warmXtrophic Project: CN Analyses

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Load in packages & data

```r
# Clear all existing data
rm(list=ls())

#Load packages
library(tidyverse)
library(bbmle)
library(lme4)
library(fitdistrplus)

# Set working directory
L1_dir<-Sys.getenv("L1DIR")

# Above .Renviron not working for PLZ; hard-coding in here
L0_dir <- "/Volumes/GoogleDrive/Shared\ drives/SpaCE_Lab_warmXtrophic/data/L0"
L1_dir <- "/Volumes/GoogleDrive/Shared\ drives/SpaCE_Lab_warmXtrophic/data/L1"
L2_dir <- "/Volumes/GoogleDrive/Shared\ drives/SpaCE_Lab_warmXtrophic/data/L2"

# Read in data
cn <- read.csv(file.path(L1_dir, "CN/CN_L1.csv"))
# Re-order "state" so gg plotting colors works better for blue=ambient; red=warmed
cn$state <- factor(cn$state,levels=c("warmed","ambient"))
cn <- cn %>%
  rename(treatment = state)
# check species in dataframe
with(cn,table(cn$site,cn$species))
```

```
##
##        Acmi Cest Popr Soca
##   kbs   139    0    0  424
##   umbs    0  275  183    0
```

```r
with(cn,table(cn$year,cn$species, cn$site))
```

```
## , ,  = kbs
##
##
##        Acmi Cest Popr Soca
##   2017   99    0    0  161
##   2018   40    0    0   60
##   2019    0    0    0   71
```

```
##    2020    0    0    0   63
##    2021    0    0    0   69
##
## , ,  = umbs
##
##
##         Acmi Cest Popr Soca
##    2017    0   69    0    0
##    2018    0   67   61    0
##    2019    0   69   58    0
##    2020    0   70   64    0
##    2021    0    0    0    0
```

```r
# Subsamples only occur in 2017; after that we did not use multiple replicate subsamples from 1 plant
with(cn,table(cn$subsample_number,cn$species, cn$year))
```

```
## , ,  = 2017
##
##
##      Acmi Cest Popr Soca
##   1   43    0    0   56
##   2   42    0    0   54
##   3   14    0    0   51
##
## , ,  = 2018
##
##
##      Acmi Cest Popr Soca
##   1    0    0    0    0
##   2    0    0    0    0
##   3    0    0    0    0
##
## , ,  = 2019
##
##
##      Acmi Cest Popr Soca
##   1    0    0    0    0
##   2    0    0    0    0
##   3    0    0    0    0
##
## , ,  = 2020
##
##
##      Acmi Cest Popr Soca
##   1    0    0    0    0
##   2    0    0    0    0
##   3    0    0    0    0
##
## , ,  = 2021
##
##
##      Acmi Cest Popr Soca
##   1    0    0    0    0
##   2    0    0    0    0
##   3    0    0    0    0
```

```
# keep sites in same dataframe for now bc unique spp at each site
```

2017 within plot and within-plant C & N variation analysis

(This section modified from code initiated by Nina Lany). In July 2017, Elizabeth Postema, an REU at KBS, analyzed the nitrogen content of *Achillea millefolium* foliage as part of a bioassay quantifying slug growth on leaves grown in warmed quadrats vs. ambient control quadrats. She collected the youngest 4 to 5 fully expanded leaves on each of five individual yarrow plants per quadrat (plot). Each leaf was placed in its own glassine envelope. The leaves were then dried. If the species had a dominant mid-rib and easily removable, the mid-rib was removed. We made the decision to pool the 4 to 5 dried leaves together, grind them, and then weigh out 2-3 replicates from the pooled tissue for each plant.

We then also collected *Solidago canadensis* leaves from KBS for warmed vs. ambient control plots and repeated the above process. For *Centaurea stoebe* at UMBS we collected basal and stem leaves per plant (one of each).

Below is the statistical analysis to pull out the sources of variation (due to the warming treatment, among individual plants within quadrats, and among individual leaves within a plant) to make informed decisions for sampling during summer 2018 and subsequent years. We focused on the nitrogen content (%N drymass) of leaves rather than the C:N ratio because this has been shown to be most relevant for nitrogen-limited folivores.

How much variation can be attributed to differences among leaves on an individual plant, individuals in a quadrat, and the warming treatment?

```
# Subset to 2017, species
cn17<-cn %>% filter(year == 2017)
cn17$plant_id <- paste(cn17$plot, cn17$plant_number, sep = "_")
cn17_soca<-cn17 %>% filter(species == "Soca")
cn17_cest<-cn17 %>% filter(species == "Cest")
cn17_acmi<-cn17 %>% filter(species == "Acmi")
#aggregate to mean and sd %N (and mean %C) of the (typically) two leaves per plant
cn17N <- cn17 %>%
    group_by(plot, site, species, treatment, insecticide) %>%
    summarize(mean_N = mean(nitrogen),sd_N = sd(nitrogen))
```

```
## `summarise()` has grouped output by 'plot', 'site', 'species', 'treatment'. You
## can override using the `.groups` argument.
```

```
cn17C <- cn17 %>%
    group_by(plot, site, species, treatment, insecticide) %>%
    summarize(mean_C = mean(carbon),sd_C = sd(carbon))
```

```
## `summarise()` has grouped output by 'plot', 'site', 'species', 'treatment'. You
## can override using the `.groups` argument.
```

```
cn17msd<-merge(cn17C,cn17N)
```

As a first pass, ignoring the nested structure of the data, we can 'eyeball' that there was a lot of variability but perhaps a small difference in the means of the warmed vs. ambient treatments per species.
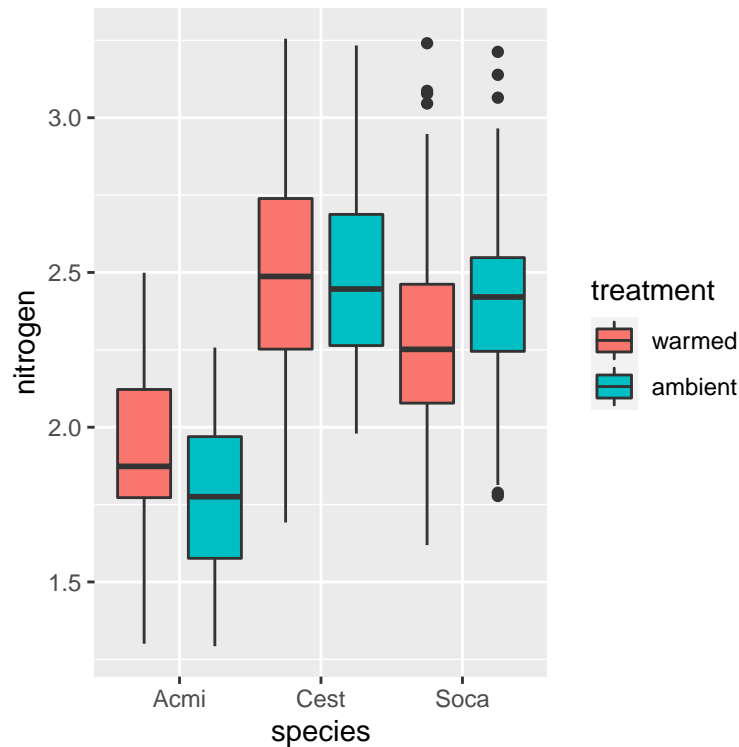
We run a mixed-effects ANOVA model with 'state' as fixed effect and 'species', 'plot' and 'plant_number' as random intercepts to quantify the variation attributable to each source. Residuals did not indicate non-normality and the data were not transformed.
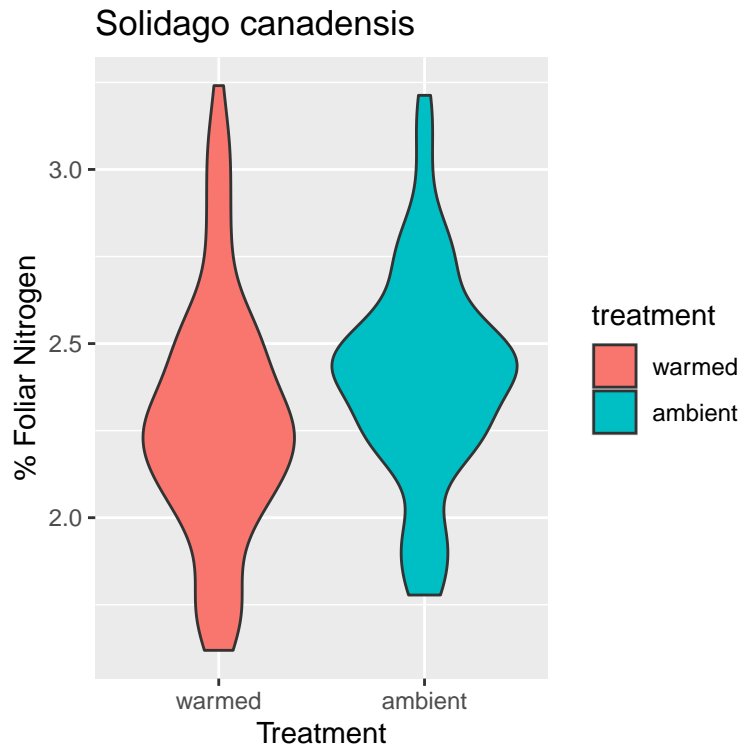
#Soca

```
library(lme4)
n.soca.m1 <- lmer(nitrogen ~ treatment + (1|plot) + (1|plant_id), data = cn17_soca)
summary(n.soca.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + (1 | plot) + (1 | plant_id)
##    Data: cn17_soca
##
## REML criterion at convergence: -37.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.3093 -0.3010 -0.0135  0.2534  3.8438
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.05473  0.2339
##  plot     (Intercept) 0.03779  0.1944
##  Residual             0.01804  0.1343
## Number of obs: 161, groups:  plant_id, 60; plot, 12
##
## Fixed effects:
##                   Estimate Std. Error t value
## (Intercept)        2.29015    0.09146  25.039
## treatmentambient   0.12186    0.12931   0.942
##
## Correlation of Fixed Effects:
```

4

```
##              (Intr)
## tretmntmbnt -0.707
```

```
plot(n.soca.m1)
```
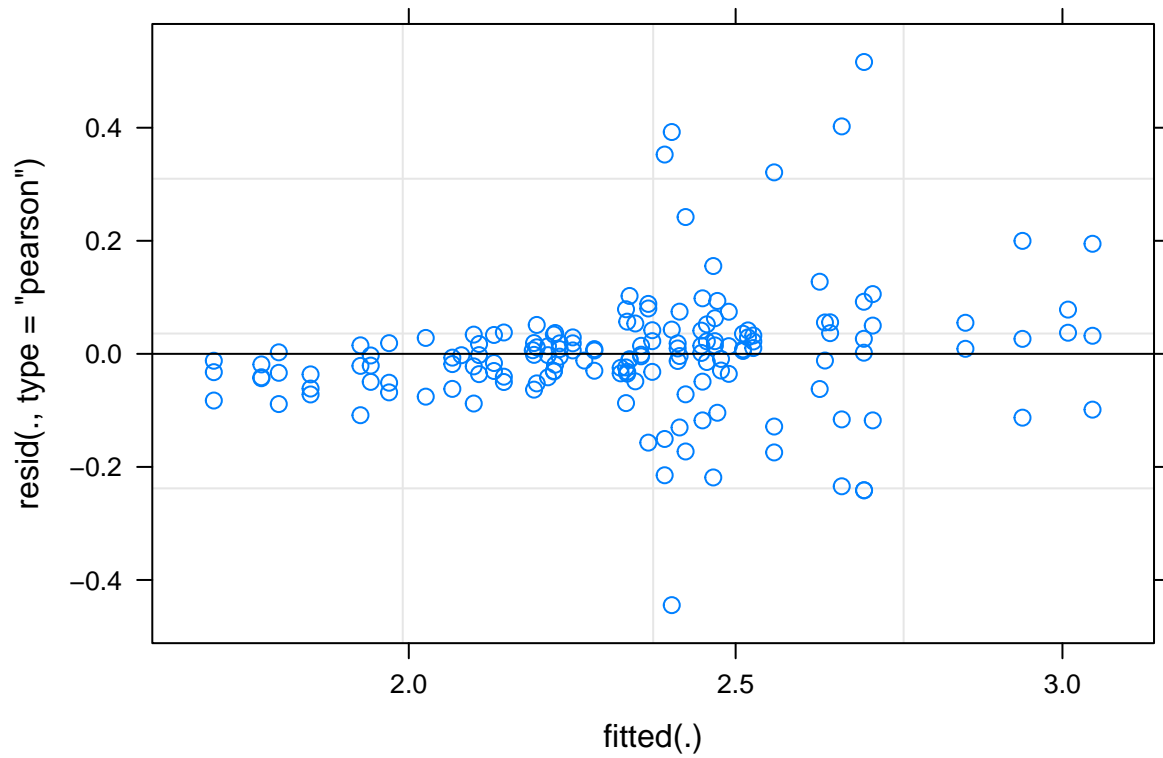

The analysis indicates that variation attributable to differences in the means of the two treatment groups was highest (0.12 %), followed by variation due to replicate runs of the pooled tissue from individual plants (0.05 %), followed by variation among individuals within a quadrat (0.04 %). This implies that variation among plots should be high. The figure below shows that variation in the mean %N among the 12 plots in the dataset was high.
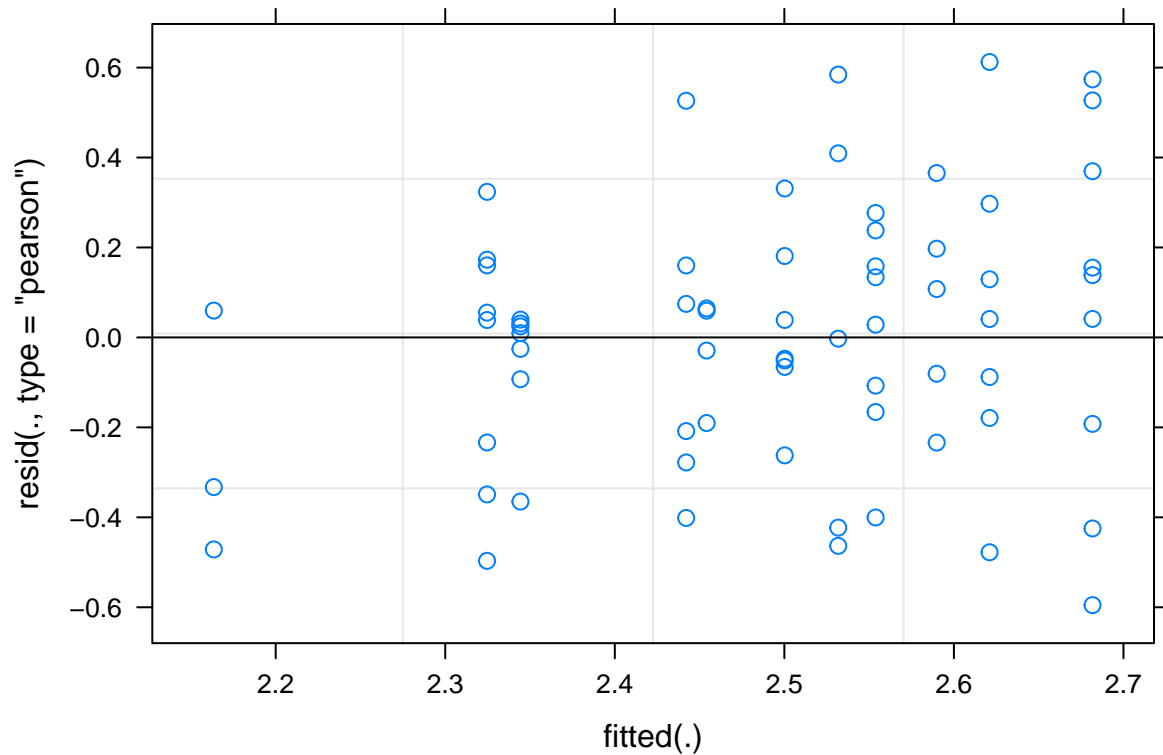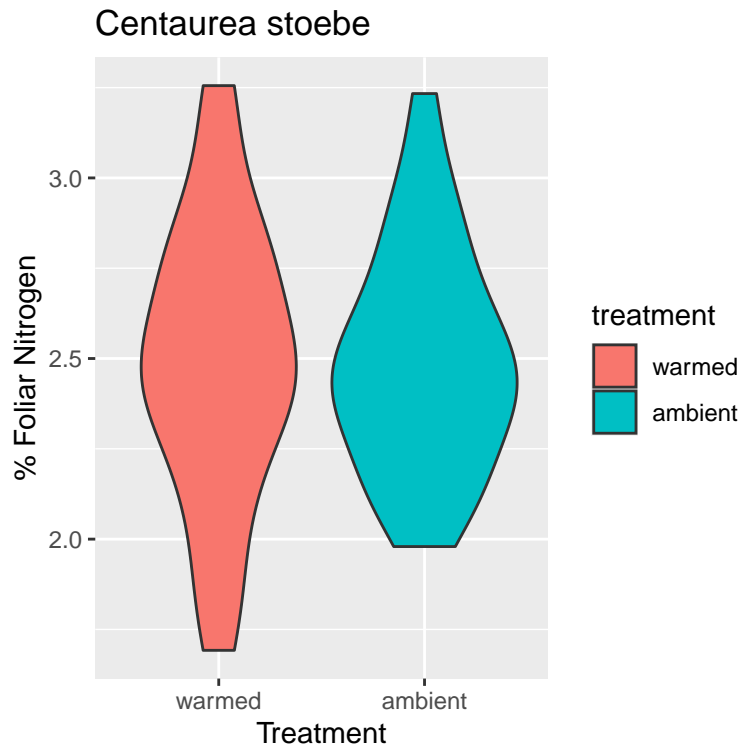
Solidago canadensis

```
library(lme4)
n.cest.m1 <- lmer(nitrogen ~ treatment + (1|plot) + (1|plant_id), data = cn17_cest)
summary(n.cest.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + (1 | plot) + (1 | plant_id)
##    Data: cn17_cest
##
## REML criterion at convergence: 47.8
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -1.9676 -0.6355  0.1023  0.5289  2.0244
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.00000  0.0000
##  plot     (Intercept) 0.03546  0.1883
##  Residual             0.09153  0.3025
## Number of obs: 69, groups:  plant_id, 44; plot, 11
##
## Fixed effects:
##                 Estimate Std. Error t value
## (Intercept)      2.45199    0.10123  24.223
## treatmentambient 0.03922    0.13683   0.287
##
## Correlation of Fixed Effects:
##             (Intr)
## tretmntmbnt -0.740
```

```
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
plot(n.cest.m1)
```


The analysis indicates that variation attributable to differences in the means of the two treatment groups was highest (0.04 %), followed by variation among individuals within a quadrat (0.035 %), followed by variation due to replicate runs of the pooled tissue from individual plants (>0.0001 %). This implies that variation among plots should be high. The figure below shows that variation in the mean %N among the 11 plots in the dataset was high.

Centaurea stoebe

#Acmi

```
library(lme4)
n.acmi.m1 <- lmer(nitrogen ~ treatment + (1|plot) + (1|plant_id), data = cn17_acmi)
summary(n.acmi.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + (1 | plot) + (1 | plant_id)
##    Data: cn17_acmi
##
## REML criterion at convergence: -114
##
## Scaled residuals:
##    Min      1Q  Median      3Q     Max
## -3.4372 -0.3214 -0.0225  0.2919  3.5491
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.050621 0.22499
##  plot     (Intercept) 0.017061 0.13062
##  Residual             0.003068 0.05539
## Number of obs: 99, groups:  plant_id, 46; plot, 11
##
## Fixed effects:
##                 Estimate Std. Error t value
## (Intercept)      1.90933    0.07983  23.916
## treatmentambient -0.14260    0.10613  -1.344
##
## Correlation of Fixed Effects:
##             (Intr)
## tretmntmbnt -0.752
```

```
plot(n.acmi.m1)
```
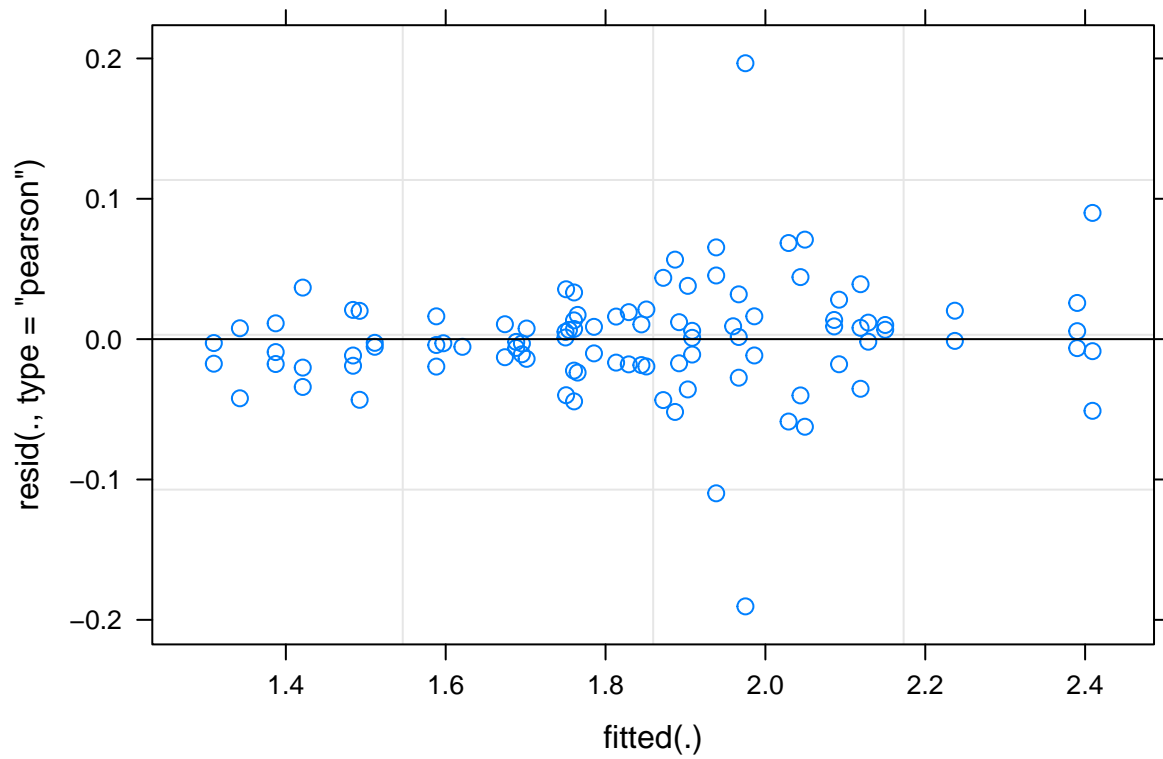

The analysis indicates that variation attributable to differences in the means of the two treatment groups was highest (0.14 %), followed by variation due to replicate runs of the pooled tissue from individual plants (0.05 %),followed by variation among individuals within a quadrat (0.02 %). This implies that variation among plots should be high. The figure below shows that variation in the mean %N among the 11 plots in the dataset was high.
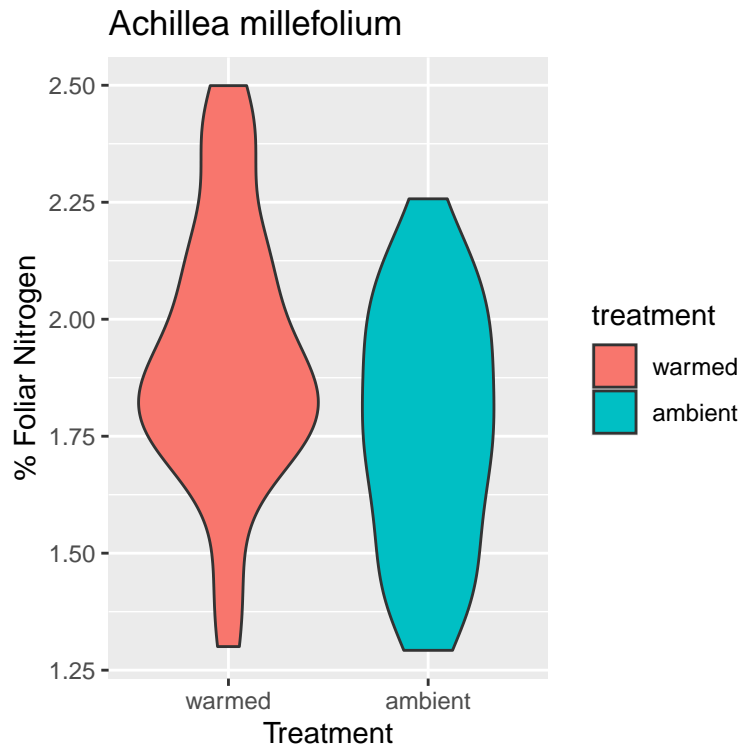
Below is a histogram of the standard deviation of %N for replicate pooled samples within individual plants. This indicates that the low variation of about foliar N, on average, among replicates within individual plants estimated by the random-effects ANOVA is reasonable per species.

```
ggplot(cn17msd, aes(x = sd_N, colour = species, fill=species)) +
  geom_histogram(alpha=0.5, position="dodge", bins=15) +
        facet_grid(vars(species))
```
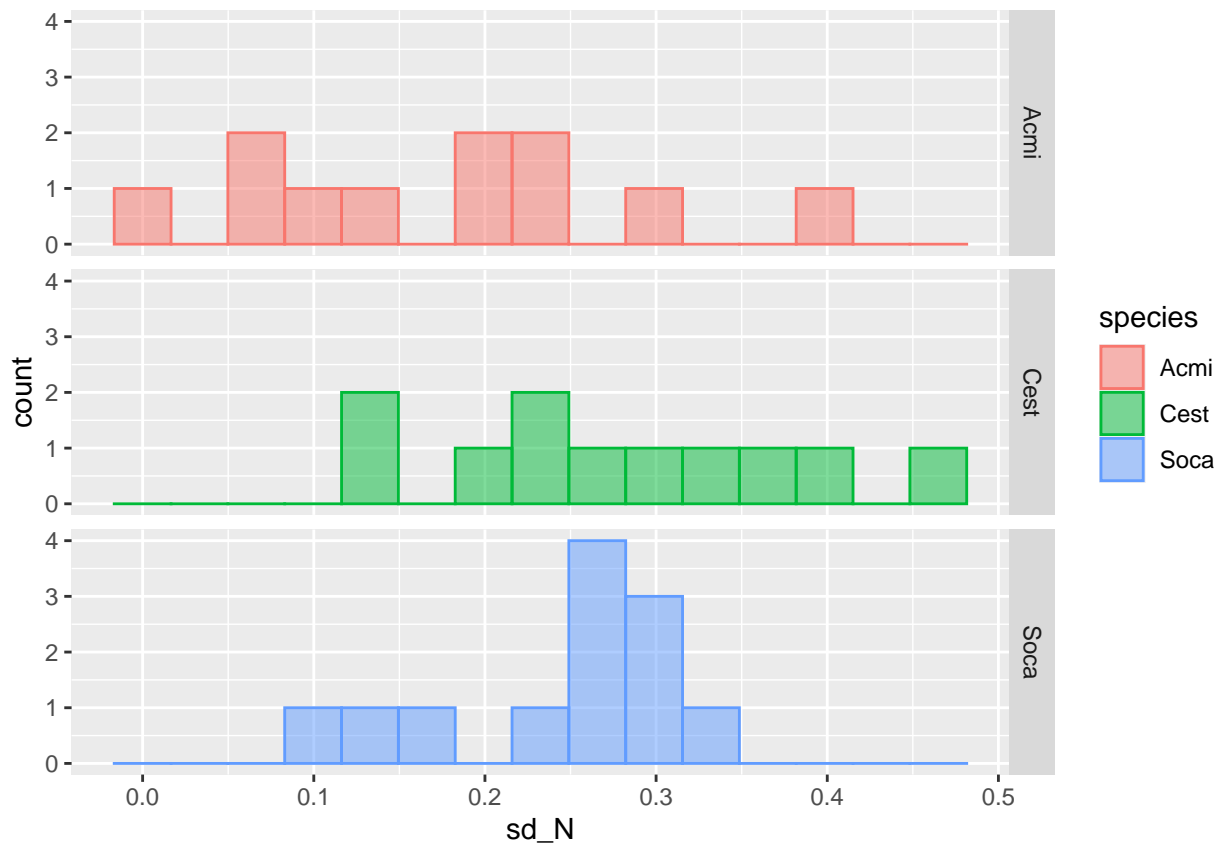
Power Analysis

So, how many samples are required to detect a difference of 0.1% drymass foliar nitrogen, given the observed variation in each species' %N? I chose a difference of 0.1% because bioassays have shown this difference has a large effect on the growth rate and survival of Lepidoptera larvae. This power analysis uses the mean %N of the two replicate subsamples of pooled leaves per plant and ignores the dependence among individual plants in the same quadrat. Thus, this is likely an underestimate of the number of samples required.

```r
library("pwr")
effect_size = 0.1/sd(cn17msd$sd_N[cn17msd$species=="Soca"])
pwr.anova.test( k=2, sig.level = 0.05, power = 0.8, f = effect_size)
```

```
##
##      Balanced one-way analysis of variance power calculation
##
##              k = 2
##              n = 3.631914
##              f = 1.286861
##      sig.level = 0.05
##          power = 0.8
##
## NOTE: n is number in each group
```

```r
effect_size = 0.1/sd(cn17msd$sd_N[cn17msd$species=="Cest"])
pwr.anova.test( k=2, sig.level = 0.05, power = 0.8, f = effect_size)
```

```
##
```

```
##      Balanced one-way analysis of variance power calculation
##
##              k = 2
##              n = 5.816709
##              f = 0.9157504
##      sig.level = 0.05
##          power = 0.8
##
## NOTE: n is number in each group
```

```
effect_size = 0.1/sd(cn17msd$sd_N[cn17msd$species=="Acmi"])
pwr.anova.test( k=2, sig.level = 0.05, power = 0.8, f = effect_size)
```

```
##
##      Balanced one-way analysis of variance power calculation
##
##              k = 2
##              n = 6.140381
##              f = 0.8847194
##      sig.level = 0.05
##          power = 0.8
##
## NOTE: n is number in each group
```

Variation among plots within a treatment was large, so definitely continue to sample all plots. Variation among replicate runs of the pooled tissue for an individual was not as large, and variation among individuals within a plot was much smaller. The power analysis for Soca indicates that approx 3.6 samples in each group, Cest = 5.8 in each group, Acmi = 6 in each group, are required to have sufficient power to detect a difference, if present. That's pretty small.

Model with all species as fixed effects; still shows plot-level variation is larger than within-plant level variation.

```
n.m1 <- lmer(nitrogen ~ treatment + species + (1|plot) + (1|plant_id), data = cn17)
summary(n.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + species + (1 | plot) + (1 | plant_id)
##    Data: cn17
##
## REML criterion at convergence: 156.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.3277 -0.6127 -0.0318  0.5894  3.2088
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.007543 0.08685
##  plot     (Intercept) 0.020749 0.14404
##  Residual             0.078936 0.28096
## Number of obs: 329, groups:  plant_id, 60; plot, 12
##
## Fixed effects:
##                   Estimate Std. Error t value
## (Intercept)       1.855102   0.070103  26.462
## treatmentambient -0.003472   0.091793  -0.038
```

```
## speciesCest        0.628147    0.045937  13.674
## speciesSoca        0.491779    0.036866  13.340
##
## Correlation of Fixed Effects:
##            (Intr) trtmnt spcsCs
## tretmntmbnt -0.667
## speciesCest -0.281  0.011
## speciesSoca -0.350  0.021  0.518
```

Based on the above investigation, we don't need to account for variation among replicate runs of the pooled tissue for an individual. So we will take the mean by plant and move forward.

```
cn$plant_id <- paste(cn$plot, cn$plant_number, sep = "_")

cn1 <- cn %>%
    group_by(plot,site,year,species,treatment, insecticide, plant_id) %>%
    summarize(nitrogen = mean(nitrogen), carbon = mean(carbon), weight_mg = mean(weight_mg))
```

```
## `summarise()` has grouped output by 'plot', 'site', 'year', 'species',
## 'treatment', 'insecticide'. You can override using the `.groups` argument.
```

```
# Take a look at these data
hist(cn1$carbon)
```


Histogram of cn1$carbon
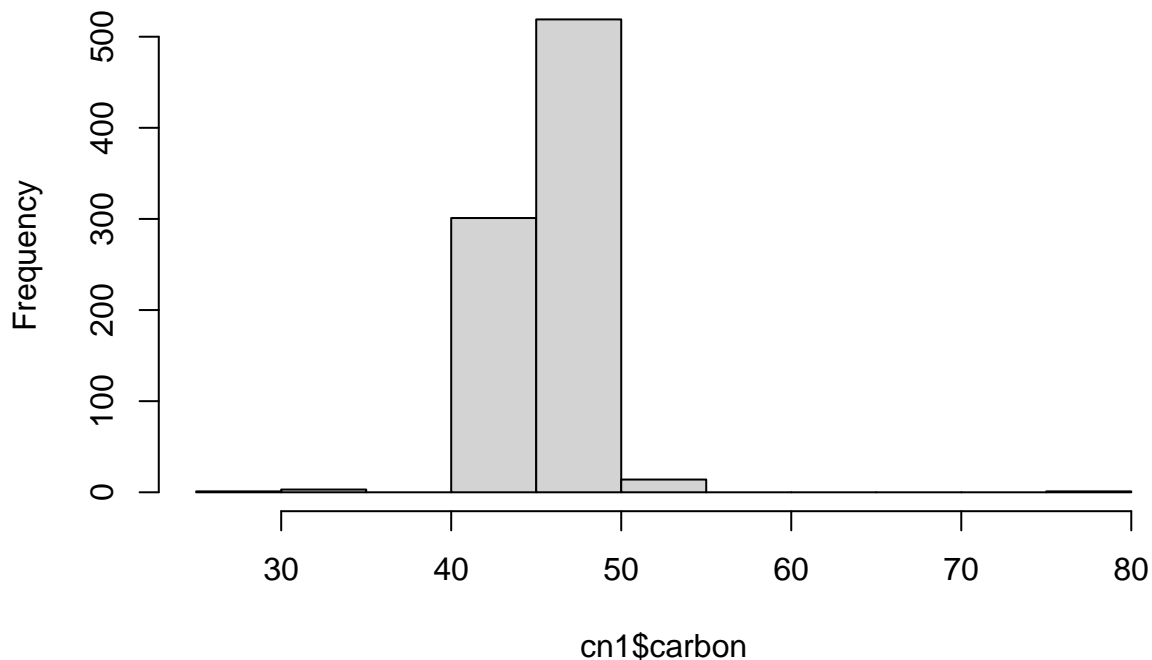
```
sort(unique(cn1$carbon))
```

```
##    [1] 28.05870 34.15057 34.62433 34.68990 40.07060 40.12880 40.31360 40.92505
##    [9] 41.09440 41.09620 41.15720 41.21150 41.33117 41.36840 41.64950 41.66580
##   [17] 41.67580 41.70130 41.74290 41.74695 41.79515 41.80480 41.80490 41.86040
##   [25] 41.91640 42.00635 42.33180 42.39485 42.40990 42.45710 42.45870 42.48690
##   [33] 42.48870 42.50403 42.50860 42.50990 42.51020 42.51160 42.54790 42.56370
##   [41] 42.57730 42.59220 42.59650 42.61800 42.66340 42.68210 42.70685 42.71467
```

```
##  [49] 42.72690 42.74020 42.74220 42.75050 42.76140 42.76260 42.77430 42.80070
##  [57] 42.81050 42.81800 42.85180 42.85490 42.86850 42.88380 42.89410 42.91443
##  [65] 42.96510 42.98130 43.00040 43.03093 43.04305 43.04520 43.07630 43.09690
##  [73] 43.10140 43.14090 43.14880 43.15420 43.15460 43.16600 43.18900 43.20160
##  [81] 43.21040 43.23870 43.24270 43.25680 43.25880 43.26210 43.27070 43.27360
##  [89] 43.31300 43.31400 43.32305 43.32500 43.33070 43.34500 43.34530 43.38030
##  [97] 43.38490 43.41260 43.43210 43.43980 43.44090 43.45870 43.45950 43.46985
## [105] 43.47320 43.47410 43.49210 43.50870 43.51090 43.51420 43.56730 43.58990
## [113] 43.61530 43.62630 43.63140 43.63150 43.63640 43.63720 43.65300 43.65830
## [121] 43.65960 43.66630 43.66840 43.67560 43.67710 43.67930 43.68090 43.68700
## [129] 43.68840 43.70060 43.70170 43.70710 43.72320 43.72500 43.72670 43.72950
## [137] 43.73060 43.73540 43.73790 43.74400 43.74950 43.74980 43.75190 43.76240
## [145] 43.78130 43.79270 43.80880 43.80980 43.81310 43.81573 43.84030 43.84980
## [153] 43.85255 43.85400 43.86570 43.87335 43.88065 43.88560 43.89280 43.90080
## [161] 43.90450 43.90470 43.91390 43.91760 43.94100 43.95030 43.95740 43.96050
## [169] 43.96420 43.96720 43.96790 43.97120 43.97890 43.97920 43.99660 44.01150
## [177] 44.01315 44.01520 44.01530 44.02270 44.04840 44.05050 44.05330 44.06200
## [185] 44.07410 44.07470 44.09080 44.10370 44.10460 44.11360 44.12010 44.12850
## [193] 44.13250 44.14120 44.14230 44.14880 44.15340 44.17240 44.17480 44.19340
## [201] 44.20580 44.20890 44.21150 44.21280 44.24425 44.25400 44.25820 44.26430
## [209] 44.26540 44.26690 44.27640 44.27930 44.28240 44.28540 44.29370 44.31700
## [217] 44.32990 44.37303 44.37880 44.37950 44.38130 44.38235 44.38800 44.39285
## [225] 44.39410 44.39580 44.40410 44.40490 44.41390 44.42470 44.43360 44.44205
## [233] 44.44500 44.44540 44.44870 44.45620 44.47440 44.48610 44.48725 44.48910
## [241] 44.49170 44.50490 44.51490 44.52170 44.53630 44.54160 44.54180 44.55660
## [249] 44.57990 44.59100 44.59520 44.59690 44.60295 44.60310 44.61200 44.62000
## [257] 44.62430 44.63530 44.64340 44.65590 44.67160 44.67480 44.68170 44.68840
## [265] 44.70250 44.70720 44.71030 44.71290 44.71330 44.73530 44.73880 44.74200
## [273] 44.74870 44.74990 44.79160 44.79460 44.80910 44.80970 44.82360 44.83130
## [281] 44.83250 44.83640 44.83880 44.84770 44.84890 44.86410 44.86970 44.87560
## [289] 44.87605 44.88240 44.88290 44.88870 44.89240 44.89470 44.89680 44.89830
## [297] 44.89890 44.90290 44.93720 44.94180 44.95780 44.98430 44.98670 44.99450
## [305] 45.00130 45.00390 45.00810 45.01560 45.03040 45.04110 45.04210 45.04820
## [313] 45.05340 45.07250 45.07370 45.07720 45.08510 45.10075 45.10590 45.10630
## [321] 45.13610 45.15430 45.18880 45.21790 45.22050 45.22660 45.23230 45.23770
## [329] 45.25780 45.26160 45.26710 45.27270 45.27915 45.28100 45.28155 45.28920
## [337] 45.29710 45.29720 45.33080 45.33730 45.33935 45.34210 45.35020 45.35240
## [345] 45.35340 45.35490 45.36190 45.37850 45.42835 45.43380 45.43430 45.44830
## [353] 45.45400 45.46010 45.46740 45.47350 45.47770 45.48710 45.50880 45.51750
## [361] 45.52430 45.56470 45.57850 45.58480 45.58670 45.60290 45.61000 45.62480
## [369] 45.65080 45.65560 45.65730 45.67040 45.67650 45.67740 45.68330 45.68480
## [377] 45.70600 45.71840 45.72010 45.73880 45.75480 45.75550 45.76200 45.76510
## [385] 45.78420 45.78820 45.79090 45.79160 45.82040 45.82900 45.83770 45.83930
## [393] 45.84350 45.85500 45.86730 45.88280 45.88600 45.88720 45.89680 45.90960
## [401] 45.91810 45.92930 45.93140 45.93400 45.93430 45.94590 45.95420 45.95960
## [409] 45.96040 45.97750 45.97840 45.98970 45.99370 45.99410 46.00360 46.01230
## [417] 46.03440 46.04100 46.04230 46.04790 46.06950 46.07197 46.08140 46.09040
## [425] 46.09410 46.09480 46.10120 46.10980 46.13760 46.15520 46.16280 46.17170
## [433] 46.21270 46.21520 46.22100 46.22310 46.25140 46.26205 46.26540 46.26570
## [441] 46.27630 46.28180 46.29260 46.29510 46.29720 46.30150 46.32060 46.32180
## [449] 46.34300 46.36090 46.38350 46.38420 46.38550 46.38990 46.39460 46.39720
## [457] 46.40660 46.40910 46.41040 46.41970 46.42050 46.42200 46.42940 46.43100
## [465] 46.45150 46.45190 46.45850 46.46210 46.46660 46.47730 46.47750 46.48490
## [473] 46.50467 46.50680 46.50867 46.51590 46.51670 46.54530 46.54750 46.55200
```

```
## [481]  46.55493 46.55780 46.55990 46.56070 46.56850 46.57180 46.59650 46.62090
## [489]  46.63630 46.63760 46.63823 46.64770 46.67430 46.69070 46.69100 46.70020
## [497]  46.71323 46.72850 46.74340 46.76260 46.77420 46.78600 46.78630 46.79490
## [505]  46.80130 46.80430 46.80450 46.81730 46.83520 46.84760 46.85400 46.87907
## [513]  46.87970 46.88300 46.88550 46.88640 46.89010 46.89130 46.90420 46.90720
## [521]  46.91390 46.91530 46.91760 46.91870 46.93580 46.93840 46.95030 46.95280
## [529]  46.95450 46.96580 46.97420 46.98000 46.98320 46.99150 47.00230 47.00340
## [537]  47.00410 47.01540 47.01660 47.02930 47.03350 47.03630 47.03720 47.04960
## [545]  47.05510 47.05550 47.05800 47.06260 47.06310 47.07170 47.08510 47.11060
## [553]  47.11180 47.11270 47.11740 47.12630 47.13070 47.14025 47.15540 47.15680
## [561]  47.16730 47.17670 47.17860 47.18390 47.18550 47.19560 47.20290 47.22520
## [569]  47.23550 47.23600 47.23910 47.24520 47.24837 47.24997 47.25230 47.25350
## [577]  47.25620 47.25640 47.25840 47.26290 47.27330 47.29930 47.30800 47.32660
## [585]  47.32870 47.33000 47.33190 47.35480 47.35840 47.36110 47.36250 47.37840
## [593]  47.38140 47.38510 47.39260 47.39670 47.40340 47.40960 47.41810 47.41930
## [601]  47.41940 47.42960 47.43120 47.43470 47.43880 47.44150 47.44850 47.46590
## [609]  47.49210 47.49240 47.49530 47.52480 47.52550 47.52763 47.53070 47.53460
## [617]  47.54060 47.54890 47.55330 47.55943 47.56220 47.56810 47.57850 47.58750
## [625]  47.59620 47.60890 47.60940 47.61110 47.61400 47.62590 47.62860 47.63600
## [633]  47.64430 47.65040 47.67440 47.67980 47.68850 47.68993 47.69810 47.70160
## [641]  47.71850 47.71900 47.72750 47.72790 47.74020 47.74300 47.75040 47.75590
## [649]  47.77910 47.78470 47.78800 47.79280 47.80440 47.81530 47.82240 47.82640
## [657]  47.82860 47.82950 47.83520 47.84330 47.85390 47.85660 47.86370 47.86930
## [665]  47.87433 47.87630 47.88860 47.90920 47.91380 47.91500 47.92190 47.92840
## [673]  47.92920 47.93430 47.93800 47.93860 47.94020 47.95730 47.96950 47.97090
## [681]  47.97150 47.99030 48.00820 48.00840 48.01370 48.01870 48.02440 48.04080
## [689]  48.04230 48.04390 48.04780 48.05040 48.05420 48.06370 48.06870 48.10110
## [697]  48.10620 48.12820 48.14020 48.15700 48.16650 48.17120 48.17400 48.17845
## [705]  48.19170 48.19630 48.19805 48.20510 48.20610 48.21377 48.23800 48.25240
## [713]  48.25710 48.26790 48.26833 48.27210 48.31010 48.32680 48.32710 48.33700
## [721]  48.34010 48.36323 48.38790 48.39057 48.39570 48.40120 48.40130 48.42490
## [729]  48.43040 48.43710 48.44687 48.45070 48.45630 48.47000 48.47500 48.48160
## [737]  48.49400 48.50310 48.52513 48.52990 48.53330 48.53743 48.54735 48.55280
## [745]  48.57270 48.59450 48.60240 48.61510 48.62710 48.63020 48.64930 48.65150
## [753]  48.65330 48.66940 48.67890 48.70530 48.73270 48.73730 48.73890 48.77070
## [761]  48.77420 48.79087 48.80960 48.81110 48.85300 48.87630 48.90120 48.90230
## [769]  48.90490 48.90890 48.92710 48.93430 48.94280 48.95080 48.95940 49.00220
## [777]  49.01640 49.06170 49.06640 49.07280 49.07850 49.09470 49.13630 49.13790
## [785]  49.15670 49.17080 49.18710 49.18970 49.21670 49.21990 49.22440 49.22600
## [793]  49.32660 49.33680 49.35860 49.36133 49.38650 49.41450 49.42880 49.45650
## [801]  49.46830 49.46980 49.47500 49.53410 49.56310 49.57990 49.58210 49.58400
## [809]  49.59760 49.62080 49.64790 49.71450 49.75120 49.85120 49.87383 49.93390
## [817]  49.93960 49.95280 49.96040 49.96740 49.99980 50.15520 50.18270 50.19450
## [825]  50.20330 50.40190 50.74400 50.74593 50.82220 50.90530 51.57830 51.64687
## [833]  51.79020 52.81883 53.50147 77.94200
```

```r
# Remove data > 55
c1<-cn1 %>% filter(carbon < 55)
#Model for Carbon
c.m1 <- lmer(carbon ~ treatment + species + (1|plot) + (1|plant_id), data = c1)
```
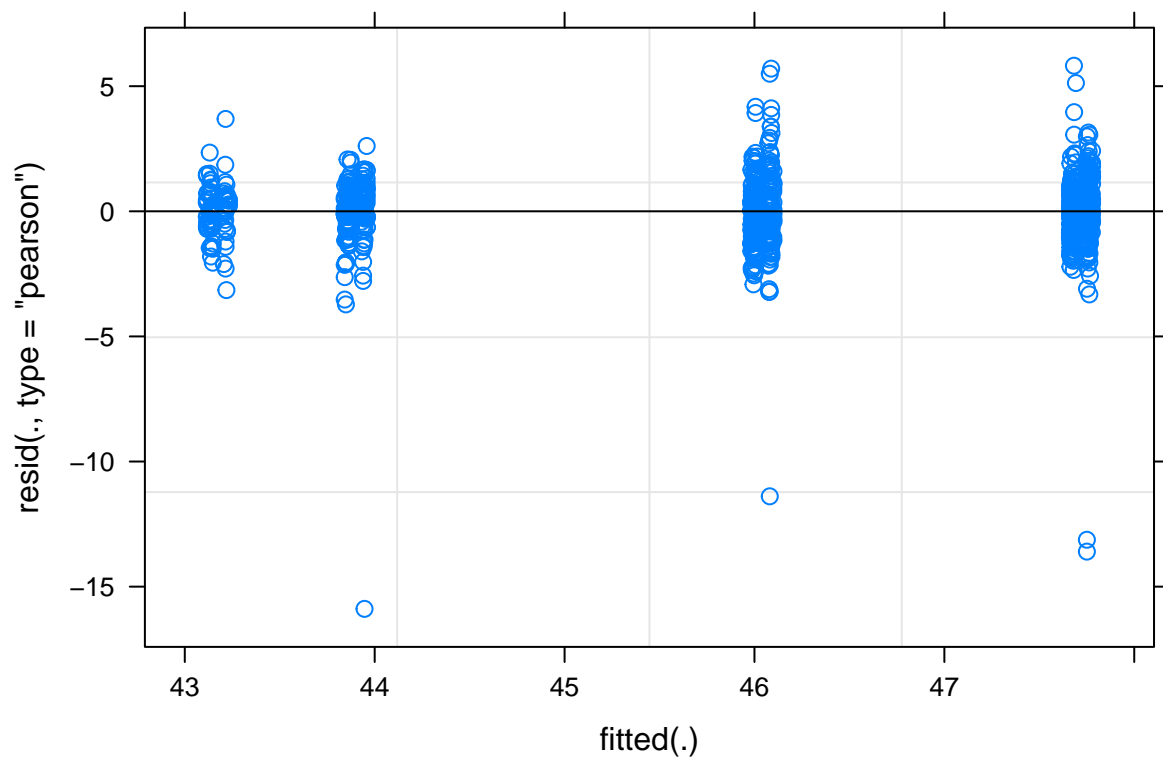
```
## boundary (singular) fit: see help('isSingular')
```
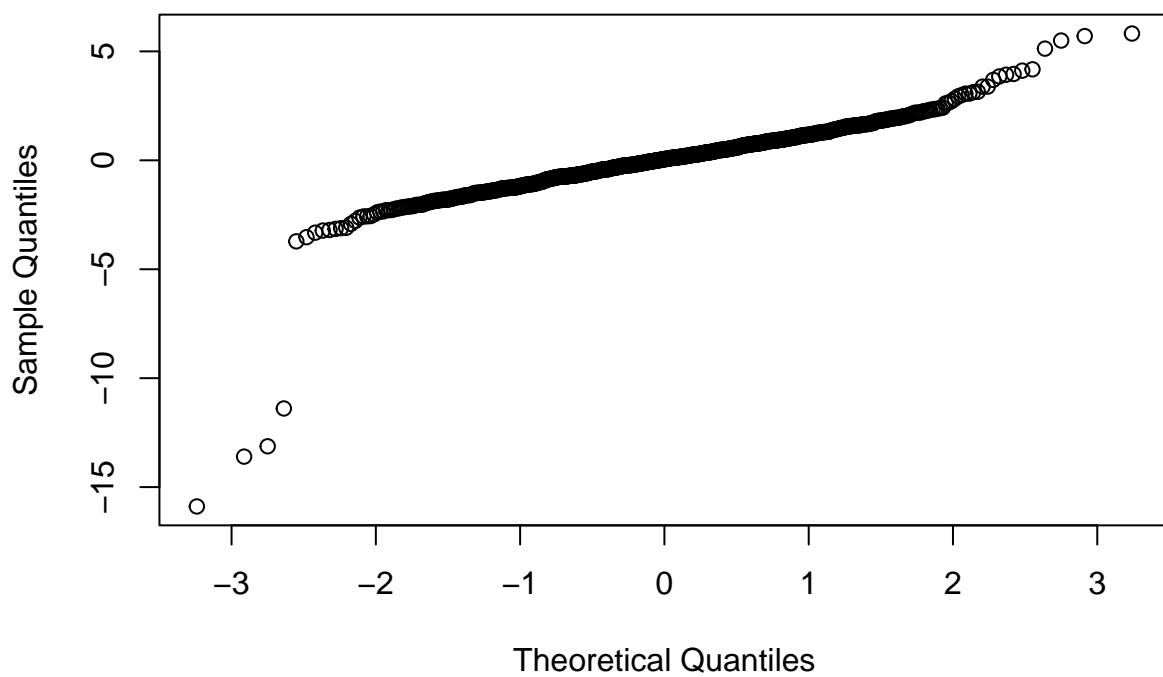
```
summary(c.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: carbon ~ treatment + species + (1 | plot) + (1 | plant_id)
##    Data: c1
##
## REML criterion at convergence: 3142.6
##
## Scaled residuals:
##      Min      1Q   Median      3Q      Max
## -10.1186  -0.4635   0.0358   0.5106   3.7059
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  plant_id (Intercept) 0.000000 0.00000
##  plot     (Intercept) 0.002461 0.04961
##  Residual             2.465411 1.57016
## Number of obs: 838, groups:  plant_id, 97; plot, 24
##
## Fixed effects:
##                    Estimate Std. Error t value
## (Intercept)        43.21720    0.18356 235.442
## treatmentambient   -0.08533    0.11081  -0.770
## speciesCest         2.86761    0.19773  14.502
## speciesPopr         0.72611    0.20661   3.514
## speciesSoca         4.54521    0.19200  23.673
##
## Correlation of Fixed Effects:
##             (Intr) trtmnt spcsCs spcsPp
## tretmntmbnt -0.367
## speciesCest -0.820  0.054
## speciesPopr -0.780  0.038  0.713
## speciesSoca -0.850  0.072  0.769  0.735
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
plot(c.m1)
```

```
qqnorm(resid(c.m1))
```

Normal Q–Q Plot


```
# Some outliers. Let's remove and re-fit.
# Remove data < 35
c1<-c1 %>% filter(carbon > 35)
```

```
#Model for Carbon
c.m1 <- lmer(carbon ~ treatment + species + (1|plot) + (1|plant_id), data = c1)
```
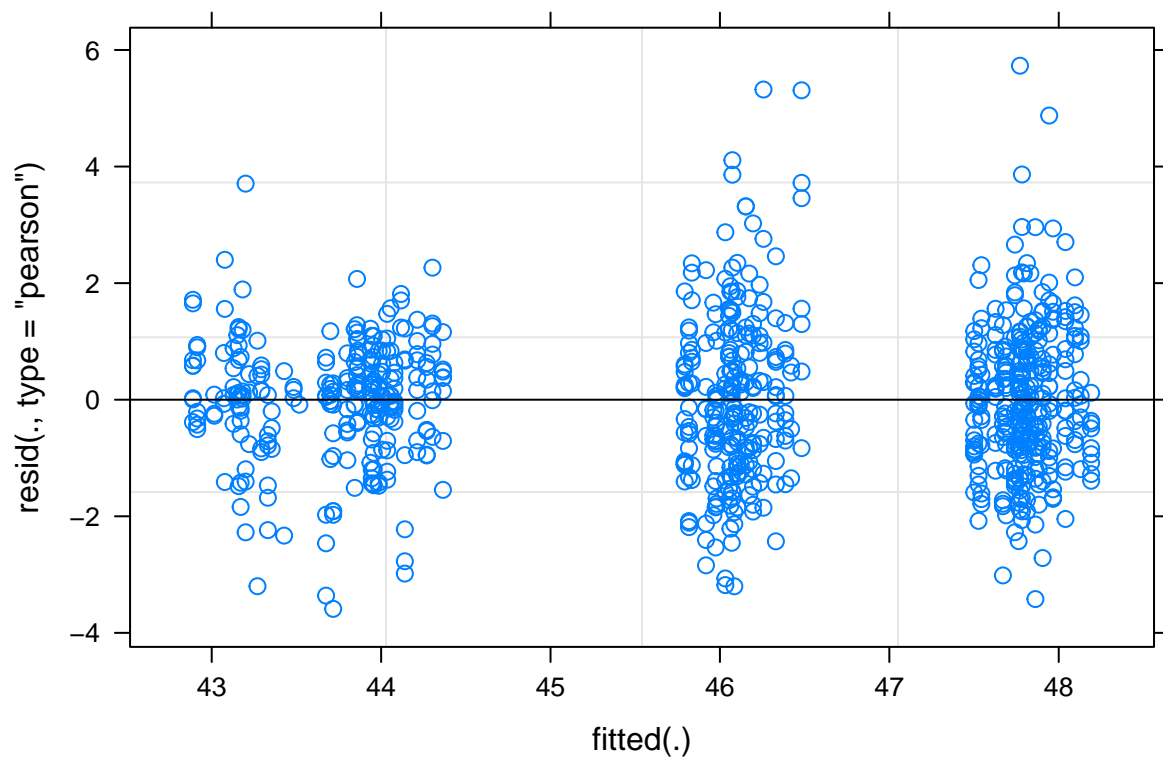
boundary (singular) fit: see help('isSingular')

```
summary(c.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: carbon ~ treatment + species + (1 | plot) + (1 | plant_id)
##    Data: c1
##
## REML criterion at convergence: 2751.1
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.8931 -0.6312  0.0202  0.5916  4.6202
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.00000  0.0000
##  plot     (Intercept) 0.04488  0.2118
##  Residual             1.53825  1.2403
## Number of obs: 834, groups:  plant_id, 97; plot, 24
##
## Fixed effects:
##                   Estimate Std. Error t value
## (Intercept)        43.3120     0.1584 273.424
## treatmentambient   -0.2204     0.1228  -1.795
## speciesCest         2.9028     0.1573  18.457
## speciesPopr         0.7858     0.1653   4.754
## speciesSoca         4.6122     0.1526  30.229
##
## Correlation of Fixed Effects:
##             (Intr) trtmnt spcsCs spcsPp
## tretmntmbnt -0.428
## speciesCest -0.758  0.034
## speciesPopr -0.723  0.023  0.715
## speciesSoca -0.786  0.048  0.770  0.735
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```
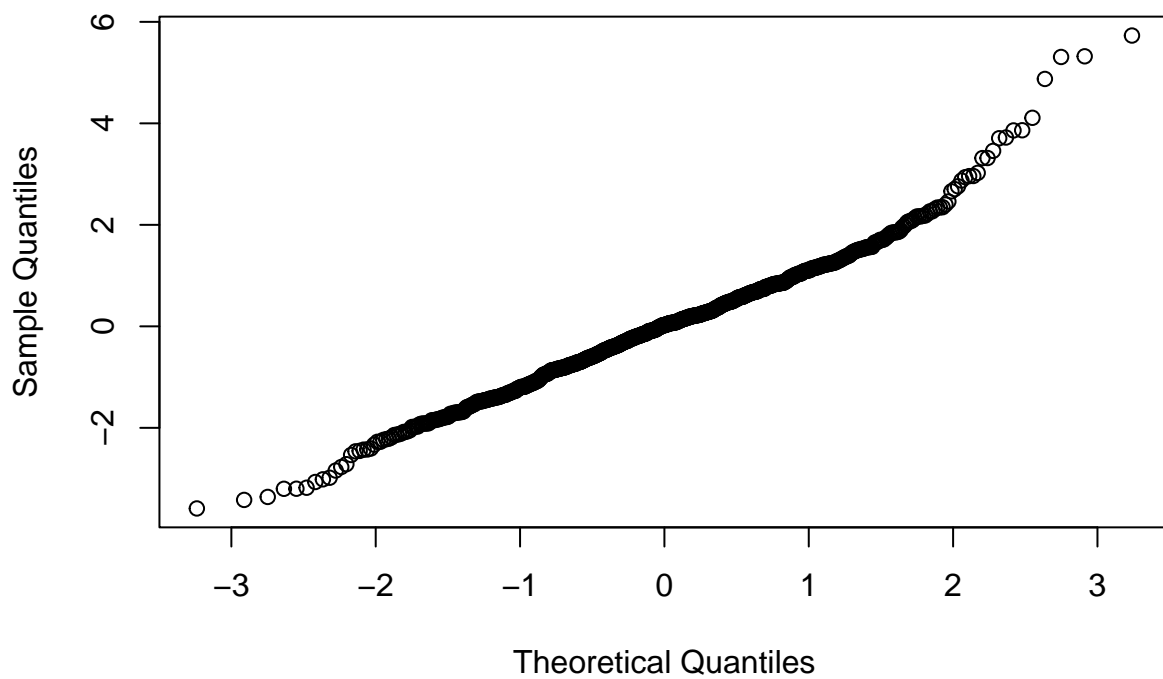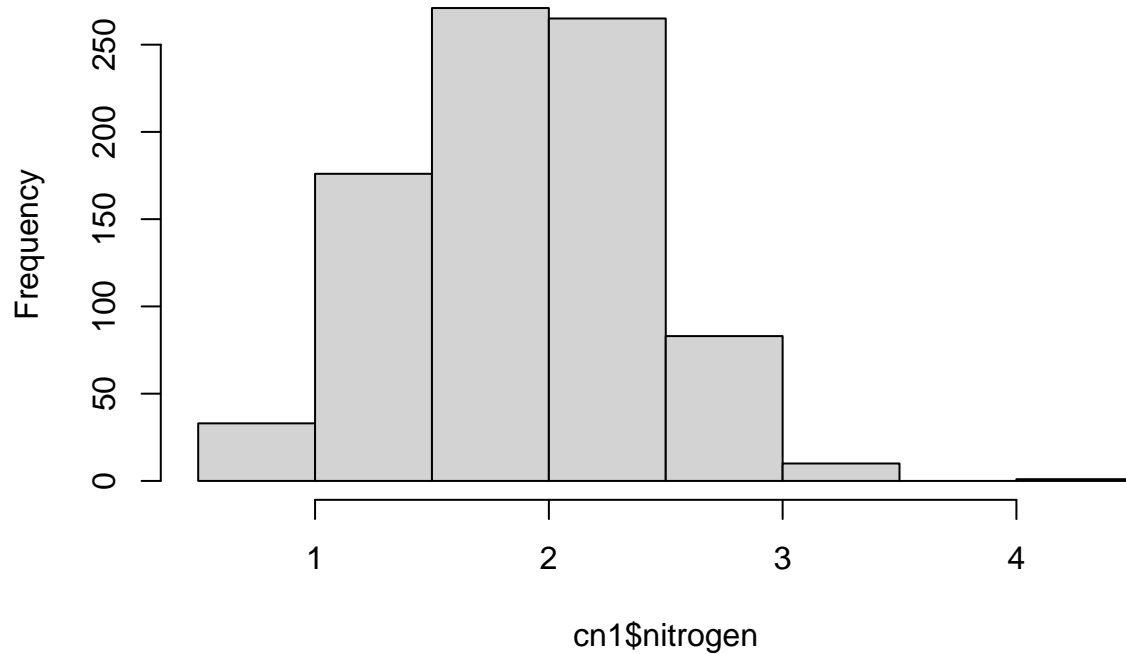
```
plot(c.m1)
```

```
qqnorm(resid(c.m1))
```

Normal Q−Q Plot


```
#Model for Nitrogen
# Take a look at these data
hist(cn1$nitrogen)
```

Histogram of cn1$nitrogen


```r
n.m1 <- lmer(nitrogen ~ treatment + species + (1|plot) + (1|plant_id), data = cn1)
```

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

```r
summary(n.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + species + (1 | plot) + (1 | plant_id)
##    Data: cn1
##
## REML criterion at convergence: 1073.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2819 -0.7297 -0.0968  0.6279  6.1130
##
## Random effects:
##  Groups   Name        Variance  Std.Dev.
##  plant_id (Intercept) 1.255e-16 1.120e-08
##  plot     (Intercept) 1.719e-03 4.146e-02
##  Residual             2.041e-01 4.518e-01
## Number of obs: 839, groups:  plant_id, 97; plot, 24
##
## Fixed effects:
##                   Estimate Std. Error t value
## (Intercept)        1.99734    0.05421  36.844
## treatmentambient   0.09861    0.03573   2.760
## speciesCest        0.05878    0.05704   1.030
## speciesPopr       -0.62871    0.05969 -10.533
## speciesSoca       -0.13192    0.05537  -2.382
```

```
## 
## Correlation of Fixed Effects:
##             (Intr) trtmnt spcsCs spcsPp
## tretmntmbnt -0.385
## speciesCest -0.802  0.046
## speciesPopr -0.764  0.031  0.715
## speciesSoca -0.832  0.063  0.770  0.736
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
plot(n.m1)
```
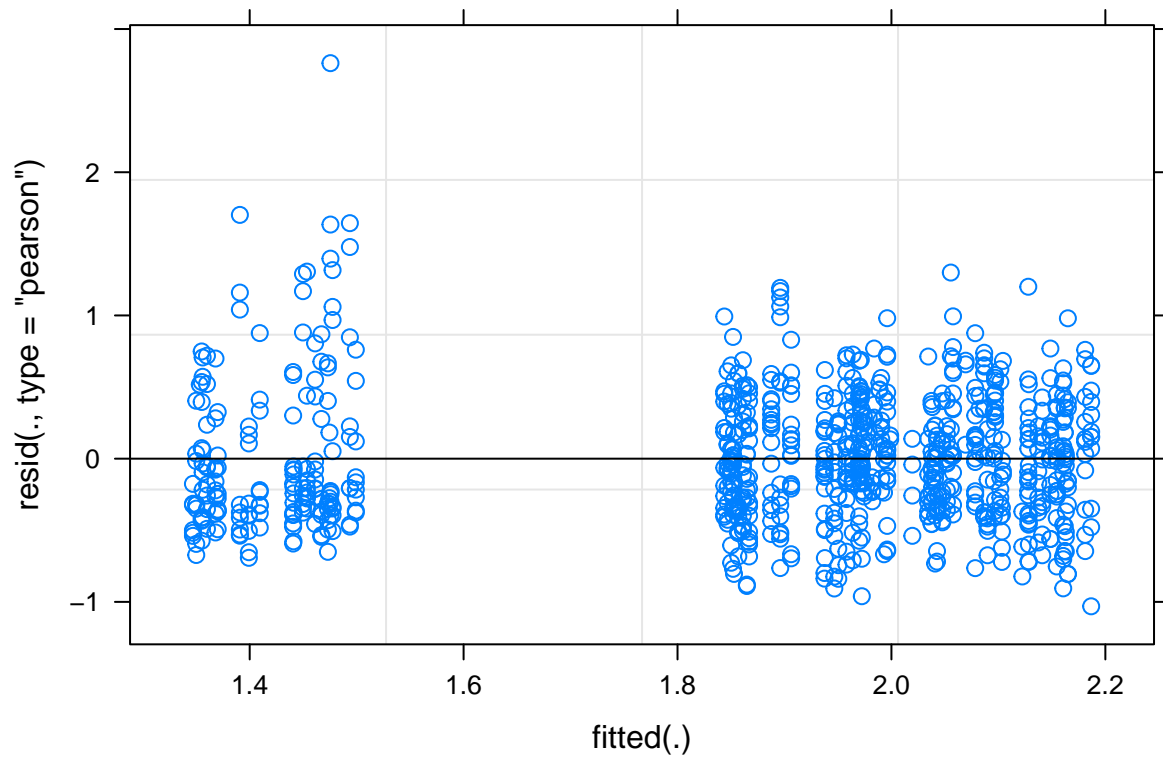


```
qqnorm(resid(n.m1))
```

Normal Q–Q Plot
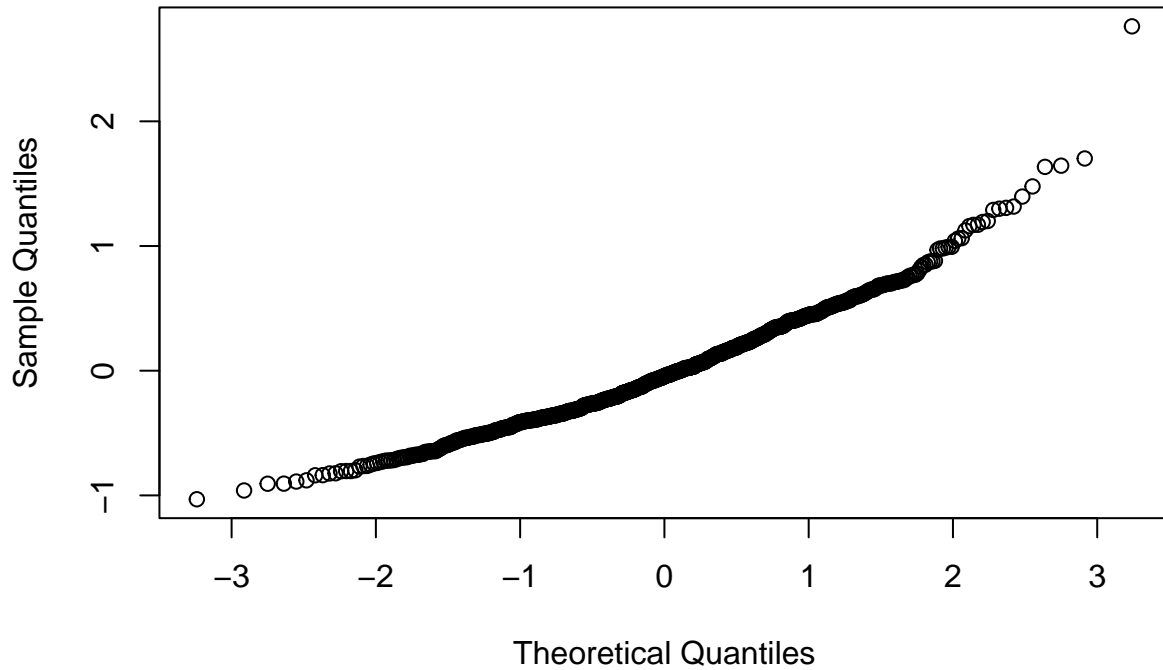

```r
# Resids suggest outlier
sort(unique(cn1$nitrogen))
```

```
##   [1]  0.677300 0.706800 0.745900 0.754800 0.782100 0.807800 0.822600 0.829600
##   [9]  0.847900 0.848000 0.852800 0.852900 0.861500 0.863600 0.870100 0.877400
##  [17]  0.894900 0.895900 0.921000 0.921300 0.928100 0.928900 0.933000 0.941500
##  [25]  0.947600 0.964200 0.971500 0.975800 0.977900 0.978400 0.984200 0.985700
##  [33]  0.988300 1.000900 1.001700 1.002000 1.005000 1.005900 1.007900 1.008300
##  [41]  1.011700 1.014000 1.015300 1.019400 1.023800 1.028500 1.028700 1.029700
##  [49]  1.031900 1.033000 1.040100 1.043500 1.046900 1.048100 1.052300 1.068600
##  [57]  1.068900 1.072800 1.074900 1.079800 1.081000 1.082200 1.082600 1.085500
##  [65]  1.086800 1.093400 1.098600 1.099700 1.102200 1.103000 1.105800 1.106800
##  [73]  1.111600 1.122300 1.122600 1.123100 1.123500 1.124100 1.129500 1.132200
##  [81]  1.133000 1.133400 1.136100 1.138700 1.139000 1.139100 1.146500 1.155800
##  [89]  1.156100 1.160700 1.170900 1.174600 1.174800 1.176100 1.177500 1.180200
##  [97]  1.184000 1.185200 1.185400 1.186500 1.189300 1.190000 1.198200 1.198800
## [105]  1.206600 1.208400 1.210300 1.216900 1.218400 1.227600 1.229500 1.238900
## [113]  1.242300 1.242700 1.242900 1.243700 1.244300 1.248900 1.249700 1.253000
## [121]  1.253900 1.254900 1.260800 1.265700 1.268300 1.273500 1.283100 1.285700
## [129]  1.287400 1.287600 1.291400 1.299300 1.299750 1.300000 1.301600 1.307000
## [137]  1.308100 1.308400 1.308600 1.311800 1.313800 1.315700 1.321600 1.322400
## [145]  1.323800 1.325350 1.330700 1.333600 1.333800 1.337100 1.337300 1.341100
## [153]  1.351200 1.356000 1.356200 1.358700 1.359700 1.360200 1.361100 1.361500
## [161]  1.365400 1.368200 1.369300 1.380800 1.381200 1.382100 1.383200 1.384900
## [169]  1.385300 1.385900 1.392200 1.393100 1.393800 1.394400 1.396500 1.397400
## [177]  1.401800 1.406600 1.407600 1.411700 1.415300 1.415800 1.419900 1.420900
## [185]  1.428500 1.438200 1.438800 1.441600 1.445000 1.446300 1.447000 1.452800
## [193]  1.453900 1.458000 1.460700 1.464600 1.466500 1.468100 1.480800 1.481500
## [201]  1.493300 1.494100 1.494900 1.495700 1.498200 1.499600 1.505500 1.507200
## [209]  1.508100 1.511900 1.514300 1.516100 1.516600 1.517600 1.522300 1.526600
```

```
## [217] 1.527800 1.528100 1.529000 1.529500 1.533200 1.535700 1.538000 1.552000
## [225] 1.553600 1.559000 1.563500 1.565800 1.567100 1.567800 1.569100 1.573200
## [233] 1.576600 1.578500 1.580300 1.582800 1.583400 1.585767 1.587700 1.588200
## [241] 1.590900 1.592500 1.593000 1.594100 1.596900 1.603000 1.611400 1.614500
## [249] 1.614800 1.615500 1.618900 1.619700 1.623700 1.623800 1.630500 1.630800
## [257] 1.635100 1.644400 1.645200 1.646900 1.648200 1.649100 1.649400 1.649500
## [265] 1.651300 1.655200 1.656800 1.659300 1.666300 1.667000 1.668000 1.670900
## [273] 1.672950 1.674100 1.674300 1.675900 1.677300 1.684150 1.684800 1.685400
## [281] 1.685800 1.686200 1.688450 1.689100 1.690800 1.692100 1.693000 1.693500
## [289] 1.695300 1.696900 1.698000 1.699100 1.699600 1.708100 1.710200 1.711300
## [297] 1.711600 1.713000 1.714400 1.716200 1.718000 1.719200 1.723800 1.725700
## [305] 1.728600 1.729300 1.731400 1.731800 1.732200 1.734800 1.734900 1.735000
## [313] 1.737500 1.739700 1.740100 1.741800 1.744100 1.744200 1.744300 1.744900
## [321] 1.745100 1.745300 1.747500 1.748450 1.750900 1.752100 1.753100 1.754700
## [329] 1.756000 1.759367 1.760800 1.761233 1.761500 1.761750 1.762200 1.763800
## [337] 1.767900 1.771400 1.773000 1.774400 1.776800 1.780800 1.781000 1.781800
## [345] 1.781900 1.784850 1.787700 1.788700 1.793200 1.794100 1.795100 1.795200
## [353] 1.798200 1.799600 1.800900 1.803100 1.803500 1.808000 1.809600 1.809800
## [361] 1.810100 1.812900 1.813000 1.813800 1.814300 1.816100 1.818200 1.821700
## [369] 1.822600 1.824300 1.824500 1.825700 1.827900 1.828800 1.829700 1.830200
## [377] 1.830700 1.836800 1.836900 1.837600 1.838300 1.838600 1.839200 1.840800
## [385] 1.840900 1.841000 1.842500 1.847400 1.850300 1.851100 1.851900 1.852600
## [393] 1.853400 1.854200 1.855600 1.859500 1.859700 1.859800 1.865700 1.867800
## [401] 1.868200 1.870700 1.872350 1.875200 1.876200 1.881100 1.881200 1.882800
## [409] 1.887800 1.889350 1.889450 1.889900 1.890300 1.893100 1.893300 1.895400
## [417] 1.898300 1.898500 1.903000 1.904000 1.904500 1.906000 1.907000 1.907400
## [425] 1.914700 1.917367 1.917700 1.918400 1.920100 1.920900 1.923900 1.924700
## [433] 1.925500 1.925800 1.927700 1.928100 1.928800 1.929000 1.934300 1.936433
## [441] 1.937200 1.938733 1.939500 1.943300 1.943500 1.949700 1.950000 1.953700
## [449] 1.959600 1.960200 1.962500 1.964600 1.965100 1.965200 1.966900 1.968667
## [457] 1.968700 1.968800 1.969700 1.970700 1.972400 1.976300 1.978100 1.985700
## [465] 1.988000 1.988450 1.991500 1.991700 1.992900 1.994100 1.998000 1.999400
## [473] 2.002150 2.004000 2.004800 2.007400 2.012300 2.012500 2.017000 2.018000
## [481] 2.018400 2.018500 2.018600 2.022500 2.024800 2.025300 2.026100 2.029600
## [489] 2.033900 2.035100 2.037667 2.039800 2.042400 2.042500 2.044800 2.045000
## [497] 2.045800 2.045950 2.047100 2.047700 2.049400 2.051400 2.053650 2.054800
## [505] 2.055500 2.057500 2.057900 2.058600 2.059000 2.059500 2.061300 2.062700
## [513] 2.065900 2.067200 2.068200 2.072000 2.074033 2.075800 2.076400 2.078000
## [521] 2.078100 2.078700 2.079100 2.079400 2.083800 2.086100 2.086300 2.088200
## [529] 2.090000 2.094900 2.095900 2.097300 2.097400 2.099500 2.100600 2.103700
## [537] 2.107800 2.109100 2.109900 2.110500 2.114500 2.120400 2.121000 2.123000
## [545] 2.124600 2.125500 2.125933 2.126200 2.126500 2.127933 2.132700 2.133700
## [553] 2.135600 2.136100 2.136200 2.137000 2.138500 2.138700 2.139800 2.142400
## [561] 2.143800 2.143900 2.149300 2.149800 2.150800 2.158300 2.158500 2.159100
## [569] 2.160700 2.164100 2.166600 2.166900 2.167400 2.168200 2.169200 2.170600
## [577] 2.172800 2.174000 2.175100 2.176267 2.176300 2.176600 2.178400 2.186600
## [585] 2.188800 2.189700 2.194700 2.196100 2.197700 2.198200 2.198900 2.199233
## [593] 2.202800 2.202833 2.208100 2.211000 2.211100 2.212867 2.213100 2.213200
## [601] 2.213300 2.213600 2.215500 2.223200 2.224700 2.227250 2.227800 2.227900
## [609] 2.230250 2.231800 2.232750 2.233900 2.235000 2.238167 2.238200 2.240400
## [617] 2.240500 2.242500 2.243900 2.246550 2.247000 2.252300 2.254400 2.256700
## [625] 2.256800 2.260100 2.261800 2.265500 2.266100 2.266400 2.266500 2.268800
## [633] 2.269900 2.270500 2.272500 2.278700 2.281200 2.286200 2.287400 2.289600
## [641] 2.293500 2.294450 2.296100 2.296500 2.298650 2.303000 2.305600 2.310100
```

```
## [649]  2.311100  2.311400  2.314300  2.319100  2.321400  2.321567  2.323000  2.330867
## [657]  2.331000  2.335600  2.338150  2.339000  2.340800  2.341300  2.341800  2.344000
## [665]  2.344400  2.349050  2.351400  2.351800  2.358167  2.360800  2.363600  2.365233
## [673]  2.366400  2.368800  2.370300  2.374600  2.375300  2.377800  2.379800  2.383267
## [681]  2.387200  2.388550  2.390600  2.394433  2.395900  2.398467  2.399233  2.400400
## [689]  2.402600  2.408800  2.410700  2.411200  2.414500  2.416567  2.417300  2.417800
## [697]  2.418200  2.419367  2.419900  2.422733  2.424300  2.426500  2.430900  2.431700
## [705]  2.432550  2.432700  2.434200  2.434600  2.437700  2.438000  2.438300  2.439900
## [713]  2.440800  2.444300  2.445700  2.448900  2.449400  2.449700  2.450800  2.453500
## [721]  2.456700  2.458350  2.459600  2.466633  2.466800  2.467900  2.472300  2.475800
## [729]  2.475900  2.478900  2.488000  2.492300  2.501733  2.502400  2.502800  2.504550
## [737]  2.507067  2.512900  2.513600  2.516400  2.516700  2.517600  2.518600  2.518700
## [745]  2.523000  2.527467  2.537100  2.541700  2.548600  2.550600  2.550700  2.554150
## [753]  2.556800  2.557900  2.558900  2.565000  2.568200  2.572900  2.576900  2.583000
## [761]  2.601200  2.602100  2.612550  2.619400  2.639000  2.642100  2.648100  2.653200
## [769]  2.654900  2.658500  2.661300  2.661600  2.662000  2.672733  2.679733  2.681100
## [777]  2.681400  2.682900  2.687700  2.690800  2.702000  2.706350  2.707700  2.710900
## [785]  2.722467  2.726400  2.728000  2.729600  2.736433  2.739100  2.739200  2.747600
## [793]  2.752800  2.753300  2.756250  2.759600  2.770400  2.786900  2.787100  2.791700
## [801]  2.793900  2.826350  2.834300  2.836400  2.836900  2.837750  2.872300  2.875900
## [809]  2.883500  2.917700  2.941100  2.954400  2.958900  2.971600  2.976700  3.021400
## [817]  3.051300  3.066350  3.088667  3.093000  3.110300  3.138000  3.145100  3.328500
## [825]  3.355000  4.237300
```

```r
# Remove data >4
n1<-cn1 %>% filter(nitrogen < 4)
hist(n1$nitrogen)
```


Histogram of n1$nitrogen
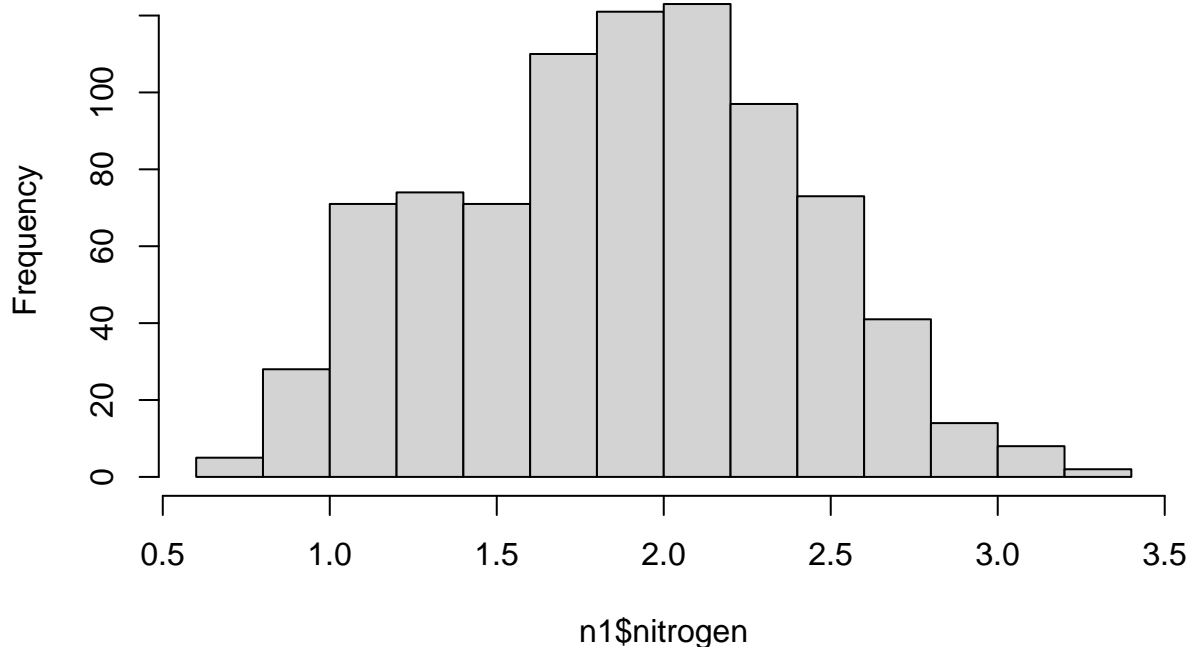
```
n.m1 <- lmer(nitrogen ~ treatment + species + (1|plot) + (1|plant_id), data = n1)
```

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

```
summary(n.m1)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: nitrogen ~ treatment + species + (1 | plot) + (1 | plant_id)
##    Data: n1
##
## REML criterion at convergence: 1034.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.3426 -0.7408 -0.0872  0.6470  3.8765
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  plant_id (Intercept) 0.000000 0.00000
##  plot     (Intercept) 0.001983 0.04453
##  Residual             0.194844 0.44141
## Number of obs: 838, groups:  plant_id, 97; plot, 24
##
## Fixed effects:
##                   Estimate Std. Error t value
## (Intercept)        2.00018    0.05328  37.544
## treatmentambient   0.09253    0.03575   2.588
## speciesCest        0.05857    0.05576   1.050
## speciesPopr       -0.64384    0.05842 -11.021
## speciesSoca       -0.13211    0.05412  -2.441
##
## Correlation of Fixed Effects:
##             (Intr) trtmnt spcsCs spcsPp
## tretmntmbnt -0.389
## speciesCest -0.798  0.045
## speciesPopr -0.760  0.031  0.715
## speciesSoca -0.828  0.061  0.770  0.736
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```
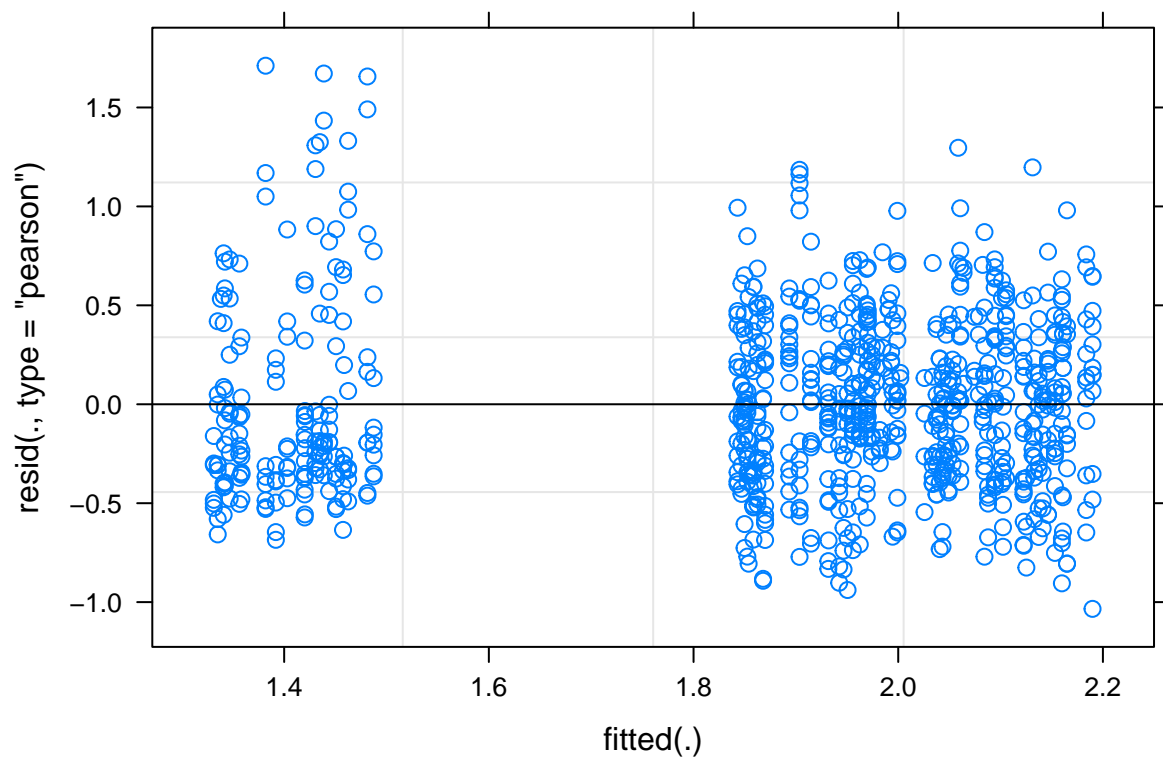
```
plot(n.m1)
```

```
qqnorm(resid(n.m1))
```

Normal Q−Q Plot