721
##           5           : 12    3rd Qu.:11.800    3rd Qu.: 6.650
##           6           : 12    Max.      :18.000    Max.      :10.400
##           (Other):247
##      leafarea1
## Min.      : 2.40
## 1st Qu.: 8.43
## Median : 22.18
## Mean     : 28.75
## 3rd Qu.: 45.46
## Max.      :105.70
##
```

```
str(LA2)
```

```
## 'data.frame': 357 obs. of 7 variables:
## $ amendment2 : Factor w/ 3 levels "BSFF","MWF","NoFrass": 1 1 1 1 1 1 1 1 1 ...
## $ time2       : Factor w/ 4 levels "21","28","35",...: 1 1 1 1 1 1 1 1 1 ...
## $ trial2      : Factor w/ 1 level "2": 1 1 1 1 1 1 1 1 1 ...
## $ plant_id2   : Factor w/ 30 levels "1","2","3","4",...: 1 2 3 4 5 6 7 8 9 10 ...
## $ leaflength2: num 4.4 3.2 3.5 3.6 4.2 3.5 3.1 4 4.5 3.2 ...
## $ leafwidth2 : num 3.7 2.5 2.7 3.2 2.2 2.5 2.6 3 4.1 2.8 ...
## $ leafarea2 : num 15.61 7.88 8.99 12.08 6.35 ...
```

```
summary(LA2)
```

```
##      amendment2  time2    trial2    plant_id2    leaflength2    leafwidth2
## BSFF      :118   21:90    2:357    1           : 12    Min.      : 2.400    Min.      : 1.600
## MWF       :119   28:90           2           : 12    1st Qu.: 4.700    1st Qu.: 3.600
## NoFrass:120   35:89           3           : 12    Median : 8.300    Median : 5.700
##           42:88           4           : 12    Mean     : 8.832    Mean     : 5.494
```

```
##           5      : 12   3rd Qu.:12.100   3rd Qu.: 7.100
##           6      : 12   Max.      :19.800   Max.      :10.300
##          (Other):285
##   leafarea2
##   Min.      : 3.77
##   1st Qu.: 14.87
##   Median : 34.16
##   Mean      : 35.48
##   3rd Qu.: 51.37
##   Max.      :103.77
##
```

```
str(LN1)
```

```
## 'data.frame':   352 obs. of  5 variables:
## $ amendment1: Factor w/ 3 levels "BSFF","MWF","NoFrass": 1 1 1 1 1 1 1 1 1 ...
## $ time1      : Factor w/ 4 levels "21","28","35",...: 1 1 1 1 1 1 1 1 1 ...
## $ trial1     : Factor w/ 1 level "1": 1 1 1 1 1 1 1 1 1 ...
## $ plant_id1  : Factor w/ 30 levels "1","2","3","4",...: 1 2 3 4 5 6 7 8 9 10 ...
## $ no_leaves1: num  5 3 2 2 3 4 4 3 3 3 ...
```

```
summary(LN1)
```

```
##   amendment1  time1  trial1  plant_id1  no_leaves1
## BSFF      :113   21:84   1:352    1      : 12   Min.      : 2.000
## MWF       :119   28:90           2      : 12   1st Qu.: 4.000
## NoFrass:120   35:88           3      : 12   Median   : 6.000
##           42:90           4      : 12   Mean      : 6.145
##           5      : 12   3rd Qu.: 8.000
##           6      : 12   Max.      :13.000
##          (Other):280
```

```
str(LN2)
```

```
## 'data.frame':   359 obs. of  5 variables:
## $ amendment2: Factor w/ 3 levels "BSFF","MWF","NoFrass": 1 1 1 1 1 1 1 1 1 ...
## $ time2      : Factor w/ 4 levels "21","28","35",...: 1 1 1 1 1 1 1 1 1 ...
## $ trial2     : Factor w/ 1 level "2": 1 1 1 1 1 1 1 1 1 ...
## $ plant_id2  : Factor w/ 30 levels "1","2","3","4",...: 1 2 3 4 5 6 7 8 9 10 ...
## $ no_leaves2: num  5 4 5 4 5 4 4 4 5 4 ...
```

```
summary(LN2)
```

```
##   amendment2  time2  trial2  plant_id2  no_leaves2
## BSFF      :119   21:90   2:359    1      : 12   Min.      : 4.000
## MWF       :120   28:90           2      : 12   1st Qu.: 5.000
## NoFrass:120   35:90           3      : 12   Median   : 6.000
##           42:89           4      : 12   Mean      : 6.735
##           5      : 12   3rd Qu.: 8.000
##           6      : 12   Max.      :13.000
##          (Other):287
```

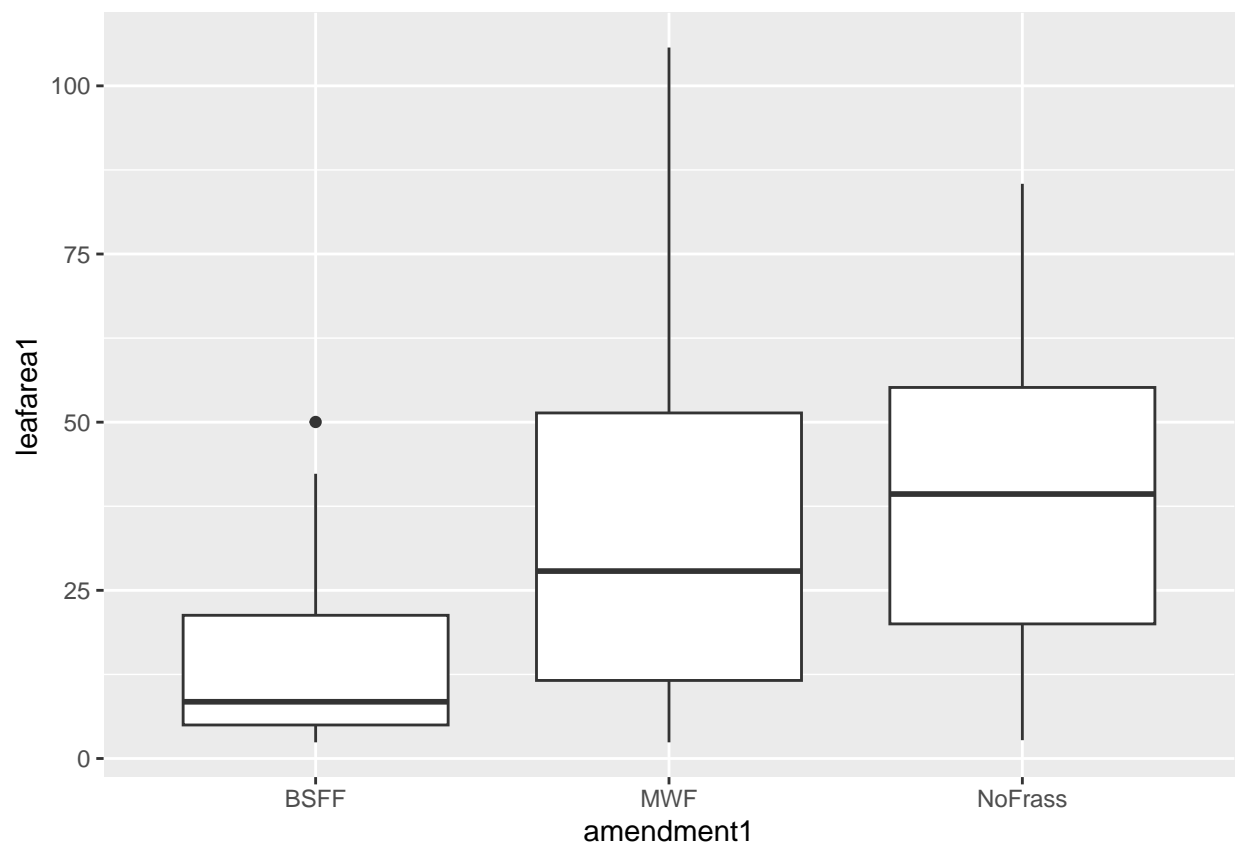
## Visualize data

### Using boxplots, Q-Q Plots, bar graphs or scatter plots

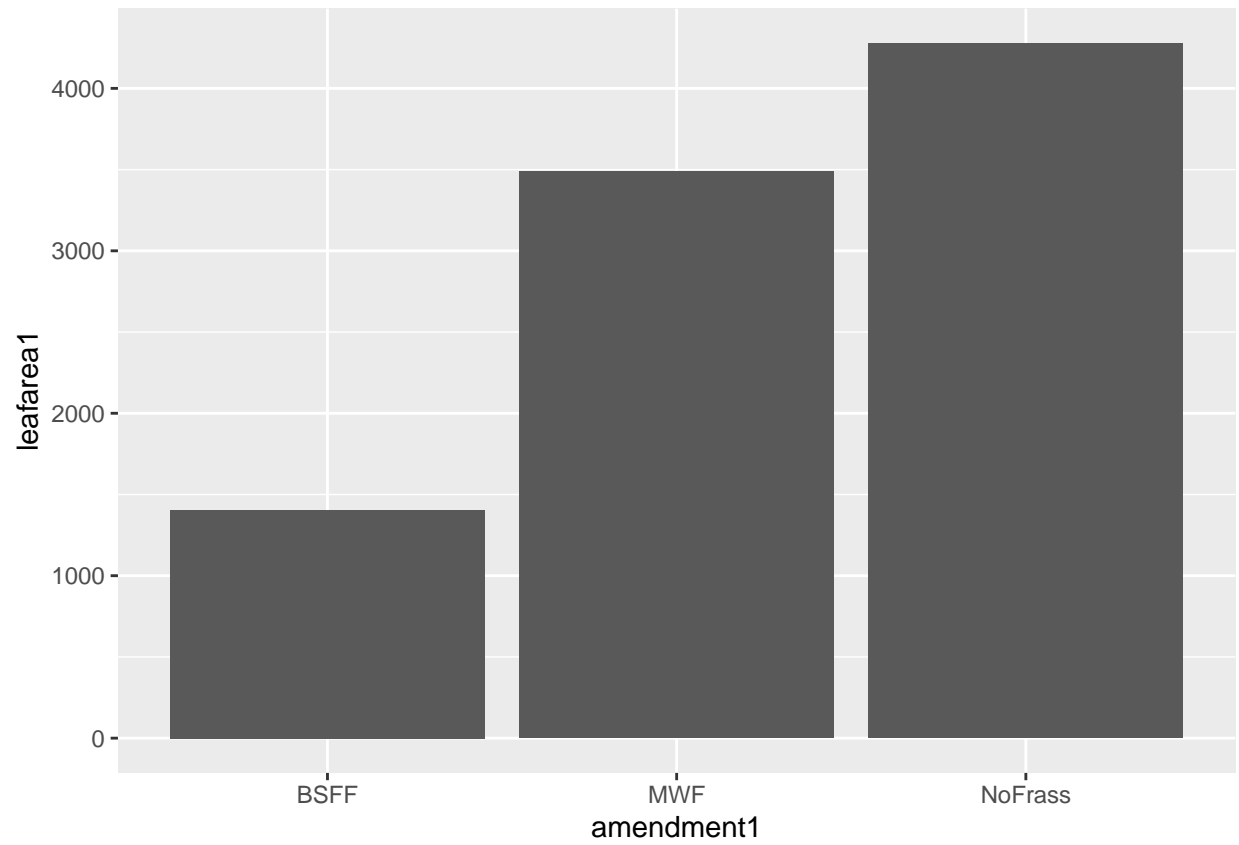
‘QQ Plot stands for Quantile vs Quantile Plot, which is exactly what it does: plotting theoretical quantiles against the actual quantiles of our variable’.

**Leaf Area (LA):** *sample sizes are very large (>30), therefore I ignore the test for normality*

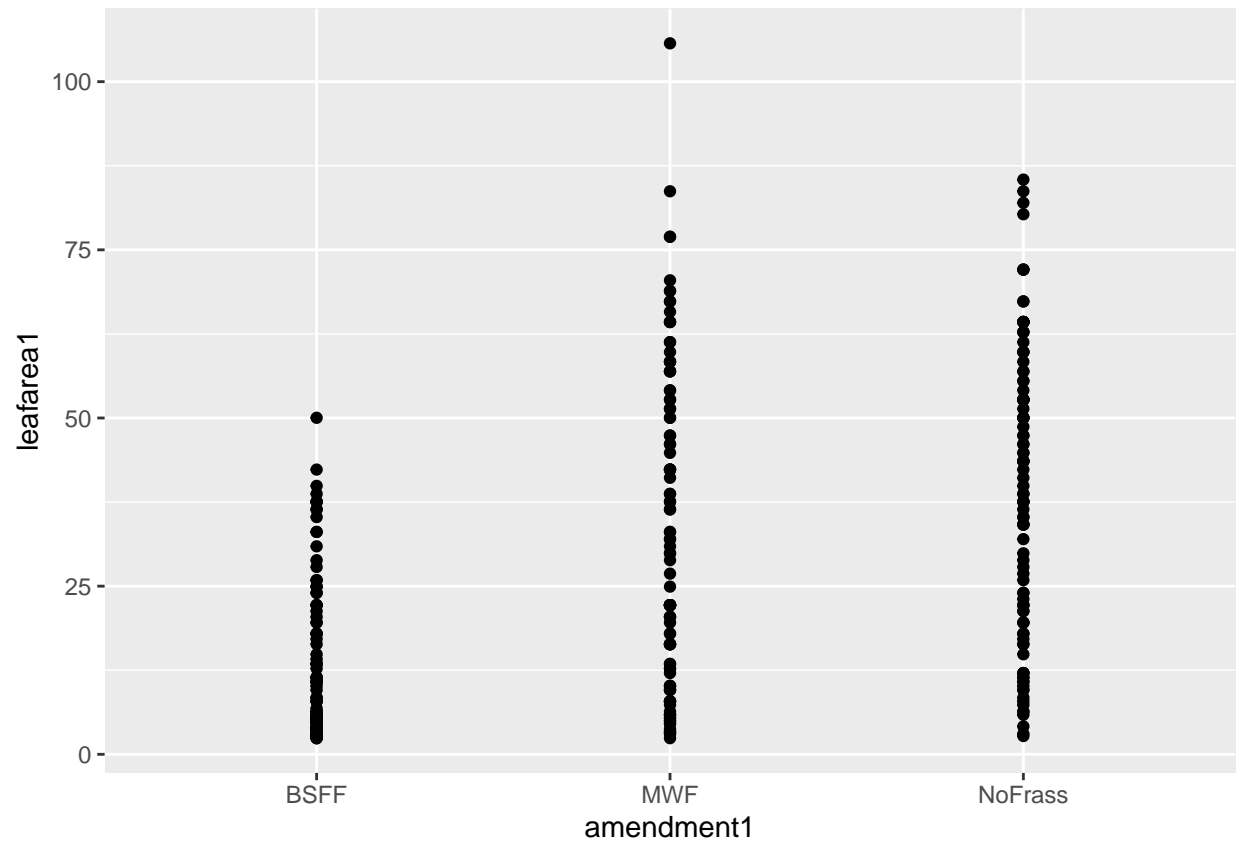
```
P <- ggplot(data = LA1, aes(x = amendment1, y = leafarea1))  
P + geom_boxplot()
```



```
P + geom_col()
```



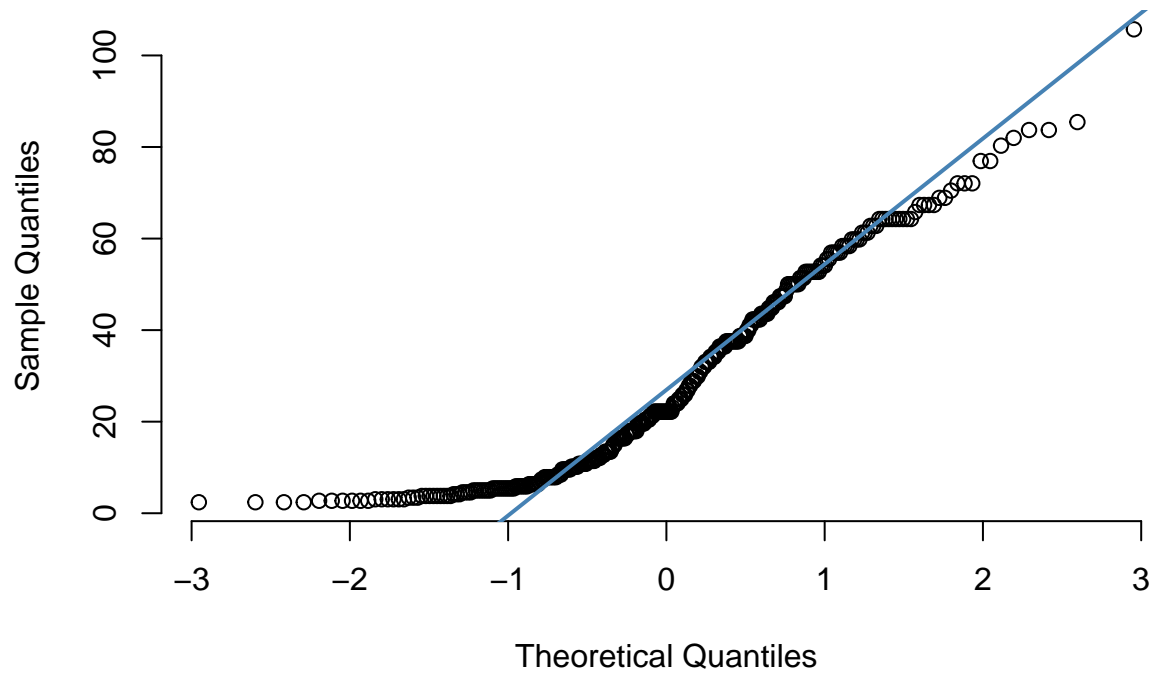
```
ggplot(data = LA1, aes(x = amendment1, y = leafarea1)) + geom_point()
```



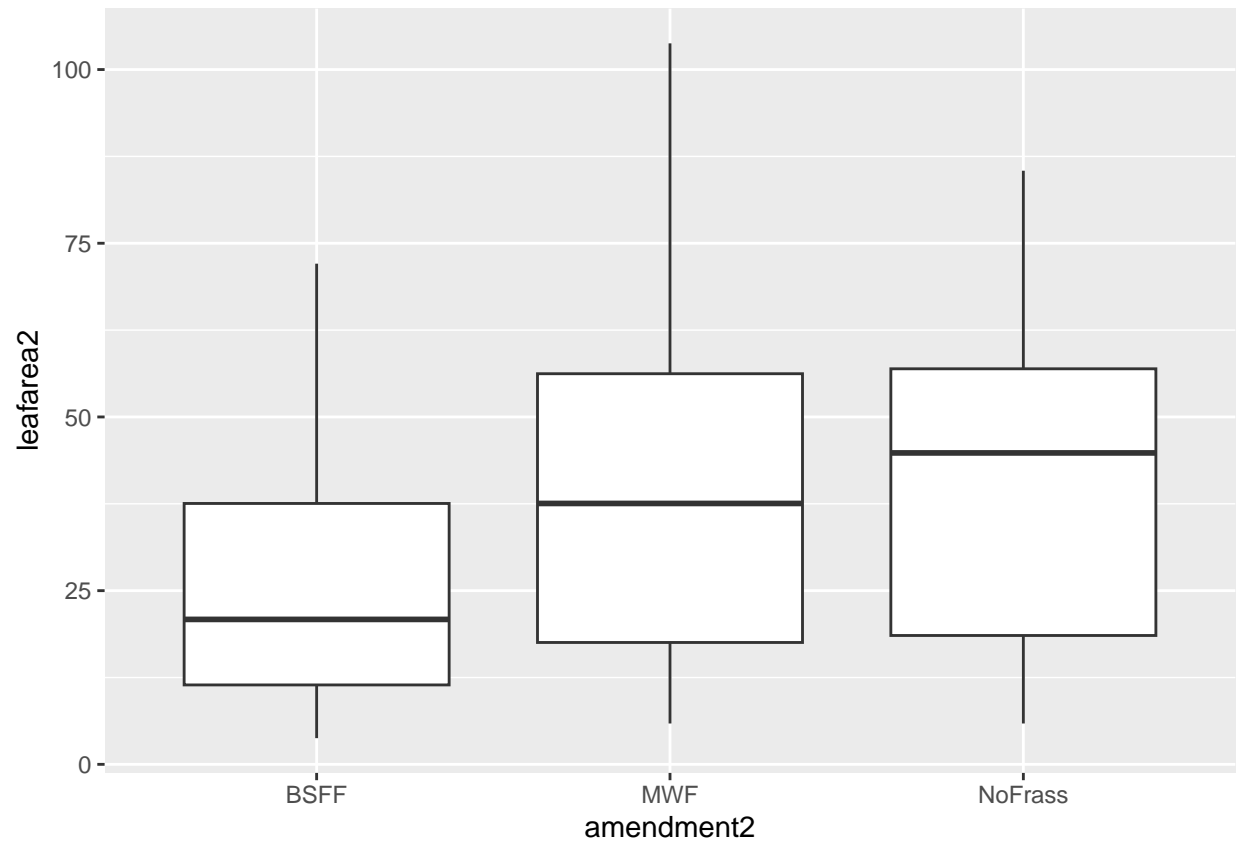
```
qqnorm(LA1$leafarea1, pch = 1, frame = FALSE)
qqline(LA1$leafarea1, col = "steelblue", lwd = 2)
```



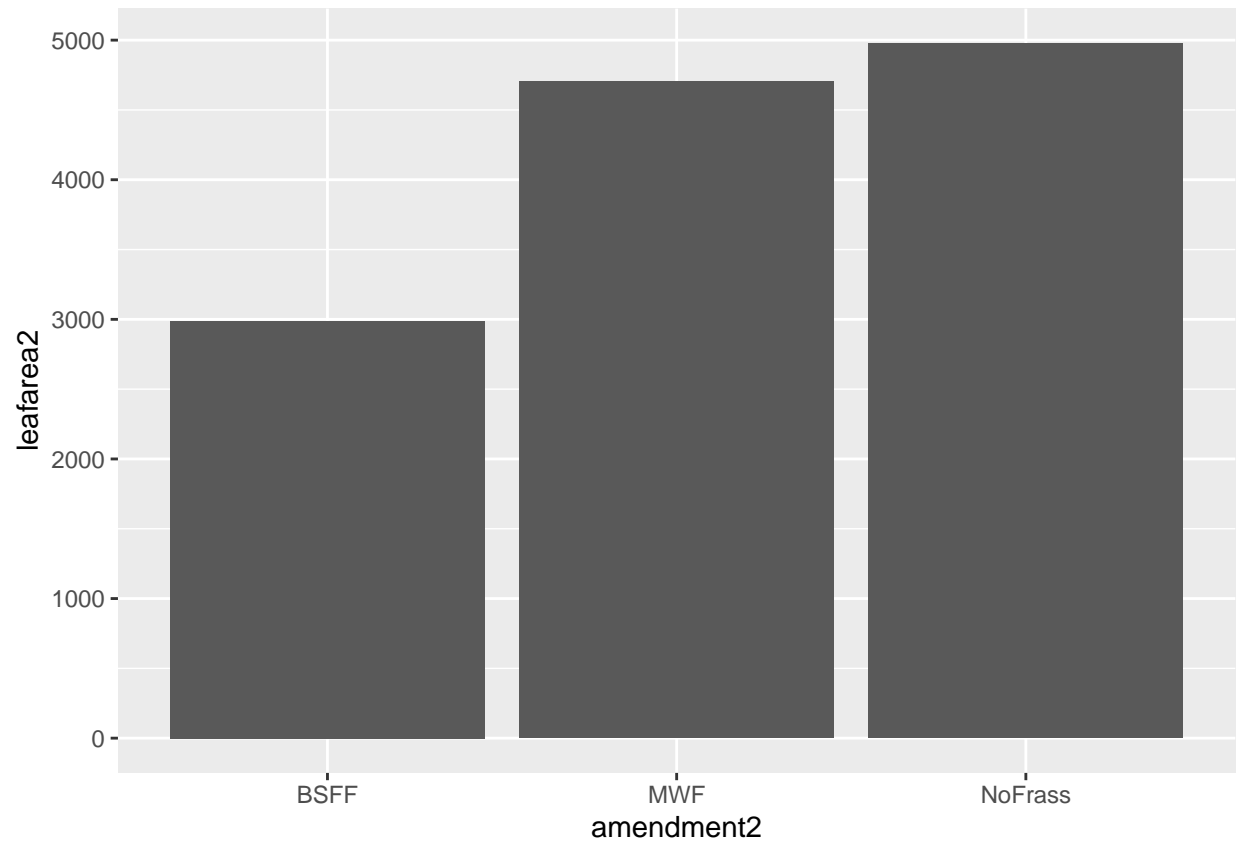
Normal Q-Q Plot



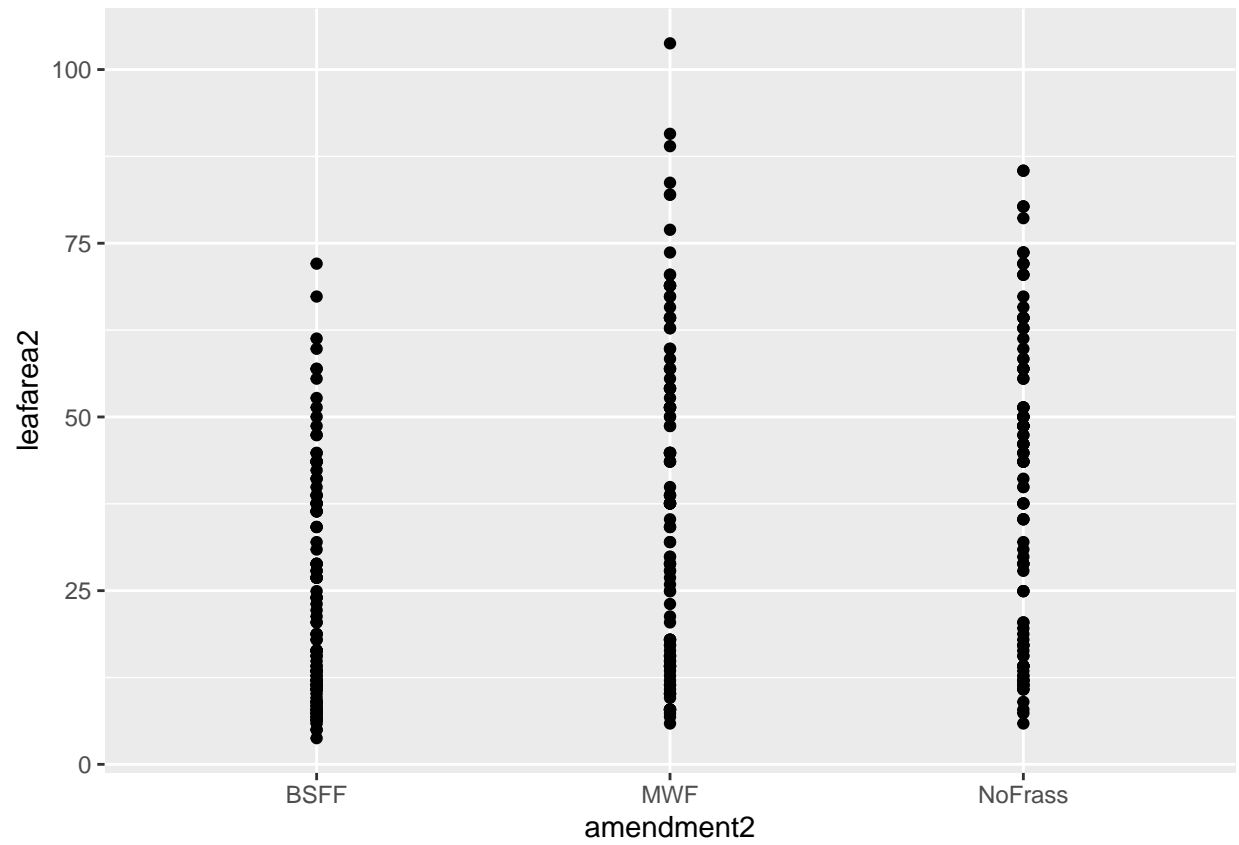
```
P1 <- ggplot(data = LA2, aes(x = amendment2, y = leafarea2))  
P1 + geom_boxplot()
```



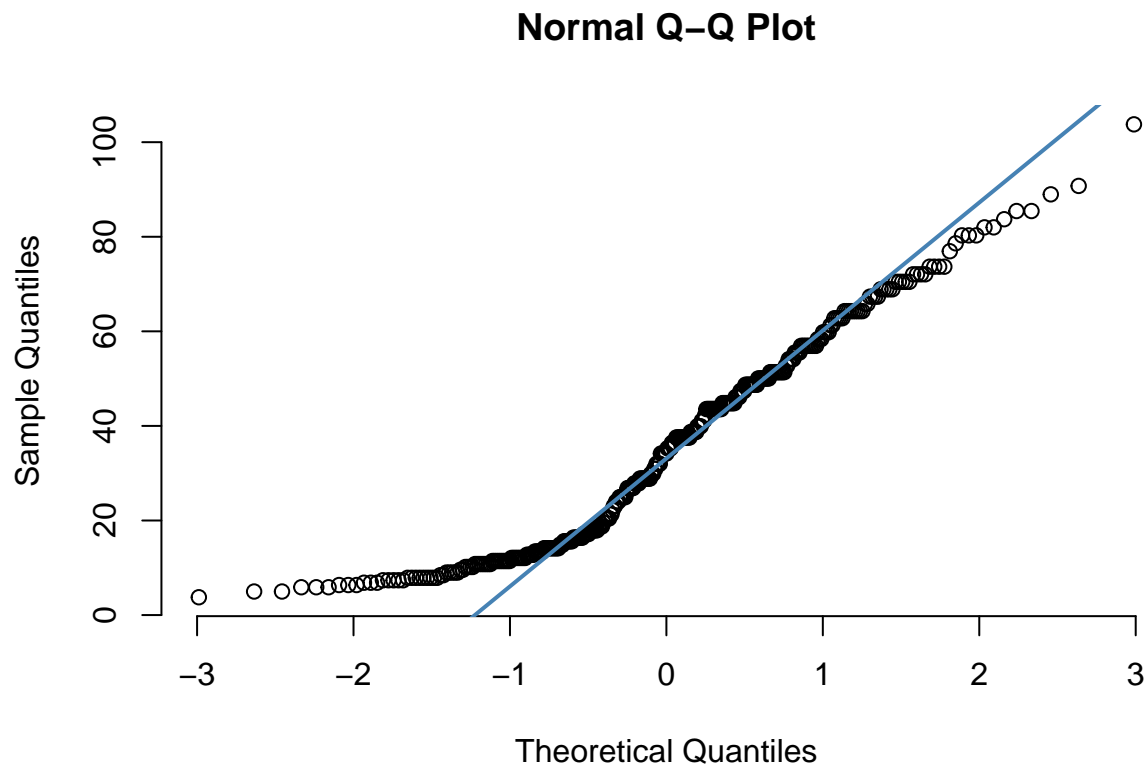
P1 + geom\_col()



```
ggplot(data = LA2, aes(x = amendment2, y = leafarea2)) + geom_point()
```

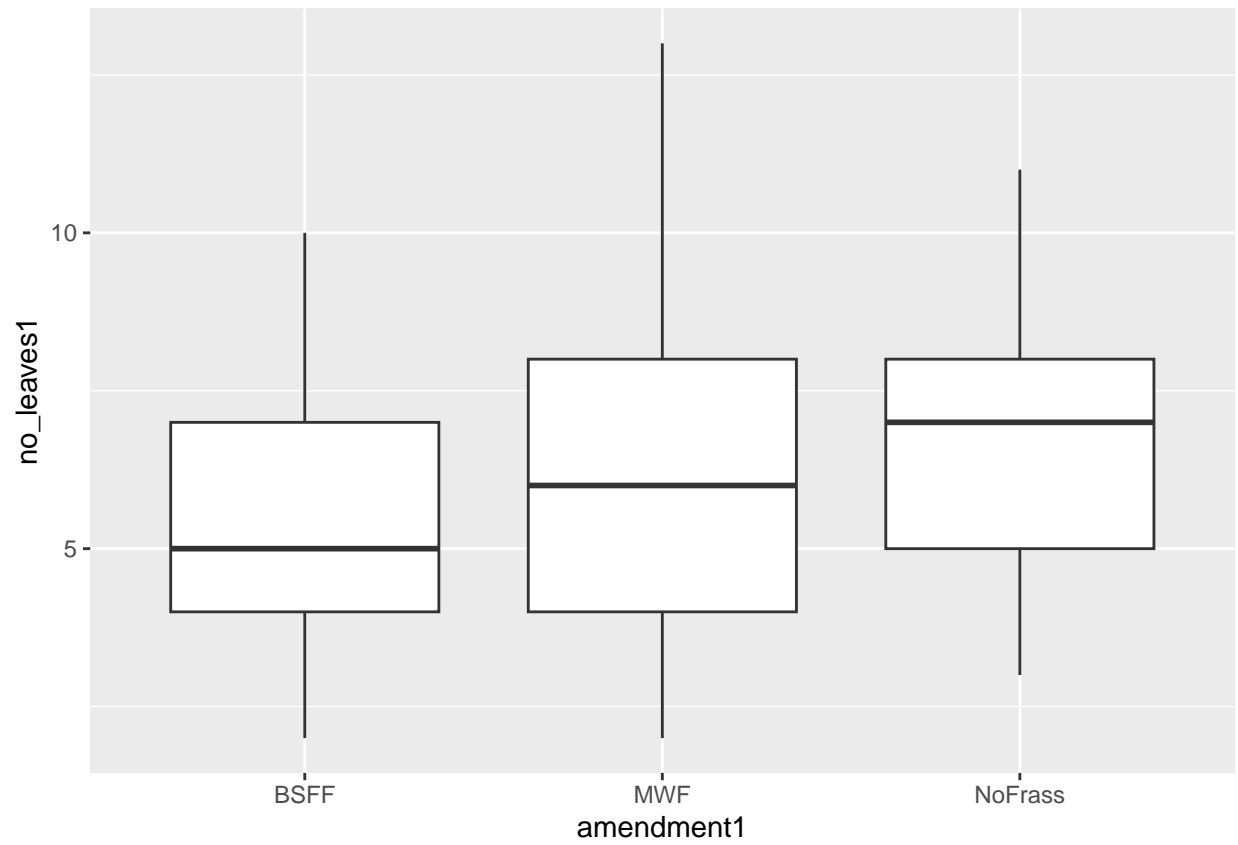


```
qqnorm(LA2$leafarea2, pch = 1, frame = FALSE)
qqline(LA2$leafarea2, col = "steelblue", lwd = 2)
```

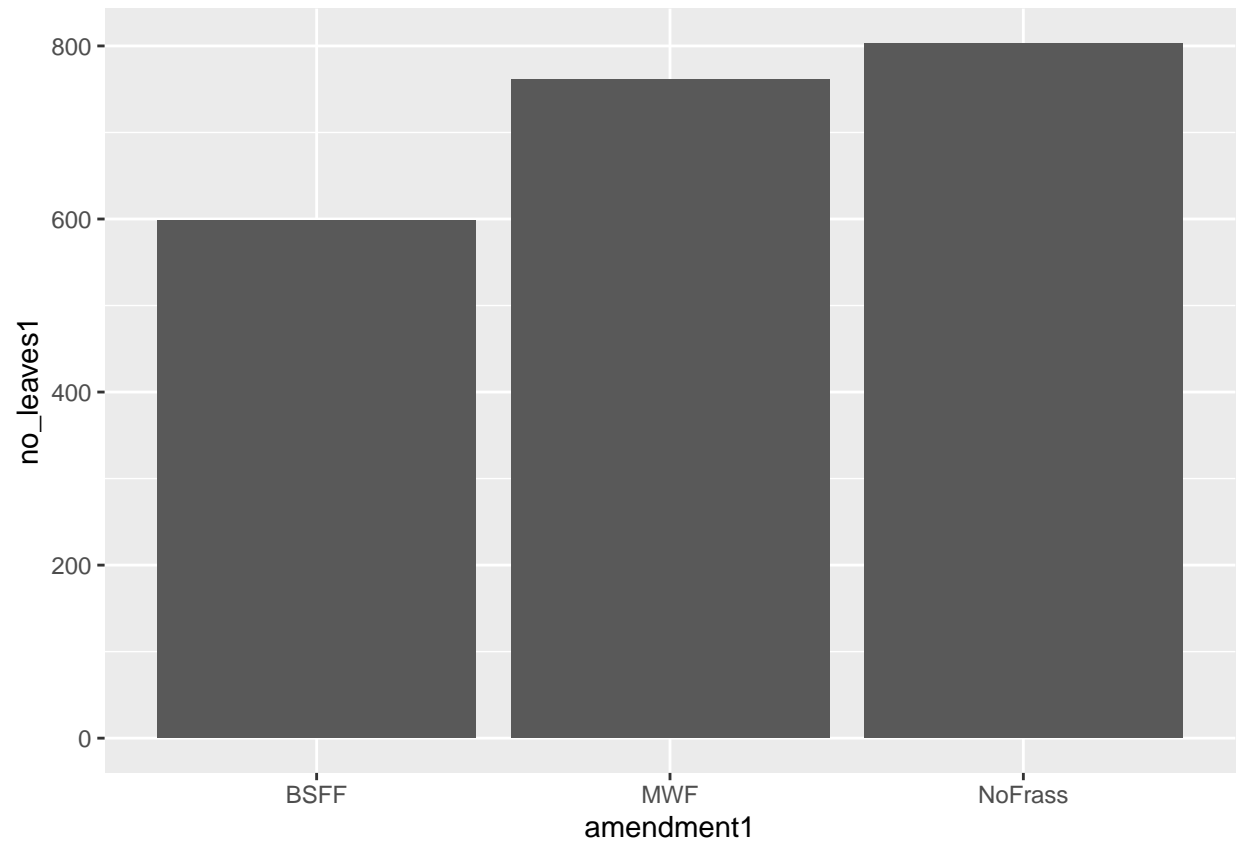


Number of leaves (LN): *sample sizes are very large (>30), therefore I ignore the test for normality*

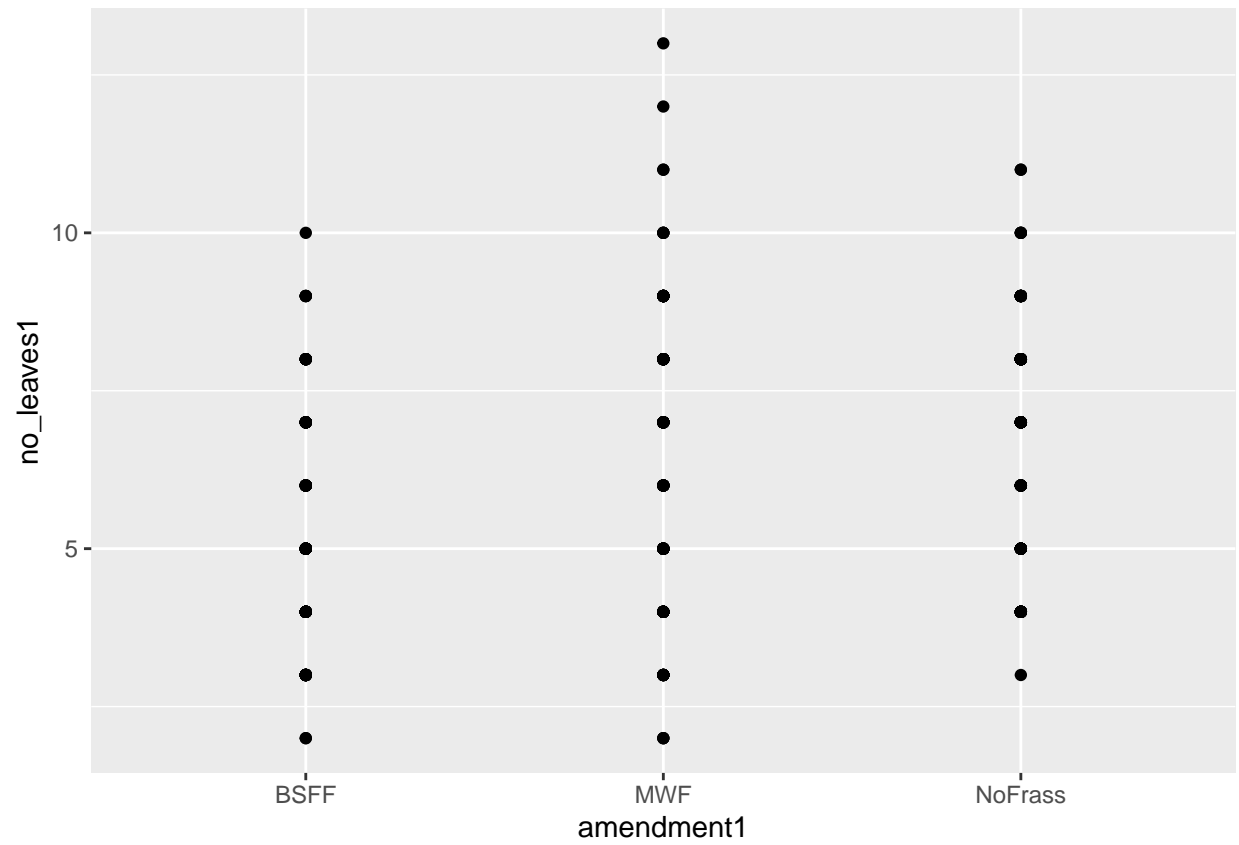
```
P2 <- ggplot(data = LN1, aes(x = amendment1, y = no_leaves1))  
P2 + geom_boxplot()
```



P2 + geom\_col()

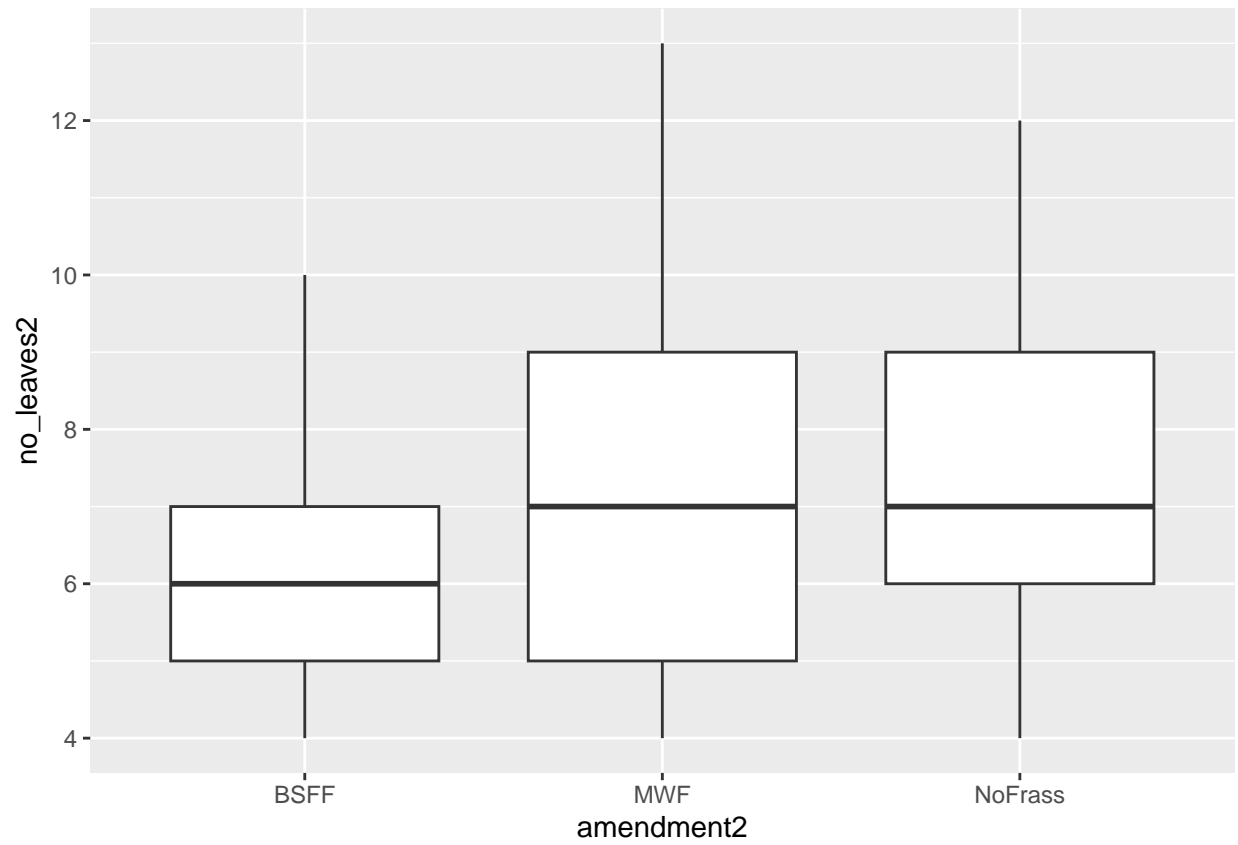


```
ggplot(data = LN1, aes(x = amendment1, y = no_leaves1)) + geom_point()
```

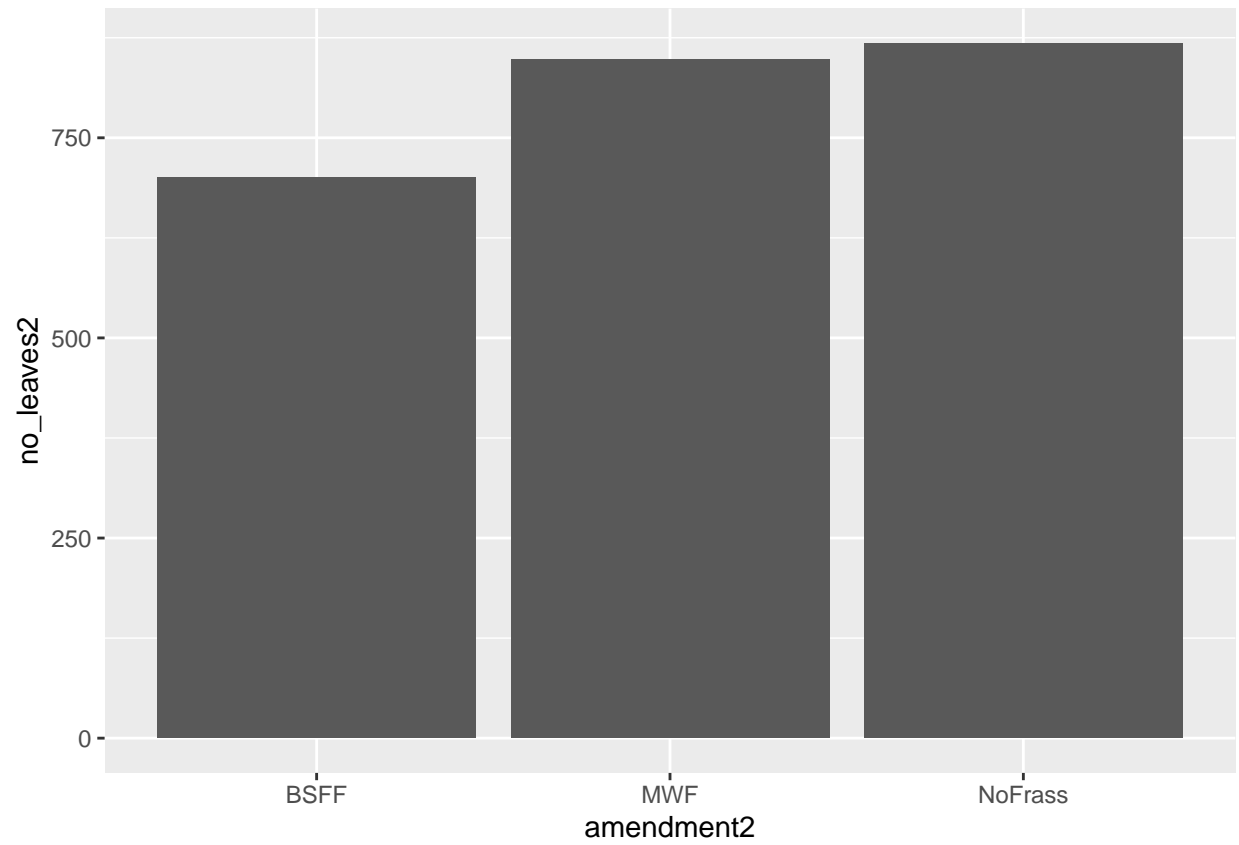


```
P3 <- ggplot(data = LN2, aes(x = amendment2, y = no_leaves2))  
P3 + geom_boxplot()
```

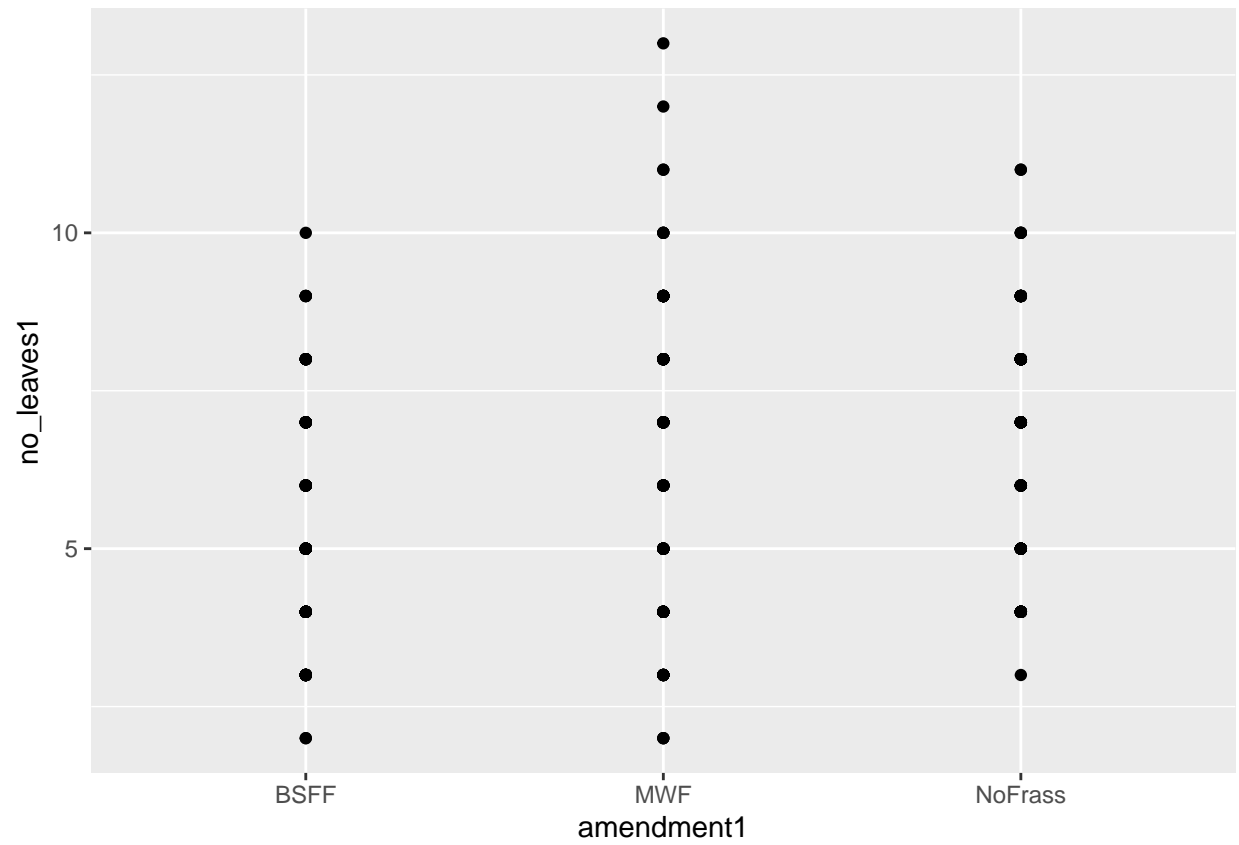




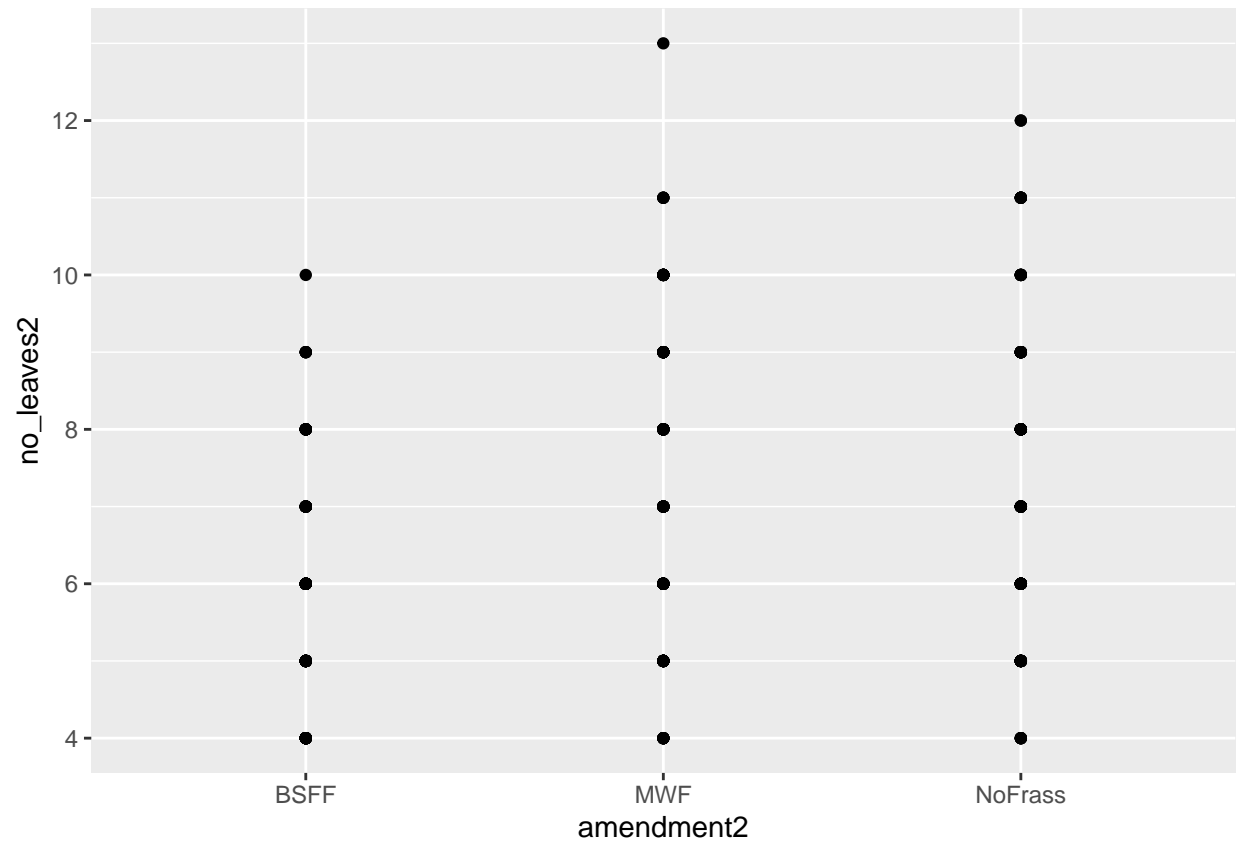
P3 + geom\_col()



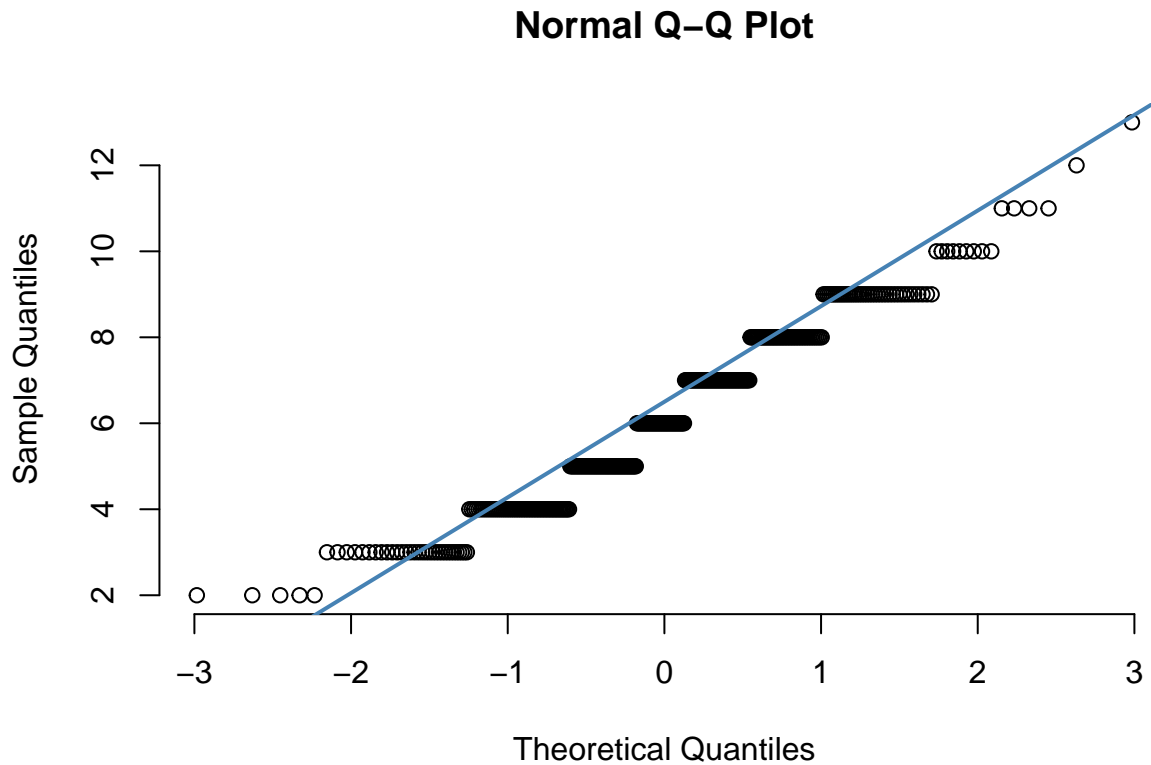
```
ggplot(data = LN1, aes(x = amendment1, y = no_leaves1)) + geom_point()
```



```
ggplot(data = LN2, aes(x = amendment2, y = no_leaves2)) + geom_point()
```



```
qqnorm(LN1$no_leaves1, pch = 1, frame = FALSE)
qqline(LN2$no_leaves2, col = "steelblue", lwd = 2)
```



## Test for homogeneity of variance

If the p-value for the 'Levene test' is greater than . 05, then the variances are not significantly different from each other (i.e., the homogeneity assumption of the variance is met). If the p-value for the Levene's test is less than . 05, then there is a Significant difference between the variances. ## Leaf Area (LA)

## Homogeneity - Number of leaves (LN)

```
leveneTest(no_leaves1 ~ amendment1, data = LN1)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value    Pr(>F)
## group  2  8.5707 0.0002324 ***
##      349
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fligner.test(no_leaves1 ~ amendment1, data = LN1)
```

```
##
```

```
## Fligner-Killeen test of homogeneity of variances
##
## data:  no_leaves1 by amendment1
## Fligner-Killeen:med chi-squared = 15.959, df = 2, p-value = 0.0003424
```

```
bartlett.test(no_leaves1 ~ amendment1, data = LN1)
```

```
##
## Bartlett test of homogeneity of variances
##
## data:  no_leaves1 by amendment1
## Bartlett's K-squared = 11.157, df = 2, p-value = 0.003779
```

```
leveneTest(no_leaves2 ~ amendment2, data = LN2)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value    Pr(>F)
## group  2  8.1081 0.0003602 ***
##      356
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
fligner.test(no_leaves2 ~ amendment2, data = LN2)
```

```
##
## Fligner-Killeen test of homogeneity of variances
##
## data:  no_leaves2 by amendment2
## Fligner-Killeen:med chi-squared = 16.666, df = 2, p-value = 0.0002405
```

```
bartlett.test(no_leaves2 ~ amendment2, data = LN2)
```

```
##
## Bartlett test of homogeneity of variances
##
## data:  no_leaves2 by amendment2
## Bartlett's K-squared = 14.073, df = 2, p-value = 0.0008791
```

## Test for Normality of variances:

If the sample size is large enough ( $n > 30$ ), we can ignore the distribution of the data and use parametric tests. The **central limit theorem** tells us that no matter what distribution things have, the sampling distribution tends to be normal if the sample is large enough ( $n > 30$ ).

Shapiro-Wilk test (recommended by Ghasemi & Zahediasl, 2002, doi: 10.5812/ijem.3505). It provides better power than K-S. It is based on the correlation between the data and the corresponding normal scores. If the p-value is less than or equal to the significance level, the decision is to reject the null hypothesis and conclude that your data do not follow a normal distribution.

## Leaf Area, Leaf number

```
shapiro.test(LA1$leafarea1)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: LA1$leafarea1  
## W = 0.91551, p-value = 2.058e-12
```

```
shapiro.test(LA2$leafarea2)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: LA2$leafarea2  
## W = 0.94408, p-value = 2.328e-10
```

```
shapiro.test(LN1$no_leaves1)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: LN1$no_leaves1  
## W = 0.95943, p-value = 2.699e-08
```

```
shapiro.test(LN2$no_leaves2)
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: LN2$no_leaves2  
## W = 0.93419, p-value = 1.683e-11
```

## Model section

*Gamma distribution is best based on the AIC values*

```
lmer.LA1 <- lmer(leafarea1 ~ amendment1 + (1|time1) + (1|plant_id1), data = LA1)  
glmmTMB.LA1 <- glmmTMB(leafarea1 ~ amendment1 + (1|time1) + (1|plant_id1), data = LA1)  
gamma.LA1 <- glmer(leafarea1 ~ amendment1 + (1|time1) + (1|plant_id1), family = Gamma(link = "log"), data = LA1)  
AIC(lmer.LA1, glmmTMB.LA1, gamma.LA1)
```

```
##           df      AIC
## lmer.LA1    6 2621.032
## glmmTMB.LA1 6 2632.832
## gamma.LA1   6 2511.769
```

```
lmer.LA2 <- lmer(leafarea2 ~ amendment2 + (1|time2) + (1|plant_id2), data = LA2)
glmmTMB.LA2 <- glmmTMB(leafarea2 ~ amendment2 + (1|time2) + (1|plant_id2), data = LA2)
gamma.LA2 <- glmer(leafarea2 ~ amendment2 + (1|time2) + (1|plant_id2), family = Gamma(link = "log"), data = LA2)
AIC(lmer.LA2, glmmTMB.LA2, gamma.LA2)
```

```
##           df      AIC
## lmer.LA2    6 2848.292
## glmmTMB.LA2 6 2859.739
## gamma.LA2   6 2721.658
```

```
lmer.LN1 <- lmer(no_leaves1 ~ amendment1 + (1|time1) + (1|plant_id1), data = LN1)
glmmTMB.LN1 <- glmmTMB(no_leaves1 ~ amendment1 + (1|time1) + (1|plant_id1), data = LN1)
gamma.LN1 <- glmer(no_leaves1 ~ amendment1 + (1|time1) + (1|plant_id1), family = Gamma(link = "log"), data = LN1)
AIC(lmer.LN1, glmmTMB.LN1, gamma.LN1)
```

```
##           df      AIC
## lmer.LN1    6 1130.220
## glmmTMB.LN1 6 1127.505
## gamma.LN1   6 1117.922
```

```
lmer.LN2 <- lmer(no_leaves2 ~ amendment2 + (1|time2) + (1|plant_id2), data = LN2)
glmmTMB.LN2 <- glmmTMB(no_leaves2 ~ amendment2 + (1|time2) + (1|plant_id2), data = LN2)
gamma.LN2 <- glmer(no_leaves2 ~ amendment2 + (1|time2) + (1|plant_id2), family = Gamma(link = "log"), data = LN2)
AIC(lmer.LN2, glmmTMB.LN2, gamma.LN2)
```

```
##           df      AIC
## lmer.LN2    6 1020.3284
## glmmTMB.LN2 6 1016.7233
## gamma.LN2   6  920.4608
```

## Data analysis

### Leaf Area and Number of leaves data

Fit a mixed model: amendment effect is fixed, time and plant\_id are random and independent.

Selected model (*Gamma distribution*)

```
gamma.LA1 <- glmer(leafarea1 ~ amendment1 + (1|time1) + (1|plant_id1), family = Gamma(link = "log"), data = LA1)
summary(gamma.LA1)
```

*effect of soil amendment was significant on leaf area and number of leaves*



```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: leafarea1 ~ amendment1 + (1 | time1) + (1 | plant_id1)
## Data: LA1
##
##      AIC      BIC   logLik deviance df.resid
## 2511.8 2534.4 -1249.9 2499.8 313
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.5291 -0.6986 -0.0653  0.5063  5.0779
##
## Random effects:
## Groups      Name      Variance Std.Dev.
## plant_id1 (Intercept) 0.02814  0.1677
## time1      (Intercept) 0.11226  0.3350
## Residual                0.31859  0.5644
## Number of obs: 319, groups: plant_id1, 30; time1, 4
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)    2.51543    0.30005   8.383  <2e-16 ***
## amendment1MWF    0.77723    0.07890   9.850  <2e-16 ***
## amendment1NoFrass 1.04710    0.07926  13.212  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) am1MWF
## amndmnt1MWF -0.137
## amndmnt1NFr -0.140  0.518
```

```
Anova(gamma.LA1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: leafarea1
##              Chisq Df Pr(>Chisq)
## amendment1 186.87  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
gamma.LA2 <- glmer(leafarea2 ~ amendment2 + (1|time2) + (1|plant_id2), family = Gamma(link = "log"), data = LA2)
summary(gamma.LA2)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: leafarea2 ~ amendment2 + (1 | time2) + (1 | plant_id2)
## Data: LA2
##
##      AIC      BIC   logLik deviance df.resid
```

```
##    2721.7    2744.9   -1354.8    2709.7        351
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -2.43271 -0.61588  0.01373  0.63682  2.42257
##
## Random effects:
##   Groups      Name      Variance Std.Dev.
## plant_id2 (Intercept) 0.01024  0.1012
## time2      (Intercept) 0.05024  0.2241
## Residual                0.11541  0.3397
## Number of obs: 357, groups: plant_id2, 30; time2, 4
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)      3.09323    0.31392   9.854  <2e-16 ***
## amendment2MWF      0.41013    0.04657   8.807  <2e-16 ***
## amendment2NoFrass  0.47530    0.04657  10.206  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) am2MWF
## amndmnt2MWF -0.075
## amndmnt2NFr -0.076  0.510
```

```
Anova(gamma.LA2)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: leafarea2
##              Chisq Df Pr(>Chisq)
## amendment2 121.68  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
gamma.LN1 <- glmer(no_leaves1 ~ amendment1 + (1|time1) + (1|plant_id1), family = Gamma(link = "log"), data = d)
summary(gamma.LN1)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: no_leaves1 ~ amendment1 + (1 | time1) + (1 | plant_id1)
## Data: LN1
##
##      AIC      BIC    logLik deviance df.resid
##  1117.9   1141.1   -553.0   1105.9     346
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.1996 -0.5523  0.0161  0.6161  2.4806
##
## Random effects:
```

```
## Groups      Name      Variance Std.Dev.
## plant_id1 (Intercept) 0.004268 0.06533
## time1      (Intercept) 0.010125 0.10062
## Residual                0.038493 0.19620
## Number of obs: 352, groups: plant_id1, 30; time1, 4
##
## Fixed effects:
##              Estimate Std. Error t value Pr(>|z|)
## (Intercept)    1.59692    0.18816   8.487 < 2e-16 ***
## amendment1MWF    0.19090    0.02500   7.636 2.25e-14 ***
## amendment1NoFrass 0.25772    0.02497  10.322 < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##              (Intr) am1MWF
## amndmnt1MWF -0.068
## amndmnt1NFr -0.069  0.514
```

```
Anova(gamma.LN1)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: no_leaves1
##              Chisq Df Pr(>Chisq)
## amendment1 113.94  2 < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
gamma.LN2 <- glmer(no_leaves2 ~ amendment2 + (1|time2) + (1|plant_id2), family = Gamma(link = "log"),
summary(gamma.LN2)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
## Approximation) [glmerMod]
## Family: Gamma ( log )
## Formula: no_leaves2 ~ amendment2 + (1 | time2) + (1 | plant_id2)
## Data: LN2
##
##      AIC      BIC    logLik deviance df.resid
##    920.5    943.8   -454.2   908.5     353
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.8438 -0.4674 -0.0623  0.6091  2.6091
##
## Random effects:
## Groups      Name      Variance Std.Dev.
## plant_id2 (Intercept) 0.001765 0.04201
## time2      (Intercept) 0.005333 0.07303
## Residual                0.017666 0.13291
## Number of obs: 359, groups: plant_id2, 30; time2, 4
##
## Fixed effects:
```

```
##               Estimate Std. Error t value Pr(>|z|)
## (Intercept)      1.74946    0.16150   10.83  <2e-16 ***
## amendment2MWF      0.16574    0.01625   10.20  <2e-16 ***
## amendment2NoFrass  0.19058    0.01624   11.74  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr) am2MWF
## amndmnt2MWF -0.050
## amndmnt2NFr -0.050  0.503
```

```
Anova(gamma.LN2)
```

```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: no_leaves2
##               Chisq Df Pr(>Chisq)
## amendment2 162.53  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

## Posthoc test

*Tukey test using the emmeans (estimated marginal means) package*

```
HSD_test <- emmeans(ref_grid(gamma.LA1, regrid = "response"), pairwise ~ amendment1)
emmeans(ref_grid(gamma.LA1, regrid = "response"), pairwise ~ amendment1)
```

```
## $emmeans
## amendment1 response      SE df asymp.LCL asymp.UCL
## BSFF          12.4   3.71 Inf      5.1      19.6
## MWF           26.9   8.06 Inf     11.1     42.7
## NoFrass       35.3  10.56 Inf     14.6     55.9
##
```

```
## Confidence level used: 0.95
##
```

```
## $contrasts
## contrast      estimate      SE df z.ratio p.value
## BSFF - MWF      -14.54  4.58 Inf  -3.173  0.0043
## BSFF - NoFrass  -22.88  7.04 Inf  -3.249  0.0033
## MWF - NoFrass    -8.34  3.45 Inf  -2.414  0.0417
##
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
HSD_test <- emmeans(ref_grid(gamma.LA2, regrid = "response"), pairwise ~ amendment2)
emmeans(ref_grid(gamma.LA2, regrid = "response"), pairwise ~ amendment2)
```

```
## $emmeans
```

```
## amendment2 response      SE  df asymp.LCL asymp.UCL
## BSFF      22.0  6.92 Inf      8.48      35.6
## MWF      33.2 10.43 Inf     12.79     53.7
## NoFrass   35.5 11.13 Inf     13.65     57.3
##
## Confidence level used: 0.95
##
## $contrasts
## contrast      estimate      SE  df z.ratio p.value
## BSFF - MWF      -11.18  3.73 Inf   -3.000  0.0076
## BSFF - NoFrass  -13.42  4.41 Inf   -3.045  0.0066
## MWF - NoFrass    -2.24  1.73 Inf   -1.293  0.3992
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
HSD_test <- emmeans(ref_grid(gamma.LN1, regrid = "response"), pairwise ~ amendment1)
emmeans(ref_grid(gamma.LN1, regrid = "response"), pairwise ~ amendment1)
```

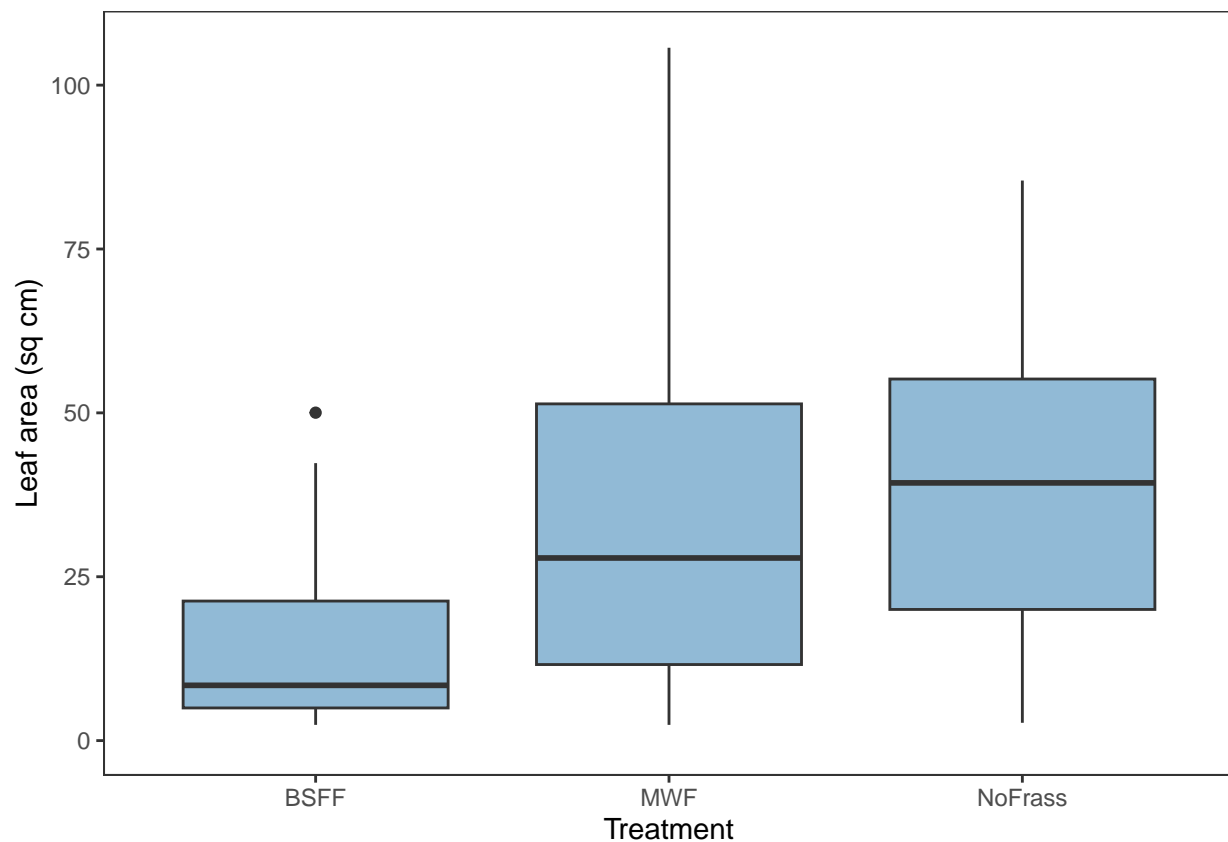
```
## $emmeans
## amendment1 response      SE  df asymp.LCL asymp.UCL
## BSFF      4.94 0.929 Inf      3.12      6.76
## MWF      5.98 1.124 Inf      3.77      8.18
## NoFrass   6.39 1.202 Inf      4.03      8.74
##
## Confidence level used: 0.95
##
## $contrasts
## contrast      estimate      SE  df z.ratio p.value
## BSFF - MWF      -1.039 0.238 Inf   -4.369 <.0001
## BSFF - NoFrass  -1.452 0.307 Inf   -4.734 <.0001
## MWF - NoFrass   -0.413 0.171 Inf   -2.417  0.0414
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
HSD_test <- emmeans(ref_grid(gamma.LN2, regrid = "response"), pairwise ~ amendment2)
emmeans(ref_grid(gamma.LN2, regrid = "response"), pairwise ~ amendment2)
```

```
## $emmeans
## amendment2 response      SE  df asymp.LCL asymp.UCL
## BSFF      5.75 0.929 Inf      3.93      7.57
## MWF      6.79 1.096 Inf      4.64      8.94
## NoFrass   6.96 1.124 Inf      4.76      9.16
##
## Confidence level used: 0.95
##
## $contrasts
## contrast      estimate      SE  df z.ratio p.value
## BSFF - MWF      -1.037 0.196 Inf   -5.295 <.0001
## BSFF - NoFrass  -1.208 0.220 Inf   -5.477 <.0001
## MWF - NoFrass   -0.171 0.115 Inf   -1.489  0.2963
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

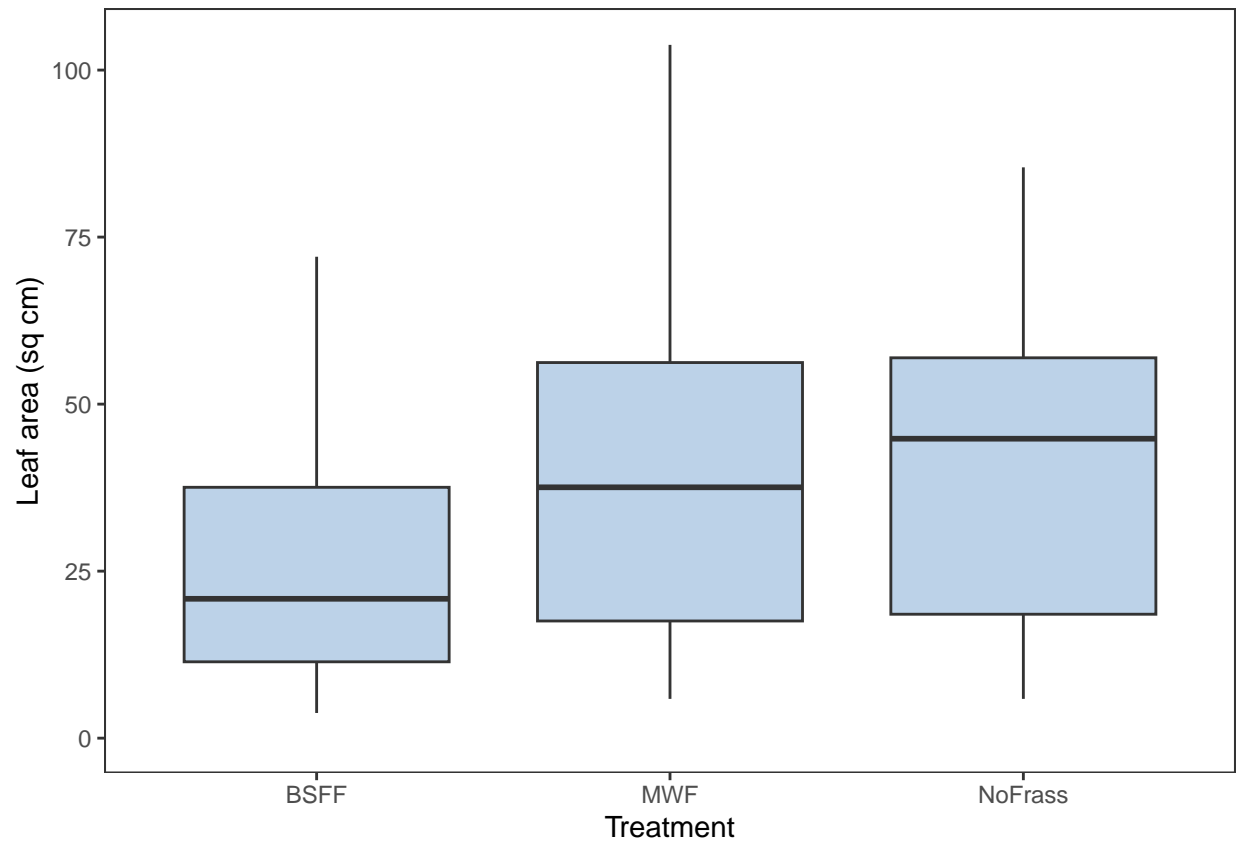
## Plots for leafarea (cm<sup>2</sup>) , Number of leaves

```
P4 <- ggplot(LA1, aes(amendment1, leafarea1))+  
  geom_boxplot(aes(fill = amendment1),  
    notch = FALSE, position = position_dodge(0.9), show.legend = FALSE) +  
  theme_minimal() + labs(x = "Treatment", y = "Leaf area (sq cm)") +  
  theme_bw() + theme(panel.grid.major = element_blank(), # removes grid background  
    panel.grid.minor = element_blank()) +  
  scale_fill_manual(values = c("#91BAD6", "#91BAD6", "#91BAD6")) # Dark Sky Blue  
P4 + ylim(0, 106)
```



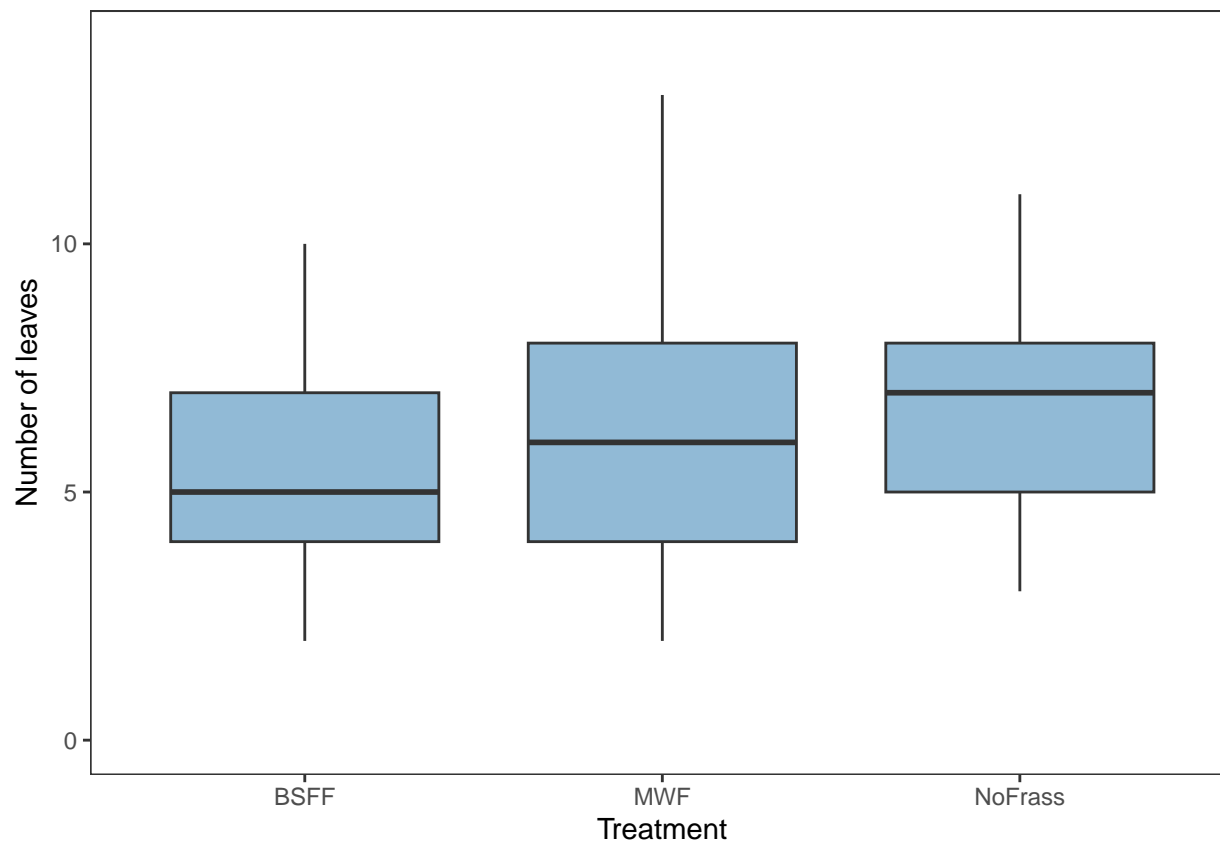
```
ggsave("boxplot_P4.jpeg", width = 4, height = 3, dpi = 1000)
```

```
P5 <- ggplot(LA2, aes(amendment2, leafarea2))+  
  geom_boxplot(aes(fill = amendment2),  
    notch = FALSE, position = position_dodge(0.9), show.legend = FALSE) +  
  theme_minimal() + labs(x = "Treatment", y = "Leaf area (sq cm)") +  
  theme_bw() + theme(panel.grid.major = element_blank(),  
    panel.grid.minor = element_blank()) +  
  scale_fill_manual(values = c("#BCD2E8", "#BCD2E8", "#BCD2E8")) # Beau Blue  
P5 + ylim(0, 104)
```



```
ggsave("boxplot_P5.jpeg", width = 4, height = 3, dpi = 1000)
```

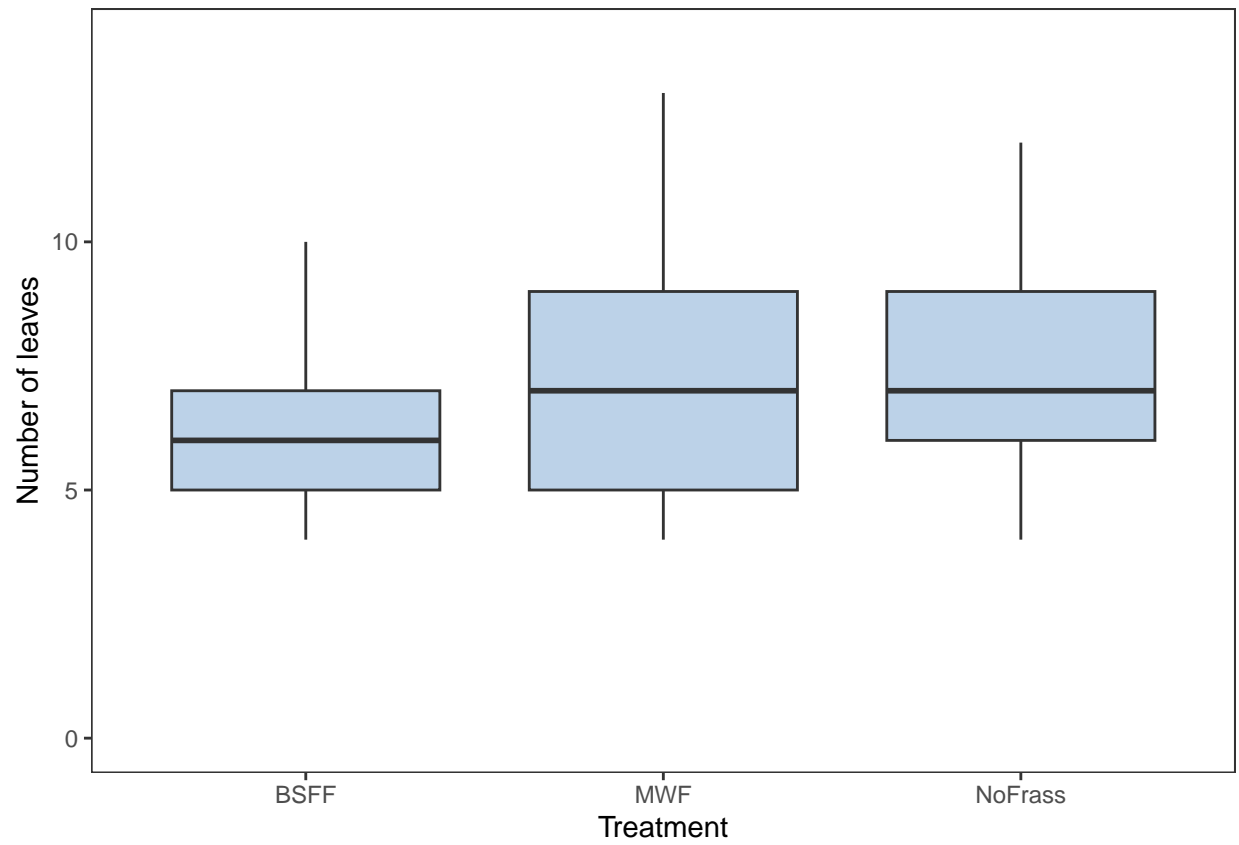
```
P6 <- ggplot(LN1, aes(amendment1, no_leaves1))+
  geom_boxplot(aes(fill = amendment1),
    notch = FALSE, position = position_dodge(0.9), show.legend = FALSE) +
  theme_minimal() + labs(x = "Treatment", y = "Number of leaves")+
  theme_bw() + theme(panel.grid.major = element_blank(),
    panel.grid.minor = element_blank())+
  scale_fill_manual(values = c("#91BAD6", "#91BAD6", "#91BAD6"))# Dark Sky Blue
P6 + ylim(0, 14)
```



```
ggsave("boxplot_P6.jpeg", width = 4, height = 3, dpi = 1000)
```

```
P7 <- ggplot(LN2, aes(amendment2, no_leaves2))+
  geom_boxplot(aes(fill = amendment2),
    notch = FALSE, position = position_dodge(0.9), show.legend = FALSE) +
  theme_minimal() + labs(x = "Treatment", y = "Number of leaves")+
  theme_bw() + theme(panel.grid.major = element_blank(),
    panel.grid.minor = element_blank())+
  scale_fill_manual(values = c("#BCD2E8", "#BCD2E8", "#BCD2E8"))# Beau Blue
P7 + ylim(0, 14)
```





```
ggsave("boxplot_P7.jpeg", width = 4, height = 3, dpi = 1000)
```