

Independent effects of tree diversity on aboveground woody and soil carbon pools after six years of experimental afforestation

Appendix S2: Statistical analyses

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Setting up the workspace

This document is meant to provide a narrative summary to the statistical analyses accompanying the manuscript “Independent effects of tree diversity on aboveground woody and soil carbon pools after six years of experimental afforestation” by Bryant et al. (in review, as of 2024-03-06). We show the R output from the models in the manuscript and test the validity of various statistical assumptions underlying our model choices. In cases when these assumptions are violated, we perform robustness checks to show at the very least that the qualitative structure of the models is supported, and to assess the plausible variation in parameter estimates.

Because this document is only meant for the technical purpose of supporting the adequacy of our statistical analyses, we do not in general make much attempt to present output in a polished form. For example, units are generally omitted from axis labels; readers are advised to look to the main text or the metadata accompanying the data repository for them. We note briefly that, as is standard in R Markdown, all figures correspond to the blocks of code that *precede* them, even if those blocks are on the previous page.

Our first step is to load a number of packages. Although this paper was a collaborative work in which multiple co-authors were working in their own R environments, all results have been checked under the package numbers listed in *R v.4.2.1*.

```
library(lme4) ## 1.1.35.1
library(lmerTest) ## 3.1.3
library(MuMin) ## 1.47.5
library(car) ## 3.1.2
library(performance) ## 0.10.9
library(piecewiseSEM) ## 2.3.0
library(MASS) ## 7.3.60.0.1
library(ggplot2) ## 3.4.4
library(emmeans) ## 1.10.0
```

Next, we go to the working directory where we saved the archived data file and read it in.

```
# setwd(".")
carbon_seq<-read.csv("carbon_sequestration.csv")
```

Relationships among aboveground woody, soil, and fine root carbon

There are three blocks in the FAB1 experiment. Adding a random intercept for block in our models tends to either yield a singular fit or negligible changes in model parameters—the exceptions being in analyses of macroaggregates or soil moisture. (For most other variables, there really does seem to be little evidence that blocks systematically vary; however, there is also a very small number of levels for estimating a random effect variance.) We generally do not show the output of models with the block random effect if it results in a singular fit or in very small changes in parameter values.

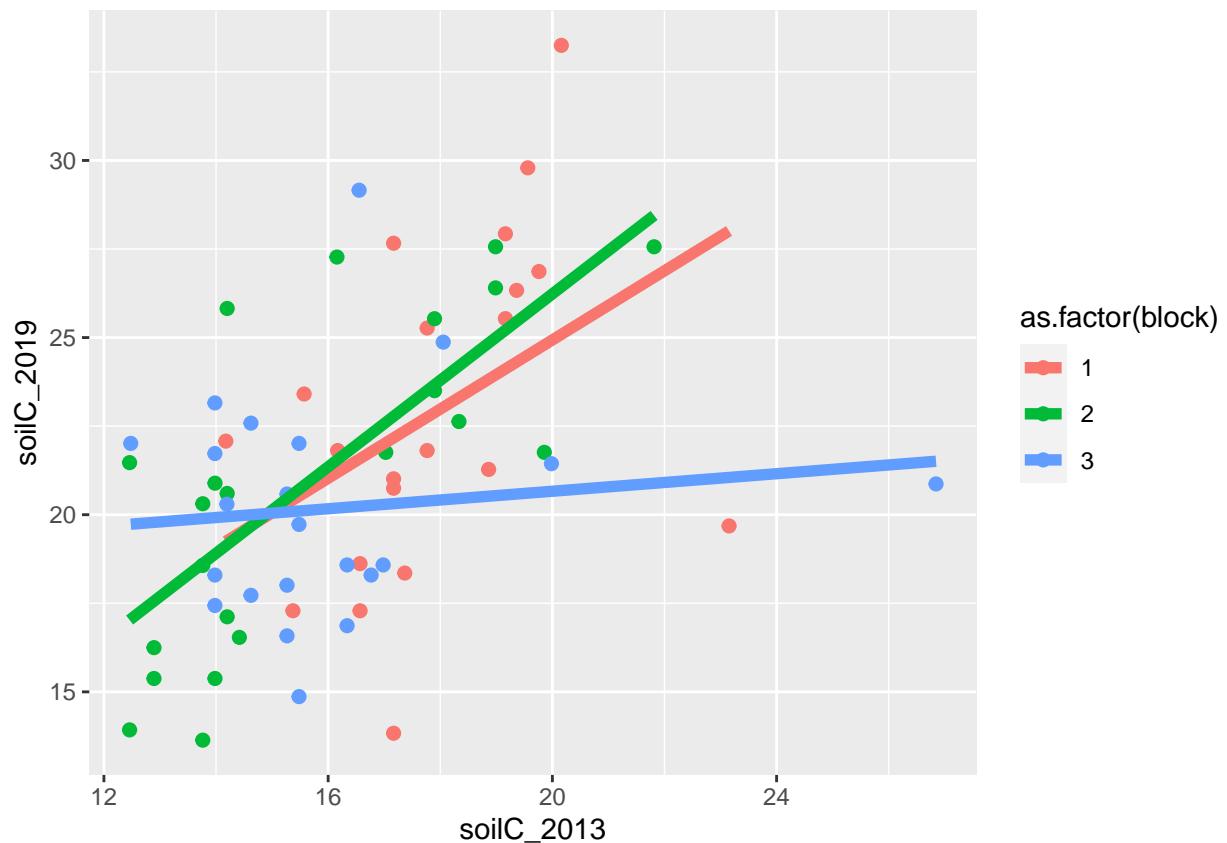
One of the first results presented in the paper is the relationship between soil C in 2013 (pre-treatment) and 2019.

```
## here we use initial and final soil C pools (which accounts for bulk density variation)
## but results are similar using the percentages

## one can add a block random intercept here with lmer, adding +(1/block)
## however, it doesn't change parameter values much
summary(lm(soilC_2019~soilC_2013,data=carbon_seq))

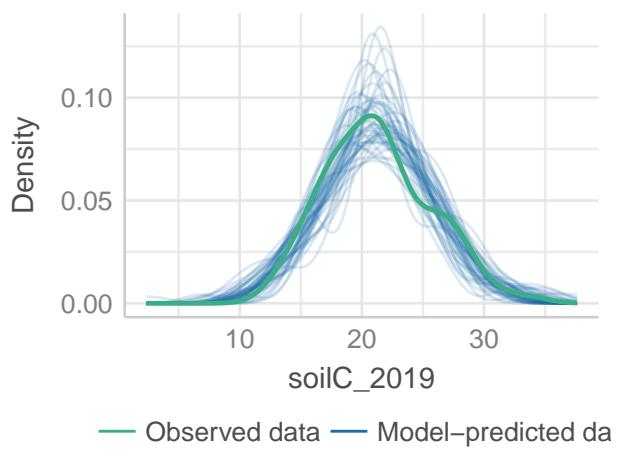
##
## Call:
## lm(formula = soilC_2019 ~ soilC_2013, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -8.1692 -2.6970 -0.0647  2.7751  9.2117 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept)  8.9527    2.8903   3.097   0.0029 **  
## soilC_2013   0.7482    0.1733   4.317   5.6e-05 *** 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.789 on 64 degrees of freedom
## (74 observations deleted due to missingness)
## Multiple R-squared:  0.2255, Adjusted R-squared:  0.2134 
## F-statistic: 18.64 on 1 and 64 DF,  p-value: 5.603e-05

## in general, we plot separate regression lines for each block just to be
## able to check the generality within/among blocks more easily
## naturally, these do not correspond to the 'overall' best-fit line
ggplot(data=carbon_seq,aes(x=soilC_2013,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)
```



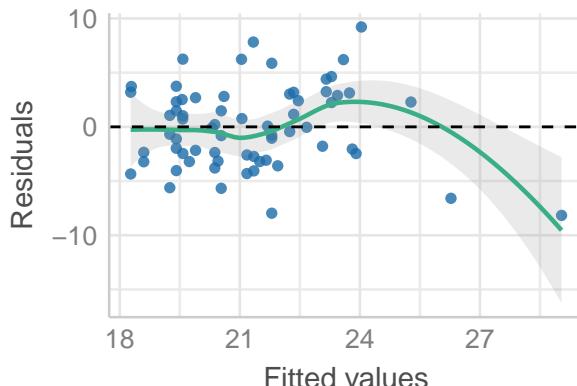
Posterior Predictive Check

Model-predicted lines should resemble observed data



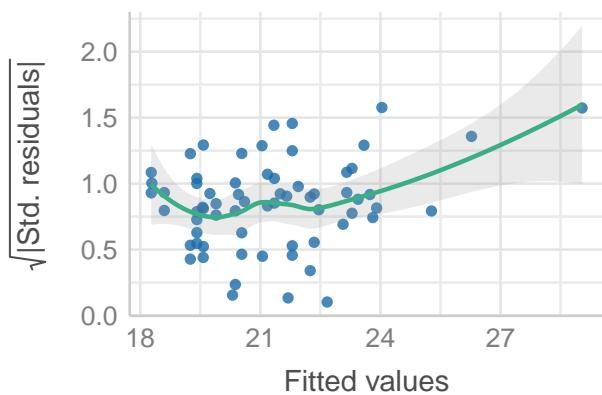
Linearity

Reference line should be flat and horizontal



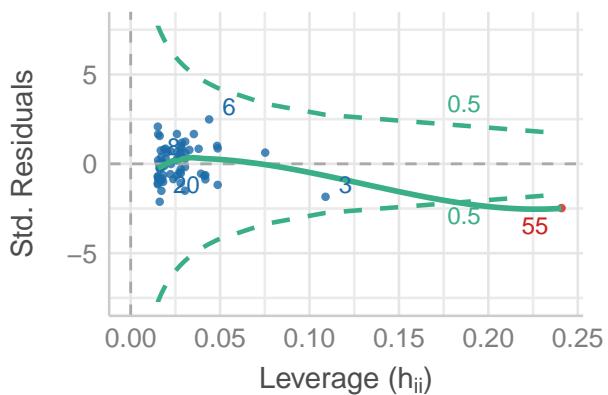
Homogeneity of Variance

Reference line should be flat and horizontal



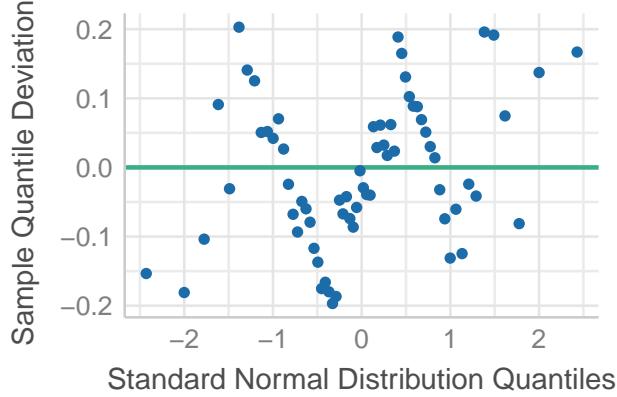
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



Despite a couple influential observations that declined in soil C, it's clear that pre-treatment and 2019 soil C are positively correlated. If we want to understand the drivers of carbon *sequestration*, we have good reason to consider the change in soil C pools rather than simply the endpoint in 2019. We might first want to check whether initial soil C determines how much C accumulates.

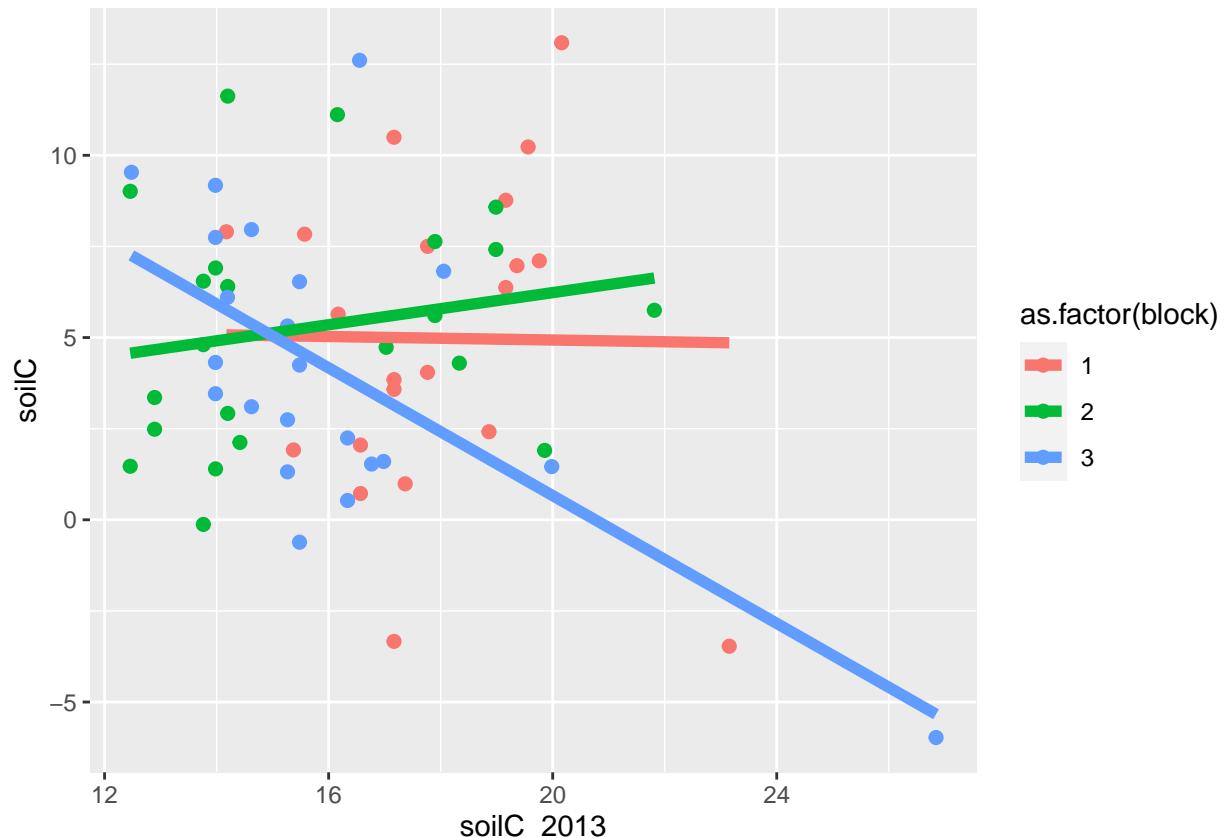
```

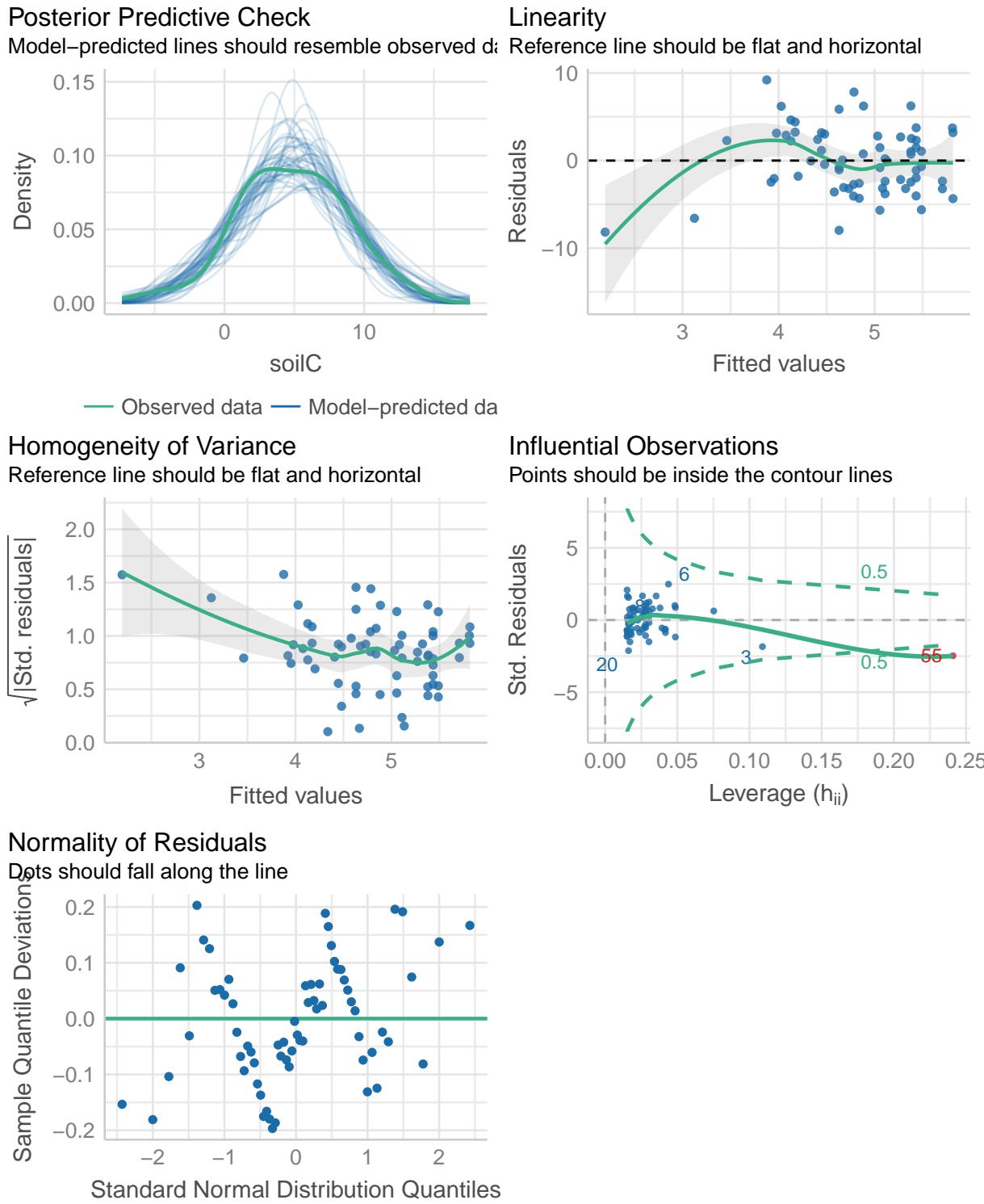
## again these results are robust to alternate ways of analyzing the data
## including considering percentages and adding block random effects
summary(lm(soilC~soilC_2013,data=carbon_seq))

##
## Call:
## lm(formula = soilC ~ soilC_2013, data = carbon_seq)
##
## Residuals:
##     Min      1Q  Median      3Q     Max 
## -8.1692 -2.6970 -0.0647  2.7751  9.2117 
## 
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept)  8.9527    2.8903   3.097   0.0029 **  
## soilC_2013   -0.2518    0.1733  -1.453   0.1512    
## ---        
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 3.789 on 64 degrees of freedom
## (74 observations deleted due to missingness)
## Multiple R-squared:  0.03192,    Adjusted R-squared:  0.01679 
## F-statistic:  2.11 on 1 and 64 DF,  p-value: 0.1512

ggplot(data=carbon_seq,aes(x=soilC_2013,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```





We see the same influential observations as before, but there remains no evidence for a relationship between initial soil C and change in soil C. We can assume that change in soil C is mainly driven by our treatments rather than be legacies of pre-existing soil conditions.

One of the key questions of the manuscript is: does change in soil C correlate with aboveground woody C?

```

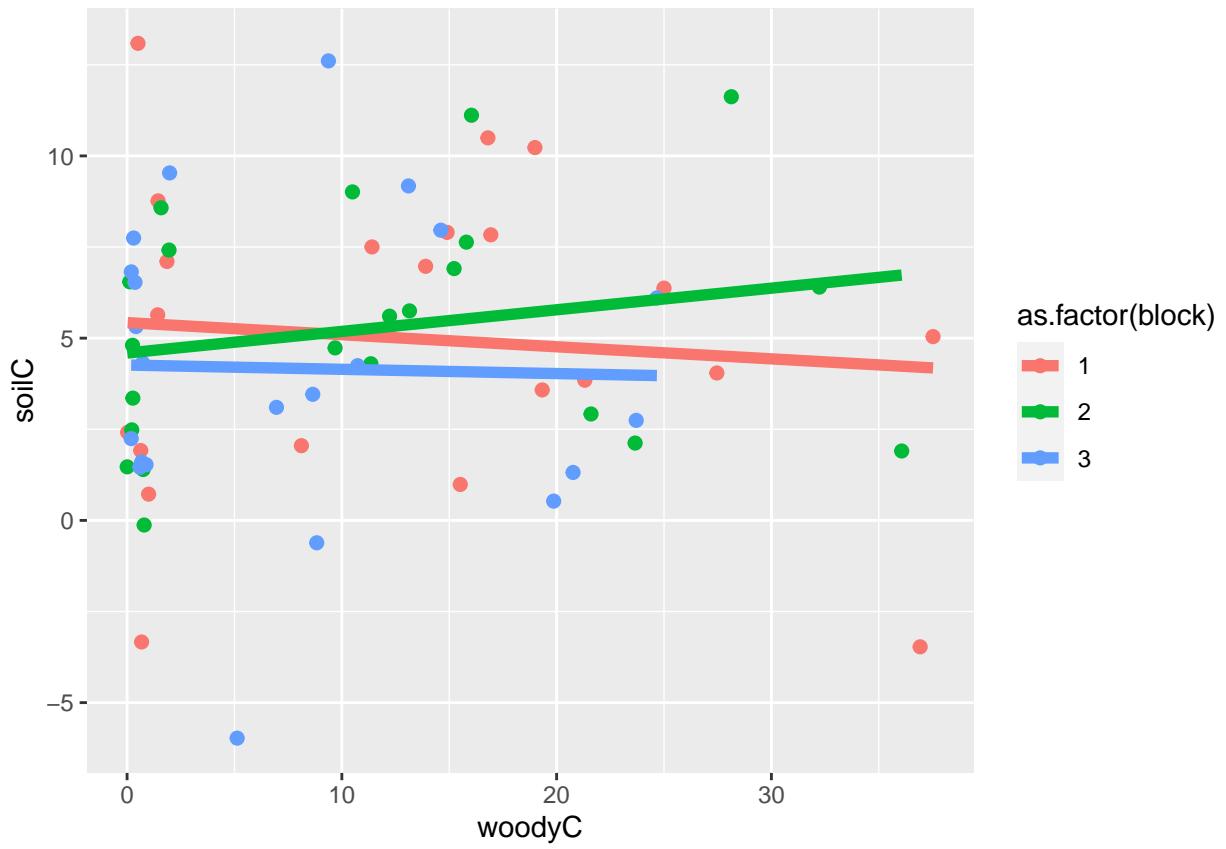
## here we'll switch to using pools so that aboveground woody and soil
## are in the same units

## note that a block random intercept results in a singular fit
summary(lm(soilC~woodyC,data=carbon_seq))

##
## Call:
## lm(formula = soilC ~ woodyC, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -10.704  -2.742  -0.100   2.720   8.424
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 4.65738   0.67854   6.864 3.22e-09 ***
## woodyC     0.01400   0.04479   0.313   0.756
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.848 on 64 degrees of freedom
## (74 observations deleted due to missingness)
## Multiple R-squared:  0.001524, Adjusted R-squared:  -0.01408
## F-statistic: 0.09767 on 1 and 64 DF,  p-value: 0.7557

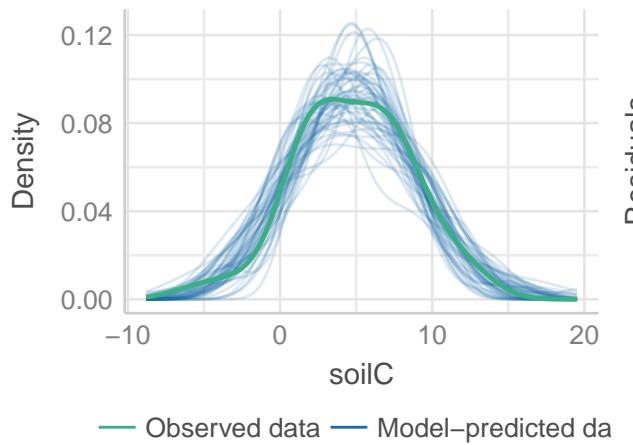
ggplot(data=carbon_seq,aes(x=woodyC,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



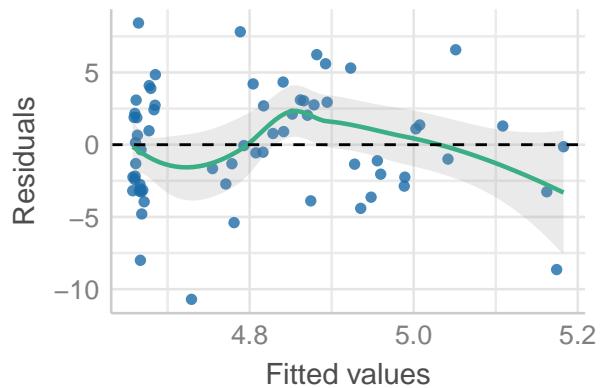
Posterior Predictive Check

Model-predicted lines should resemble observed data



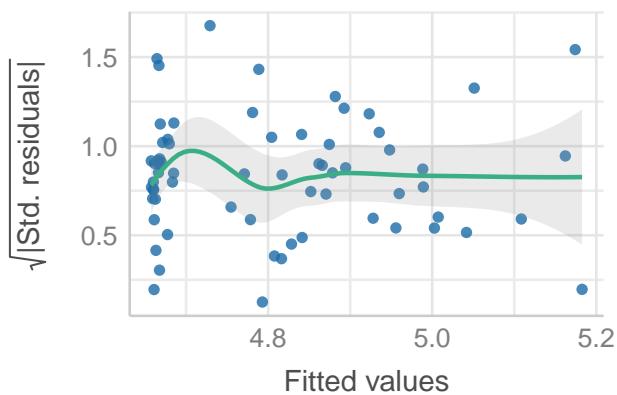
Linearity

Reference line should be flat and horizontal



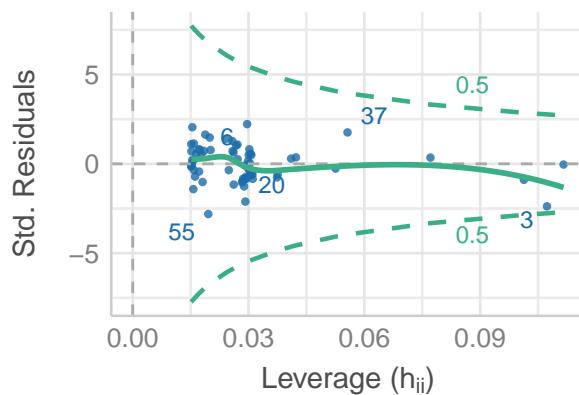
Homogeneity of Variance

Reference line should be flat and horizontal



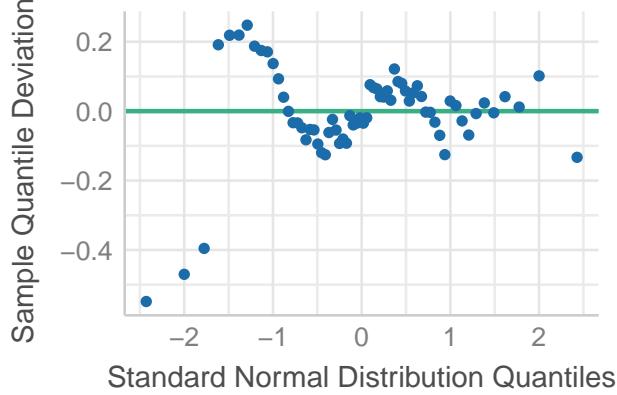
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line

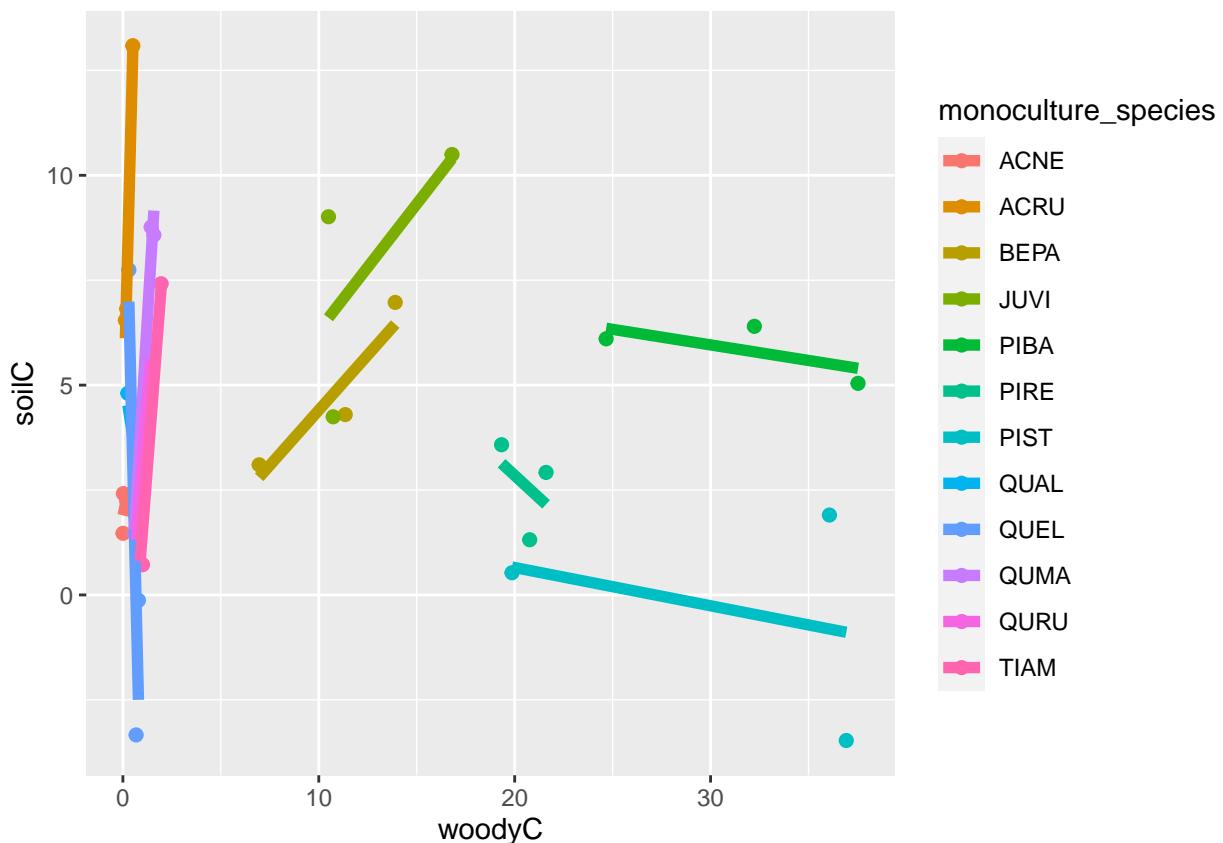


It is clear that there is no relationship between the two variables. However, as we report in the paper, if we subset the data to monoculture plots only, we see a significant interaction between species ID and aboveground woody C on soil C.

```
summary(aov(soilC~woodyC*monoculture_species,data=carbon_seq))
```

```
##                                     Df Sum Sq Mean Sq F value    Pr(>F)
## woodyC                               1   4.86   4.864   1.108 0.31320
## monoculture_species                  11 238.30  21.663   4.935 0.00523 **
## woodyC:monoculture_species          11 165.90  15.082   3.436 0.02209 *
## Residuals                            12  52.67   4.389
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 104 observations deleted due to missingness
```

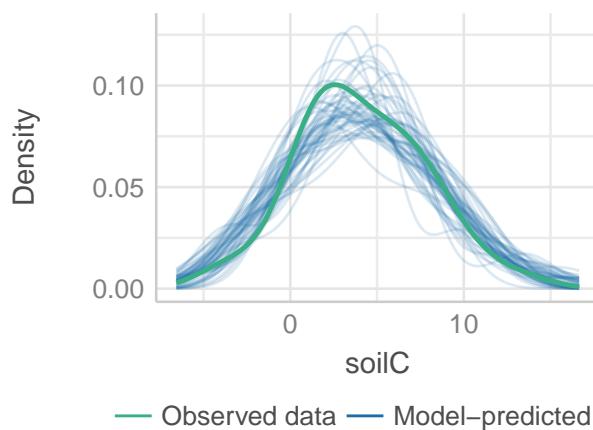
```
ggplot(data=carbon_seq[which(!is.na(carbon_seq$monoculture_species)),],
       aes(x=woodyC,y=soilC,color=monoculture_species))++
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)
```



```
check_model(aov(soilC~log(woodyC)*monoculture_species,data=carbon_seq))
```

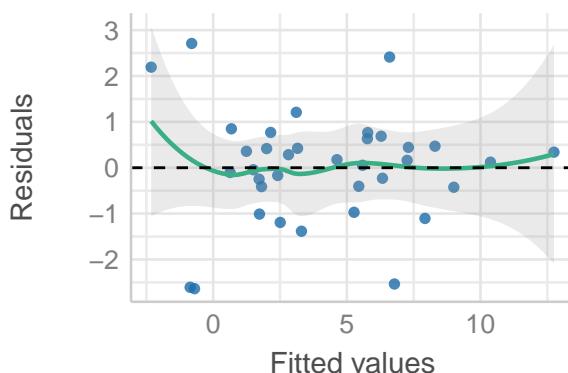
Posterior Predictive Check

Model-predicted lines should resemble observed data



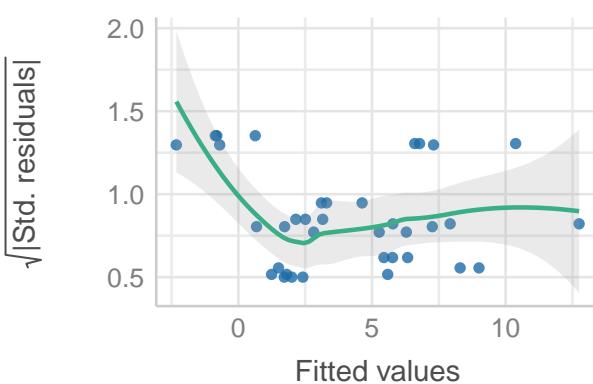
Linearity

Reference line should be flat and horizontal



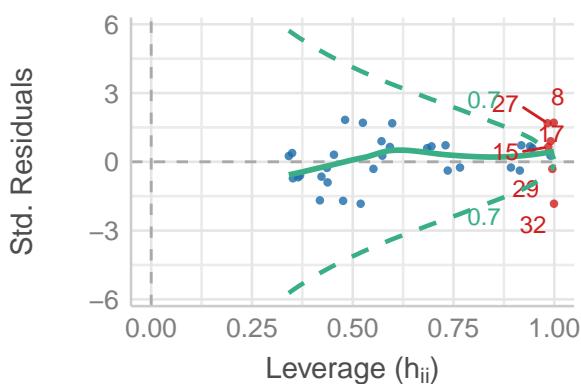
Homogeneity of Variance

Reference line should be flat and horizontal



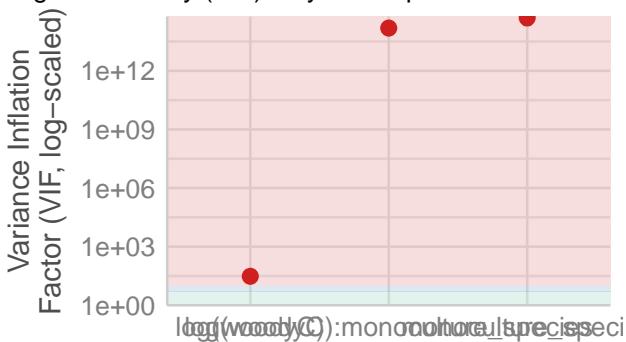
Influential Observations

Points should be inside the contour lines



Collinearity

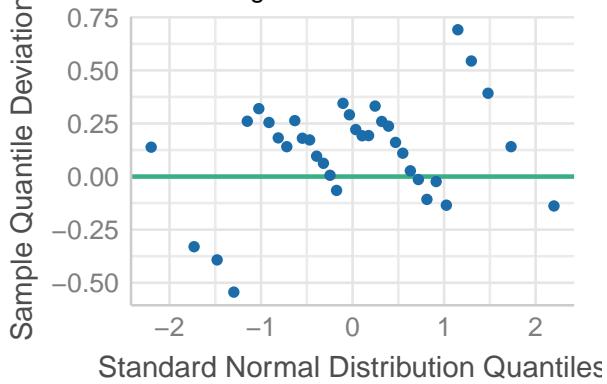
High collinearity (VIF) may inflate parameter uncertainty



♦ High (= 10)

Normality of Residuals

Dots should fall along the line



```
## note that although the VIFs here seem astronomical, the
## generalized VIFs implemented with the vif() function in package
## car are much more suitable for multilevel categorical data
vif(aov(soilC~log(woodyC)*monoculture_species,data=carbon_seq))
```

```

## there are higher-order terms (interactions) in this model
## consider setting type = 'predictor'; see ?vif

##                                     GVIF Df GVIF^(1/(2*Df))
## log(woodyC)                  3.037724e+01  1      5.511555
## monoculture_species          5.173756e+14 11     4.664546
## log(woodyC):monoculture_species 1.543947e+14 11     4.415072
## moderately high but much less worrisome!

```

Lastly, we look at the third pool we measured: fine root C. First of all, how does fine root C relate to soil C accumulation?

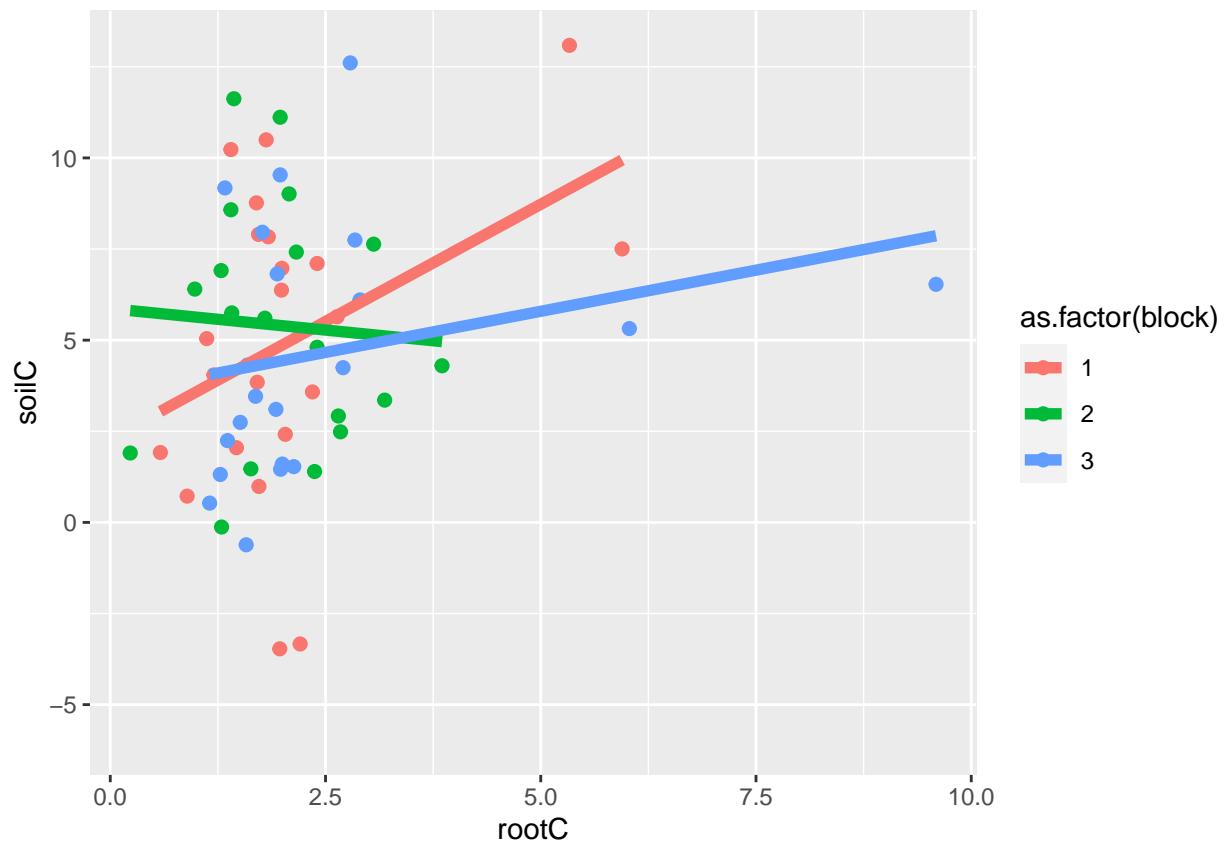
```

## note that a block random intercept results in a singular fit
summary(lm(soilC~rootC,data=carbon_seq))

##
## Call:
## lm(formula = soilC ~ rootC, data = carbon_seq)
##
## Residuals:
##    Min      1Q  Median      3Q      Max
## -8.3431 -2.5301 -0.3742  2.4080  7.2747
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 3.7722    0.8464   4.457  3.7e-05 ***
## rootC       0.5598    0.3245   1.725   0.0897 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.609 on 60 degrees of freedom
## (78 observations deleted due to missingness)
## Multiple R-squared:  0.04726,    Adjusted R-squared:  0.03138
## F-statistic: 2.976 on 1 and 60 DF,  p-value: 0.08965

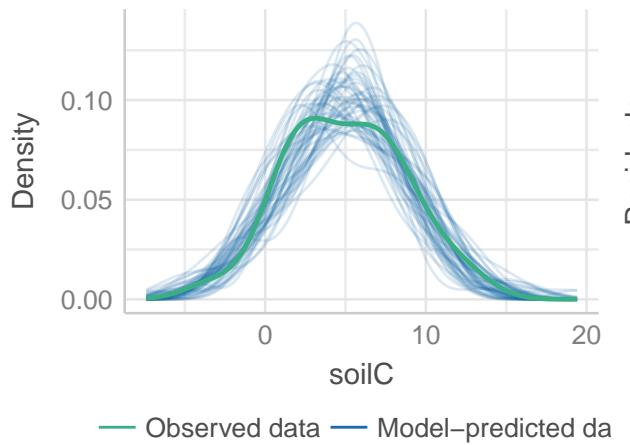
ggplot(data=carbon_seq,aes(x=rootC,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



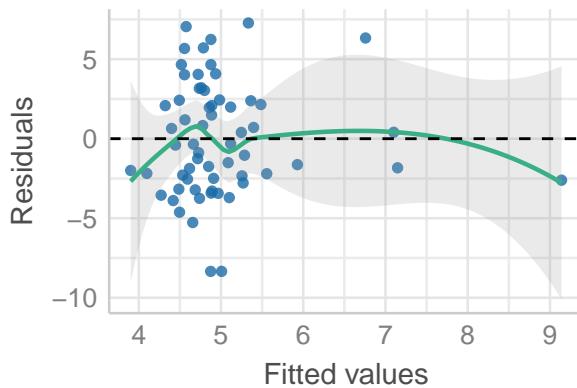
Posterior Predictive Check

Model-predicted lines should resemble observed data



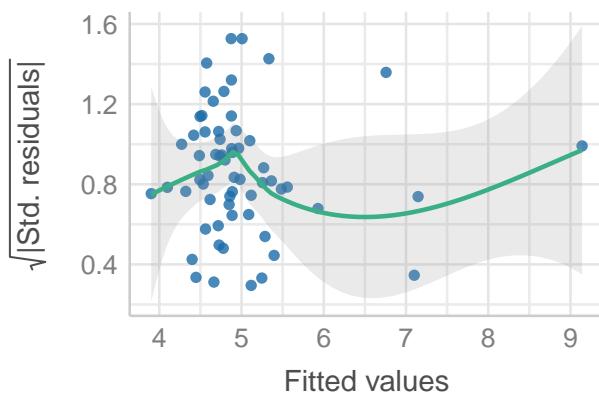
Linearity

Reference line should be flat and horizontal



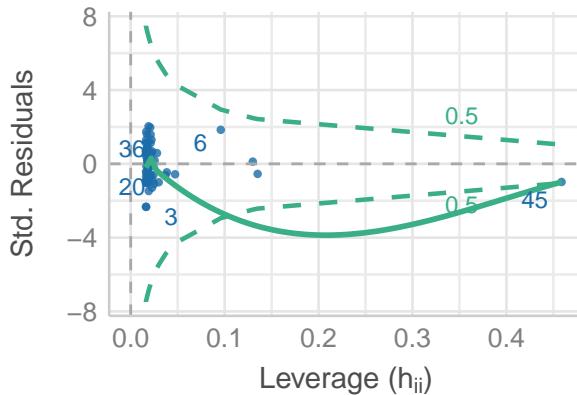
Homogeneity of Variance

Reference line should be flat and horizontal



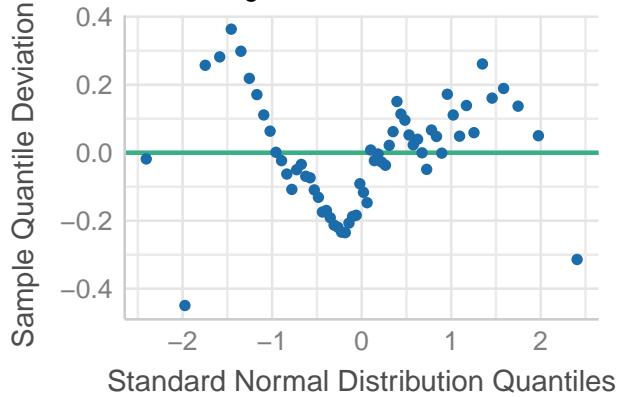
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



There is fairly weak evidence for a positive relationship between fine root C and soil C accumulation. Next, we check the relationship with aboveground woody C.

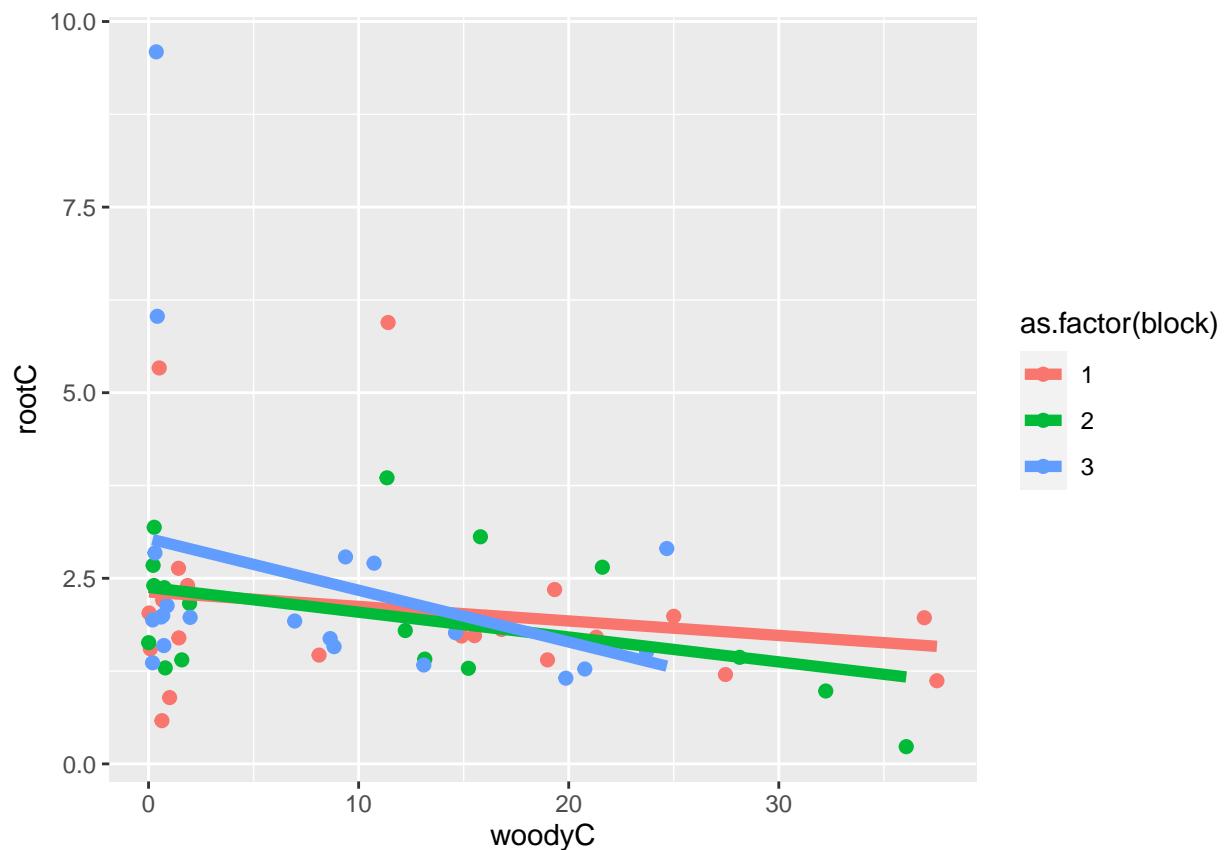
```

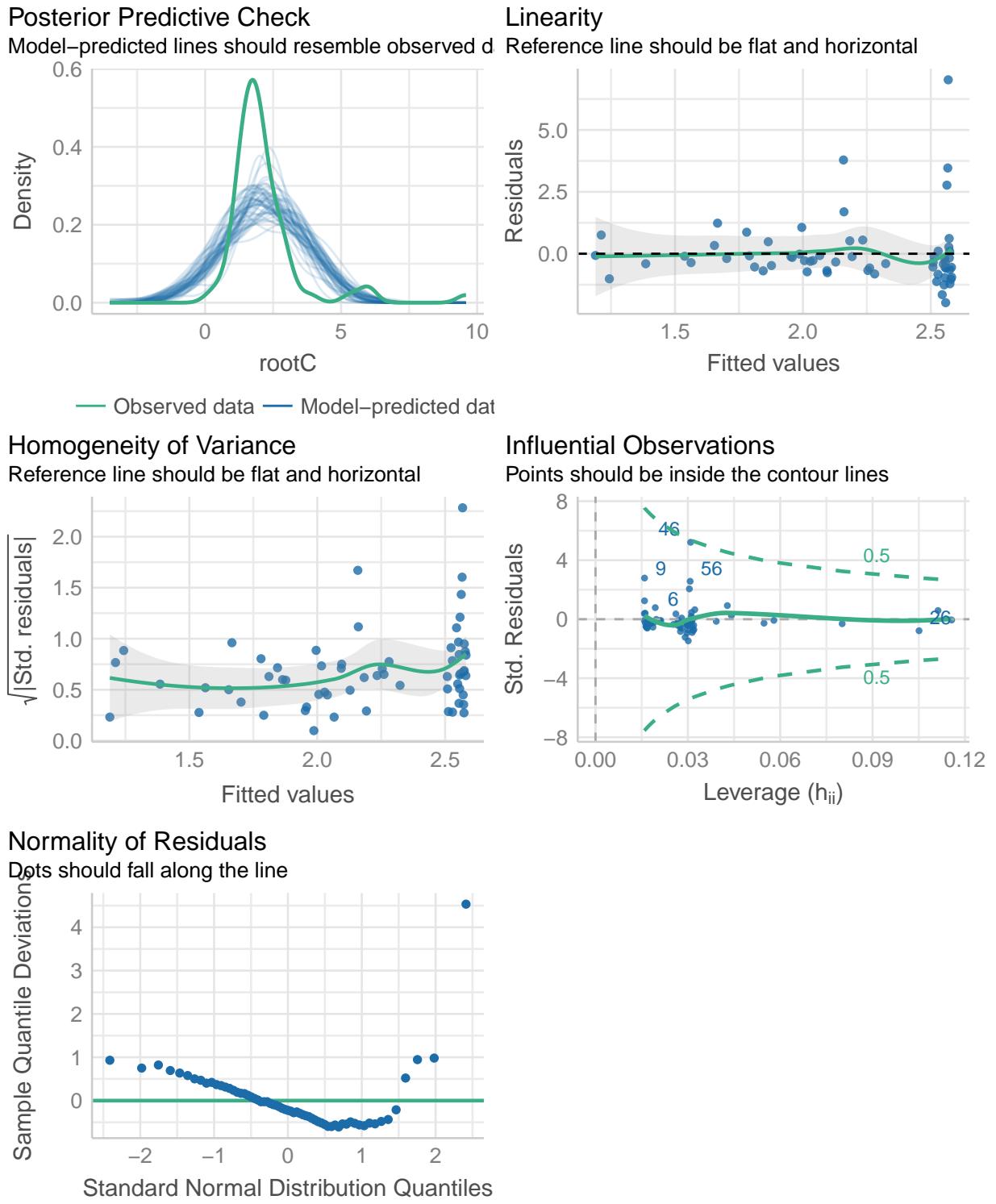
## note that a block random intercept results in a singular fit
summary(lm(rootC~woodyC,data=carbon_seq))

##
## Call:
## lm(formula = rootC ~ woodyC, data = carbon_seq)
##
## Residuals:
##     Min      1Q  Median      3Q     Max 
## -1.9760 -0.6554 -0.3069  0.1031  7.0232 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 2.58183   0.24467 10.552 2.19e-15 ***
## woodyC      -0.03711   0.01615 -2.299   0.025 *  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 1.368 on 61 degrees of freedom
## (77 observations deleted due to missingness)
## Multiple R-squared:  0.07971,    Adjusted R-squared:  0.06463 
## F-statistic: 5.284 on 1 and 61 DF,  p-value: 0.02497

ggplot(data=carbon_seq,aes(x=woodyC,y=rootC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```





Due to the highly skewed distribution of root C, this model evidently violates the normality of residuals assumption of OLS. Although we have good reasons to want to keep root C in its original units, we could try the model with log-transformed root C as a robustness check:

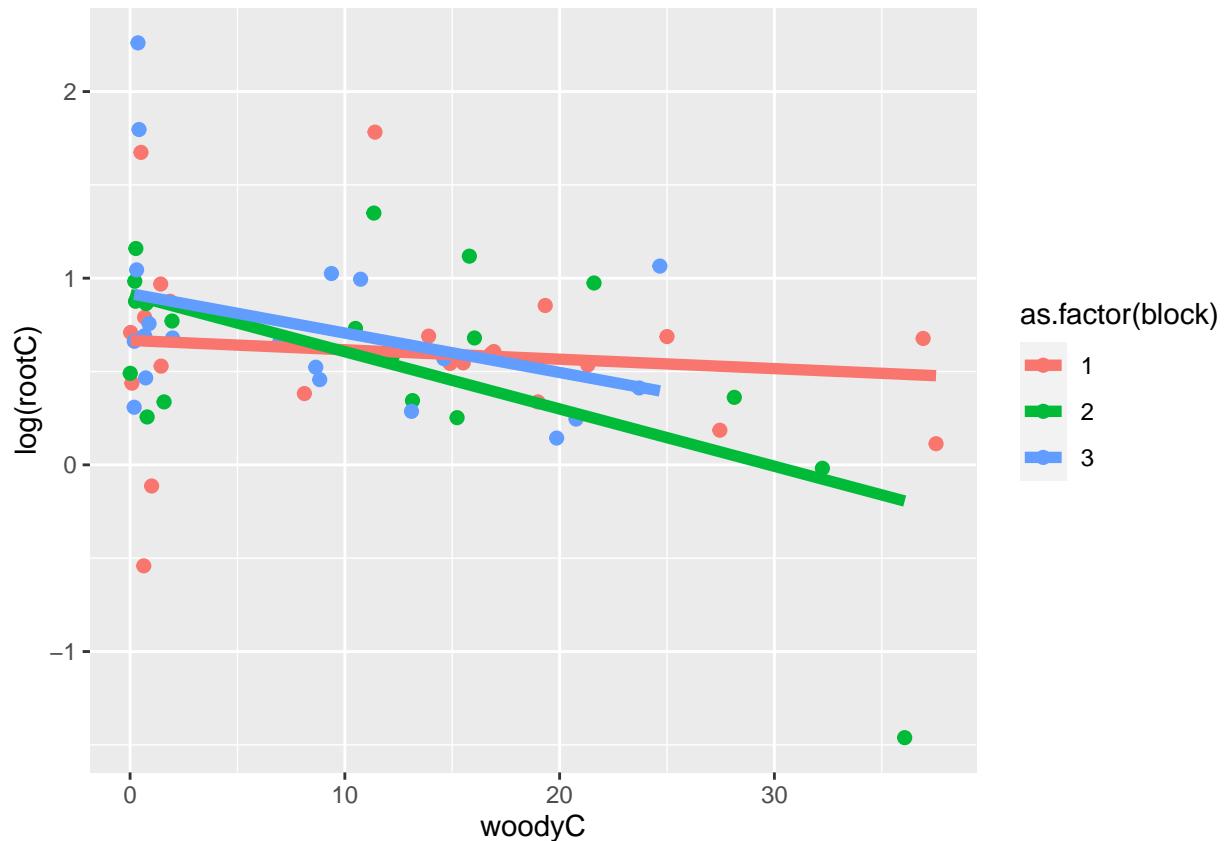
```

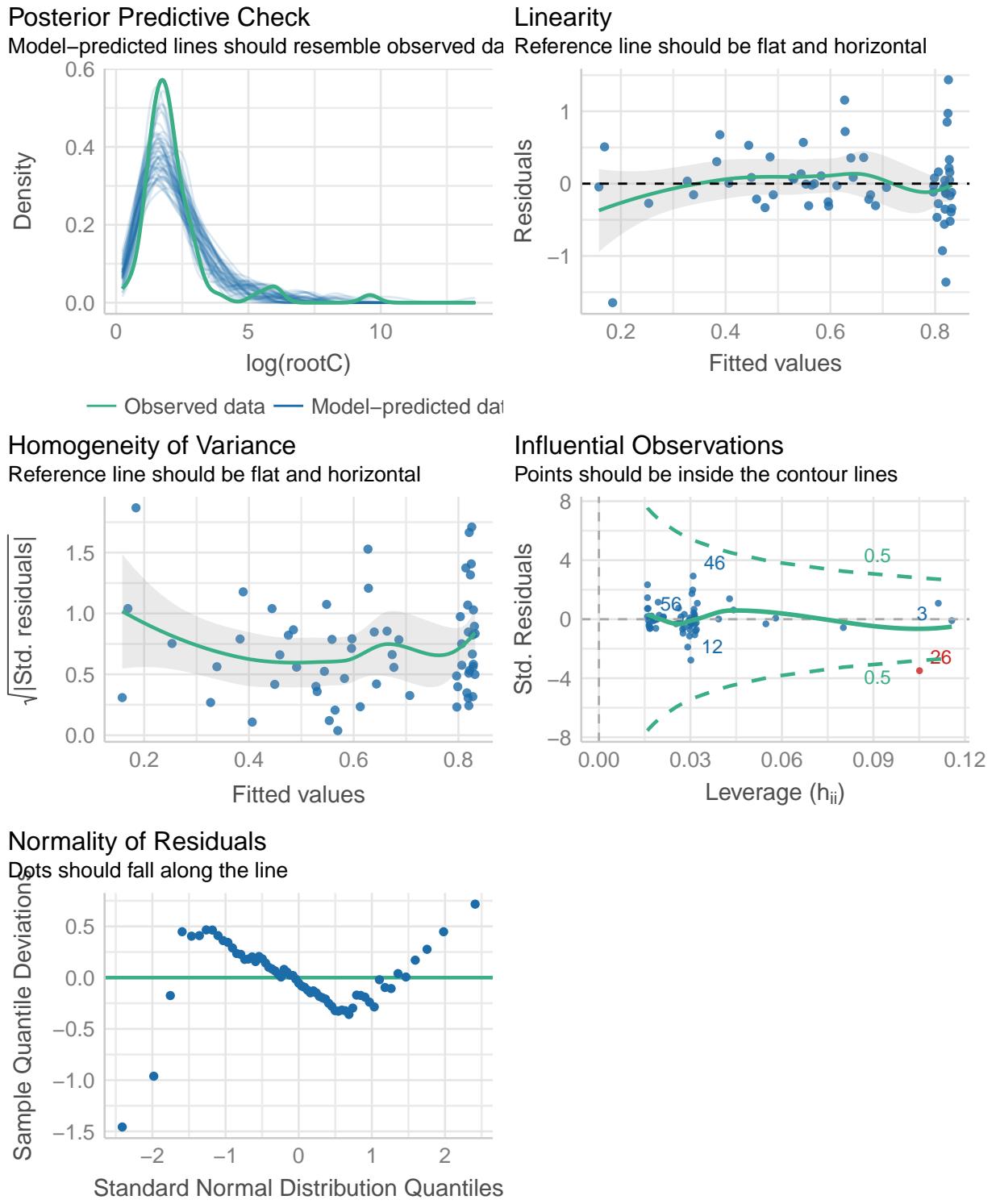
## note that a block random intercept results in a singular fit
summary(lm(log(rootC) ~ woodyC, data=carbon_seq))

##
## Call:
## lm(formula = log(rootC) ~ woodyC, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -1.64577 -0.23411 -0.02634  0.15896  1.43547 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept)  0.831995  0.089143  9.333 2.33e-13 ***
## woodyC      -0.017959  0.005882 -3.053  0.00335 **  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.4985 on 61 degrees of freedom
## (77 observations deleted due to missingness)
## Multiple R-squared:  0.1325, Adjusted R-squared:  0.1183 
## F-statistic: 9.321 on 1 and 61 DF,  p-value: 0.003354

ggplot(data=carbon_seq,aes(x=woodyC,y=log(rootC),color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```





Although the functional form of the relationship now evidently differs, the coefficient is still negative and the model does somewhat better at meeting the assumptions.

Changes in soil C in relation to other soil characteristics

We measured three other edaphic properties that we consider here for their relationships with each other and with soil C:

macroaggregates (250 µm diameter and up) soil moisture *pH

First of all, how do these three correlate with each other? Macroaggregates are commonly associated with the ability to retain moisture, so we can test the relationship between macroaggregates and soil moisture.

```
summary(lmer(soil_moisture~macro250+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soil_moisture ~ macro250 + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 174.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.32277 -0.71385 -0.03953  0.58157  2.94246
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block    (Intercept) 0.2087   0.4569
##   Residual           0.7259   0.8520
## Number of obs: 65, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) -5.54921   1.37804 33.53552 -4.027 0.000305 ***
## macro250     0.15561   0.01838 41.56302  8.464 1.38e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## macro250 -0.979

## for F-statistics of mixed-effects models, one can run:
# anova(lmer(soil_moisture~macro250+(1/block),data=carbon_seq))
## and for Nakagawa & Schielzeth Rc2 and Rm2, one can run:
# r.squaredGLMM(lmer(soil_moisture~macro250+(1/block),data=carbon_seq))

## another possibility is to simply treat block
## as a fixed effect to avoid endogeneity problems
## since blocks vary in macroaggregates
## however, this yields very similar results
summary(lm(soil_moisture~macro250+as.factor(block),data=carbon_seq))

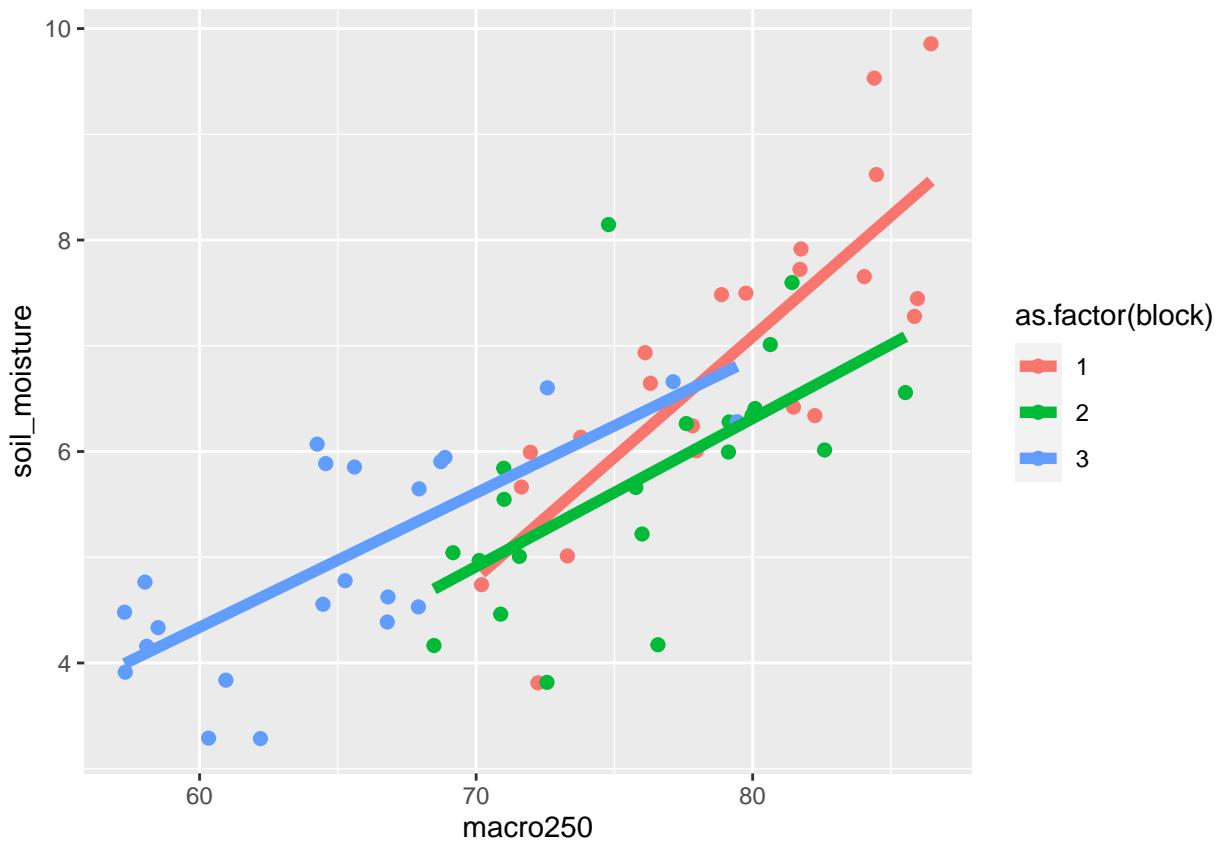
##
## Call:
## lm(formula = soil_moisture ~ macro250 + as.factor(block), data = carbon_seq)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.32277 -0.71385 -0.03953  0.58157  2.94246
```

```

## -1.95032 -0.63758 -0.00113  0.50498  2.58852
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) -5.95126   1.58488 -3.755 0.000389 ***
## macro250     0.16216   0.01993  8.138 2.55e-11 ***
## as.factor(block)2 -0.61830   0.26727 -2.313 0.024086 *
## as.factor(block)3  0.37926   0.37748  1.005 0.318997
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8522 on 61 degrees of freedom
## (75 observations deleted due to missingness)
## Multiple R-squared:  0.6629, Adjusted R-squared:  0.6463
## F-statistic: 39.98 on 3 and 61 DF,  p-value: 2.053e-14

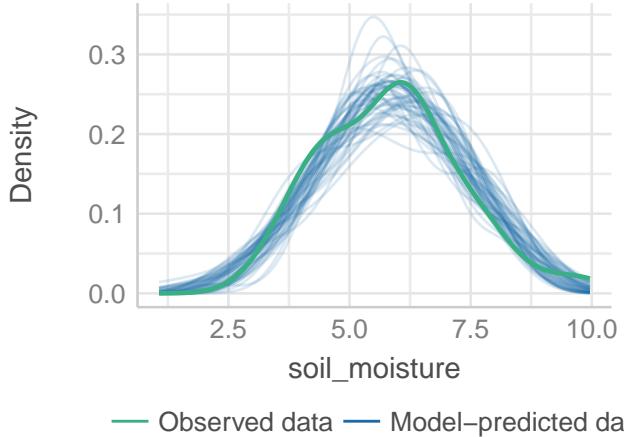
ggplot(data=carbon_seq,aes(x=macro250,y=soil_moisture,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



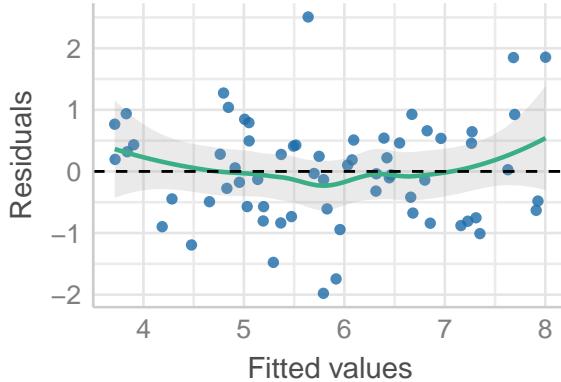
Posterior Predictive Check

Model-predicted lines should resemble observed data



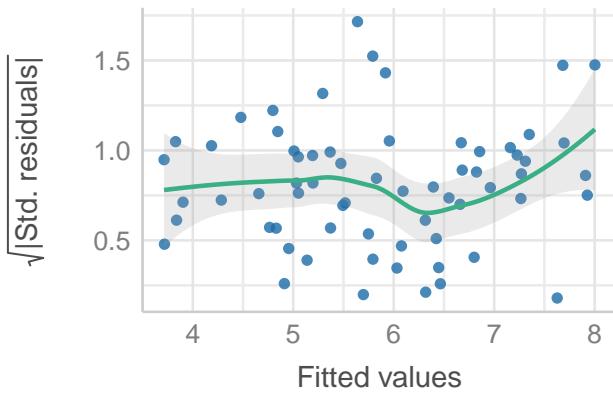
Linearity

Reference line should be flat and horizontal



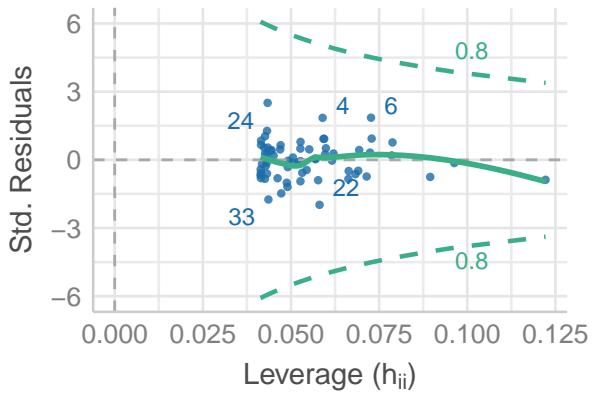
Homogeneity of Variance

Reference line should be flat and horizontal



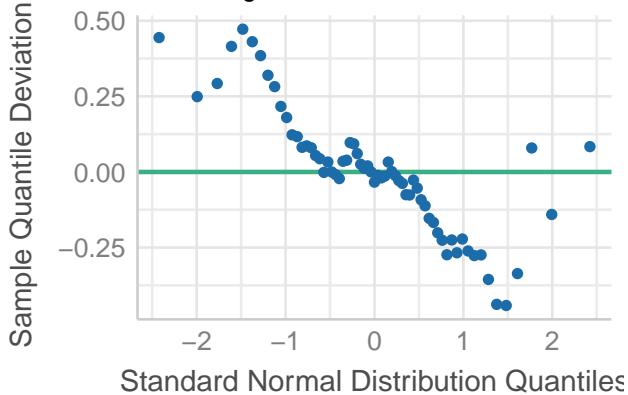
Influential Observations

Points should be inside the contour lines



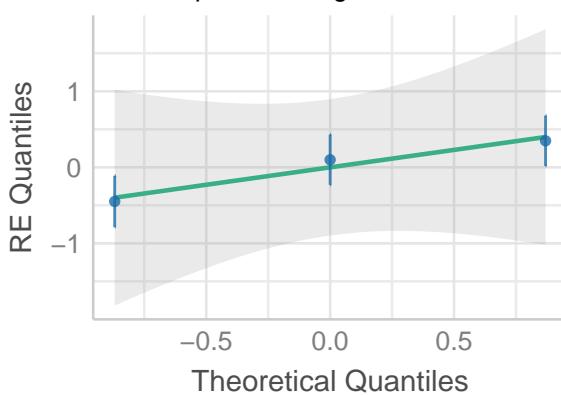
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (block)

Dots should be plotted along the line



There is evidently a close relationship between soil moisture and macroaggregate fraction. How about pH? As it turns out, it isn't correlated with soil moisture but is correlated with macroaggregates.

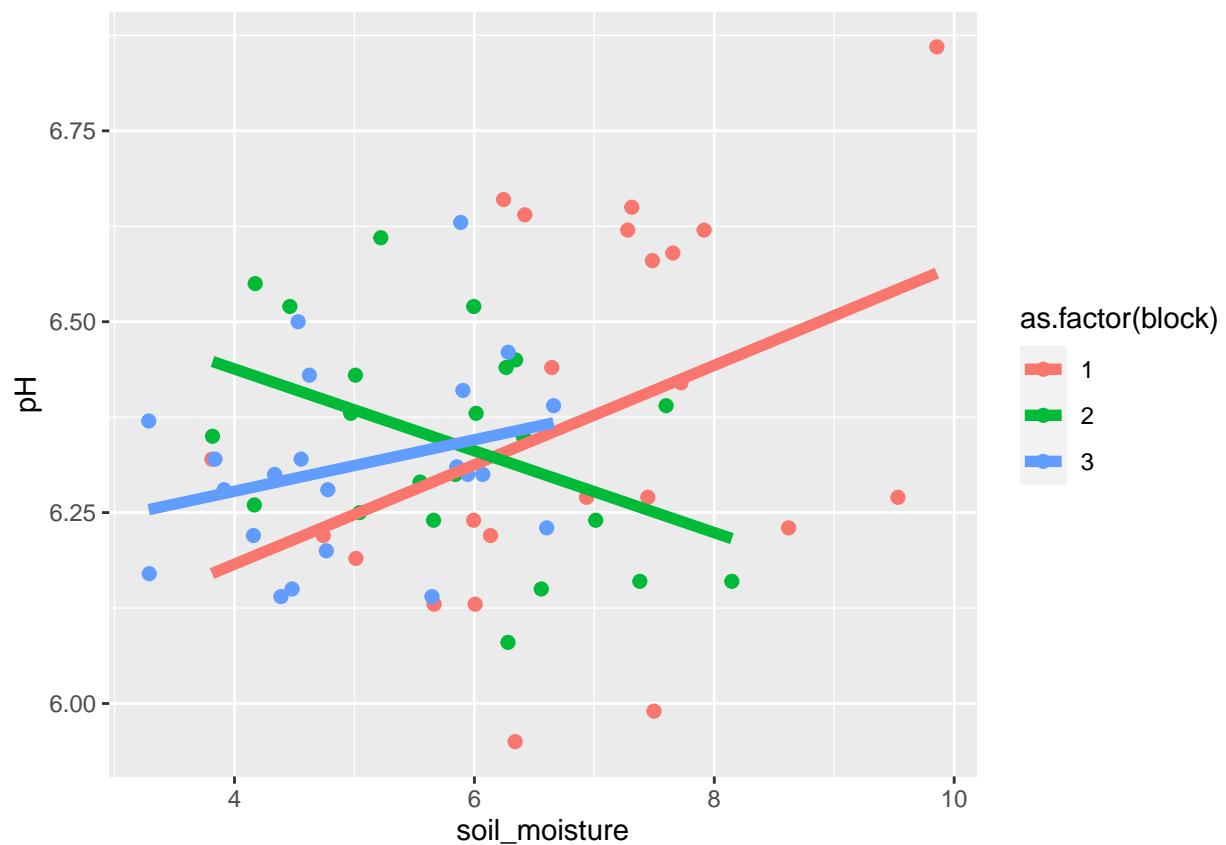
```

## block random intercept yields singular fit
summary(lm(pH~soil_moisture,data=carbon_seq))

##
## Call:
## lm(formula = pH ~ soil_moisture, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -0.40207 -0.11956 -0.01069  0.10361  0.41899 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept)  6.19171   0.09125  67.857 <2e-16 ***
## soil_moisture 0.02529   0.01501   1.685   0.0968 .  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1748 on 65 degrees of freedom
## (73 observations deleted due to missingness)
## Multiple R-squared:  0.04185,    Adjusted R-squared:  0.02711 
## F-statistic: 2.839 on 1 and 65 DF,  p-value: 0.09678

ggplot(data=carbon_seq,aes(x=soil_moisture,y=pH,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



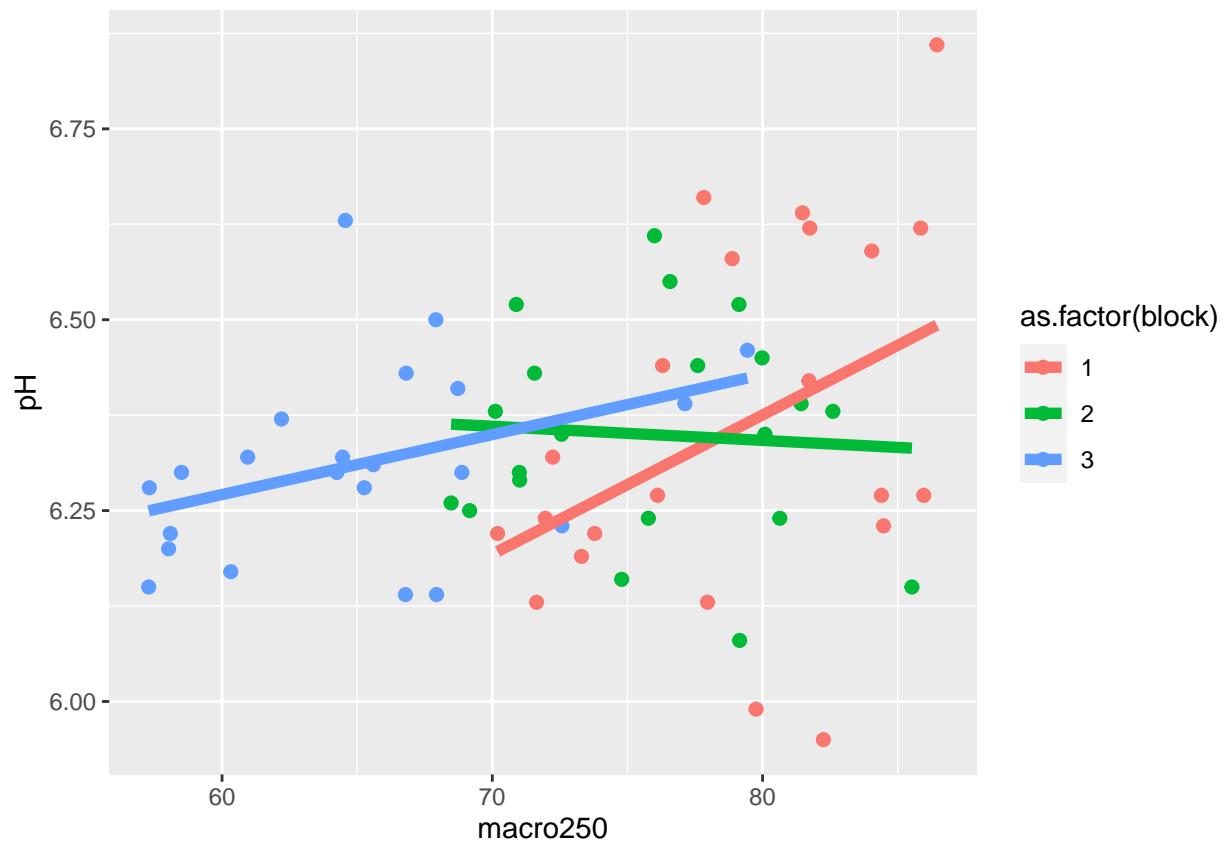
```

## block random intercept yields singular fit
summary(lm(pH~macro250,data=carbon_seq))

##
## Call:
## lm(formula = pH ~ macro250, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -0.43960 -0.10507 -0.01157  0.09340  0.44673 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 5.926046  0.194069 30.536 <2e-16 ***
## macro250    0.005636  0.002632  2.142  0.0361 *  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1697 on 63 degrees of freedom
## (75 observations deleted due to missingness)
## Multiple R-squared:  0.06787, Adjusted R-squared:  0.05307 
## F-statistic: 4.587 on 1 and 63 DF, p-value: 0.03609

ggplot(data=carbon_seq,aes(x=macro250,y=pH,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

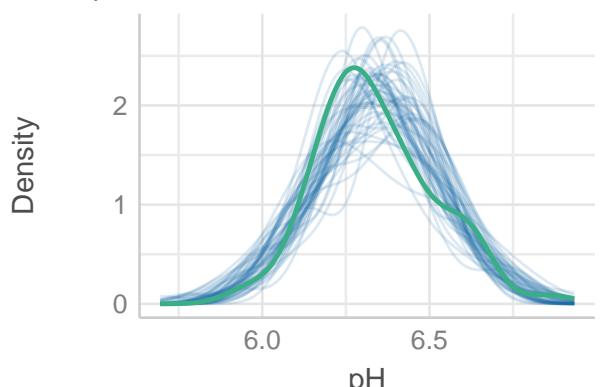
```



```
check_model(lm(pH~macro250,data=carbon_seq))
```

Posterior Predictive Check

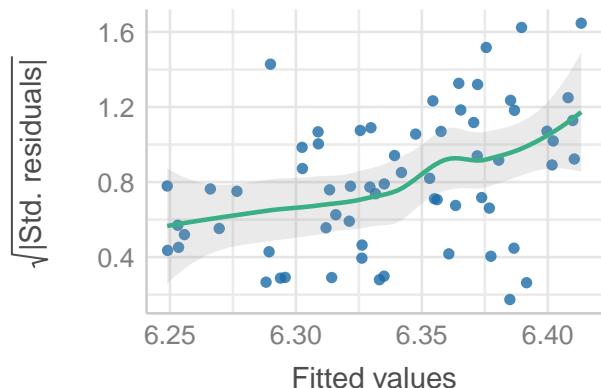
Model-predicted lines should resemble observed data



— Observed data — Model-predicted data

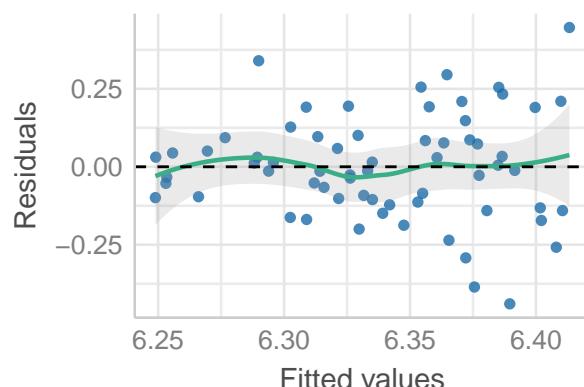
Homogeneity of Variance

Reference line should be flat and horizontal



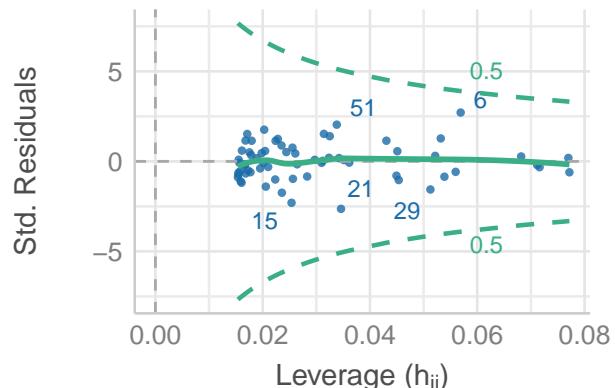
Linearity

Reference line should be flat and horizontal



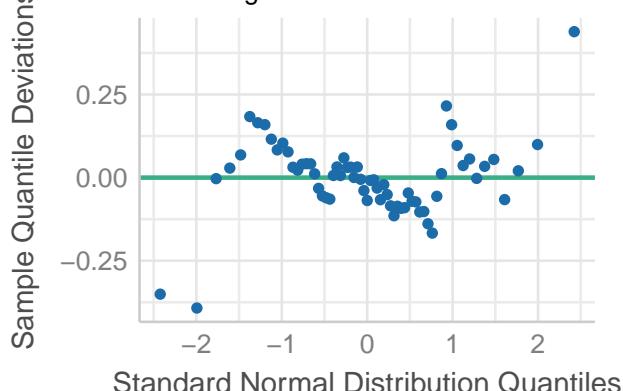
Influential Observations

Points should be inside the contour lines

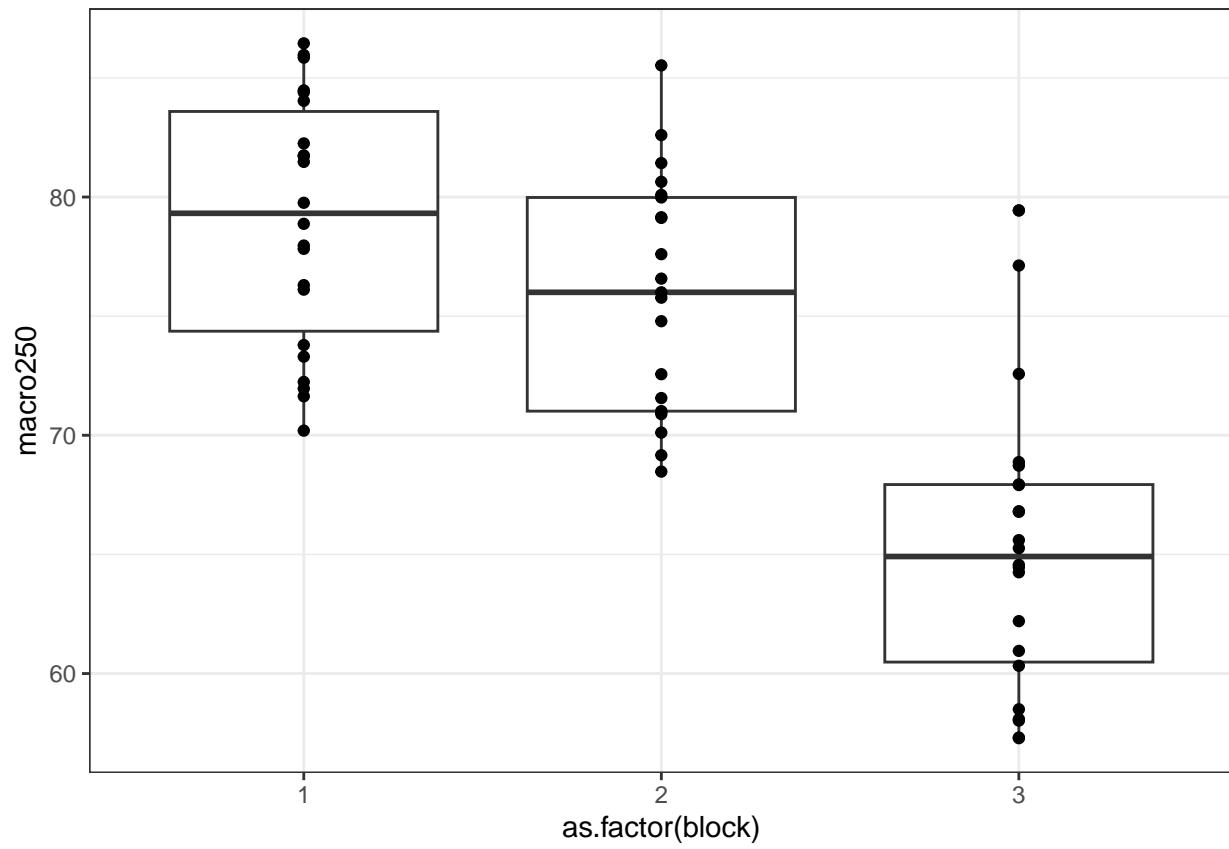


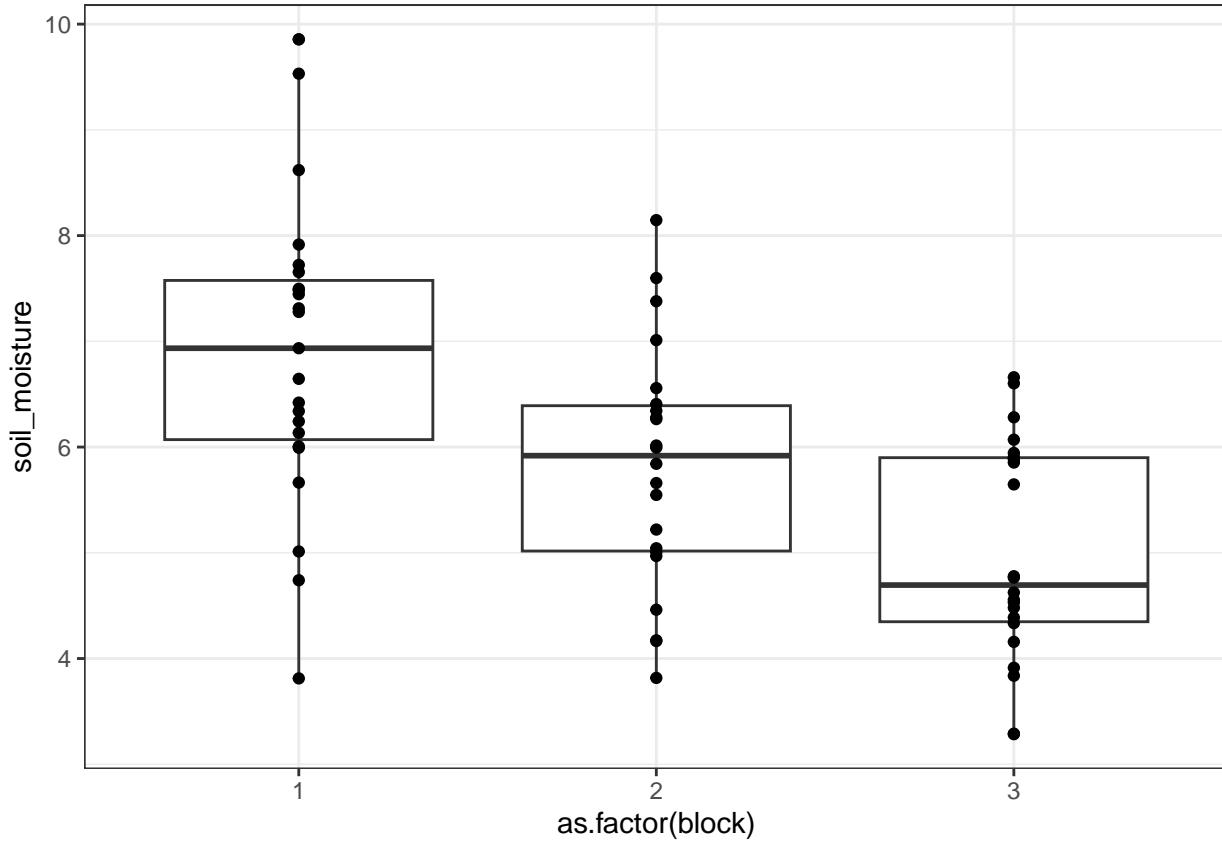
Normality of Residuals

Dots should fall along the line



These plots make it rather clear that macroaggregates and soil moisture show considerable block-level variation. Indeed, Block 3 has a much lower macroaggregate fraction than the other two, and Blocks 2 and 3 have lower soil moisture than Block 1.





Are any of macroaggregates, soil moisture, or pH influenced by tree species richness? Apparently not, as shown below.

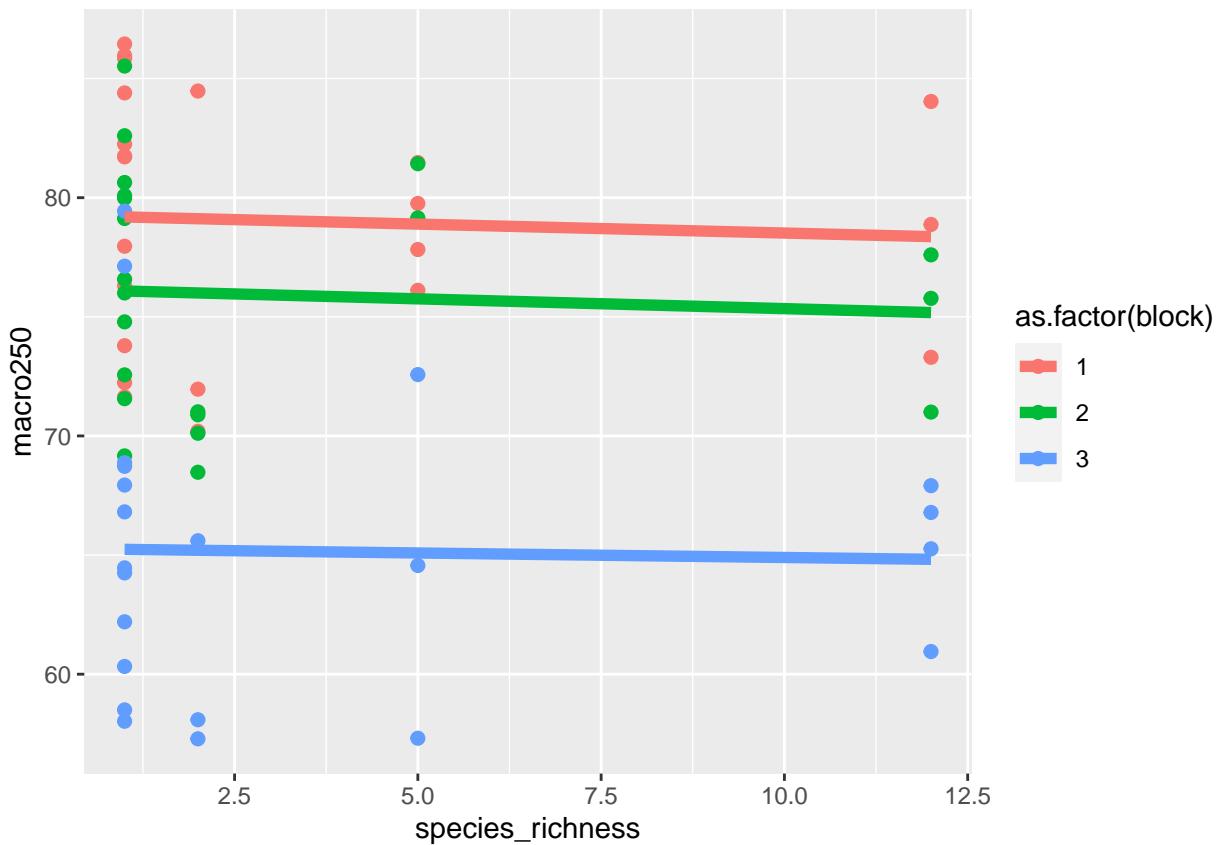
```
summary(lmer(macro250 ~ species_richness + (1 | block), data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [ 
## lmerModLmerTest]
## Formula: macro250 ~ species_richness + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 411.3
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.60168 -0.89578  0.08311  0.61296  2.54386
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 51.49     7.175
## Residual           29.92     5.470
## Number of obs: 65, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)    
## (Intercept) 73.57210  4.23945  2.08133 17.354 0.00276 ***
## species_richness -0.06492  0.17416 61.01154 -0.373  0.71062
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```

## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## spcs_rchnss -0.140
ggplot(data=carbon_seq,aes(x=species_richness,y=macro250,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```

## we note in passing that there appear to be multiple influential observations here
## that may influence these results, but examining the plots it strikes us as unlikely
## that any alternate 'more appropriate' model would return a significant relationship

```

```
summary(lmer(soil_moisture~species_richness+(1|block),data=carbon_seq))
```

```

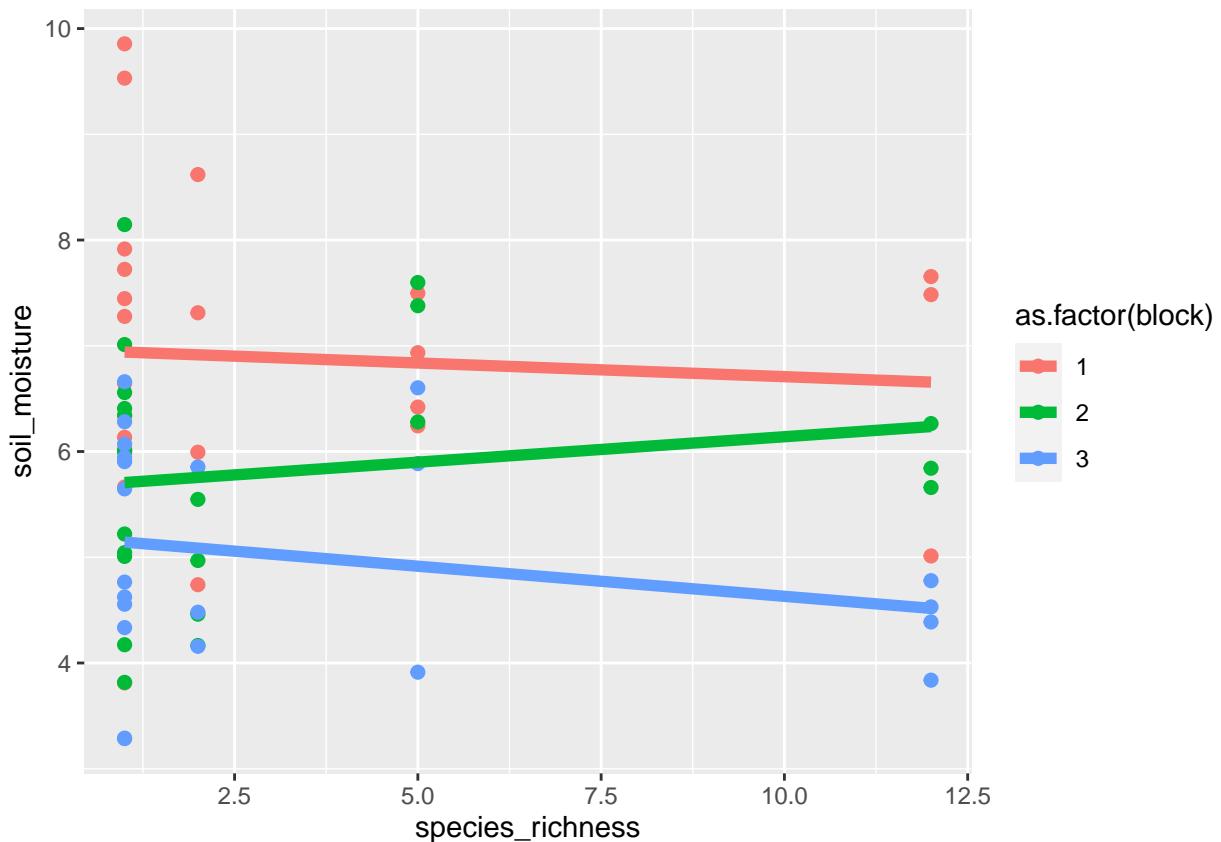
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soil_moisture ~ species_richness + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 227.4
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max 
## -2.46993 -0.67087 -0.02065  0.65685  2.45327 
##
## Random effects:

```

```

##  Groups     Name        Variance Std.Dev.
##  block      (Intercept) 0.8273   0.9095
##  Residual           1.5071   1.2276
## Number of obs: 67, groups: block, 3
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 5.94869  0.56202 2.24549 10.584 0.00583 ***
## species_richness -0.01541  0.03898 63.02794 -0.395 0.69391
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## spcs_rchnss -0.236
ggplot(data=carbon_seq,aes(x=species_richness,y=soil_moisture,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```
summary(lm(pH~species_richness,data=carbon_seq)) ## singular fit with block
```

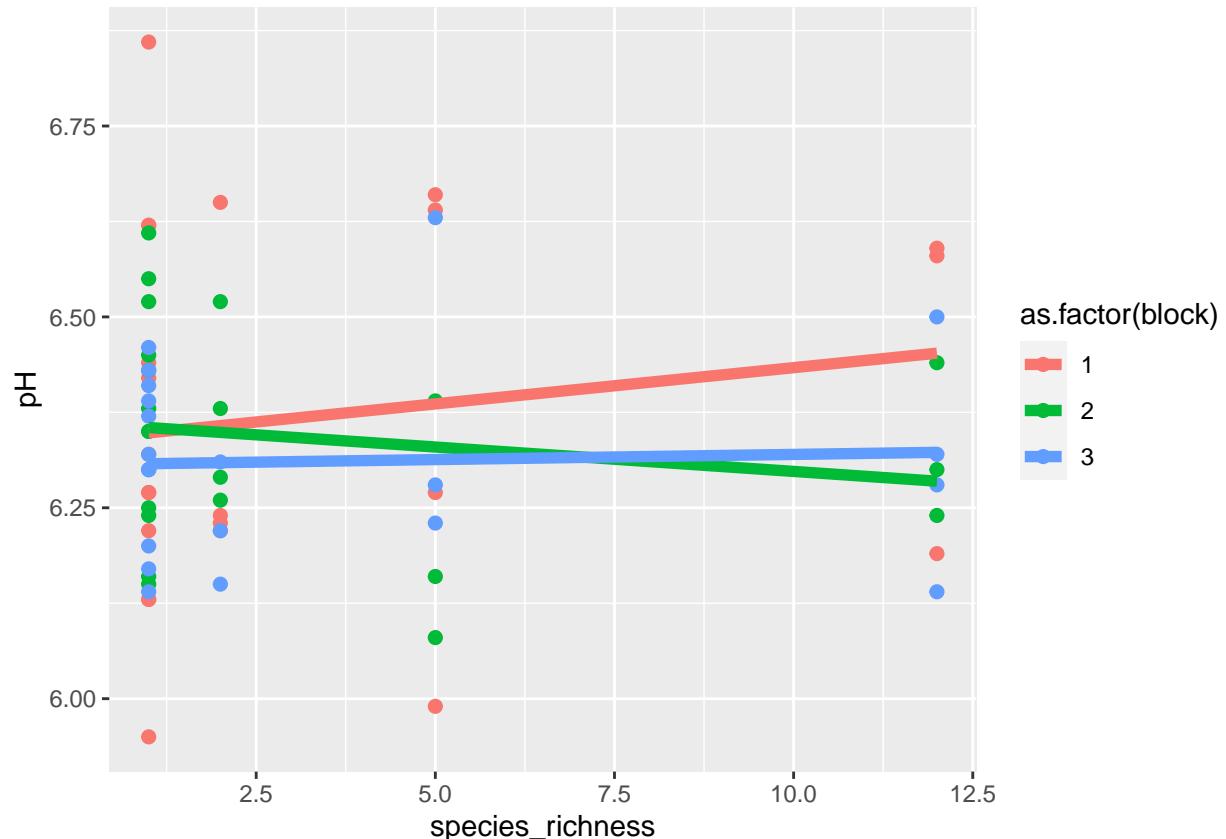
```

##
## Call:
## lm(formula = pH ~ species_richness, data = carbon_seq)
##
## Residuals:
##    Min     1Q  Median     3Q    Max 
## -1.50  -0.75  -0.25  0.25  1.50 
##
```

```

## -0.3882 -0.1157 -0.0382  0.0968  0.5218
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 6.336957   0.029106 217.72 <2e-16 ***
## species_richness 0.001245   0.005663    0.22    0.827
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1785 on 65 degrees of freedom
## (73 observations deleted due to missingness)
## Multiple R-squared:  0.0007432, Adjusted R-squared:  -0.01463
## F-statistic: 0.04834 on 1 and 65 DF, p-value: 0.8267
ggplot(data=carbon_seq,aes(x=species_richness,y=pH,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



A lot of recent work in ecosystem ecology links the accumulation of stable soil C to the formation of macroaggregates. We could ask: does the percentage of macroaggregates (in 2019) correlate with soil carbon in 2013 (pre-treatment) or 2019? (Here, we put soil C variables on the left side of the formula because we expect the causality to be that macroaggregate formation increases the stability of soil C. This is important because the random intercept terms are quite important. In analyses of soil moisture and pH, we keep soil C variables on the left side even though the causality is less straightforward; however, it is less consequential due to the lack of random intercept terms.)

```

## note that these relationships would be significant in the absence
## of the block random intercept

```

```

summary(lmer(soilC_2013~macro250+(1|block), data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2013 ~ macro250 + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 312.1
##
## Scaled residuals:
##    Min     1Q Median     3Q    Max
## -1.4538 -0.6916 -0.0738  0.4413  4.0100
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block     (Intercept) 0.8442   0.9188
##   Residual            6.5752   2.5642
## Number of obs: 65, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 10.15177  3.85797 21.17731  2.631  0.0155 *
## macro250    0.08554  0.05192 23.59887  1.647  0.1127
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## macro250 -0.987

# summary(lmer(perC_2013~macro250+(1/block), data=carbon_seq))

## another possibility is to simply treat block
## as a fixed effect to avoid endogeneity problems
## since blocks vary in macroaggregates
## however, this yields the same results
summary(lm(soilC_2013~macro250+as.factor(block), data=carbon_seq))

##
## Call:
## lm(formula = soilC_2013 ~ macro250 + as.factor(block), data = carbon_seq)
##
## Residuals:
##    Min     1Q Median     3Q    Max
## -3.4685 -1.7696 -0.4127  1.0165 10.2026
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 10.89574  4.77818  2.280  0.0261 *
## macro250    0.08666  0.06007  1.443  0.1543
## as.factor(block)2 -1.94888  0.80578 -2.419  0.0186 *
## as.factor(block)3 -0.54369  1.13804 -0.478  0.6345
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

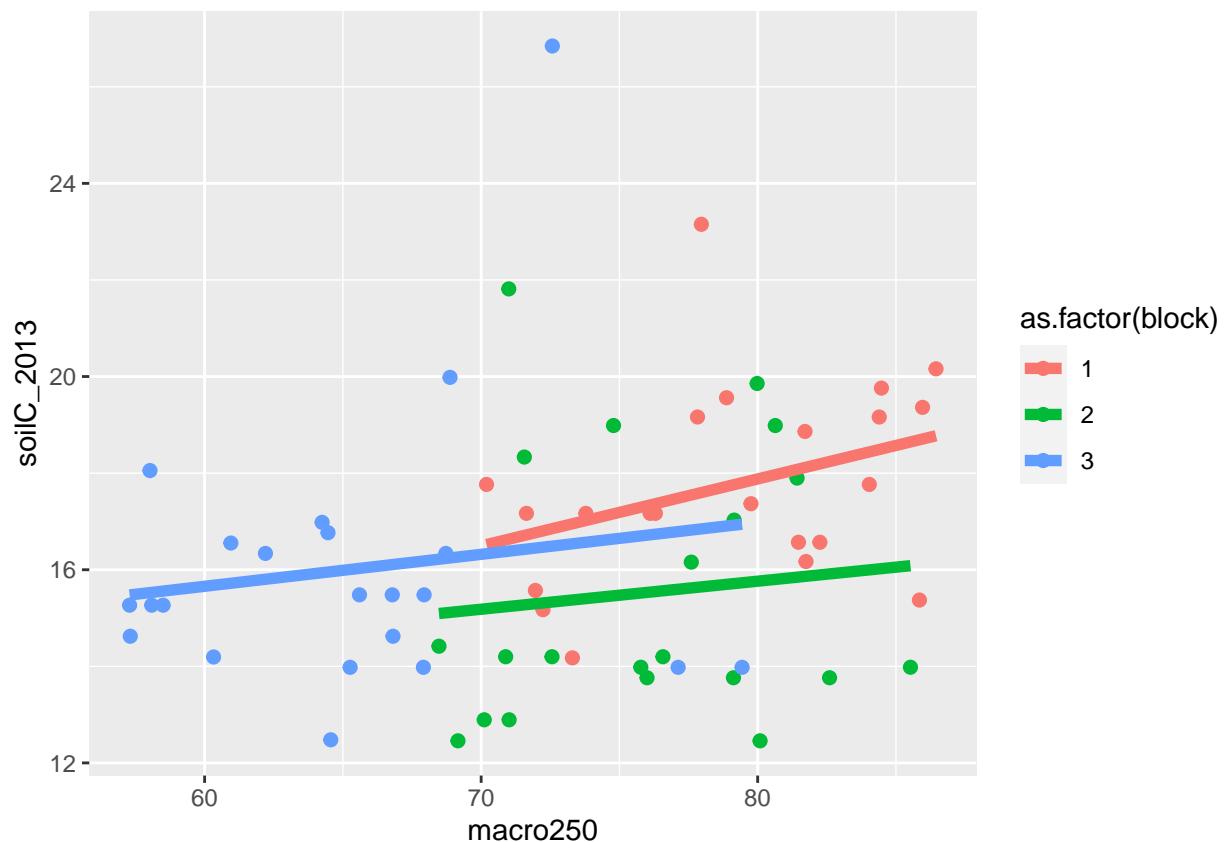
```

```

## 
## Residual standard error: 2.569 on 61 degrees of freedom
##   (75 observations deleted due to missingness)
## Multiple R-squared:  0.1535, Adjusted R-squared:  0.1118 
## F-statistic: 3.686 on 3 and 61 DF,  p-value: 0.0166 

ggplot(data=carbon_seq,aes(x=macro250,y=soilC_2013,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```

## note that these relationships would be significant/marginal in the absence
## of the block random intercept
summary(lmer(soilC_2019~macro250+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [ 
## lmerModLmerTest]
## Formula: soilC_2019 ~ macro250 + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 371.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.0382 -0.7215 -0.1137  0.6404  2.4799
##
## Random effects:
##   Groups   Name       Variance Std.Dev.
##   block    (Intercept) 0.1458  0.3818

```

```

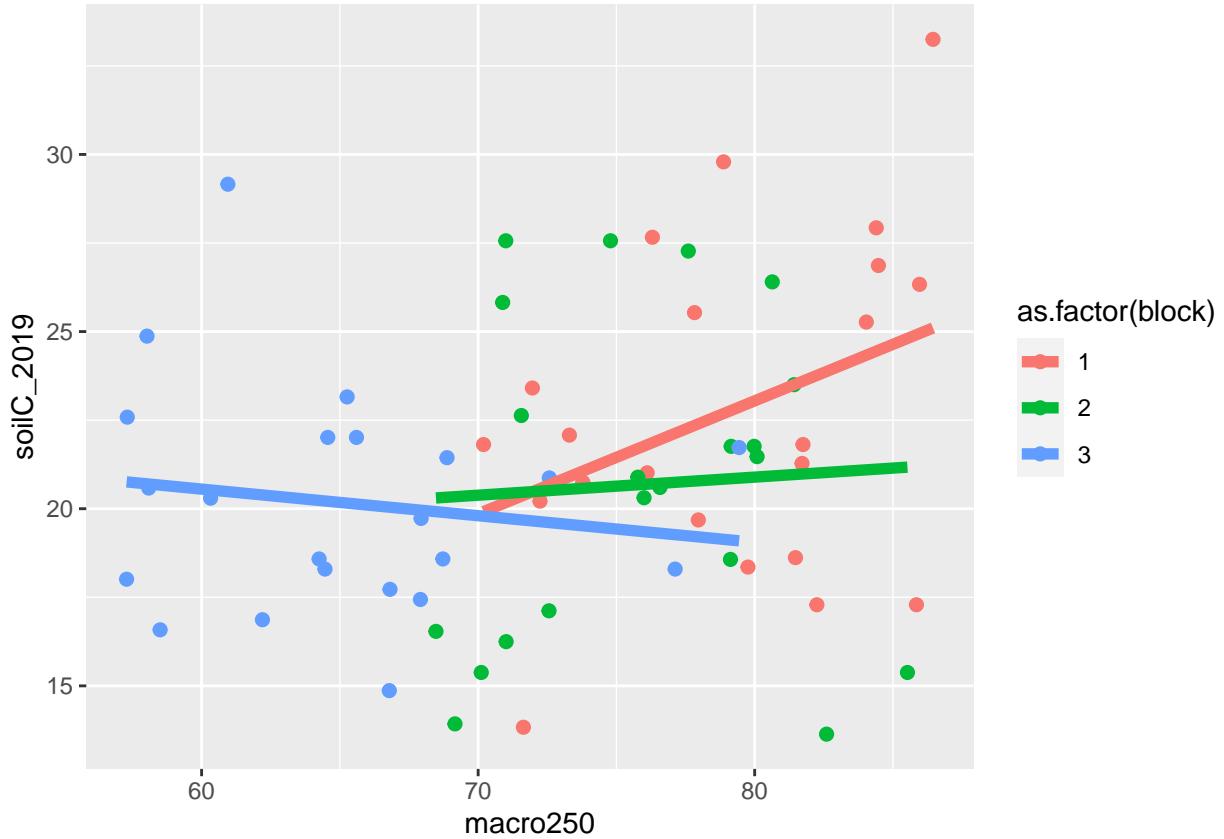
##  Residual           17.5092  4.1844
## Number of obs: 65, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|) 
## (Intercept) 12.61086   5.01076  5.50575  2.517   0.049 * 
## macro250    0.11715   0.06791  5.64541  1.725   0.138  
## --- 
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## macro250 -0.994
# summary(lmer(perc_2019~macro250+(1/block),data=carbon_seq))

## another possibility is to simply treat block
## as a fixed effect to avoid endogeneity problems
## since blocks vary in macroaggregates
## however, this yields the same results
summary(lm(soilC_2019~macro250+as.factor(block),data=carbon_seq))

##
## Call:
## lm(formula = soilC_2019 ~ macro250 + as.factor(block), data = carbon_seq)
##
## Residuals:
##      Min      1Q      Median      3Q      Max 
## -8.2952 -2.9559 -0.1855  2.3672  9.9105 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 16.26744   7.80624  2.084   0.0414 *  
## macro250    0.08179   0.09814  0.833   0.4079    
## as.factor(block)2 -1.79306   1.31642 -1.362   0.1782    
## as.factor(block)3 -1.42749   1.85924 -0.768   0.4456    
## --- 
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.198 on 61 degrees of freedom
##   (75 observations deleted due to missingness)
## Multiple R-squared:  0.07948,    Adjusted R-squared:  0.03421 
## F-statistic: 1.756 on 3 and 61 DF,  p-value: 0.1651

ggplot(data=carbon_seq,aes(x=macro250,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

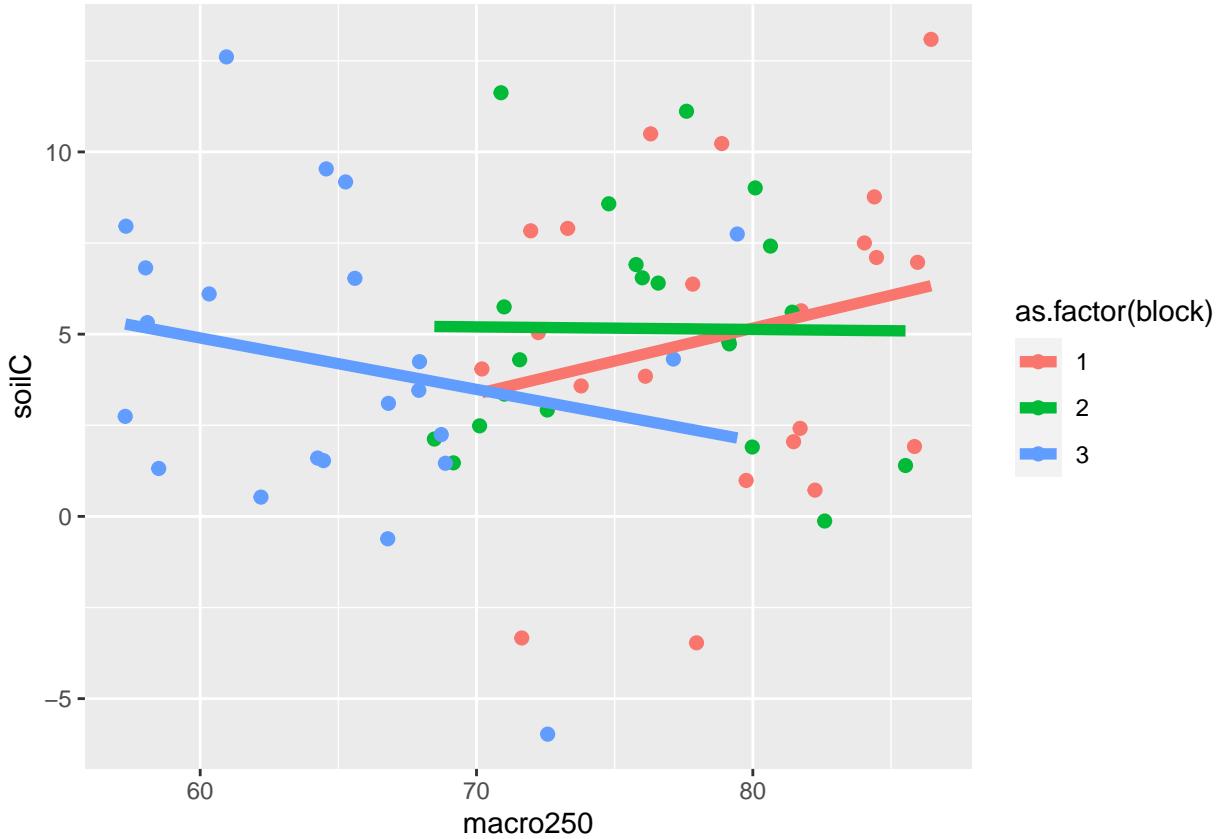
```



```
## when looking at change in soil C, the block effect reaches a singular fit
summary(lm(soilC~macro250,data=carbon_seq))
```

```
##
## Call:
## lm(formula = soilC ~ macro250, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -10.7145  -2.8438  -0.2396  2.3915  8.2794 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 2.16897   4.40823   0.492   0.624    
## macro250    0.03542   0.05977   0.593   0.556    
## 
## Residual standard error: 3.854 on 63 degrees of freedom
## (75 observations deleted due to missingness)
## Multiple R-squared:  0.005543, Adjusted R-squared:  -0.01024 
## F-statistic: 0.3511 on 1 and 63 DF,  p-value: 0.5556
```

```
ggplot(data=carbon_seq,aes(x=macro250,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)
```



These plots offer scant evidence that macroaggregates have much relation to soil C, accounting for the non-independence of plots from the same block. From a more basic soil science perspective, we might wish to see whether there is a correlation between soil percent C and macroaggregates across the full dataset (without block random or fixed effects). There is, although this relationship is perhaps surprisingly weak, and (even more surprisingly) is stronger when examining percent C in 2013 than 2019:

```
summary(lm(perC_2013 ~ macro250, data=carbon_seq))

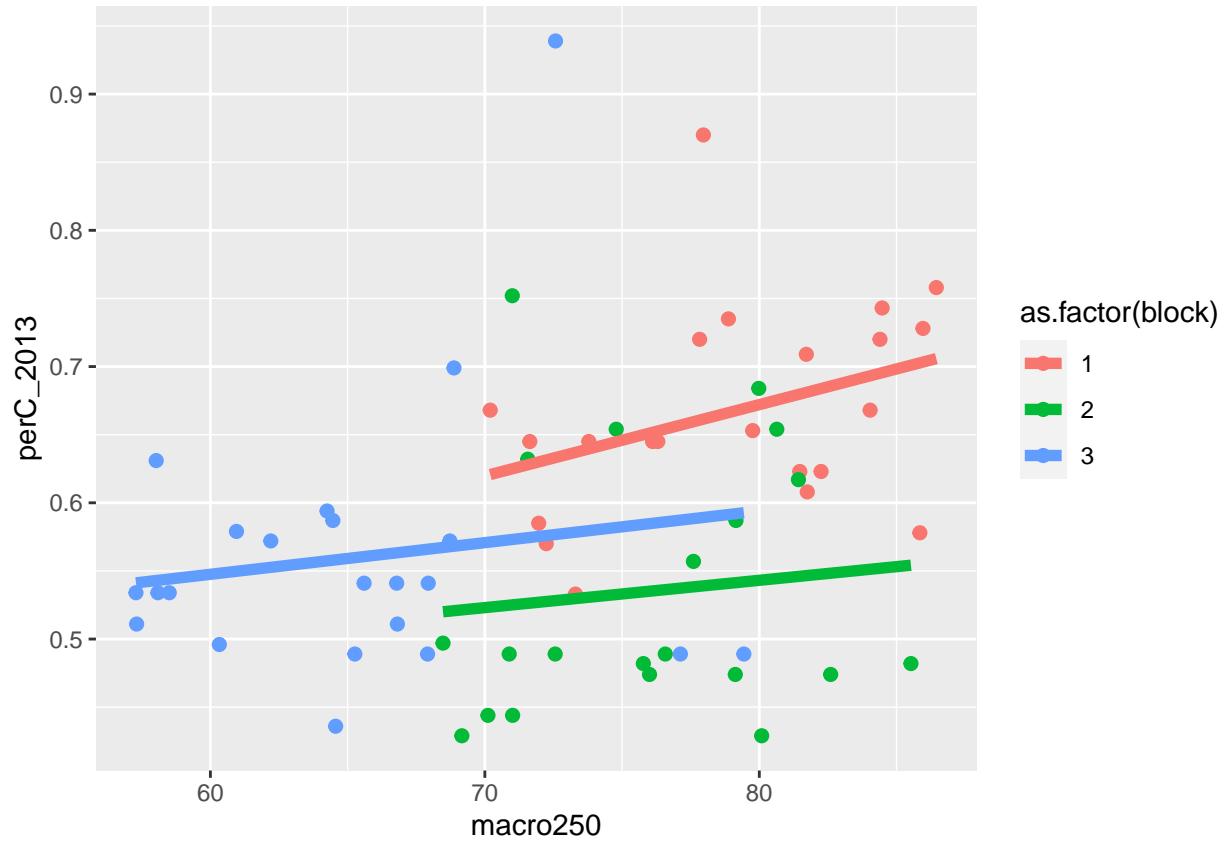
##
## Call:
## lm(formula = perC_2013 ~ macro250, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -0.18807 -0.07005 -0.00004  0.05507  0.35429 
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 0.272147  0.116918   2.328   0.0232 *  
## macro250    0.004307  0.001585   2.717   0.0085 ** 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1022 on 63 degrees of freedom
##   (75 observations deleted due to missingness)
## Multiple R-squared:  0.1049, Adjusted R-squared:  0.09065 
## F-statistic:  7.38 on 1 and 63 DF,  p-value: 0.008505
```

```

ggplot(data=carbon_seq,aes(x=macro250,y=perC_2013,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 75 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 75 rows containing missing values (`geom_point()`).

```



```

summary(lm(perC_2019~macro250,data=carbon_seq))

##
## Call:
## lm(formula = perC_2019 ~ macro250, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -0.3437 -0.1020 -0.0239  0.1080  0.4133 
## 
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) 0.320852  0.180165  1.781   0.0798 .  
## macro250    0.005967  0.002443  2.442   0.0174 *  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 
## 
## Residual standard error: 0.1575 on 63 degrees of freedom

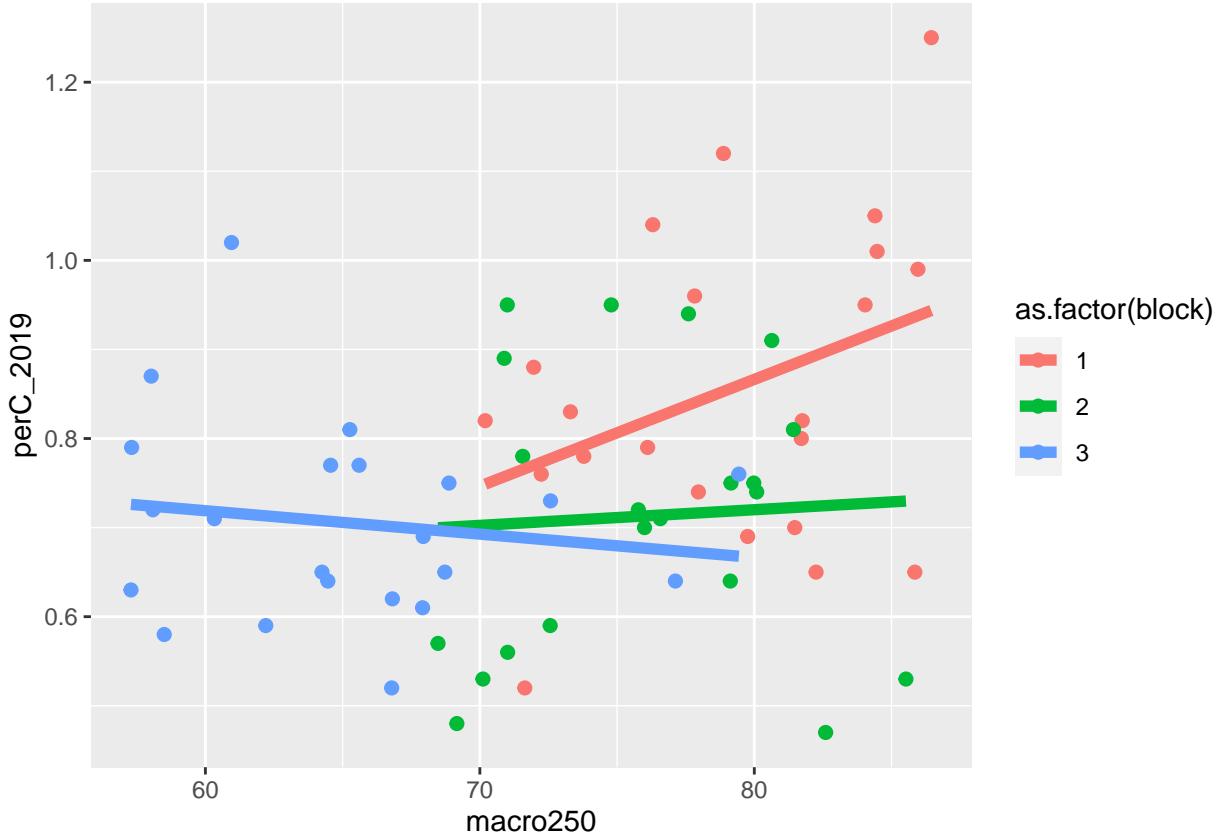
```

```

##      (75 observations deleted due to missingness)
## Multiple R-squared:  0.0865, Adjusted R-squared:  0.072
## F-statistic: 5.966 on 1 and 63 DF,  p-value: 0.0174
ggplot(data=carbon_seq,aes(x=macro250,y=perC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 75 rows containing non-finite values (`stat_smooth()`).
## Removed 75 rows containing missing values (`geom_point()`).

```



To the extent that there appears to be a relationship across the full dataset, it appears largely driven by the fact that Block 1 has higher macroaggregates than Block 3 in particular, and greater initial and final percent C than Blocks 2 and 3. (These differences in percent C are largely offset by lower bulk density when calculating the top 20 cm C pool, such that there remain no post-treatment block differences in soil C pools or soil C accumulation.)

Although soil C pools and macroaggregates are not strongly related, it turns out that soil C and soil moisture are, based on pools (or percent C) in both 2013 and 2019:

```

## this also yields positive relationships using initial and 2019 percent C
summary(lmer(soilC_2013~soil_moisture+(1|block),data=carbon_seq))

```

```

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2013 ~ soil_moisture + (1 | block)
##   Data: carbon_seq
##

```

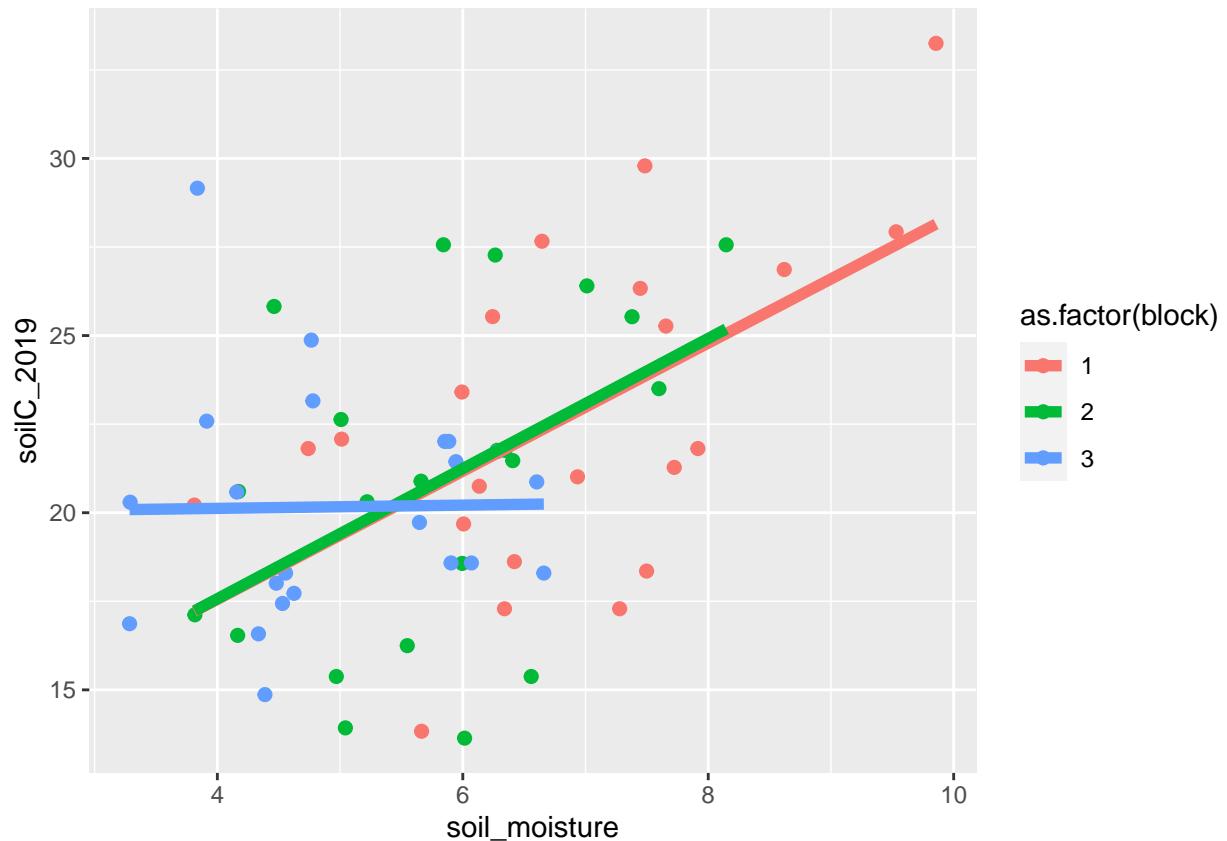
```

## REML criterion at convergence: 307.2
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.7258 -0.5444 -0.0945  0.3223  4.0595
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block    (Intercept) 0.2019   0.4494
##   Residual           5.6512   2.3772
## Number of obs: 67, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error    df t value Pr(>|t|) 
## (Intercept) 11.4088   1.3514 23.2259 8.442 1.56e-08 ***
## soil_moisture 0.8546   0.2192 33.7577 3.899 0.000436 *** 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## soil_moistr -0.958
## singular fit
summary(lm(soilC_2019~soil_moisture,data=carbon_seq))

##
## Call:
## lm(formula = soilC_2019 ~ soil_moisture, data = carbon_seq)
##
## Residuals:
##      Min     1Q Median     3Q    Max
## -7.8059 -2.4716 -0.1146  2.1767 10.7522
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|) 
## (Intercept) 13.0578   1.9935  6.550 1.14e-08 ***
## soil_moisture 1.3941   0.3291  4.236 7.42e-05 *** 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.804 on 64 degrees of freedom
##   (74 observations deleted due to missingness)
## Multiple R-squared:  0.219, Adjusted R-squared:  0.2068
## F-statistic: 17.95 on 1 and 64 DF, p-value: 7.423e-05
## these findings can easily be reproduced with block as a fixed effect
## with minimal changes to parameter estimates

ggplot(data=carbon_seq,aes(x=soil_moisture,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

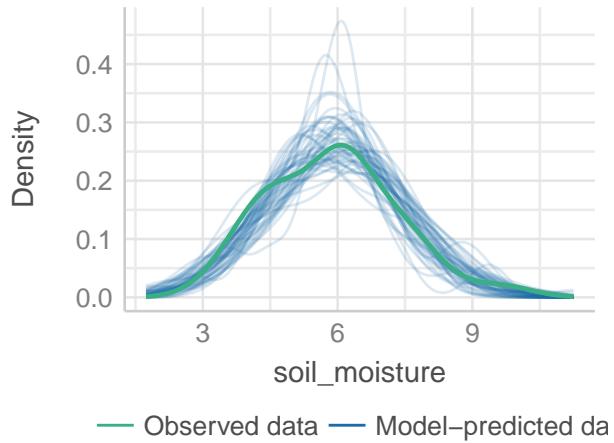
```



```
check_model(lmer(soil_moisture~soilC_2019+(1|block),data=carbon_seq))
```

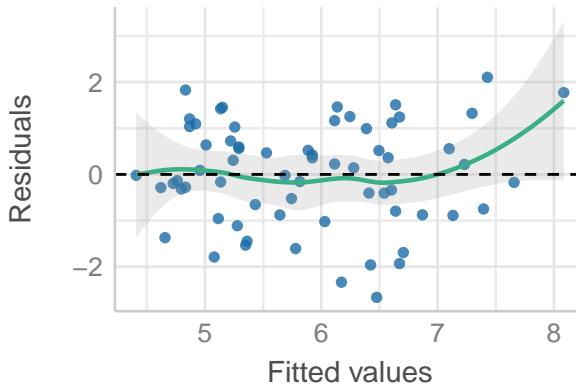
Posterior Predictive Check

Model-predicted lines should resemble observed data



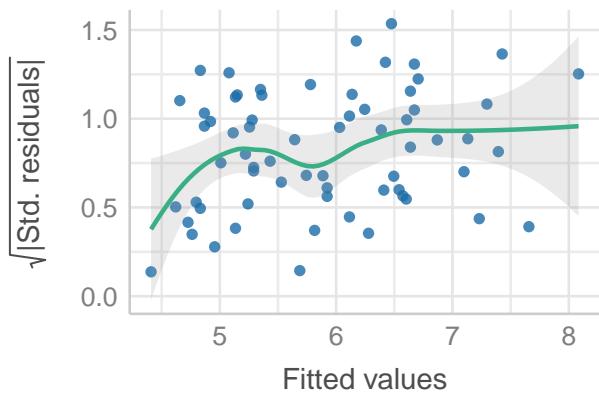
Linearity

Reference line should be flat and horizontal



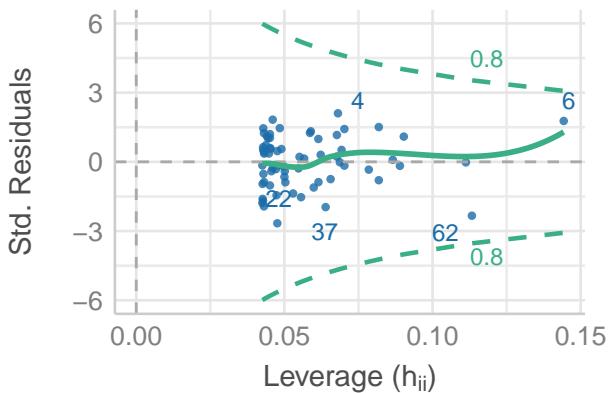
Homogeneity of Variance

Reference line should be flat and horizontal



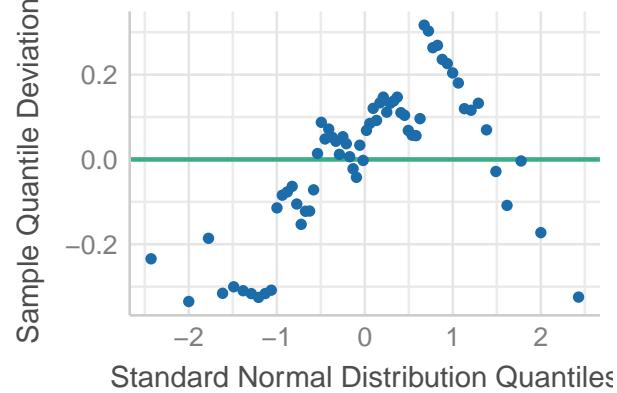
Influential Observations

Points should be inside the contour lines



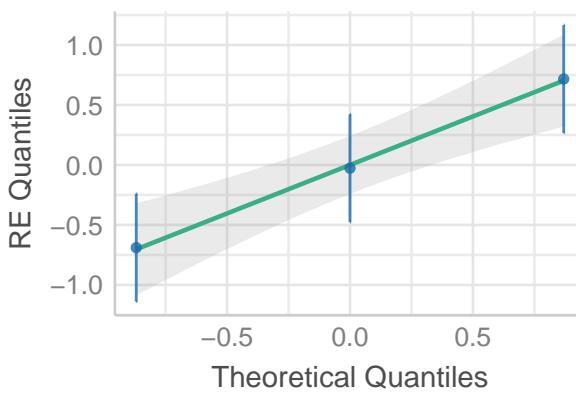
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (block)

Dots should be plotted along the line



However, there is no relationship between soil moisture and soil C accumulation.

```
## singular fit
summary(lm(soilC~soil_moisture,data=carbon_seq))
```

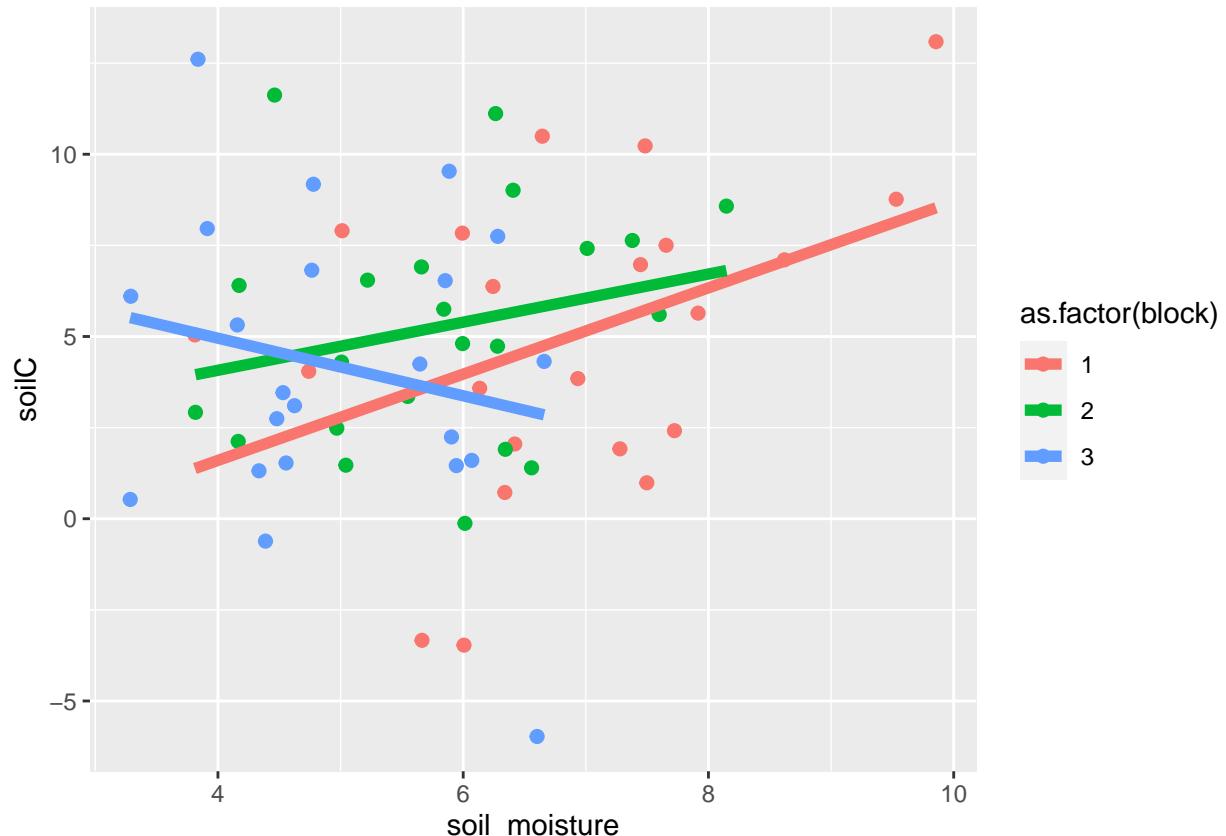
```
##
```

```

## Call:
## lm(formula = soilC ~ soil_moisture, data = carbon_seq)
##
## Residuals:
##      Min       1Q   Median       3Q      Max 
## -11.1521  -2.6684  -0.1297  2.4115  8.8549 
## 
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept)  1.7751    1.9796   0.897   0.373    
## soil_moisture 0.5153    0.3268   1.577   0.120    
## 
## Residual standard error: 3.778 on 64 degrees of freedom
##   (74 observations deleted due to missingness)
## Multiple R-squared:  0.0374, Adjusted R-squared:  0.02236 
## F-statistic: 2.487 on 1 and 64 DF,  p-value: 0.1198 

ggplot(data=carbon_seq,aes(x=soil_moisture,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



As it turns out, soil pH is positively correlated both with soil C accumulation and (more weakly) with the soil C pool in 2019 (but not in 2013).

```

## block random intercept yields singular fit
summary(lm(soilC~pH,data=carbon_seq))

```

```

## 
## Call:

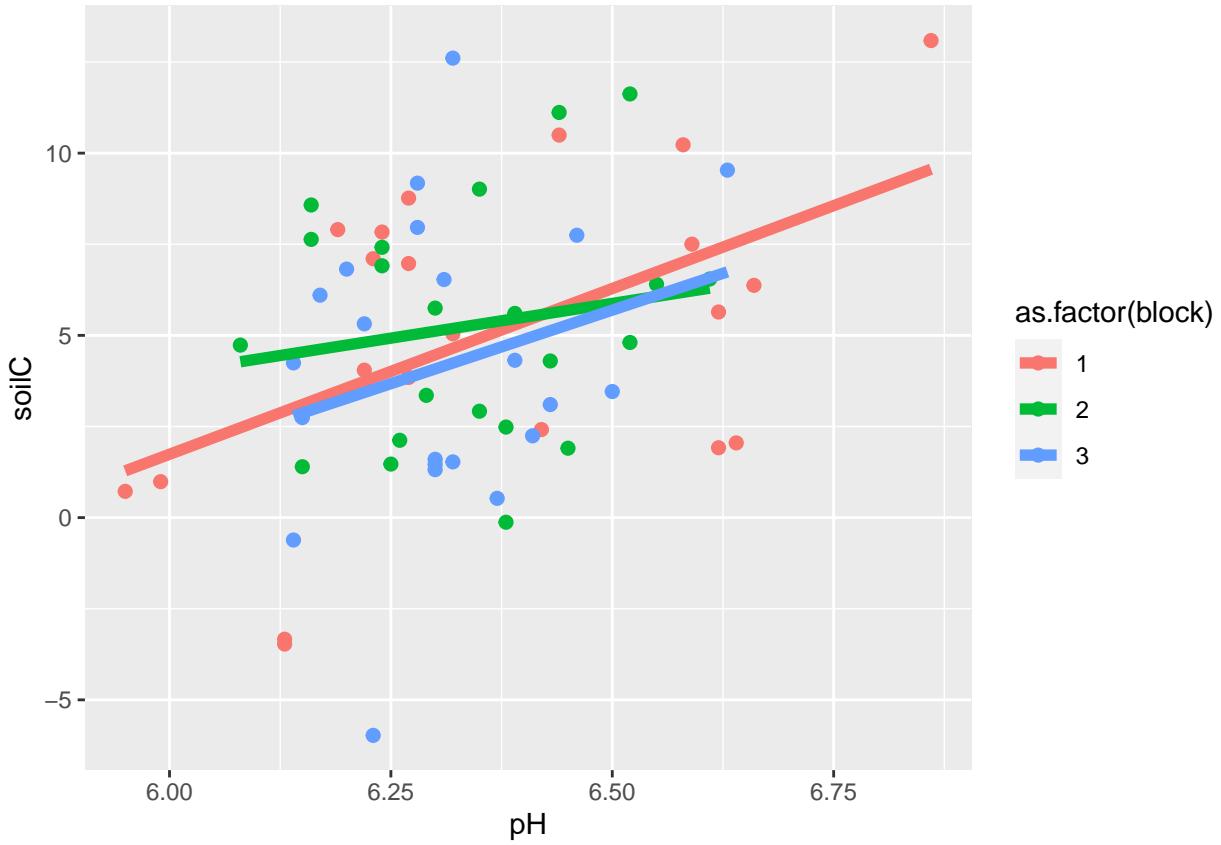
```

```

## lm(formula = soilC ~ pH, data = carbon_seq)
##
## Residuals:
##   Min     1Q Median     3Q    Max 
## -9.940 -2.653 -0.368  3.034  7.929 
## 
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)    
## (Intercept) -45.387     16.185  -2.804  0.00667 ** 
## pH            7.922      2.553   3.103  0.00285 ** 
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Residual standard error: 3.59 on 64 degrees of freedom
## (74 observations deleted due to missingness)
## Multiple R-squared:  0.1307, Adjusted R-squared:  0.1172 
## F-statistic: 9.626 on 1 and 64 DF, p-value: 0.002854

ggplot(data=carbon_seq,aes(x=pH,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```

## block random intercept yields singular fit
summary(lm(soilC_2013~pH,data=carbon_seq))

```

```

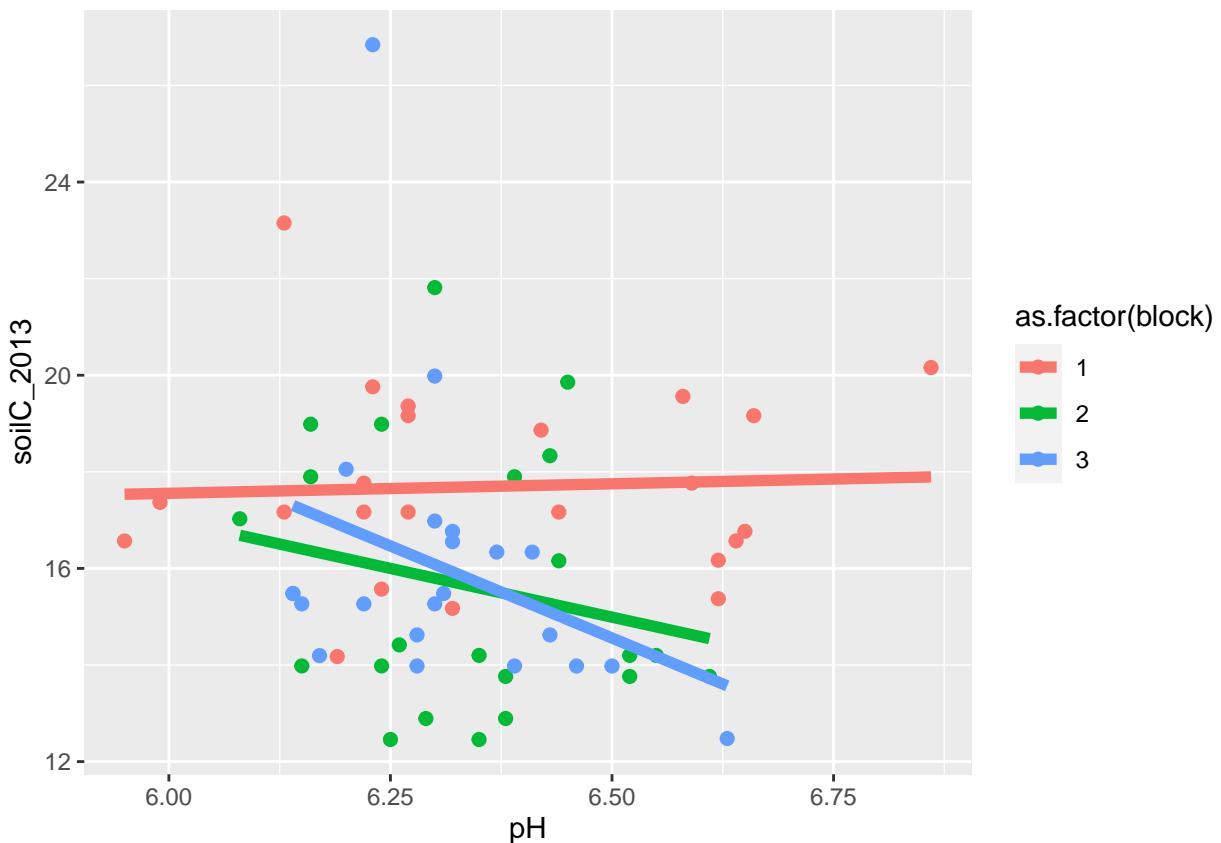
## 
## Call:
## lm(formula = soilC_2013 ~ pH, data = carbon_seq)

```

```

##
## Residuals:
##   Min     1Q Median     3Q    Max
## -4.1176 -2.1997 -0.0886  1.4560 10.2439
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 24.328     11.905   2.044   0.0451 *
## pH          -1.240      1.877  -0.661   0.5110
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.702 on 65 degrees of freedom
## (73 observations deleted due to missingness)
## Multiple R-squared:  0.006677, Adjusted R-squared:  -0.008605
## F-statistic: 0.4369 on 1 and 65 DF, p-value: 0.511
ggplot(data=carbon_seq,aes(x=pH,y=soilC_2013,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```

## block random intercept yields singular fit
summary(lm(soilC_2019~pH,data=carbon_seq))

```

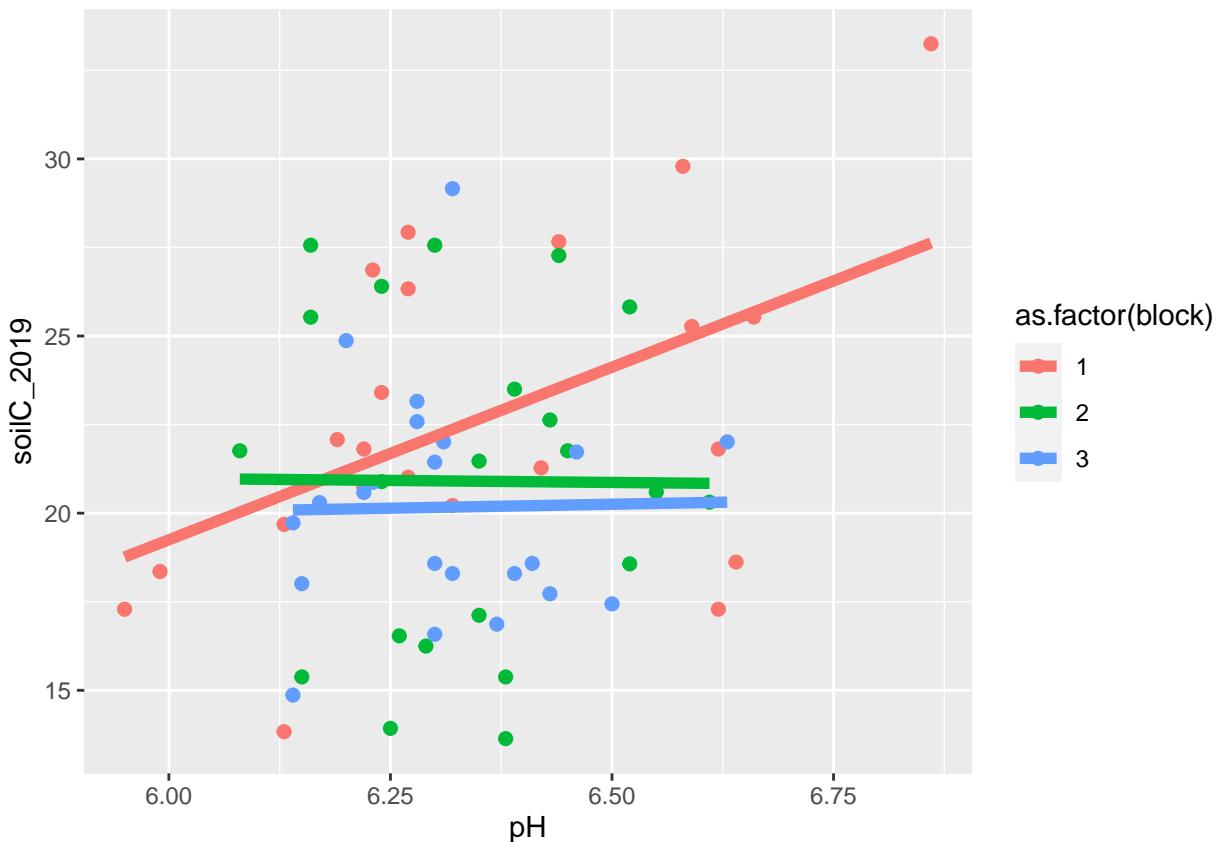
```

##
## Call:
## lm(formula = soilC_2019 ~ pH, data = carbon_seq)
## 
```

```

## Residuals:
##      Min       1Q   Median      3Q      Max
## -7.9162 -3.2835 -0.0722  2.2404  8.5414
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -20.377    18.697  -1.090  0.2799
## pH           6.572     2.950   2.228  0.0294 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.147 on 64 degrees of freedom
## (74 observations deleted due to missingness)
## Multiple R-squared:  0.07199,    Adjusted R-squared:  0.05749
## F-statistic: 4.965 on 1 and 64 DF,  p-value: 0.02939
ggplot(data=carbon_seq,aes(x=pH,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



```

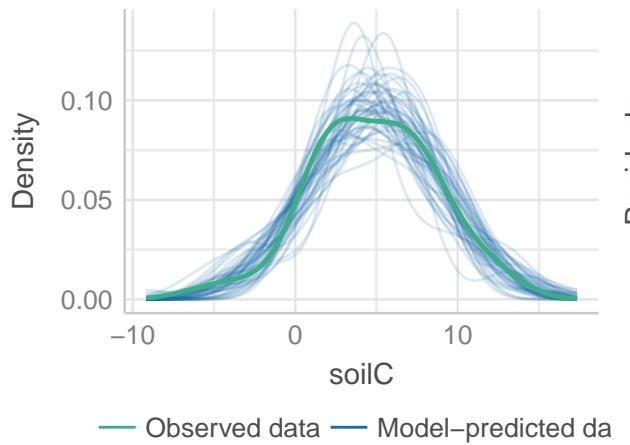
## this model with the 2019 C pool may be
## influenced by a high-leverage point (plot 12)

check_model(lm(soilC~pH,data=carbon_seq))

```

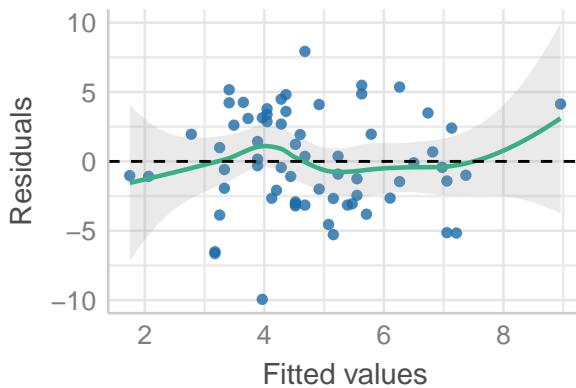
Posterior Predictive Check

Model-predicted lines should resemble observed data



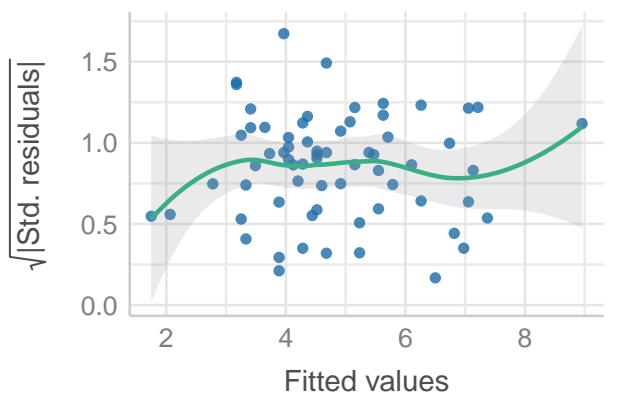
Linearity

Reference line should be flat and horizontal



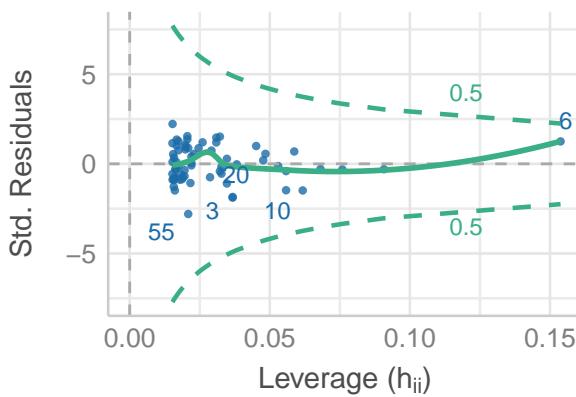
Homogeneity of Variance

Reference line should be flat and horizontal



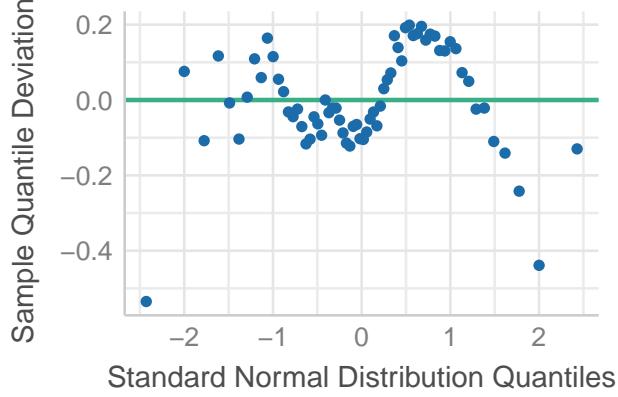
Influential Observations

Points should be inside the contour lines



Normality of Residuals

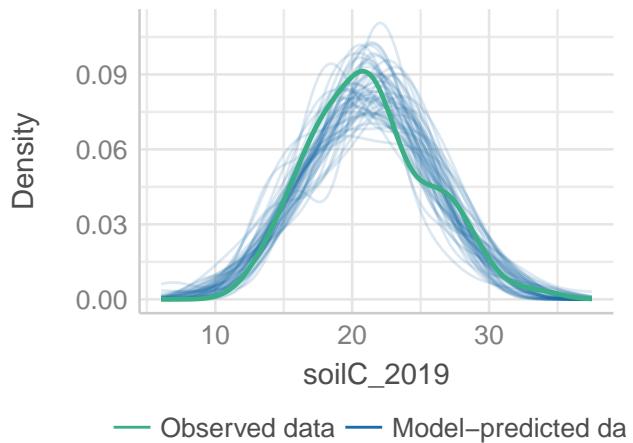
Dots should fall along the line



```
check_model(lm(soilC_2019~pH, data=carbon_seq))
```

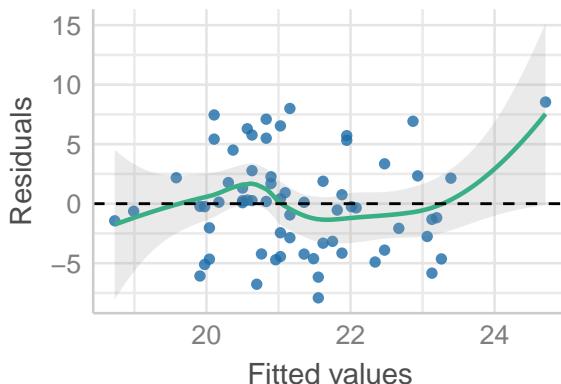
Posterior Predictive Check

Model-predicted lines should resemble observed data



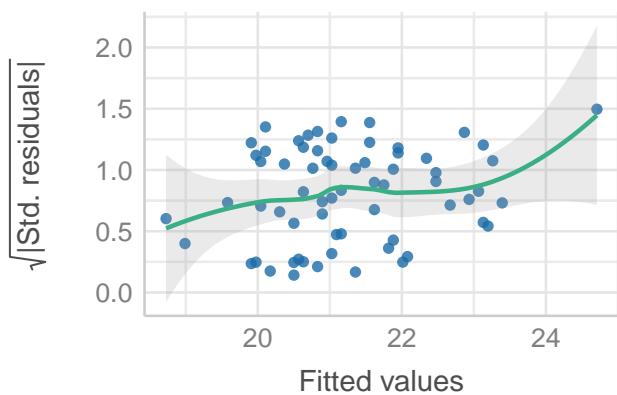
Linearity

Reference line should be flat and horizontal



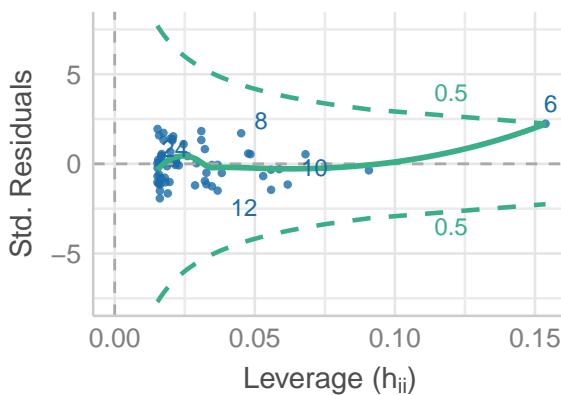
Homogeneity of Variance

Reference line should be flat and horizontal



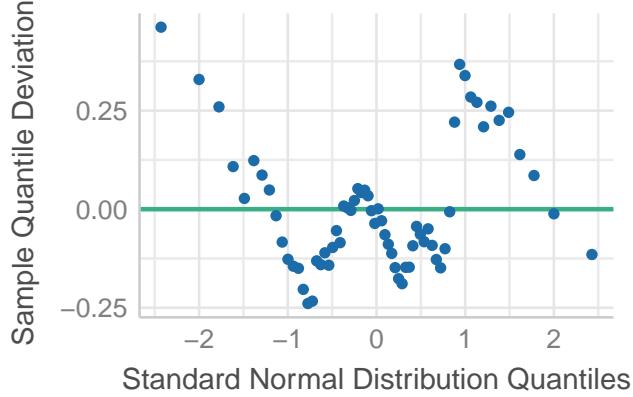
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



We might wonder whether macroaggregates (or soil moisture, or pH) are correlated with fine root C. Once again, the answer is no: we show this for macroaggregates below.

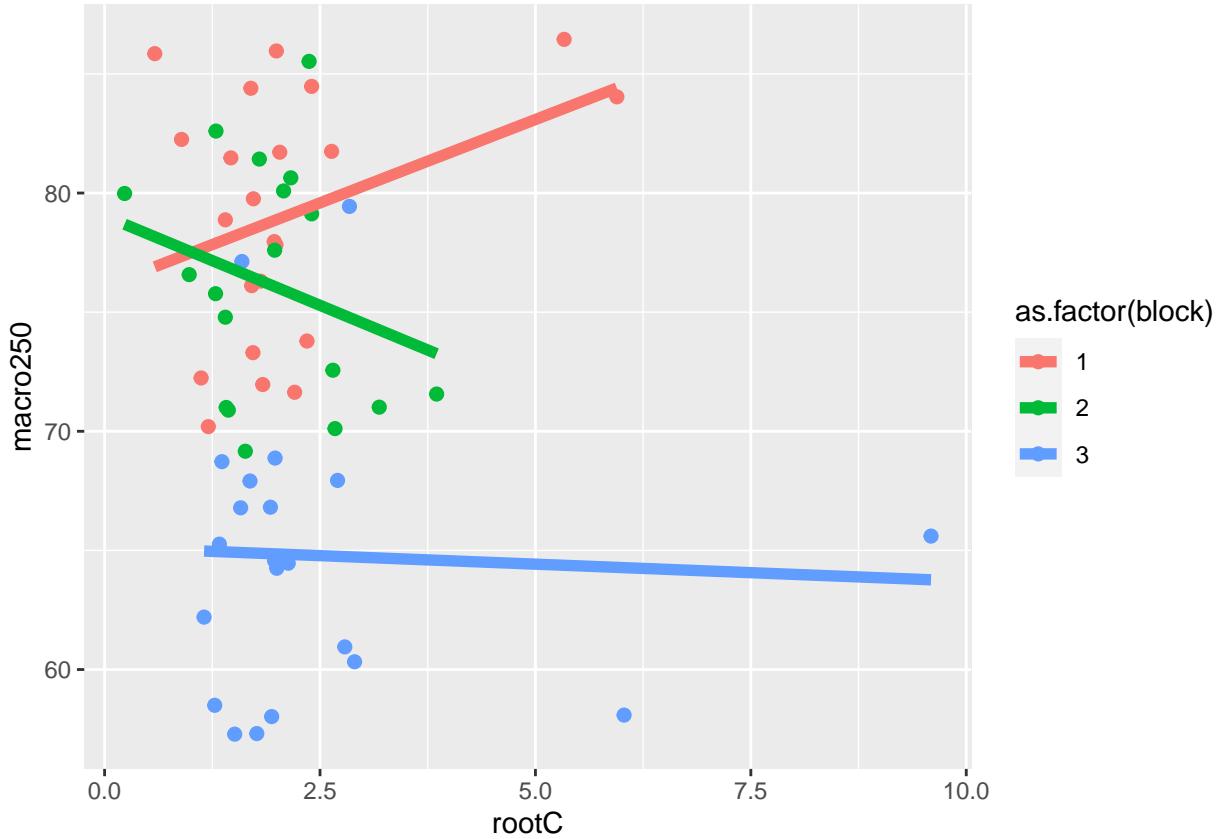
```
summary(lmer(macro250 ~ rootC + (1 | block), data=carbon_seq))
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
```

```

## lmerModLmerTest]
## Formula: macro250 ~ rootC + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 384
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.56867 -0.91252 -0.03593  0.70785  2.63446
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block    (Intercept) 55.74    7.466
##   Residual           29.86    5.465
## Number of obs: 61, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error    df t value Pr(>|t|)    
## (Intercept) 73.0389    4.4994  2.2551 16.233 0.00221 ***
## rootC       0.1244    0.4991 57.0805   0.249  0.80405  
## ---      
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## rootC -0.241
ggplot(data=carbon_seq,aes(x=rootC,y=macro250,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

```



Lastly, we present a result that we show in the Discussion: plots with higher aboveground woody C have lower soil moisture (and also fewer macroaggregates, although this is wrapped up into the structural equation model below).

```
summary(lmer(soil_moisture ~ woodyC + (1 | block), data=carbon_seq))
```

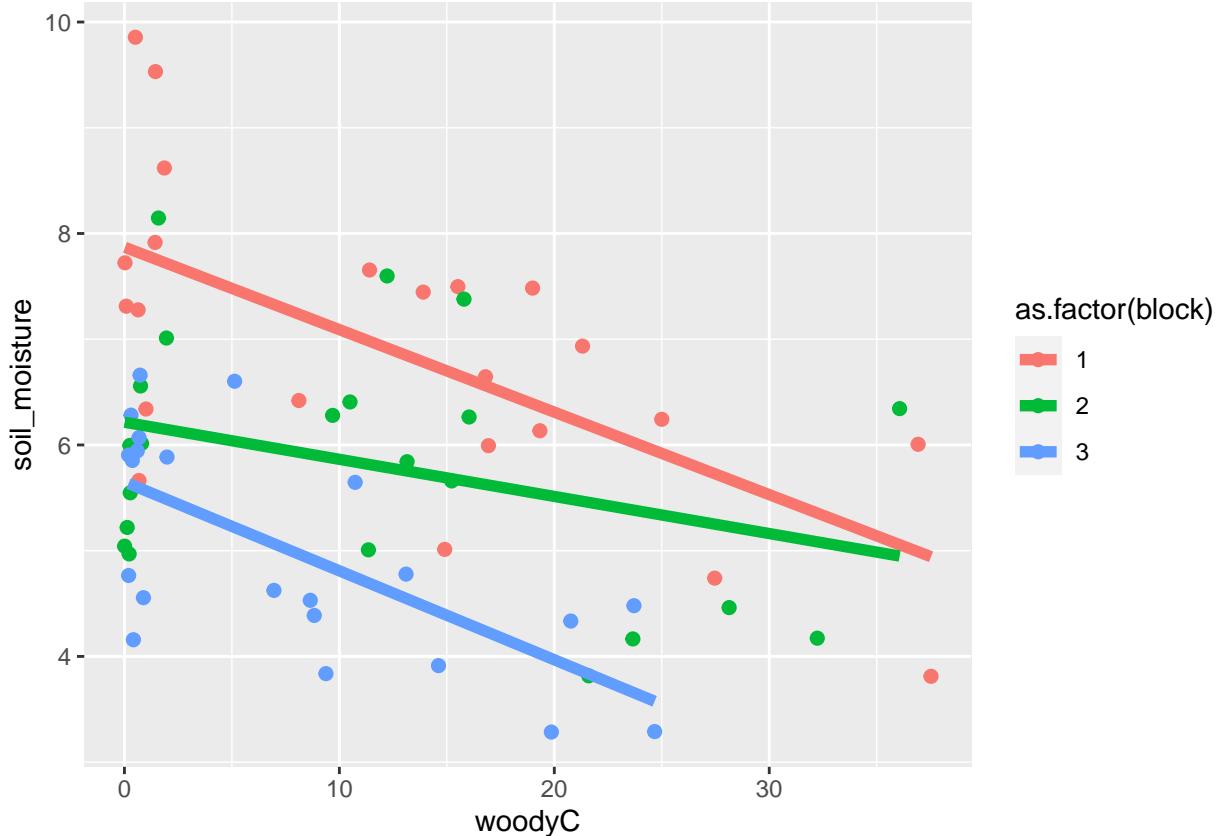
```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: soil_moisture ~ woodyC + (1 | block)  
## Data: carbon_seq  
##  
## REML criterion at convergence: 207.3  
##  
## Scaled residuals:  
##      Min    1Q   Median    3Q   Max  
## -1.87910 -0.70804  0.07241  0.64348  2.19792  
##  
## Random effects:  
## Groups   Name        Variance Std.Dev.  
## block    (Intercept) 1.158     1.076  
## Residual           1.052     1.026  
## Number of obs: 67, groups: block, 3  
##  
## Fixed effects:  
##             Estimate Std. Error       df t value Pr(>|t|)  
## (Intercept) 6.56362   0.64681  2.16309 10.148 0.00731 **  
## woodyC     -0.06266   0.01207 63.18655 -5.193 2.35e-06 ***
```

```

## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##      (Intr)
## woodyC -0.199
ggplot(data=carbon_seq,aes(x=woodyC,y=soil_moisture,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 73 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 73 rows containing missing values (`geom_point()`).

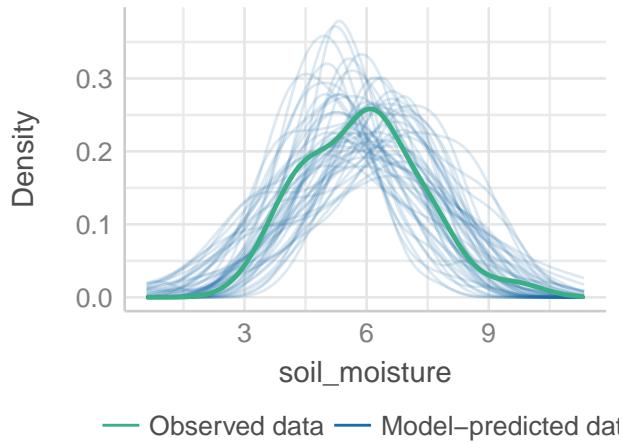
```



```
check_model(lmer(soil_moisture~woodyC+(1|block),data=carbon_seq))
```

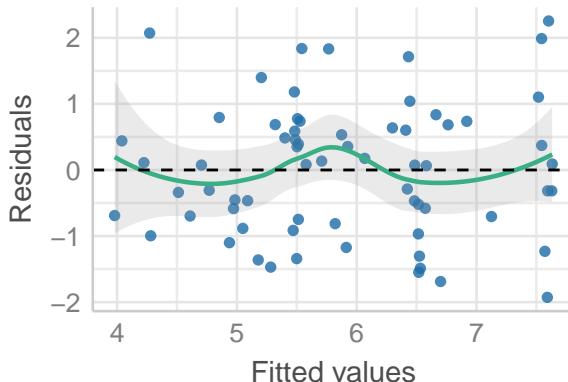
Posterior Predictive Check

Model-predicted lines should resemble observed data



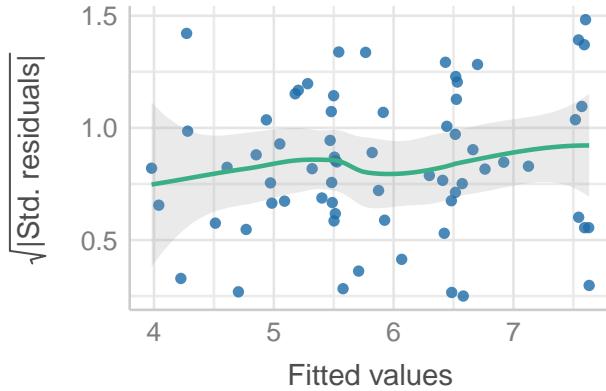
Linearity

Reference line should be flat and horizontal



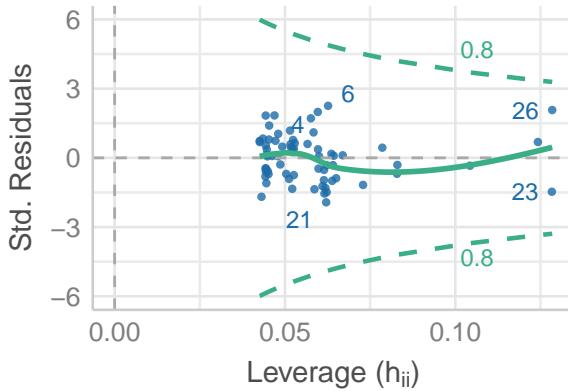
Homogeneity of Variance

Reference line should be flat and horizontal



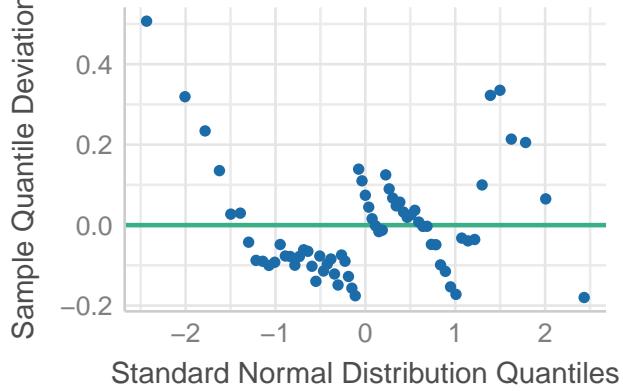
Influential Observations

Points should be inside the contour lines



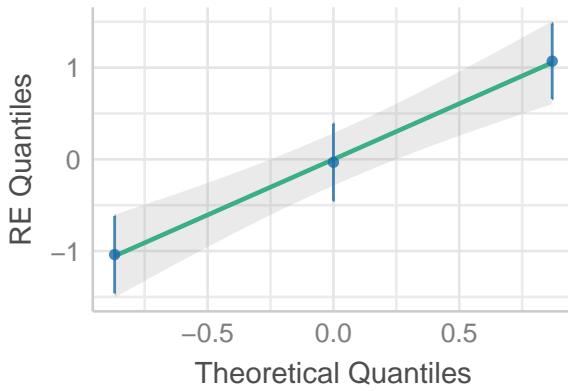
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (block)

Dots should be plotted along the line



Tree biodiversity effects on aboveground woody and belowground C accumulation

One of the core goals of our paper is to show how carbon storage in our experimental plots was influenced by the diversity of planted trees in the first six years of the experiment. We estimated carbon storage across three pools:

- aboveground wood
- soil (0-20 cm)–expressed as change from pre-treatment soil carbon
- fine roots (0-20 cm)

Besides these pools, we also calculated the net biodiversity effect (NBE) for mixture plots as the difference between the observed pools and monoculture-based expectations. (The NBE is synonymous with overyielding, and we use the abbreviation OY in variable names because that was what we chose in early stages of the project.) For aboveground wood, we further partitioned the NBE into complementarity and selection effects following Loreau & Hector (2001).

We considered three facets of diversity:

- species richness
- Laliberté and Legendre (2010)’s functional dispersion (FDis), as reported by Grossman et al. (2017)
- Helmus et al. (2007)’s phylogenetic species variability (PSV), as reported by Grossman et al. (2017)

The traits that went into the calculation of FDis include: leaf mass per area, leaf nitrogen concentration, leaf habit (categorical), wood density, mycorrhizal type (categorical), leaf calcium concentration, and indices of shade, drought, and waterlogging tolerance from Niinemets & Valladares (2006). Full details are found in the paper by Grossman et al. (2017).

The functional and phylogenetic diversity metrics chosen are designed not to be dependent on species richness. However, FDis and PSV are both 0 for monocultures–by default for FDis and as an analysis choice for PSV. As a result, they do end up somewhat correlated with each other and with species richness. As we will see, multicollinearity does not end up being a severe problem in most cases.

In keeping with the dominant paradigm in biodiversity-ecosystem function (BEF) research, we proposed that planted tree diversity would positively influence all three carbon pools. However, we did not pose hypotheses about which specific facets of tree diversity would matter most. As a result, we used an AICc-based framework to test all models nested within a model that includes species richness, FDis, and PSV. We select the most parsimonious model with a $\Delta AICc < 2$ from the model with the lowest AICc. (In effect, this is much like standard null hypothesis significance testing, where $AICc < 2$ corresponds to $p > 0.05$.) When there are two or more equally parsimonious such models, we selected the one with the lower AICc.

Aboveground woody carbon

We can start with aboveground woody carbon:

```
mwt<-lm(woodyC~species_richness+FDis+PSV,data=carbon_seq)
mwt_block<-lmer(woodyC~species_richness+FDis+PSV+(1|block),
                 data=carbon_seq, REML=F)

options(na.action = "na.fail") # required for dredge to run
mwt_dredge <- dredge(mwt,beta = "none",evaluate = T, rank = AICc)
mwt_block_dredge <- dredge(mwt_block,beta = "none",evaluate = T,rank = AICc)
options(na.action = "na.omit")

## instead of selecting a 'best' model, one could instead perform model averaging
# summary(model.avg(mwt_dredge, subset = delta <= 2))
```

The `dredge` function tests all nested submodels of the specified model. Here we apply it to models both with and without block random intercepts.

We can examine our AIC table, select our ‘best’ model, and do some basic checks of model assumptions as follows:

```
mwt_dredge ## we'll select the second model

## Global model call: lm(formula = woodyC ~ species_richness + FDis + PSV, data = carbon_seq)
```

```

## ---
## Model selection table
##   (Int)    FDs    PSV  spc_rch df  logLik  AICc delta weight
## 8  7.941 -5.549 20.870  0.62920 5 -499.261 1009.0  0.00  0.704
## 4  8.494 -4.251 19.140           4 -501.209 1010.7  1.74  0.294
## 3  6.563           9.453       3 -507.897 1022.0 13.00  0.001
## 7  6.662           9.560 -0.05419 4 -507.880 1024.1 15.09  0.000
## 2  8.863  1.263           3 -517.217 1040.6 31.64  0.000
## 1 10.690           0.32500  2 -518.636 1041.4 32.39  0.000
## 5  9.826           0.32500  3 -518.053 1042.3 33.31  0.000
## 6  8.788  1.147  0.09113  4 -517.182 1042.7 33.69  0.000
## Models ranked by AICc(x)
mwt_block_dredge ## parameters are very similar with block

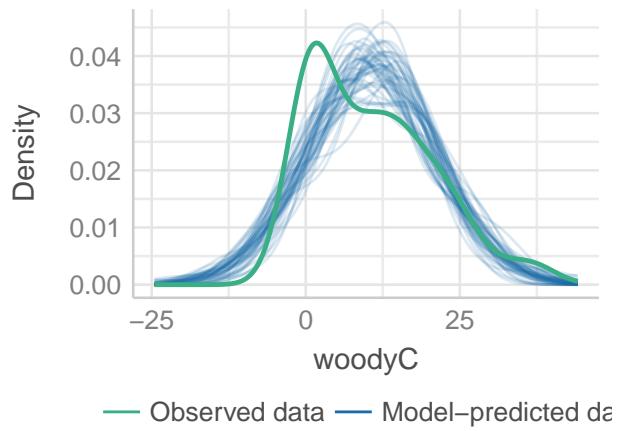
## Global model call: lmer(formula = woodyC ~ species_richness + FDis + PSV + (1 |
##   block), data = carbon_seq, REML = F)
## ---
## Model selection table
##   (Int)    FDs    PSV  spc_rch df  logLik  AICc delta weight
## 8  7.932 -5.541 20.810  0.63690 6 -498.787 1010.2  0.00  0.719
## 4  8.492 -4.229 19.070           5 -500.822 1012.1  1.89  0.280
## 3  6.572           9.434       4 -507.564 1023.4 13.22  0.001
## 7  6.656           9.524 -0.04602 5 -507.551 1025.5 15.35  0.000
## 2  8.860  1.265           4 -516.984 1042.3 32.06  0.000
## 1 10.690           0.33140  3 -518.428 1043.0 32.83  0.000
## 5  9.809           0.33140  4 -517.814 1043.9 33.72  0.000
## 6  8.779  1.139  0.09905  5 -516.942 1044.3 34.13  0.000
## Models ranked by AICc(x)
## Random terms (all models):
##   1 | block

mwt_model<-get.models(mwt_dredge, subset = 2)[[1]]
check_model(mwt_model)

```

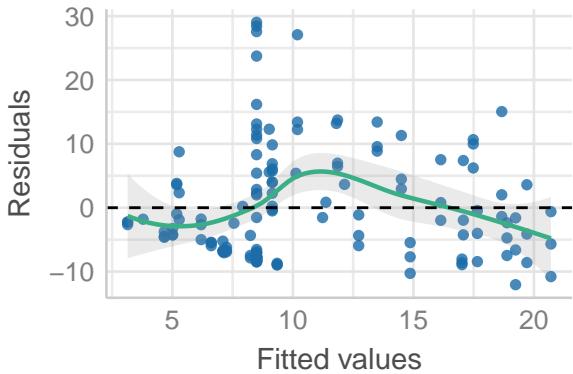
Posterior Predictive Check

Model-predicted lines should resemble observed data



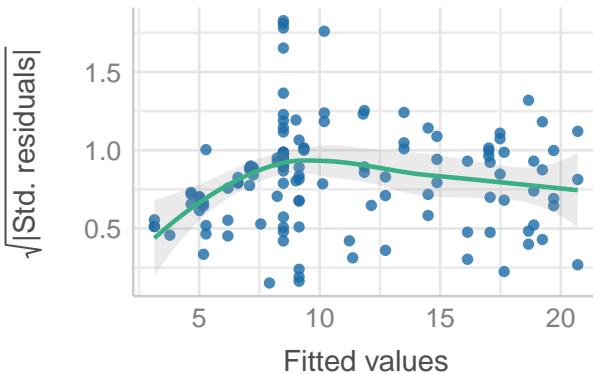
Linearity

Reference line should be flat and horizontal



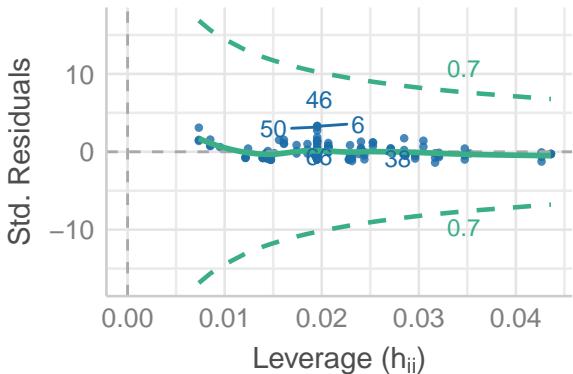
Homogeneity of Variance

Reference line should be flat and horizontal



Influential Observations

Points should be inside the contour lines



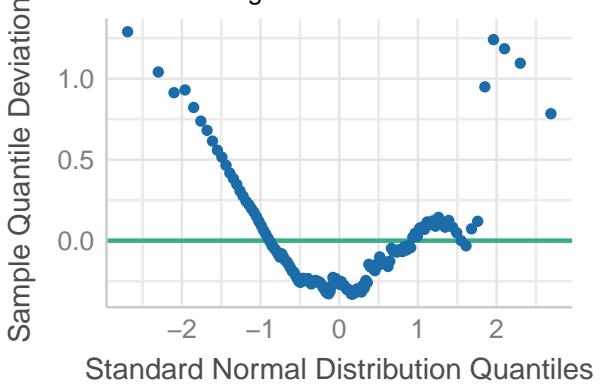
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



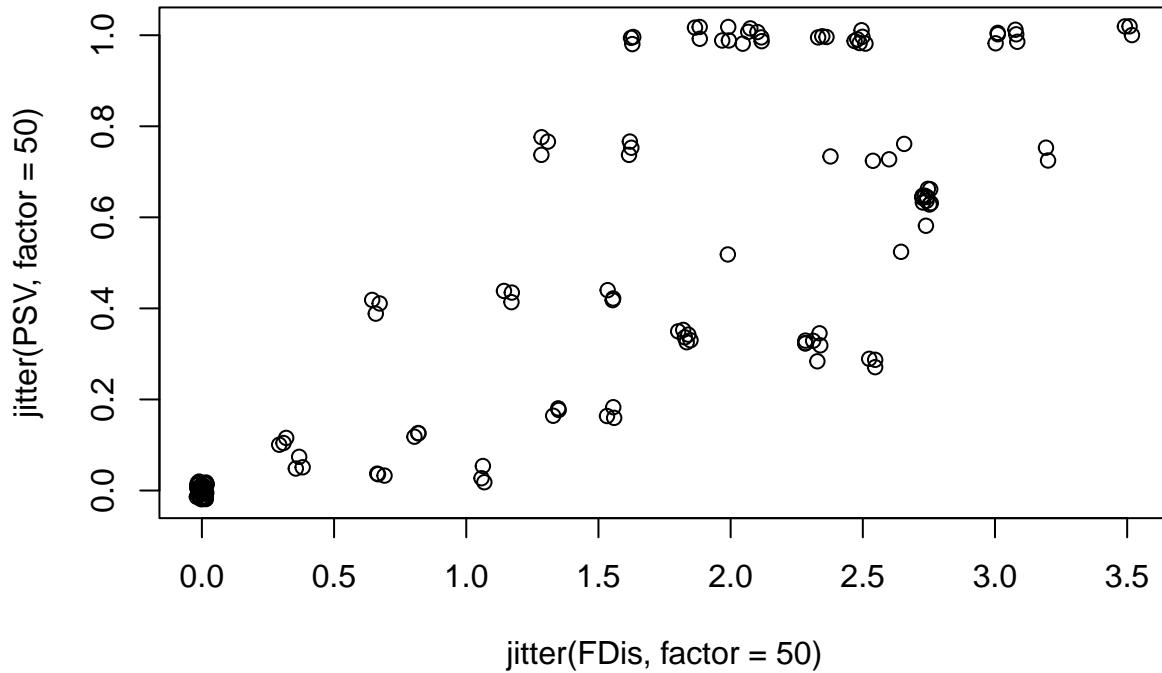
Normality of Residuals

Dots should fall along the line



● Low (< 5)

A few things are apparent here. First of all, the two retained predictors (FDis and PSV) have opposite-sign coefficients. These predictors have a positive correlation with each other across the full dataset, as shown below:



Therefore, their “effects” partly offset each other. However, their variance inflation factor (VIF) is not so high that it would ordinarily raise concerns about multicollinearity.

Perhaps more troubling are the other checks: the posterior predictive check plot, the homoskedasticity plot, and the Q-Q (normality of residuals) plot. It is common for data from BEF experiments not to strictly meet these assumptions of ordinary least-squares regression because the variance is often greatest among monocultures. To assure ourselves that our results are robust, we can use robust regression through iterated re-weighted least squares to reduce the influence of outliers.

```
mwt_robust<-rlm(woodyC~species_richness+FDis+PSV,data=carbon_seq)

options(na.action = "na.fail")
mwt_robust_dredge <- dredge(mwt_robust,beta = "none",evaluate = T,rank = AICc)
options(na.action = "na.omit")

mwt_robust_dredge

## Global model call: rlm(formula = woodyC ~ species_richness + FDis + PSV, data = carbon_seq)
## ---
## Model selection table
##   (Int)    FDs    PSV spc_rch df  logLik  AICc delta weight
## 8  5.734 -4.794 20.85  0.6500  5 -501.023 1012.5  0.00  0.571
## 4  6.656 -3.558 19.15          4 -502.384 1013.1  0.57  0.429
## 3  3.924          11.82          3 -510.742 1027.7 15.17  0.000
## 7  3.564          11.57  0.1425  4 -511.200 1030.7 18.20  0.000
## 1 10.380          2 -518.709 1041.5 29.01  0.000
## 2  7.361  1.794  3 -517.864 1041.9 29.41  0.000
```

```

## 5 9.306          0.3876 3 -518.165 1042.5 30.01 0.000
## 6 7.285 1.643    0.1125 4 -517.818 1043.9 31.44 0.000
## Models ranked by AICc(x)

```

Here, the ‘best’ model selected involves the same variables with similar (but not exactly the same) parameter values to when we were using OLS.

Net biodiversity effect in aboveground woody carbon

Let’s repeat the same procedure for the NBE in aboveground woody carbon.

```

## for the dredge function to work, we need to remove plots with NAs
carbon_seq_woody0Y<-carbon_seq[which(!is.na(carbon_seq$woody0Y)),]

## we'll leave out models with block random intercept
## because they all result in a singular fit
mw<-lm(woody0Y~species_richness+FDis+PSV,
       data=carbon_seq_woody0Y)

## as before, the posterior predictive check and Q-Q plot
## for the OLS model reveal violations of assumptions
mw_robust<-rlm(woody0Y~species_richness+FDis+PSV,data=carbon_seq_woody0Y)

options(na.action = "na.fail")
mw_dredge <- dredge(mw, beta = "none", evaluate = T, rank = AICc)
mw_robust_dredge <- dredge(mw_robust, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

mw_dredge ## we select the first model

## Global model call: lm(formula = woody0Y ~ species_richness + FDis + PSV, data = carbon_seq_woody0Y)
## ---
## Model selection table
##   (Int)   FDs   PSV spc_rch df   logLik   AICc delta weight
## 6 -2.1370 1.2200      0.2501 4 -269.594 547.6  0.00  0.450
## 8 -2.2040 0.9463  0.897  0.2729 5 -269.329 549.3  1.68  0.194
## 7 -1.4580      2.262  0.3643 4 -270.706 549.8  2.22  0.148
## 2 -2.0320 1.5830      3 -271.887 550.0  2.42  0.134
## 4 -2.0420 1.5400  0.159  4 -271.879 552.2  4.57  0.046
## 5 -0.1805      0.3808  3 -273.559 553.4  5.77  0.025
## 3 -0.3916      2.463   3 -276.153 558.5 10.95  0.002
## 1  1.0570      2 -279.205 562.5 14.94  0.000
## Models ranked by AICc(x)
mw_robust_dredge ## we select the first model

## Global model call: rlm(formula = woody0Y ~ species_richness + FDis + PSV, data = carbon_seq_woody0Y)
## ---
## Model selection table
##   (Int)   FDs   PSV spc_rch df   logLik   AICc delta weight
## 6 -1.9010 1.0570      0.2476 4 -269.724 547.9  0.00  0.454
## 8 -1.9020 0.8079  0.7843  0.2619 5 -269.472 549.6  1.70  0.194
## 7 -1.2420      2.0290  0.3304 4 -270.797 550.0  2.15  0.155
## 2 -1.7270 1.3700      3 -272.086 550.4  2.56  0.126
## 4 -1.7240 1.3200  0.1664  4 -272.082 552.6  4.71  0.043
## 5 -0.2588      0.3588  3 -273.683 553.6  5.75  0.026
## 3 -0.3801      2.3410  3 -276.176 558.6 10.74  0.002

```

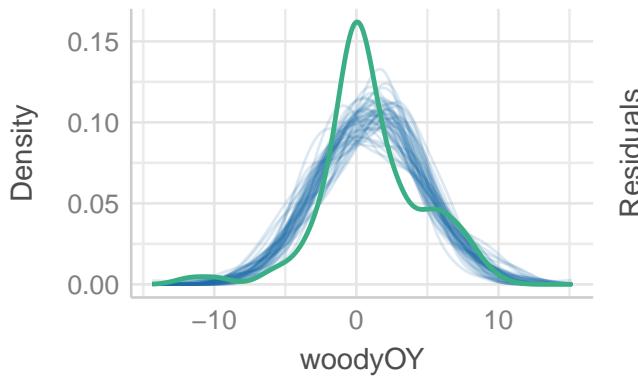
```

## 1 0.9372
## Models ranked by AICc(x)
mw_model<-get.models(mw_dredge, subset = 1)[[1]]
check_model(mw_model)

```

Posterior Predictive Check

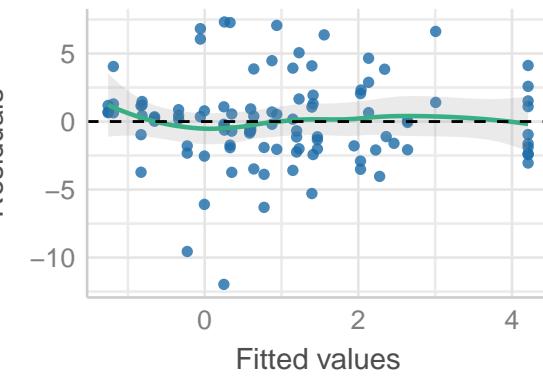
Model-predicted lines should resemble observed data



— Observed data — Model-predicted data

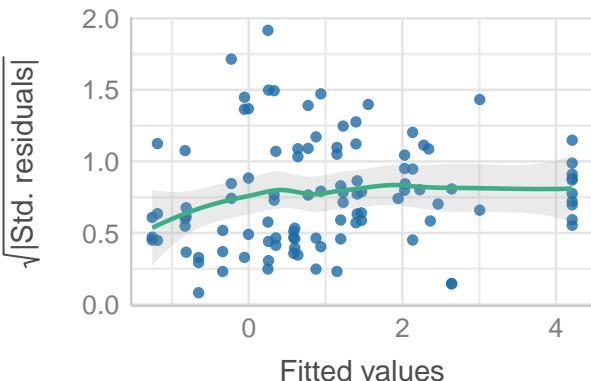
Linearity

Reference line should be flat and horizontal



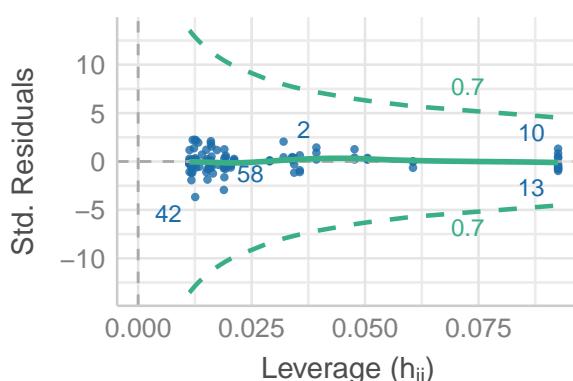
Homogeneity of Variance

Reference line should be flat and horizontal



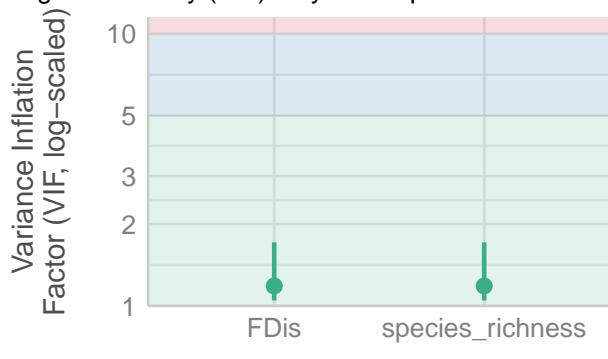
Influential Observations

Points should be inside the contour lines



Collinearity

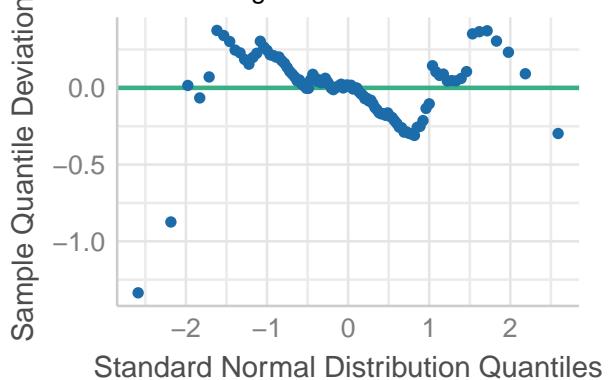
High collinearity (VIF) may inflate parameter uncertainty



● Low (< 5)

Normality of Residuals

Dots should fall along the line

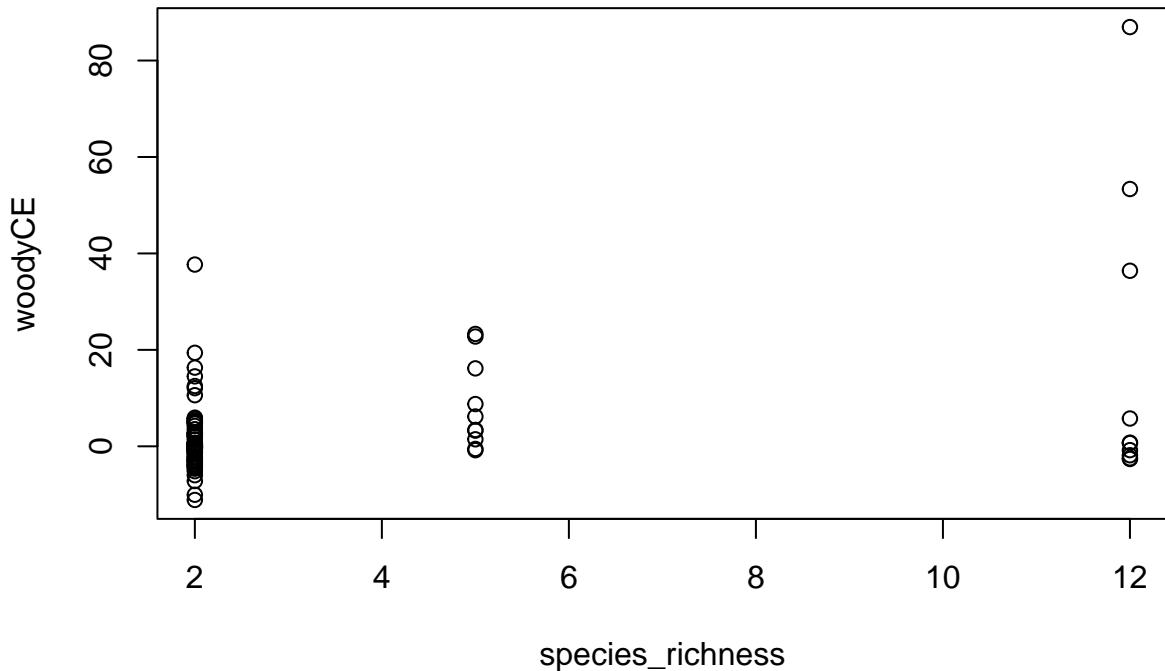


Robust regression slightly shrinks parameter estimates here, but yields a qualitatively very similar model.

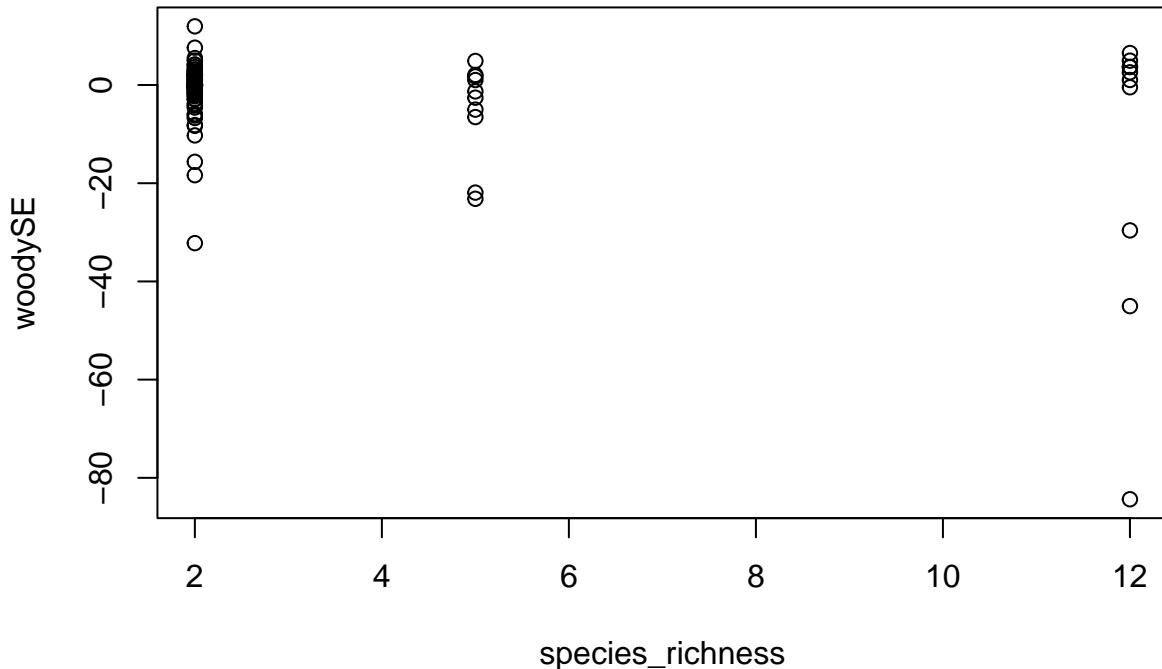
The NBE is often partitioned via Loreau & Hector (2001)'s method into complementarity and selection effects. Complementarity effects (CE) are based on the mean of species' *relative* yields and are often taken to indicate synergistic interactions like niche partitioning or facilitation. Selection (SE) effects describe how more productive species tend to show greater (or lesser) relative boosts in productivity than average in mixtures.

Although CE and SE must sum to NBE, it is possible for one to be greater than the NBE (or even total plot biomass) if the other is negative. (It is also possible for total overyielding to be negative, in which case it may be called underyielding.) Although CE and SE are expressed in the same units as NBE (here, kg biomass per ha), it can be hard to make sense of extreme CE or SE values in these terms. For these reasons, we choose to focus on whether they are generally positive or negative than on their actual magnitudes. Starting with the Loreau & Hector (2001) paper itself, it is common for BEF researchers to expect positive CE and non-positive SE on average across plots. This would imply that (1) species tend to overperform in mixture and (2) it is not only the most productive species that overperform in mixture. It may help to look at the data quickly before proceeding.

```
plot(woodyCE~species_richness,data=carbon_seq_woody0Y)
```



```
plot(woodySE~species_richness,data=carbon_seq_woody0Y)
```



These plots reveal that there are a couple outliers with highly positive CE and negative SE. The most extreme are plots 14 and 50, both 12-species plots. Because of the odd distribution of CE and SE values, we'll conduct both a parametric t-test and a non-parametric Wilcoxon rank sum test to check whether CE or SE differ from 0. Most research does not concern itself with whether inferences about CE or SE are driven by extreme values, so the t-test is probably more consistent with how results are usually reported. However, the Wilcoxon test may be more appropriate given what we see in the plots above.

```
t.test(carbon_seq_woodY$woodyCE)
```

```
##  
## One Sample t-test  
##  
## data: carbon_seq_woodY$woodyCE  
## t = 2.8862, df = 103, p-value = 0.00475  
## alternative hypothesis: true mean is not equal to 0  
## 95 percent confidence interval:  
## 1.087102 5.862464  
## sample estimates:  
## mean of x  
## 3.474783
```

```
t.test(carbon_seq_woodY$woodySE)
```

```
##  
## One Sample t-test  
##  
## data: carbon_seq_woodY$woodySE  
## t = -2.1868, df = 103, p-value = 0.03102
```

```

## alternative hypothesis: true mean is not equal to 0
## 95 percent confidence interval:
## -4.6108083 -0.2250558
## sample estimates:
## mean of x
## -2.417932
wilcox.test(carbon_seq_woody0Y$woodyCE)

##
## Wilcoxon signed rank test with continuity correction
##
## data: carbon_seq_woody0Y$woodyCE
## V = 3315, p-value = 0.05804
## alternative hypothesis: true location is not equal to 0
wilcox.test(carbon_seq_woody0Y$woodySE)

##
## Wilcoxon signed rank test with continuity correction
##
## data: carbon_seq_woody0Y$woodySE
## V = 2630, p-value = 0.747
## alternative hypothesis: true location is not equal to 0

```

There is some evidence that CE differs from 0, but not much evidence that SE does.

Soil C accumulation and net biodiversity effects

We can now do the same for soil C accumulation:

```

carbon_seq_soilC<-carbon_seq[which(!is.na(carbon_seq$soilC)),]
mst<-lm(soilC~species_richness+FDis+PSV,data=carbon_seq_soilC)

options(na.action = "na.fail")
mst_dredge <- dredge(mst, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

mst_dredge ## we pick the first one

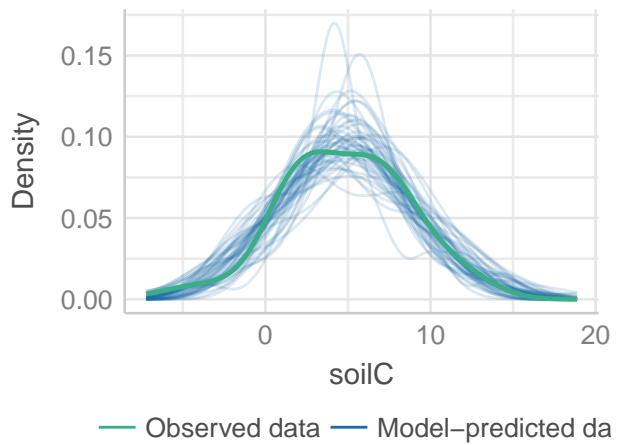
## Global model call: lm(formula = soilC ~ species_richness + FDis + PSV, data = carbon_seq_soilC)
## ---
## Model selection table
##   (Int)     FDs      PSV spc_rch df    logLik  AICc delta weight
## 5 3.881          0.2712  3 -178.975 364.3  0.00  0.356
## 3 4.099          2.5760  3 -180.022 366.4  2.09  0.125
## 7 3.839          0.5298  0.2406  4 -178.939 366.5  2.20  0.119
## 2 4.109  0.6608          3 -180.088 366.6  2.23  0.117
## 6 3.901 -0.1126          0.3000  4 -178.959 366.6  2.24  0.116
## 1 4.809          2 -181.616 367.4  3.09  0.076
## 8 3.823 -0.8537  2.7680  0.3294  5 -178.663 368.3  3.99  0.048
## 4 4.074  0.2645  1.6490          4 -179.983 368.6  4.28  0.042
## Models ranked by AICc(x)

mst_model<-get.models(mst_dredge, subset = 1)[[1]]
check_model(mst_model)

```

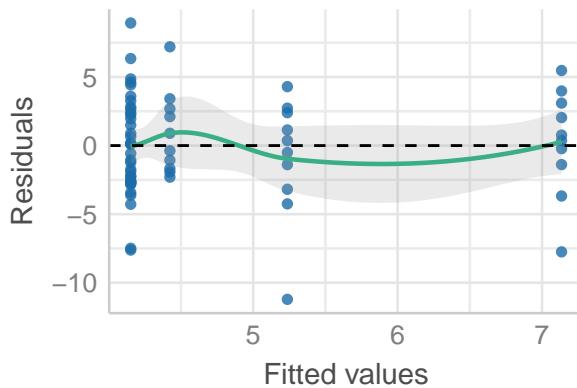
Posterior Predictive Check

Model-predicted lines should resemble observed data



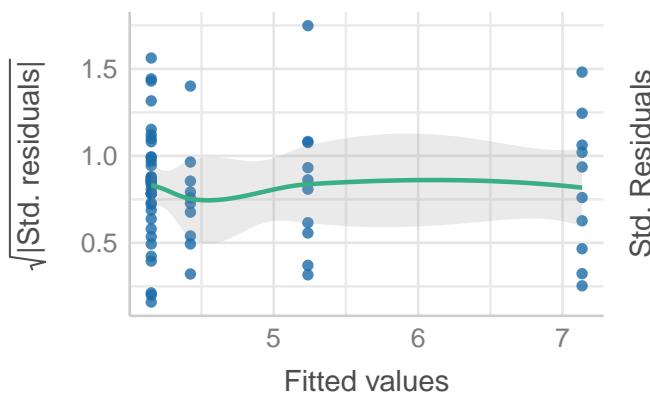
Linearity

Reference line should be flat and horizontal



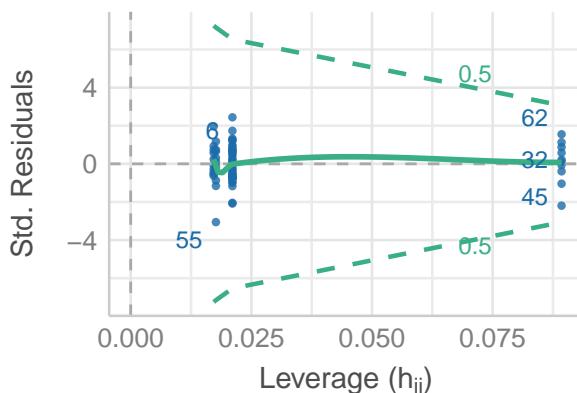
Homogeneity of Variance

Reference line should be flat and horizontal



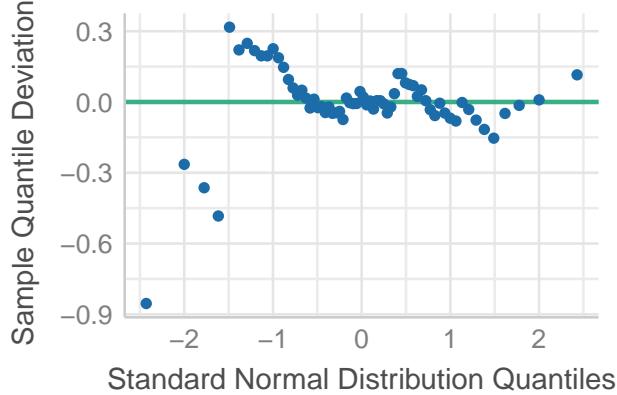
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



And for NBE in soil C accumulation:

```
carbon_seq_soil0Y<-carbon_seq[which(!is.na(carbon_seq$soil0Y)),]
ms<-lm(soil0Y~species_richness+FDis+PSV,data=carbon_seq_soil0Y)
```

```

options(na.action = "na.fail")
ms_dredge <- dredge(ms, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

ms_dredge ## we pick the second one

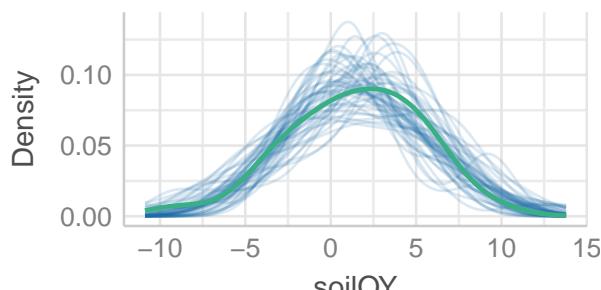
## Global model call: lm(formula = soilOY ~ species_richness + FDis + PSV, data = carbon_seq_soilOY)
## ---
## Model selection table
## (Int)      FDs      PSV spc_rch df  logLik  AICc delta weight
## 5 -0.57840          0.3115  3 -81.153 169.2  0.00  0.325
## 1  1.39500          2 -82.987 170.4  1.19  0.179
## 7  2.12400         -4.879  0.3522  4 -80.519 170.6  1.41  0.161
## 2 -2.08600   1.4920          3 -82.271 171.5  2.24  0.106
## 6  0.05418  -0.3653          0.3462  4 -81.129 171.9  2.63  0.087
## 3  3.05200          -2.732          3 -82.805 172.5  3.30  0.062
## 4 -0.20830   1.6920  -3.863          4 -81.899 173.4  4.17  0.040
## 8  2.62100  -0.2960  -4.851          5 -80.503 173.5  4.28  0.038
## Models ranked by AICc(x)

ms_model<-get.models(ms_dredge, subset = 2)[[1]]
check_model(ms_model)

```

Posterior Predictive Check

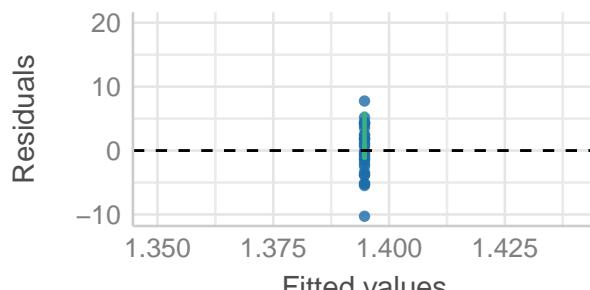
Model-predicted lines should resemble observed c



— Observed data — Model-predicted data

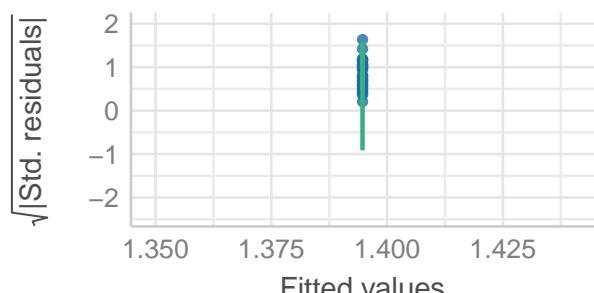
Linearity

Reference line should be flat and horizontal



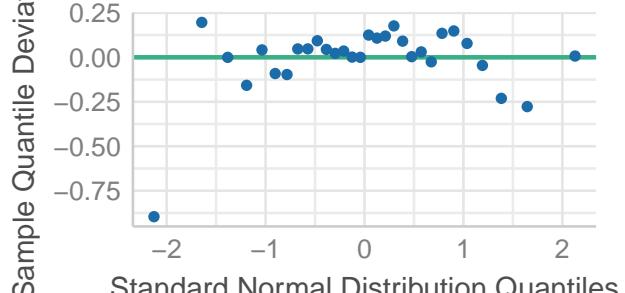
Homogeneity of Variance

Reference line should be flat and horizontal



Normality of Residuals

Dots should fall along the line



Both of these models seem to meet the assumptions quite well.

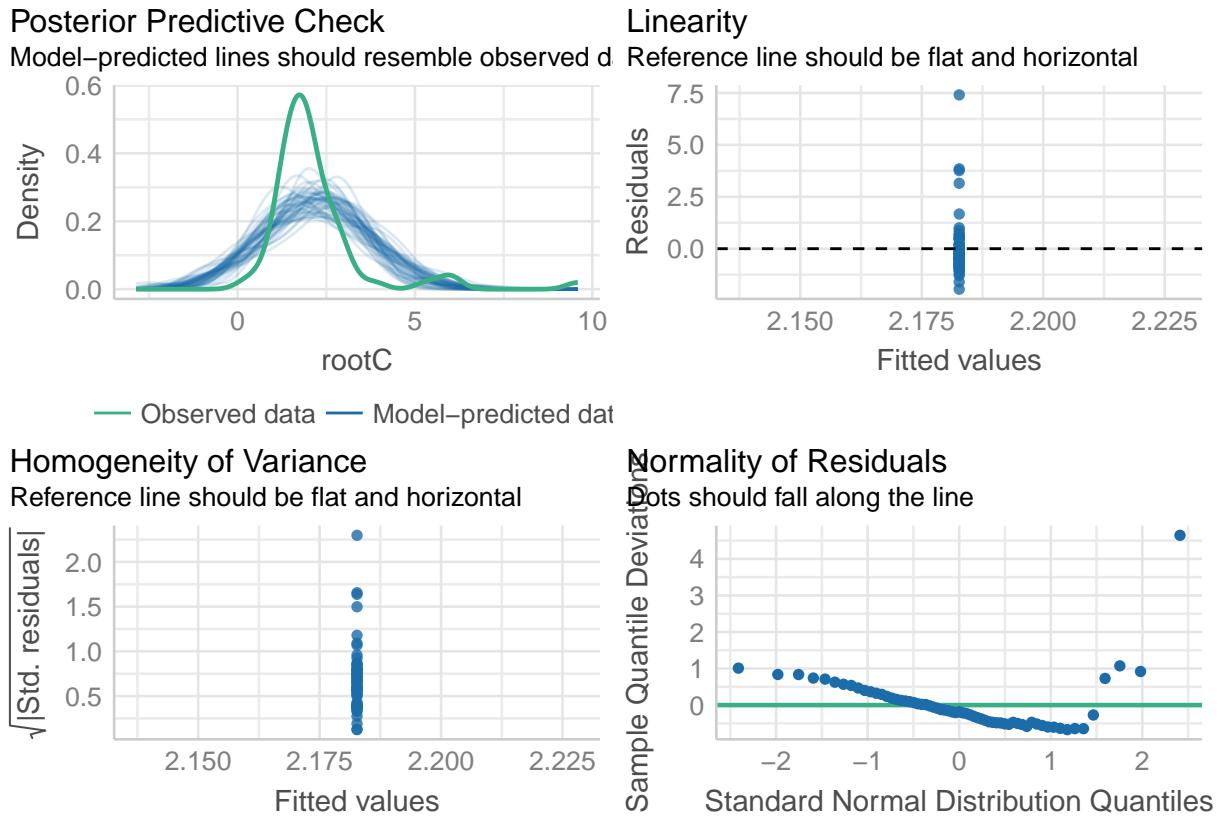
Fine roots and net biodiversity effects

```
## total root C
carbon_seq_rootC<-carbon_seq[which(!is.na(carbon_seq$rootC)),]
mrt<-lm(rootC~species_richness+FDis+PSV,data=carbon_seq_rootC)

options(na.action = "na.fail")
mrt_dredge <- dredge(mrt, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

mrt_dredge ## we select the first model

## Global model call: lm(formula = rootC ~ species_richness + FDis + PSV, data = carbon_seq_rootC)
## ---
## Model selection table
##   (Int)    FDs      PSV    spc_rch df logLik AICc delta weight
## 1 2.183                2 -110.745 225.7  0.00  0.237
## 8 2.191 1.1200 -2.75600 -0.128900 5 -107.450 226.0  0.26  0.208
## 4 2.098 0.7036 -2.44400                 4 -108.930 226.5  0.86  0.155
## 2 2.056 0.1228                 3 -110.389 227.2  1.50  0.112
## 6 2.131 0.4149      -0.110000 4 -109.351 227.4  1.70  0.101
## 5 2.204            -0.006146 3 -110.736 227.9  2.19  0.080
## 3 2.165            0.06906   3 -110.738 227.9  2.19  0.079
## 7 2.186            0.26150 -0.021100 4 -110.685 230.1  4.37  0.027
## Models ranked by AICc(x)
mrt_model<-get.models(mrt_dredge, subset = 1)[[1]]
check_model(mrt_model)
```



The selected model (with no predictors) does a poor job of meeting assumptions, but even changing out `rootC` for `log(rootC)`, which meets the assumptions better, yields a 'best' model with no predictor variables. The same is true of robust regression. We move on to the NBE.

```
## overyielding in root C
carbon_seq_root0Y<-carbon_seq[which(!is.na(carbon_seq$root0Y)),]
mr<-lm(root0Y~species_richness+FDis+PSV,data=carbon_seq_root0Y)
mr_robust<-rlm(root0Y~species_richness+FDis+PSV,data=carbon_seq_root0Y)

options(na.action = "na.fail")
mr_dredge <- dredge(mr, beta = "none", evaluate = T, rank = AICc)
mr_robust_dredge <- dredge(mr_robust, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

mr_dredge ## we select the first model

## Global model call: lm(formula = root0Y ~ species_richness + FDis + PSV, data = carbon_seq_root0Y)
## ---
## Model selection table
##   (Int)      FDs     PSV    spc_rch df logLik AICc delta weight
## 3 3.5240        -5.098       3 -54.654 116.3  0.00  0.506
## 7 3.5700        -4.823 -0.03245  4 -54.568 118.9  2.57  0.140
## 4 3.8510 -0.18630 -4.920       4 -54.605 118.9  2.64  0.135
## 1 0.5177          2 -57.648 119.8  3.47  0.089
## 5 1.1020        -0.09082  3 -57.036 121.1  4.76  0.047
## 2 1.8690 -0.58290       3 -57.223 121.4  5.14  0.039
## 8 3.6110 -0.02526 -4.819 -0.03009  5 -54.568 121.9  5.56  0.031
```

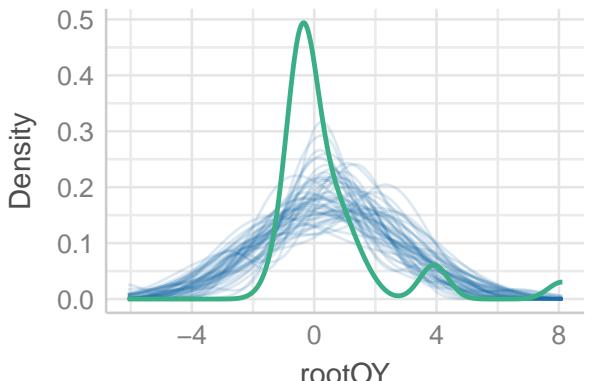
```

## 6 1.3670 -0.15540      -0.07601 4 -57.021 123.8 7.47 0.012
## Models ranked by AICc(x)
mr_model<-get.models(mr_dredge, subset = 1)[[1]]
check_model(mr_model)

```

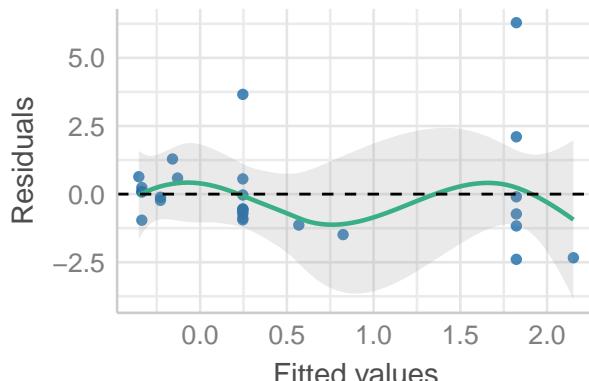
Posterior Predictive Check

Model-predicted lines should resemble observed data



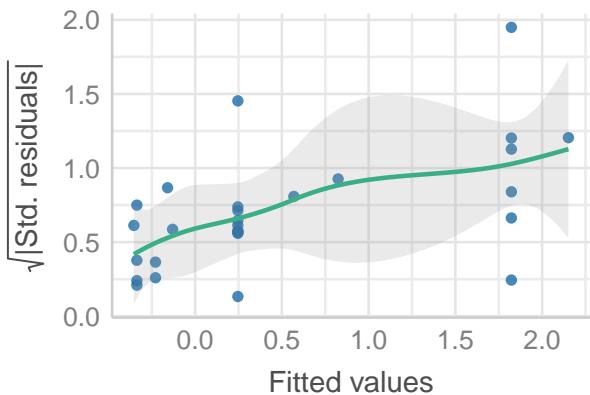
Linearity

Reference line should be flat and horizontal



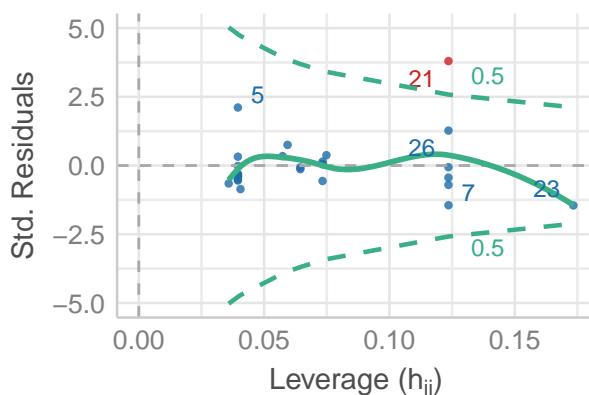
Homogeneity of Variance

Reference line should be flat and horizontal



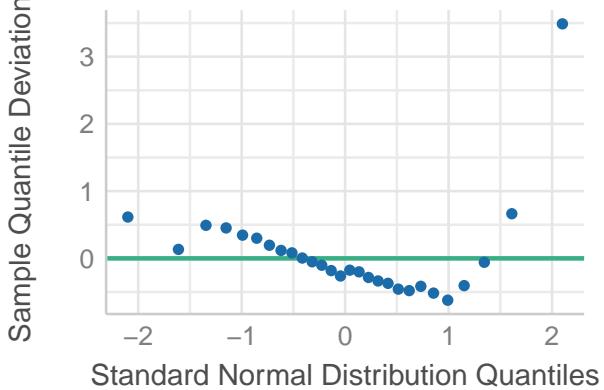
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



Evidently, this model also does a poor job of meeting assumptions. Thus, we show the robust regression results to buttress the claim that the same model is selected even when high-leverage points are not allowed to be as influential.

```
mr_robust<-rlm(root0Y~species_richness+FDis+PSV,data=carbon_seq_root0Y)

options(na.action = "na.fail")
mr_robust_dredge <- dredge(mr_robust, beta = "none", evaluate = T, rank = AICc)
options(na.action = "na.omit")

mr_robust_dredge

## Global model call: rlm(formula = root0Y ~ species_richness + FDis + PSV, data = carbon_seq_root0Y)
## ---
## Model selection table
##   (Int)      FDs      PSV  spc_rch df  logLik  AICc delta weight
## 3 1.6910      -2.632          3 -56.054 119.1  0.00  0.445
## 1 0.0654          2 -58.423 121.3  2.22  0.147
## 7 1.7780      -2.492 -0.02781 4 -55.955 121.6  2.54  0.125
## 4 1.8470 -0.079010 -2.585          4 -56.005 121.8  2.64  0.119
## 5 0.3619          -0.04437 3 -57.960 122.9  3.81  0.066
## 2 0.5933 -0.226700          3 -58.165 123.3  4.22  0.054
## 8 1.6320  0.099930 -2.543 -0.03692 5 -55.959 124.6  5.54  0.028
## 6 0.3433  0.009522          -0.04512 4 -57.967 125.7  6.57  0.017
## Models ranked by AICc(x)
```

PSV is still the only variable selected, but its parameter estimate shrinks quite a bit.

Tree composition and soil C accumulation

In this section we first test the influence of leaf type and genus on soil C accumulation:

```
anova(lm(soilC~leaf_type,data=carbon_seq))

## Analysis of Variance Table
##
## Response: soilC
##             Df Sum Sq Mean Sq F value Pr(>F)
## leaf_type    2  18.64  9.3201  0.6312 0.5353
## Residuals  63 930.25 14.7658
## one could run the following to incorporate the block effect
# anova(lmer(soilC~leaf_type+(1/block),data=carbon_seq))
# but it's a singular fit here

## only comparing Quercus and Pinus plots
anova(lm(soilC~major_genus,data=subset(carbon_seq,major_genus!="N")))
```

```
## Analysis of Variance Table
##
## Response: soilC
##             Df Sum Sq Mean Sq F value Pr(>F)
## major_genus   1  3.811  3.8109  0.3149 0.5813
## Residuals  19 229.970 12.1037
```

And the same for root C:

```

anova(lm(rootC~leaf_type,data=carbon_seq))

## Analysis of Variance Table
##
## Response: rootC
##             Df  Sum Sq Mean Sq F value Pr(>F)
## leaf_type     2    7.534  3.7672  1.9393 0.1527
## Residuals   60 116.552  1.9425
anova(lm(rootC~major_genus,data=subset(carbon_seq,major_genus!="N")))

```

```

## Analysis of Variance Table
##
## Response: rootC
##             Df  Sum Sq Mean Sq F value Pr(>F)
## major_genus   1  0.435  0.43497  0.7674  0.392
## Residuals   19 10.769  0.56680

```

These outputs reveal no differences in soil or root C based on leaf type or genus (*Quercus* vs. *Pinus*). However, we do report that both influence macroaggregates:

```

macro_leaf<-lmer(macro250~leaf_type+(1|block),data=carbon_seq)
anova(macro_leaf)

```

```

## Type III Analysis of Variance Table with Satterthwaite's method
##             Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## leaf_type 295.38 147.69      2     60  0.013  5.7856 0.005039 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
pairs(emmeans(macro_leaf,specs=~leaf_type))

```

```

## contrast estimate   SE df t.ratio p.value
## B - C      3.61 1.71 60   2.111  0.0961
## B - D     -1.60 1.51 60  -1.062  0.5412
## C - D     -5.22 1.54 60  -3.395  0.0035
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
## strong evidence that conifer and deciduous plots differ

```

```

macro_genus<-lmer(macro250~major_genus+(1|block),data=subset(carbon_seq,major_genus!="N"))
anova(macro_genus)

```

```

## Type III Analysis of Variance Table with Satterthwaite's method
##             Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## major_genus 288.32 288.32      1     17  10.938 0.004165 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

We can check the diagnostic plots of both models as well:

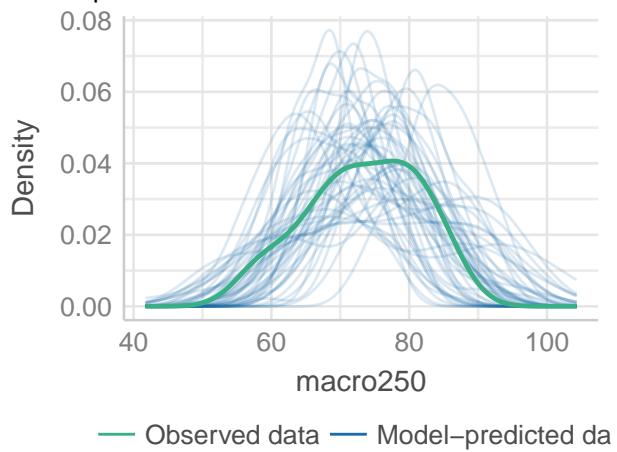
```

check_model(macro_leaf)

```

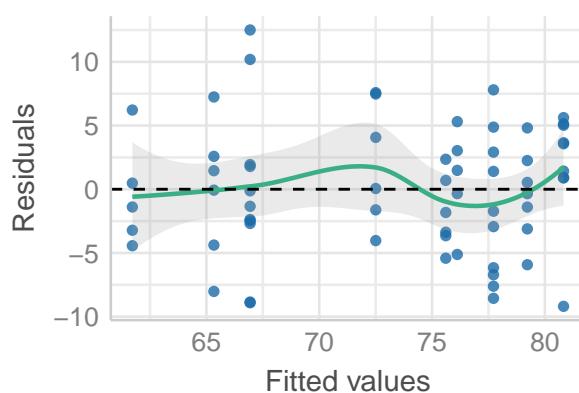
Posterior Predictive Check

Model-predicted lines should resemble observed data



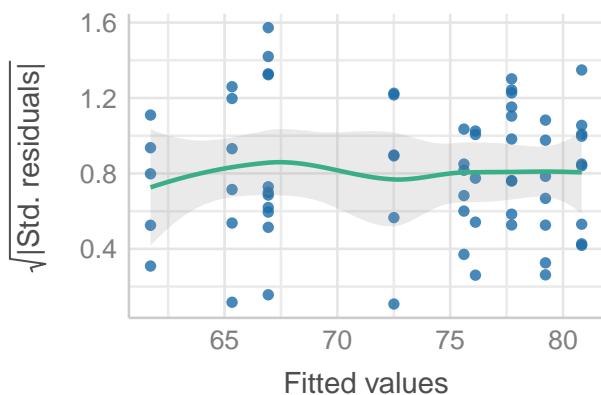
Linearity

Reference line should be flat and horizontal



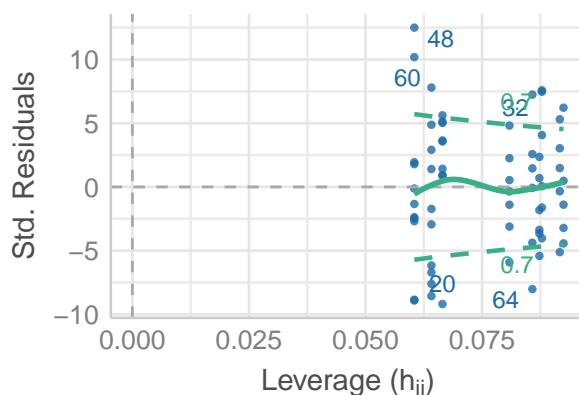
Homogeneity of Variance

Reference line should be flat and horizontal



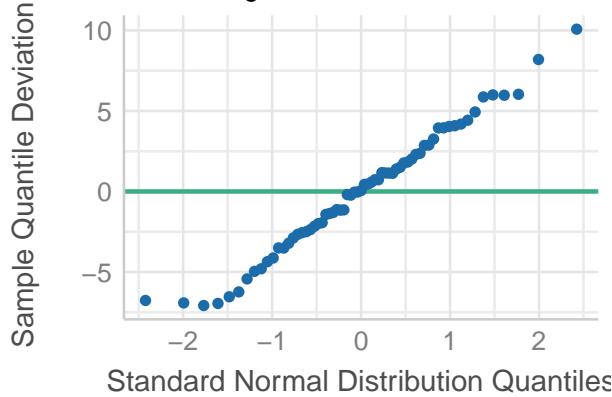
Influential Observations

Points should be inside the contour lines



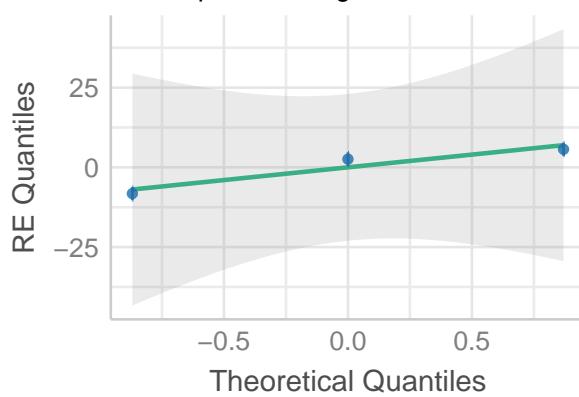
Normality of Residuals

Dots should fall along the line

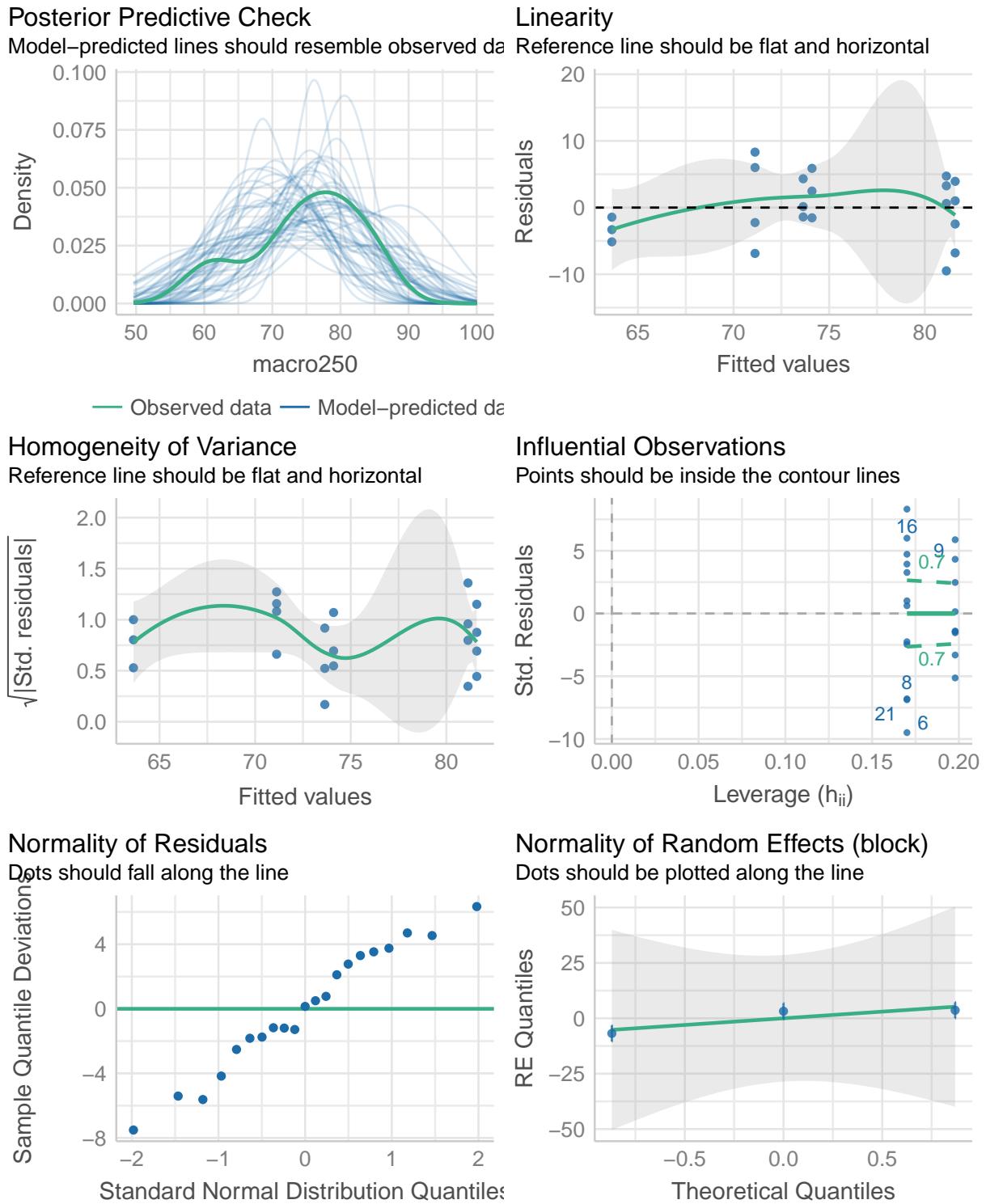


Normality of Random Effects (block)

Dots should be plotted along the line



```
check_model(macro_genus)
```



And the same for soil moisture:

```
moisture_leaf <- lmer(soil_moisture ~ leaf_type + (1 | block), data = carbon_seq)
anova(moisture_leaf)
```

```
## Type III Analysis of Variance Table with Satterthwaite's method
```

```

##           Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## leaf_type 26.119   13.06      2  62.014  11.751 4.707e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
pairs(emmeans(moisture_leaf,specs=~leaf_type))

##   contrast estimate   SE df t.ratio p.value
##   B - C      1.094 0.352 62   3.107  0.0079
##   B - D     -0.446 0.307 62  -1.452  0.3211
##   C - D     -1.540 0.319 62  -4.834 <.0001
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
## strong evidence that conifer and deciduous plots differ
## as well as 'both' vs conifer

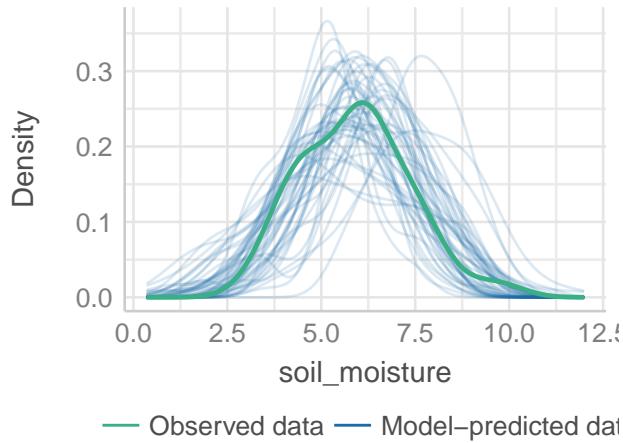
moisture_genus<-lmer(soil_moisture~major_genus+(1|block),data=subset(carbon_seq,major_genus!="N"))
anova(moisture_genus)

## Type III Analysis of Variance Table with Satterthwaite's method
##           Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
## major_genus 26.29   26.29      1     17  23.089 0.0001651 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
check_model(moisture_leaf)

```

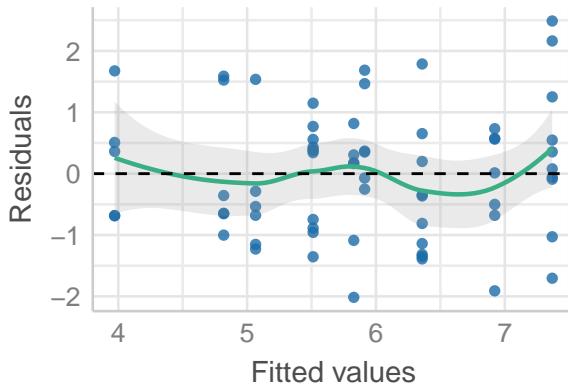
Posterior Predictive Check

Model-predicted lines should resemble observed data



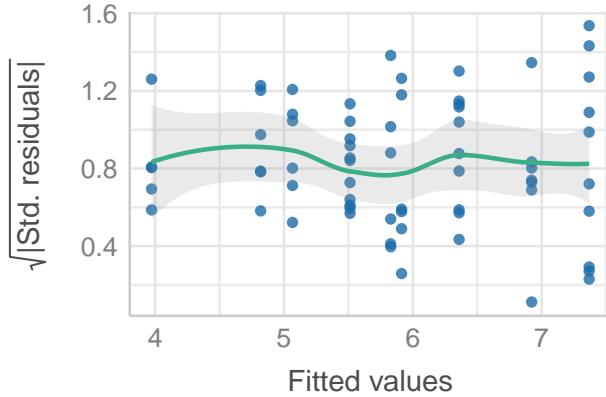
Linearity

Reference line should be flat and horizontal



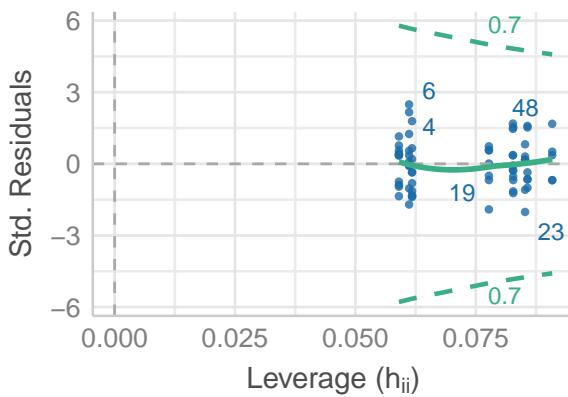
Homogeneity of Variance

Reference line should be flat and horizontal



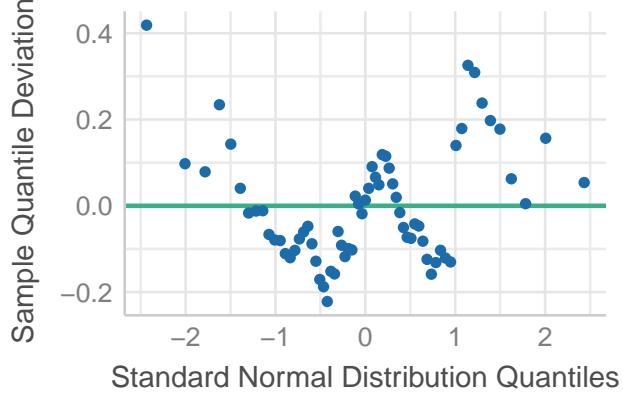
Influential Observations

Points should be inside the contour lines



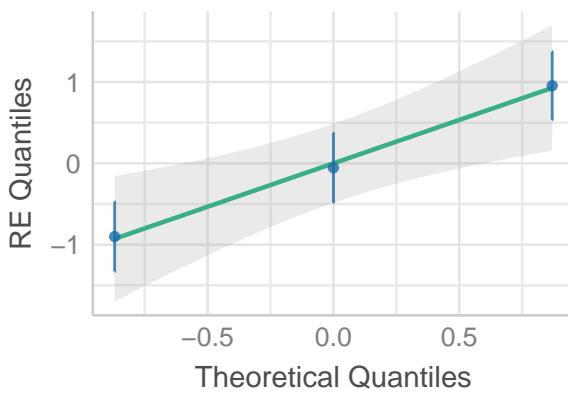
Normality of Residuals

Dots should fall along the line

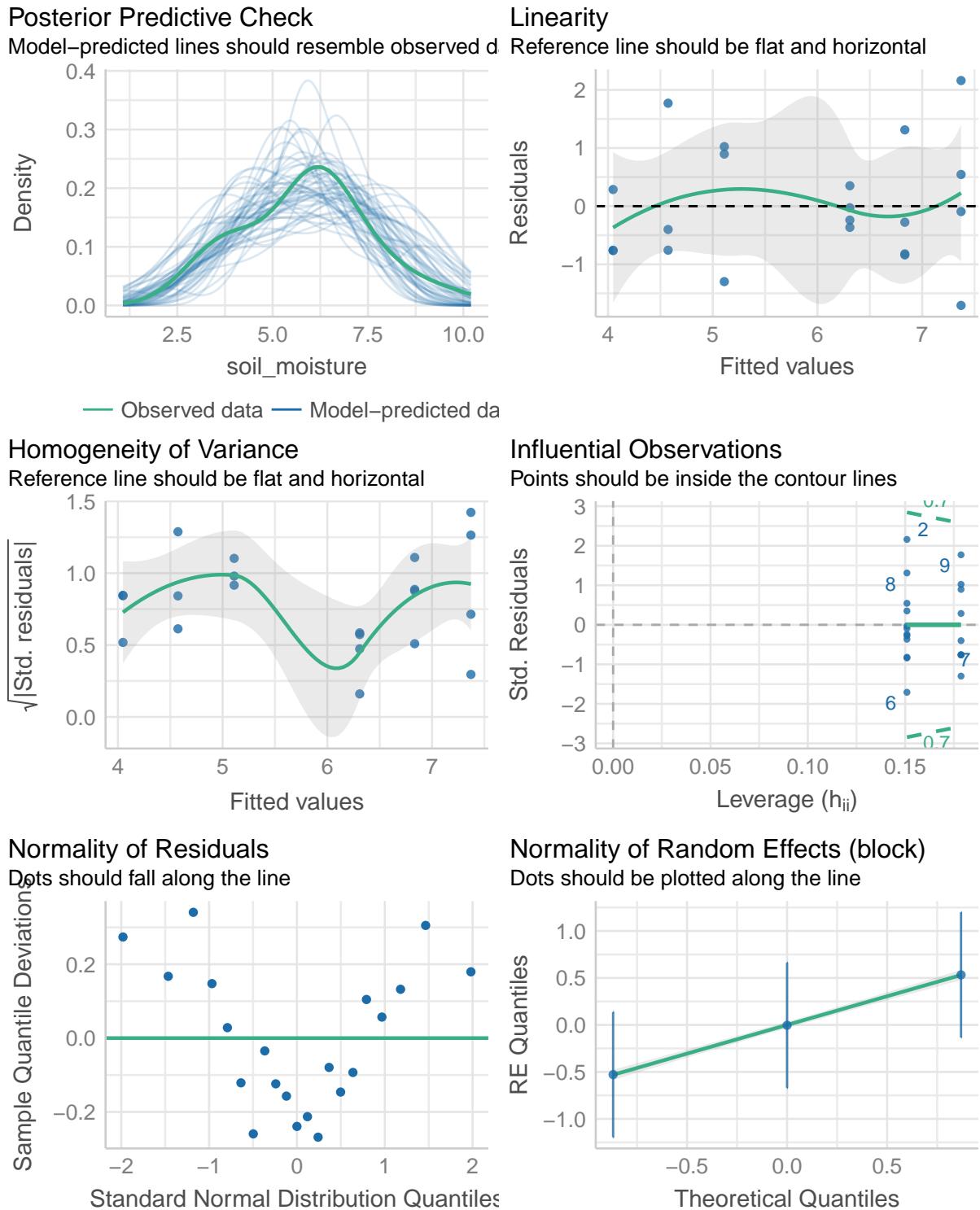


Normality of Random Effects (block)

Dots should be plotted along the line



```
check_model(moisture_genus)
```



Lastly, we explore the effects of mycotype on soil properties. Here, we *do* see an effect on soil C accumulation:

```
## block effect once again singular
soilC_mycoco<-lm(soilC~mycotype, data=carbon_seq)
anova(soilC_mycoco)
```

```

## Analysis of Variance Table
##
## Response: soilC
##           Df Sum Sq Mean Sq F value    Pr(>F)
## mycotype     2   88.28  44.141   3.2313 0.04614 *
## Residuals  63  860.60   13.660
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
pairs(emmeans(soilC_myco,specs=-mycotype))

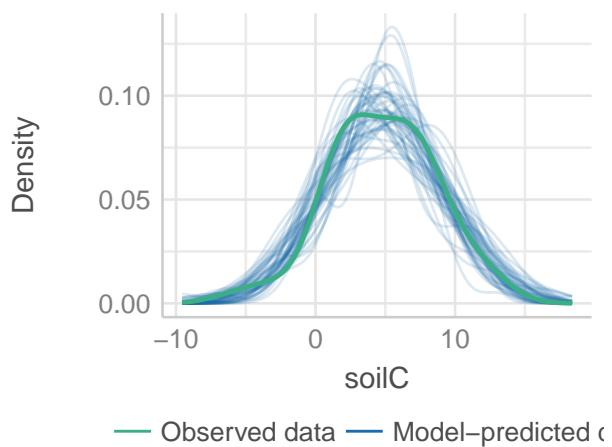
##   contrast estimate    SE df t.ratio p.value
##   A - B      0.619 1.410 63   0.439  0.8994
##   A - E      2.777 1.416 63   1.961  0.1304
##   B - E      2.158 0.979 63   2.204  0.0782
##
## P value adjustment: tukey method for comparing a family of 3 estimates
## no pairwise contrasts are significant

check_model(soilC_myco)

```

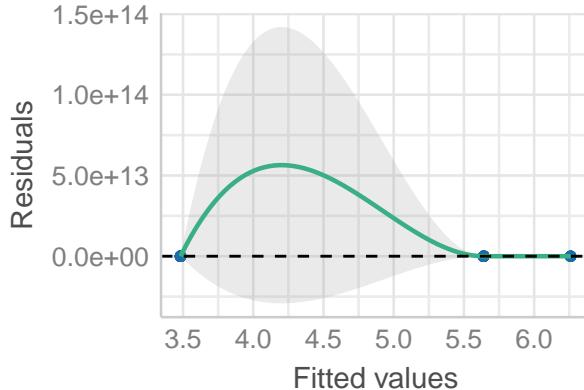
Posterior Predictive Check

Model-predicted lines should resemble observed data



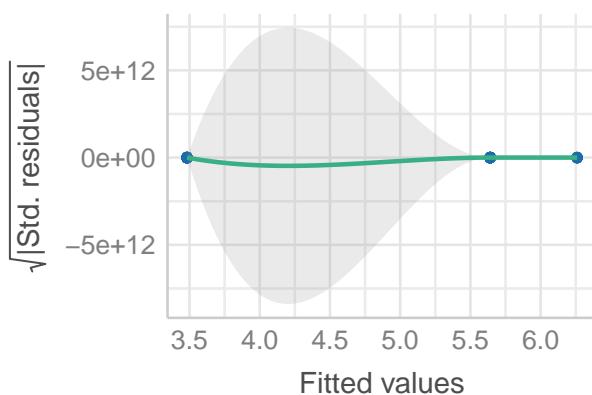
Linearity

Reference line should be flat and horizontal



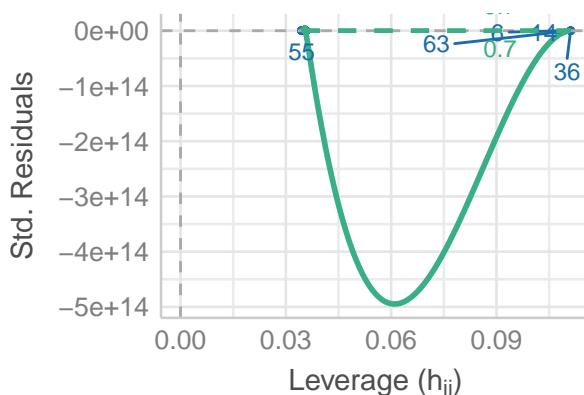
Homogeneity of Variance

Reference line should be flat and horizontal



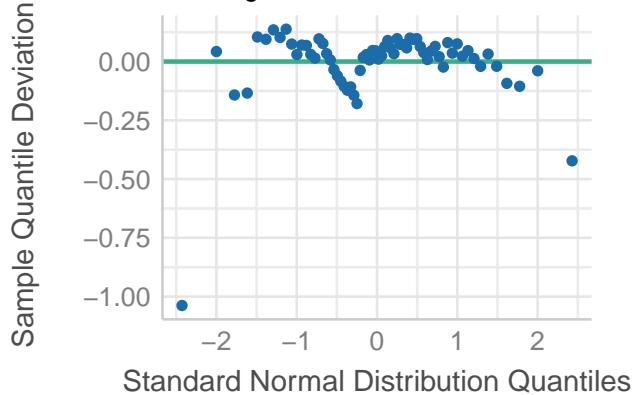
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



But not on macroaggregates or root C:

```
## block effect once again singular
## log-transforming root C does not change results
rootC_myco<-lm(rootC~mycotype,data=carbon_seq)
anova(rootC_myco)
```

```

## Analysis of Variance Table
##
## Response: rootC
##          Df  Sum Sq Mean Sq F value Pr(>F)
## mycotype    2   4.728  2.3641  1.1884 0.3118
## Residuals 60 119.358  1.9893
macro_myco<-lmer(macro250~mycotype+(1|block),data=carbon_seq)
anova(macro_myco)

## Type III Analysis of Variance Table with Satterthwaite's method
##          Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
## mycotype 127.02  63.509      2     60 20.004 2.2372 0.1156

```

Microbial composition and soil C accumulation

We conducted an exploratory analysis of the relationships between various PLFAs (measured in 2016) and either the change in soil C pools or the final pool in 2019. The idea here is that microbial activity could be a driver of how much of the C inputs are actually retained in the soil. However, we don't have the temporal sampling needed to support a clean causal story here, so these correlations are best interpreted as just that: correlations. On a statistical level, it may be worth noting the large deviations from the normality of residuals assumption in some of these models, especially those with a random effect.

Although many PLFAs were measured (and Grossman et al. 2019 has further details), we selected certain PLFAs for examination in the analyses below.

```
summary(lmer(soilC~X15.0_iso.pp+(1|block),data=carbon_seq))
```

```

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [ 
## lmerModLmerTest]
## Formula: soilC ~ X15.0_iso.pp + (1 | block)
## Data: carbon_seq
##
## REML criterion at convergence: 175.7
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -1.69728 -0.78218  0.01349  0.61349  1.80321
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 0.4711   0.6864
## Residual           12.6766   3.5604
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)    
## (Intercept)  5.0273    1.4664 18.7918  3.428  0.00285 ** 
## X15.0_iso.pp  0.2396    0.3176 29.1179  0.754  0.45673  
## ---      
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr)
## X15.0_is.pp -0.865

```

```

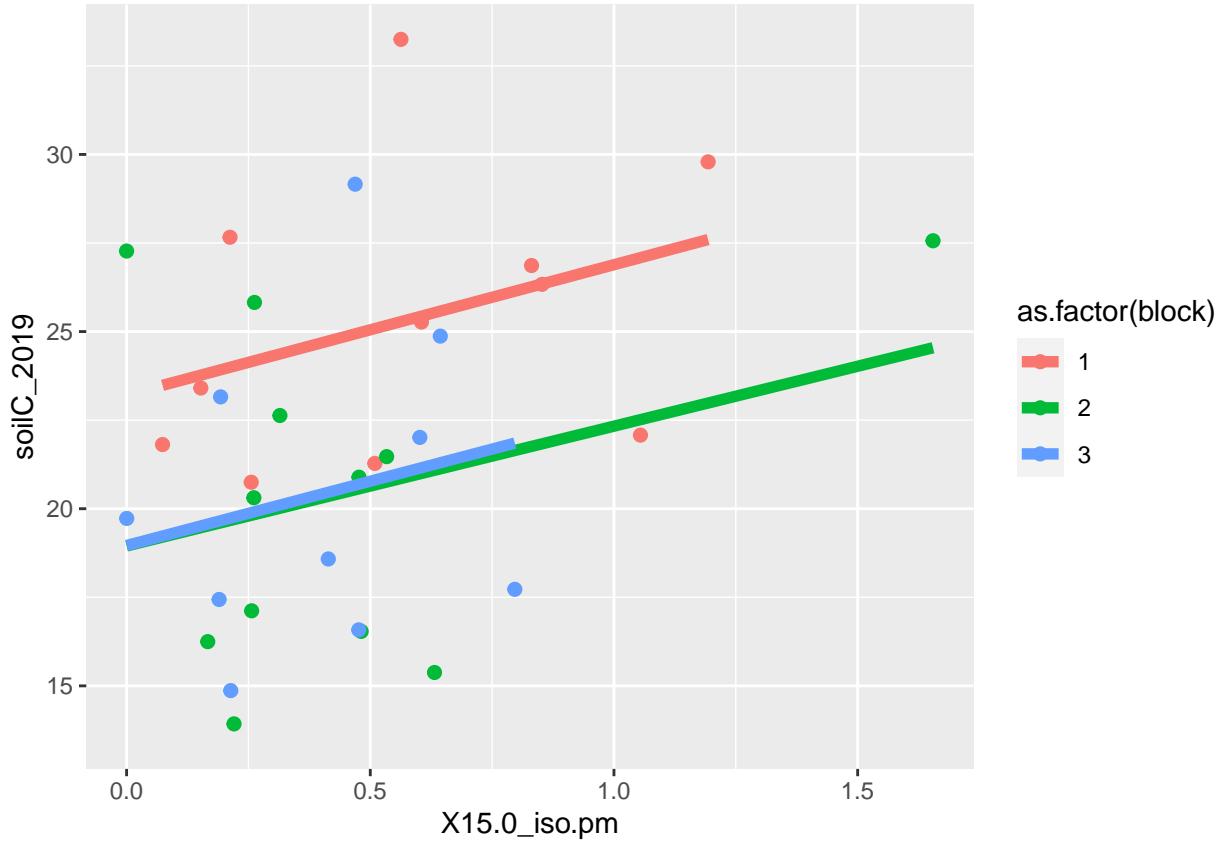
summary(lmer(soilC_2019~X15.0_iso.pm+(1|block), data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2019 ~ X15.0_iso.pm + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 185.2
##
## Scaled residuals:
##      Min    1Q Median    3Q   Max
## -1.45213 -0.71541  0.07136  0.54293  2.05434
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block     (Intercept) 4.589    2.142
##   Residual           18.038   4.247
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error    df t value Pr(>|t|)
## (Intercept) 20.245     1.756  4.211 11.528 0.000242 ***
## X15.0_iso.pm 3.845     2.131 29.634   1.805 0.081267 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## X15.0_is.pm -0.571

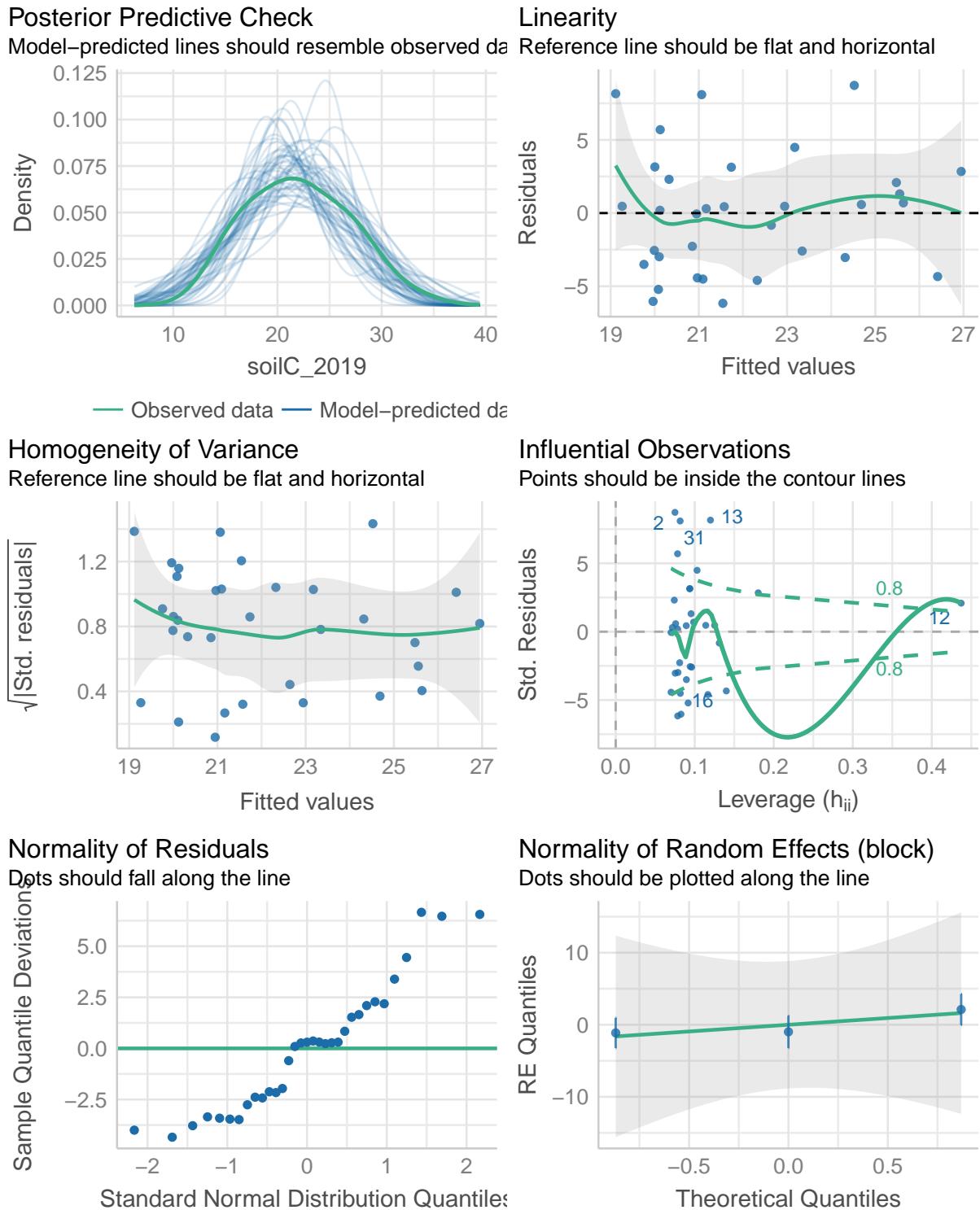
ggplot(data=carbon_seq, aes(x=X15.0_iso.pm, y=soilC_2019, color=as.factor(block))) +
  geom_point(size=2) + geom_smooth(method="lm", se=F, linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 107 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 107 rows containing missing values (`geom_point()`).

```



```
check_model(lmer(soilC_2019 ~ X15.0_iso.pm + (1 | block), data=carbon_seq))
```



```
summary(lmer(soilC~X17.0_anteiso.pp+(1|block),data=carbon_seq))
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [  
## lmerModLmerTest]  
## Formula: soilC ~ X17.0_anteiso.pp + (1 | block)
```

```

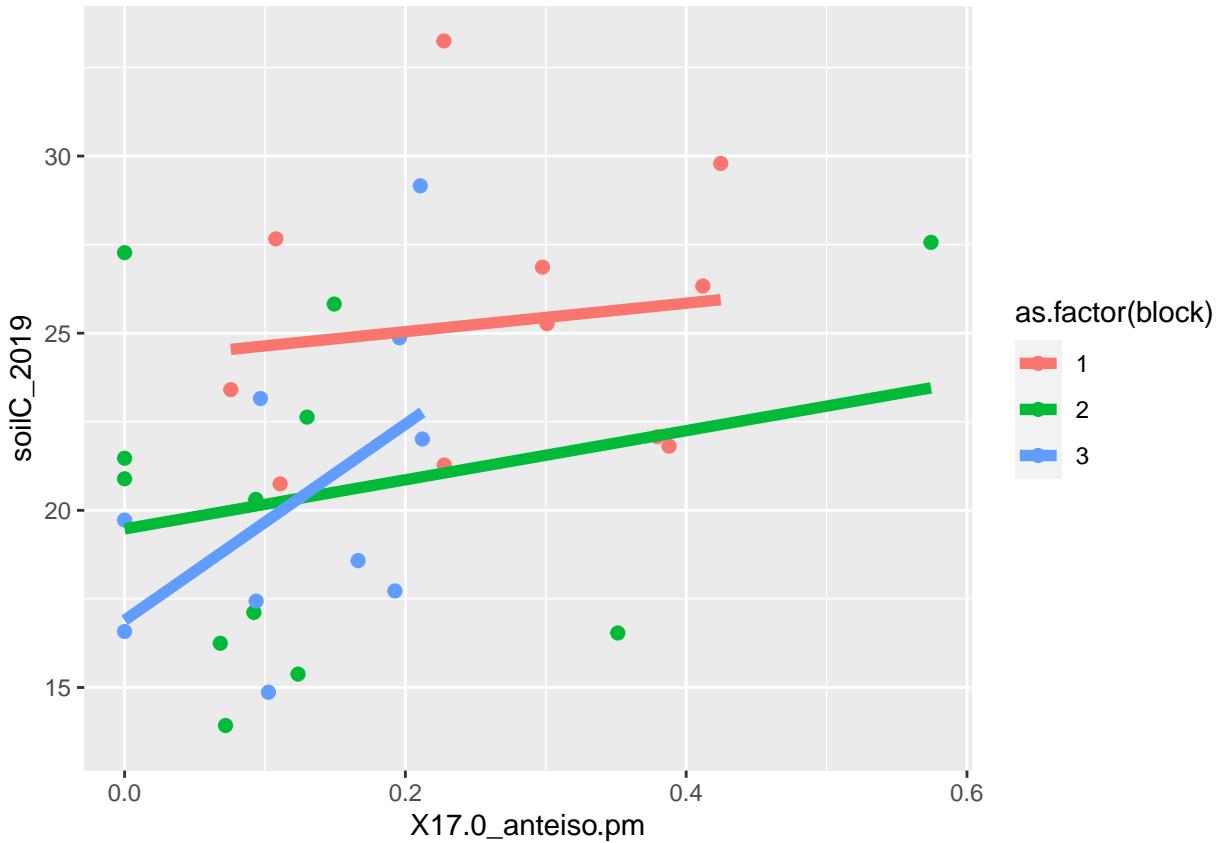
##      Data: carbon_seq
##
## REML criterion at convergence: 173.4
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.82090 -0.80076 -0.09776  0.72258  1.79534
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 0.1089  0.330
## Residual           12.5780  3.547
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##                  Estimate Std. Error     df t value Pr(>|t|)
## (Intercept)      4.7659    1.2280 14.1883  3.881 0.00162 **
## X17.0_anteiso.pp 0.8183    0.6992 30.7896  1.170 0.25081
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## X17.0_nts.p -0.850
summary(lmer(soilC_2019~X17.0_anteiso.pm+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2019 ~ X17.0_anteiso.pm + (1 | block)
## Data: carbon_seq
##
## REML criterion at convergence: 183.1
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.5587 -0.7614  0.0550  0.5029  2.1667
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 2.387   1.545
## Residual           18.496   4.301
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##                  Estimate Std. Error     df t value Pr(>|t|)
## (Intercept)      20.100    1.535  4.262 13.097 0.000133 ***
## X17.0_anteiso.pm 10.990    5.612 30.295  1.958 0.059481 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## X17.0_nts.p -0.651

```

```
ggplot(data=carbon_seq,aes(x=X17.0_anteiso.pm,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)
```

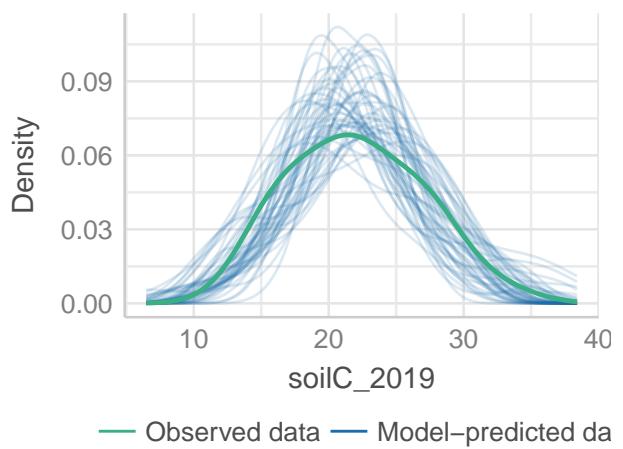
```
## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 107 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 107 rows containing missing values (`geom_point()`).
```



```
check_model(lmer(soilC_2019~X17.0_anteiso.pm+(1|block),data=carbon_seq))
```

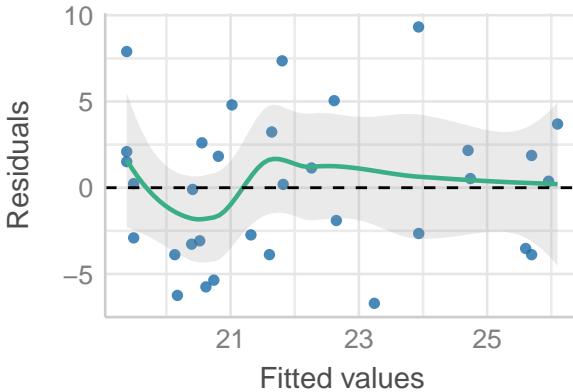
Posterior Predictive Check

Model-predicted lines should resemble observed data



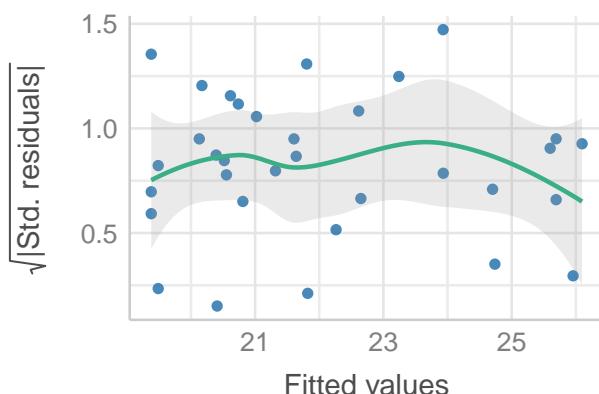
Linearity

Reference line should be flat and horizontal



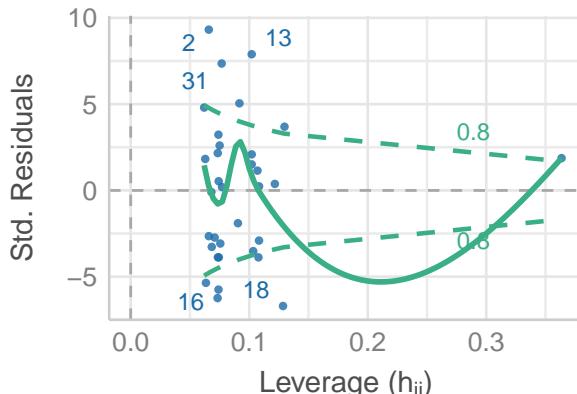
Homogeneity of Variance

Reference line should be flat and horizontal



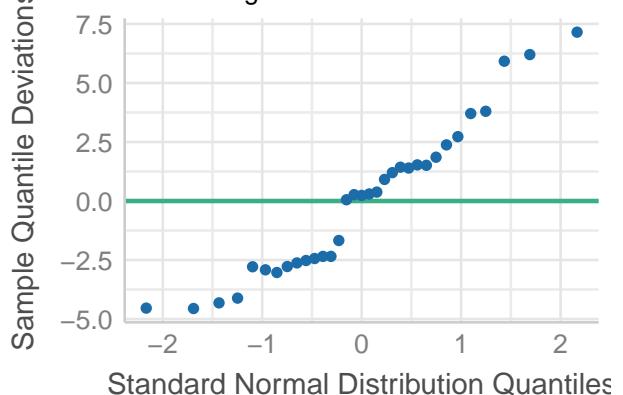
Influential Observations

Points should be inside the contour lines



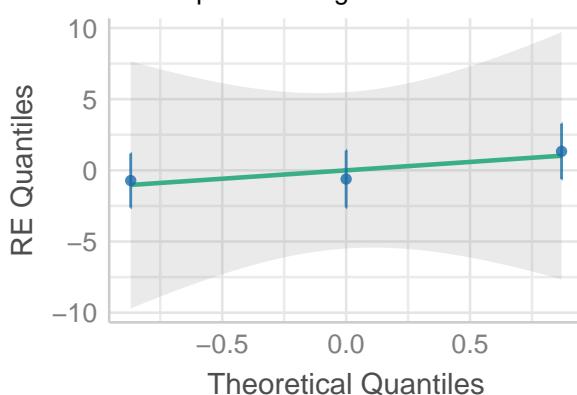
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (block)

Dots should be plotted along the line



```
## block yields singular fit here
summary(lm(soilC~X18.0_10me.pp, data=carbon_seq))
```

```
##
## Call:
```

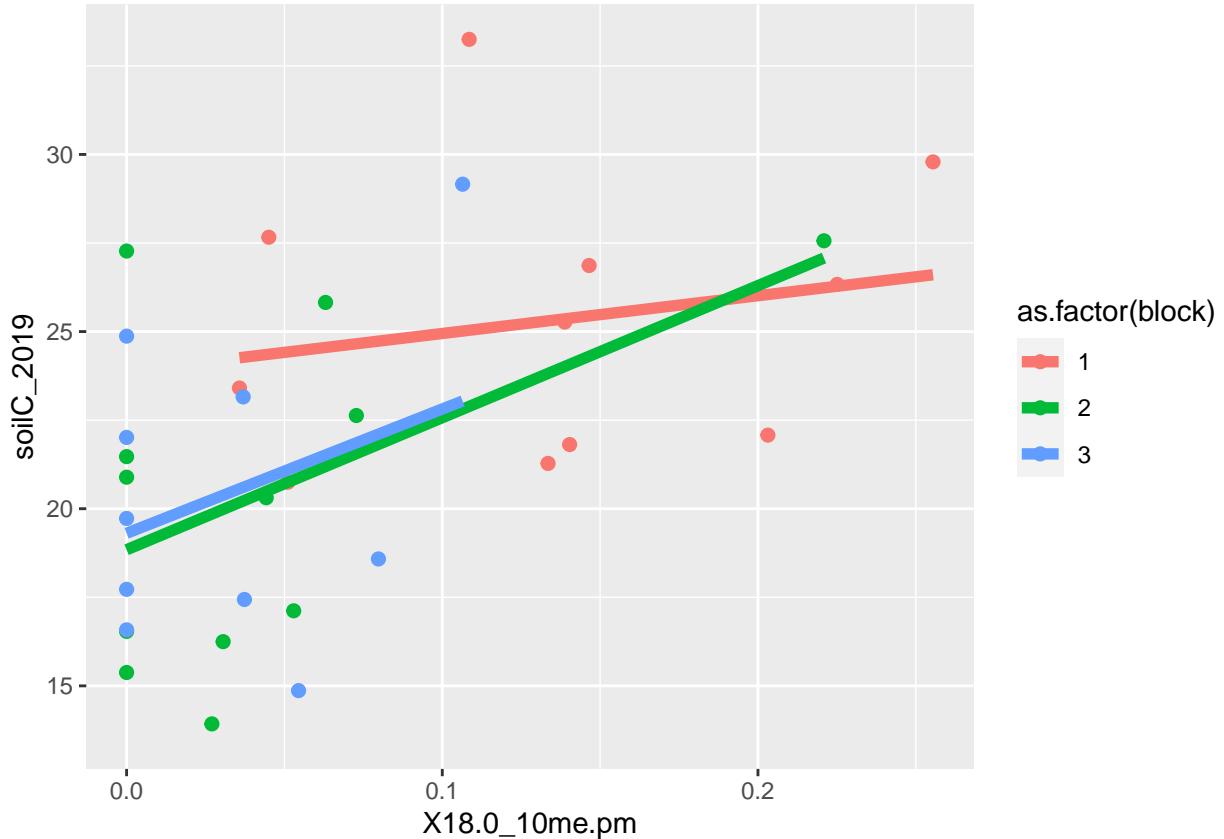
```

## lm(formula = soilC ~ X18.0_10me.pp, data = carbon_seq)
##
## Residuals:
##   Min     1Q Median     3Q    Max
## -6.8063 -2.7873 -0.2353  2.5633  6.2004
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  4.9156    0.9813  5.009 2.09e-05 ***
## X18.0_10me.pp 1.6880    1.2031  1.403   0.171
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.527 on 31 degrees of freedom
##   (107 observations deleted due to missingness)
## Multiple R-squared:  0.05971, Adjusted R-squared:  0.02938
## F-statistic: 1.969 on 1 and 31 DF, p-value: 0.1705
summary(lm(soilC_2019~X18.0_10me.pm,data=carbon_seq))

##
## Call:
## lm(formula = soilC_2019 ~ X18.0_10me.pm, data = carbon_seq)
##
## Residuals:
##   Min     1Q Median     3Q    Max
## -6.6603 -3.1264  0.0614  2.2310  9.8797
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 19.664     1.000  19.66 < 2e-16 ***
## X18.0_10me.pm 34.140     9.839   3.47  0.00155 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 4.167 on 31 degrees of freedom
##   (107 observations deleted due to missingness)
## Multiple R-squared:  0.2798, Adjusted R-squared:  0.2565
## F-statistic: 12.04 on 1 and 31 DF, p-value: 0.001553
ggplot(data=carbon_seq,aes(x=X18.0_10me.pm,y=soilC_2019,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 107 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 107 rows containing missing values (`geom_point()`).

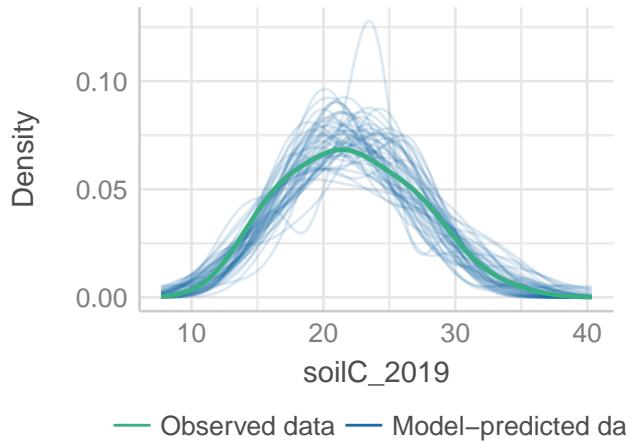
```



```
check_model(lm(soilC_2019~X18.0_10me.pm, data=carbon_seq))
```

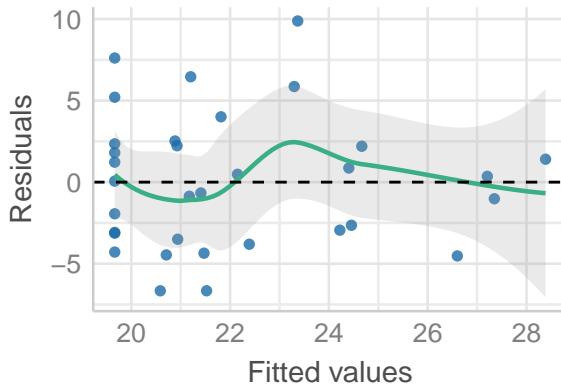
Posterior Predictive Check

Model-predicted lines should resemble observed data



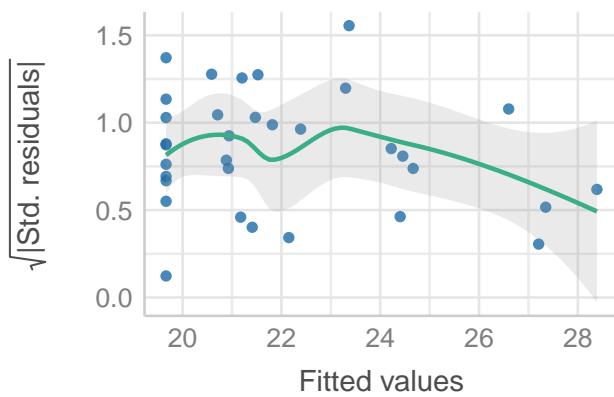
Linearity

Reference line should be flat and horizontal



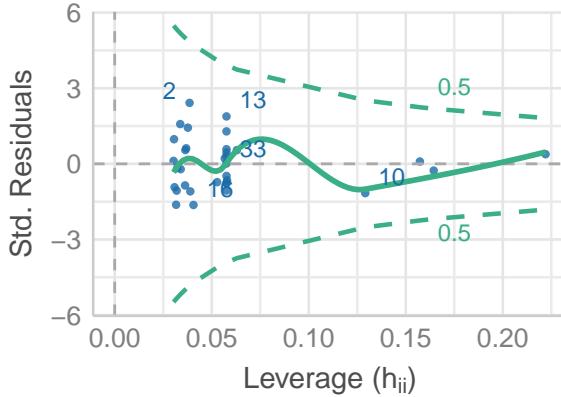
Homogeneity of Variance

Reference line should be flat and horizontal



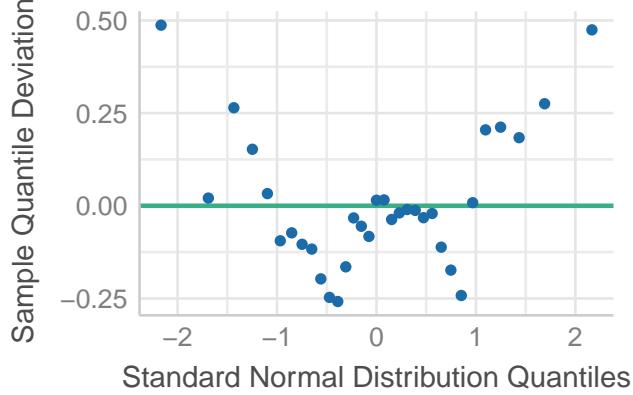
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



```
## block yields singular fit for the first of these
summary(lm(soilC~X18.2_w6.9c.pp,data=carbon_seq))
```

```
##
## Call:
```

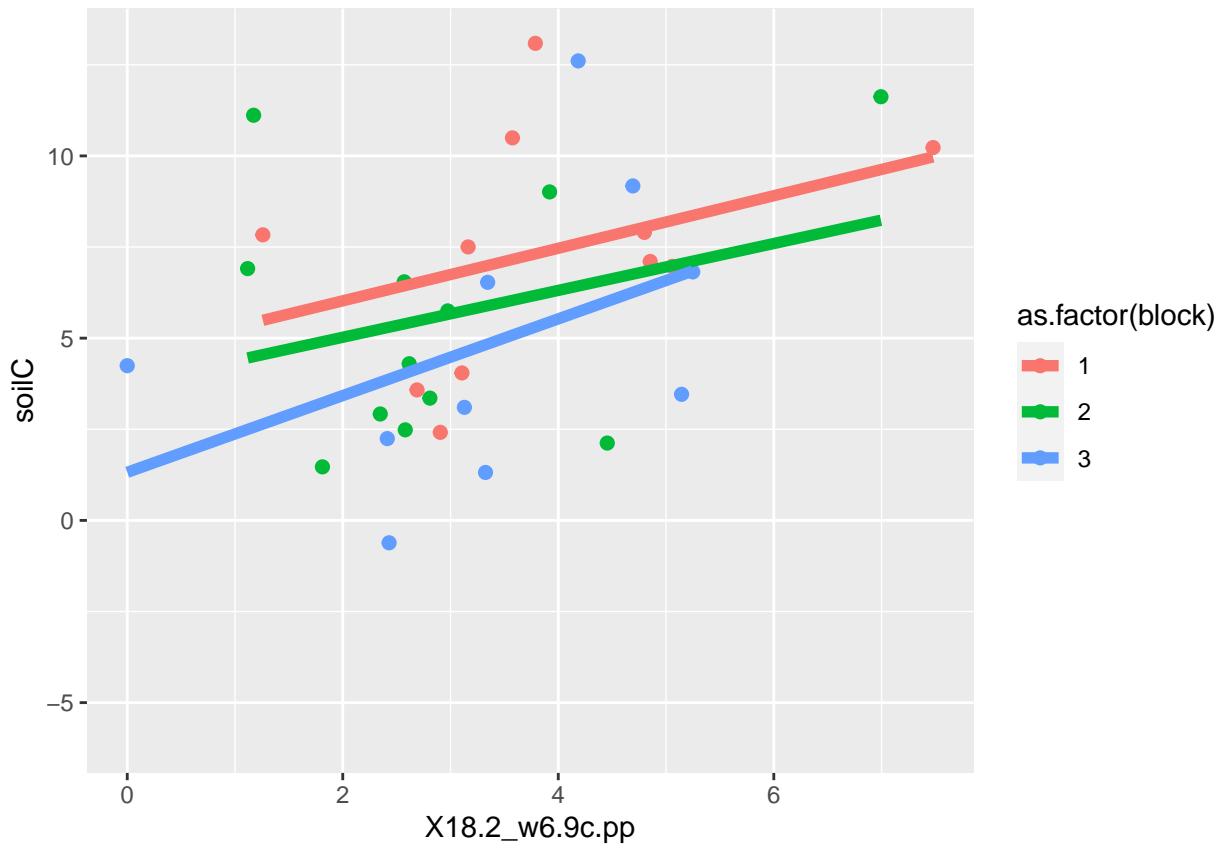
```

## lm(formula = soilC ~ X18.2_w6.9c.pp, data = carbon_seq)
##
## Residuals:
##   Min     1Q Median     3Q    Max
## -5.7818 -2.6623 -0.1339  2.0764  7.0234
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.0902    1.3884   2.226  0.0334 *
## X18.2_w6.9c.pp  0.8547    0.3711   2.303  0.0282 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 3.361 on 31 degrees of freedom
##   (107 observations deleted due to missingness)
## Multiple R-squared:  0.1461, Adjusted R-squared:  0.1185
## F-statistic: 5.303 on 1 and 31 DF,  p-value: 0.02816
summary(lmer(soilC_2019~X18.2_w6.9c.pm+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2019 ~ X18.2_w6.9c.pm + (1 | block)
## Data: carbon_seq
##
## REML criterion at convergence: 188.9
##
## Scaled residuals:
##   Min     1Q Median     3Q    Max
## -1.4481 -0.7358 -0.1612  0.5788  2.0213
##
## Random effects:
##   Groups   Name        Variance Std.Dev.
##   block   (Intercept) 5.935    2.436
##   Residual           19.428    4.408
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 21.5710    1.7433  2.7873 12.374 0.00161 **
## X18.2_w6.9c.pm  0.8801    1.2456 29.4019  0.707  0.48538
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## X18.2_w6.9. -0.393
ggplot(data=carbon_seq,aes(x=X18.2_w6.9c.pp,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 107 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 107 rows containing missing values (`geom_point()`).

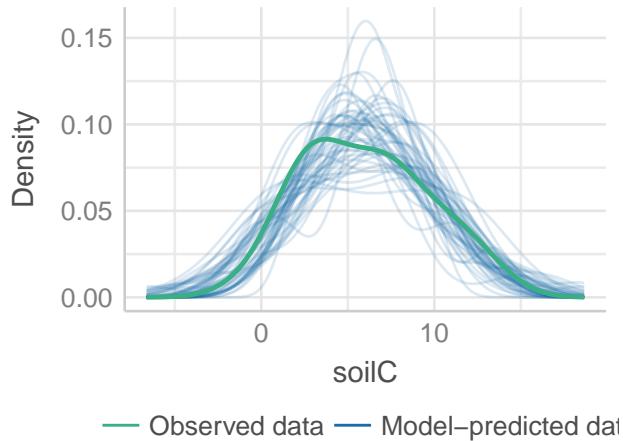
```



```
check_model(lm(soilC~X18.2_w6.9c.pp,data=carbon_seq))
```

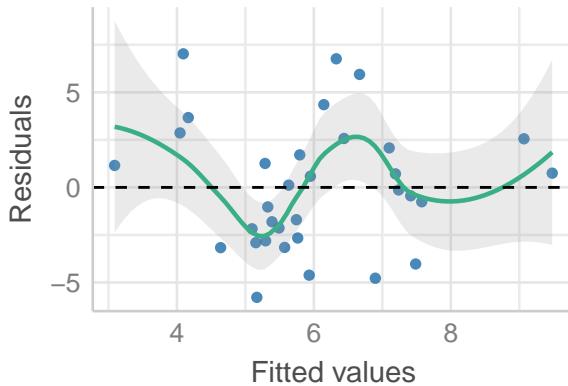
Posterior Predictive Check

Model-predicted lines should resemble observed data



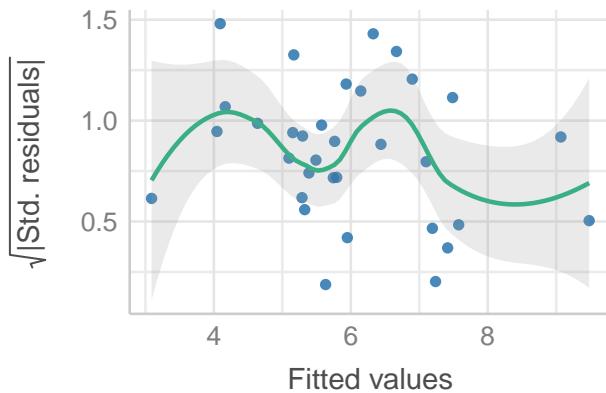
Linearity

Reference line should be flat and horizontal



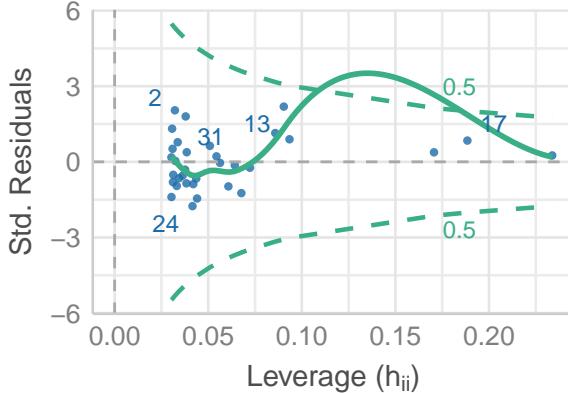
Homogeneity of Variance

Reference line should be flat and horizontal



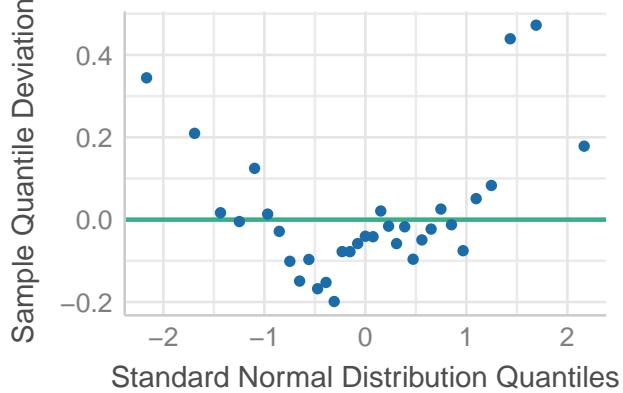
Influential Observations

Points should be inside the contour lines



Normality of Residuals

Dots should fall along the line



Besides these taxon-specific PLFAs, we were interested in showing whether total PLFA concentration correlated with soil C or macroaggregates. It does not:

```

## singular fit for the first of these
summary(lmer(soilC~Biomass.pm+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC ~ Biomass.pm + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 180
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.7273 -0.7567  0.1593  0.4219  1.9625
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 0.3332   0.5773
## Residual           12.8193  3.5804
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)    
## (Intercept) 5.58835   0.94587  5.80432  5.908  0.00118 ** 
## Biomass.pm   0.02547   0.04030 30.54715   0.632  0.53212  
## ---      
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## Biomass.pm -0.664

summary(lmer(soilC_2019~Biomass.pm+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC_2019 ~ Biomass.pm + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 195.6
##
## Scaled residuals:
##      Min     1Q Median     3Q    Max
## -1.4732 -0.7589 -0.1817  0.5723  1.9933
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 6.173    2.484
## Residual           19.578    4.425
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)    
## (Intercept) 21.69933   1.80574  3.00948 12.017  0.00122 ** 
## Biomass.pm   0.02302   0.05043 29.58150   0.456  0.65144  

```

```

## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## Biomass.pm -0.432
summary(lmer(macro250~Biomass.pm+(1|block),data=carbon_seq))

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: macro250 ~ Biomass.pm + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 202.6
##
## Scaled residuals:
##     Min      1Q  Median      3Q      Max
## -1.89533 -0.64800  0.05047  0.73189  1.74772
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept) 46.75    6.837
## Residual           22.01    4.691
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##             Estimate Std. Error      df t value Pr(>|t|)
## (Intercept) 71.46798  4.11558  2.15891 17.365 0.00233 **
## Biomass.pm   0.04234  0.05370 29.10275  0.788  0.43682
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## Biomass.pm -0.201

```

However, the percent of fungal PLFAs has a marginally significant correlation with soil C accumulation.

```

## singular fit for the first of these
summary(lmer(soilC~gen_fungi_pct+(1|block),data=carbon_seq))

```

```

## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: soilC ~ gen_fungi_pct + (1 | block)
##   Data: carbon_seq
##
## REML criterion at convergence: 174.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q      Max
## -1.71659 -0.74177  0.02309  0.63062  1.98055
##
## Random effects:
## Groups   Name        Variance Std.Dev.
## block    (Intercept)  0.14    0.3742

```

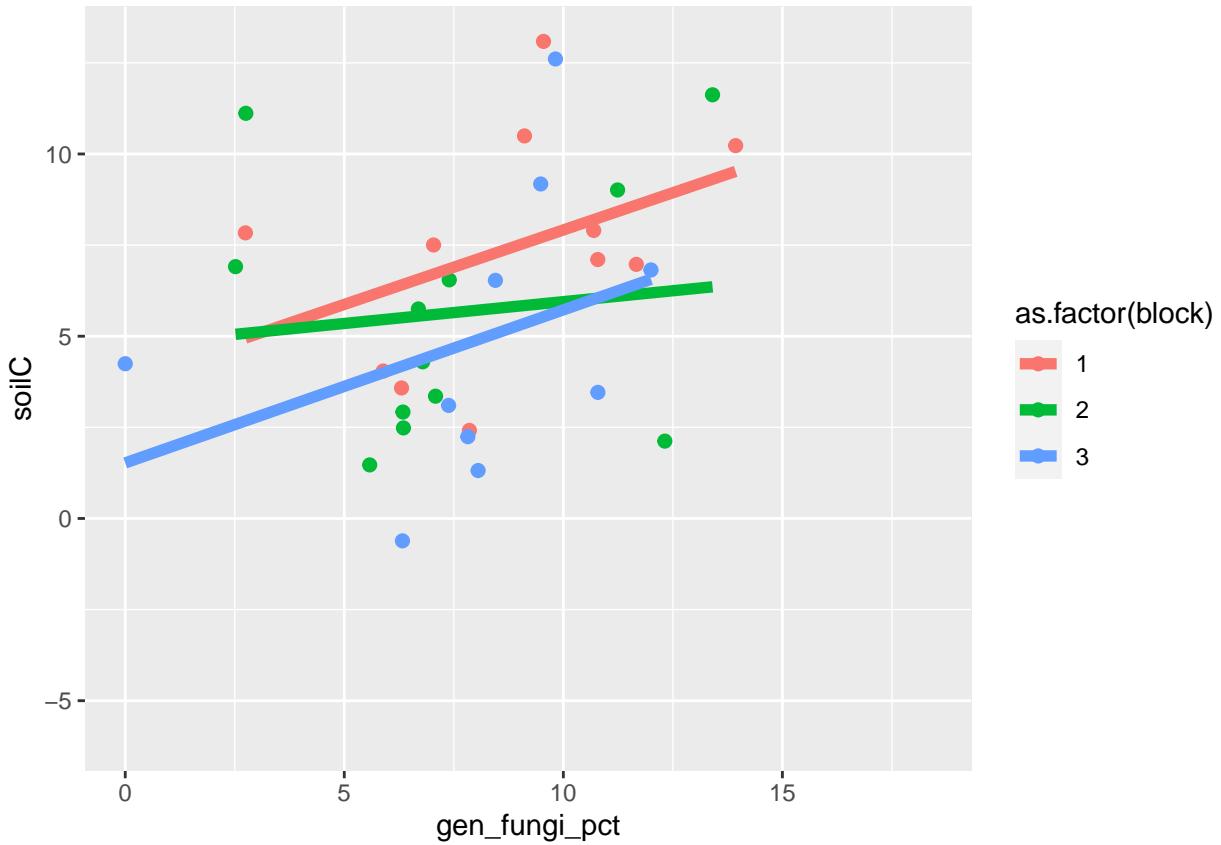
```

##  Residual           11.98    3.4609
## Number of obs: 33, groups: block, 3
##
## Fixed effects:
##              Estimate Std. Error      df t value Pr(>|t|)
## (Intercept)   3.3848    1.6536 25.0264   2.047  0.0513 .
## gen_fungi_pct 0.3251    0.1904 30.2885   1.707  0.0980 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##          (Intr)
## gen_fng_pct -0.922

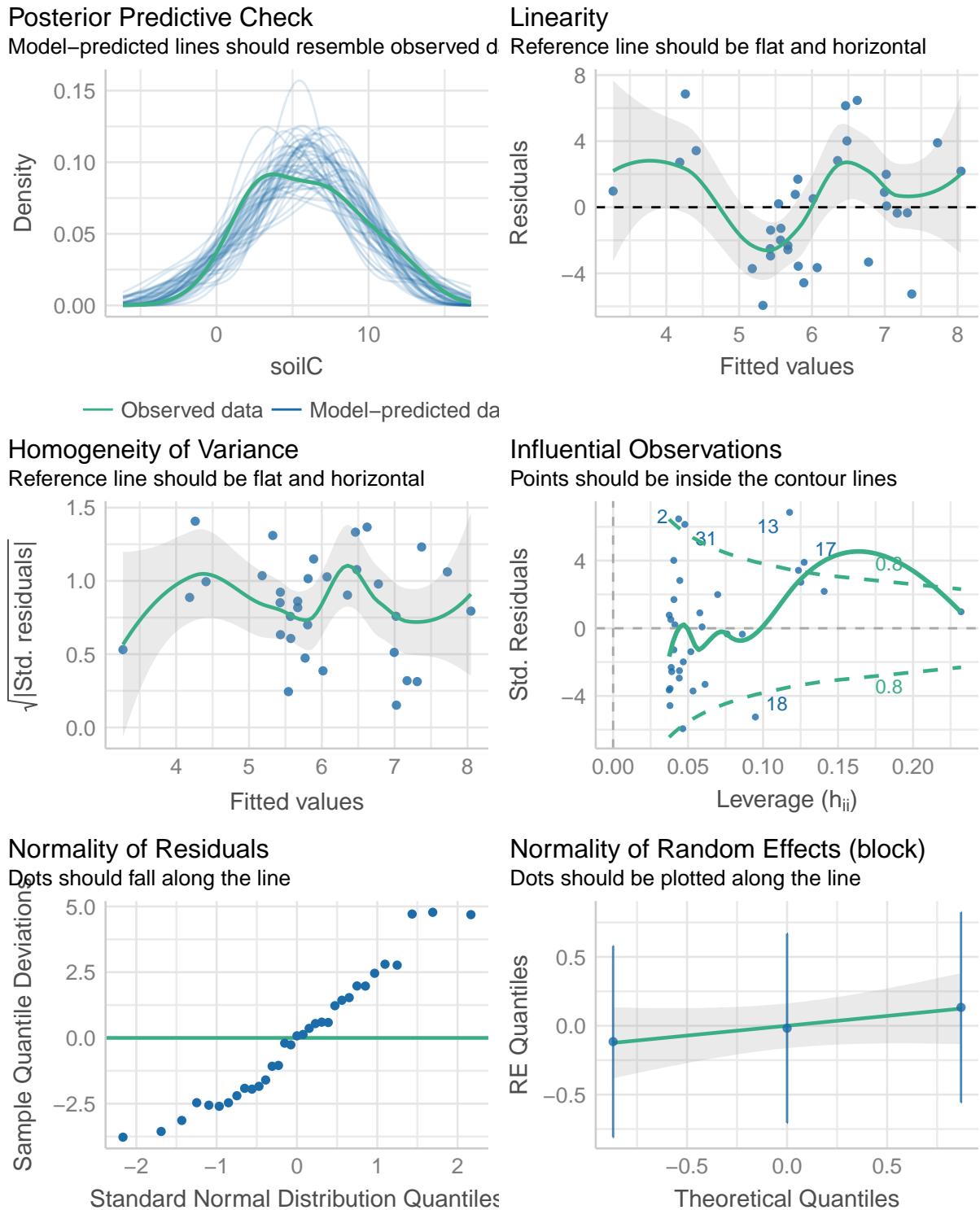
ggplot(data=carbon_seq,aes(x=gen_fungi_pct,y=soilC,color=as.factor(block)))+
  geom_point(size=2)+geom_smooth(method="lm",se=F,linewidth=2)

## `geom_smooth()` using formula = 'y ~ x'
## Warning: Removed 107 rows containing non-finite values (`stat_smooth()`).
## Warning: Removed 107 rows containing missing values (`geom_point()`).

```



```
check_model(lmer(soilC~gen_fungi_pct+(1|block),data=carbon_seq))
```



Structural equation modeling of aboveground woody and soil C

We use structural equation modeling (SEM) to tie together our hypotheses in a more integrative manner. Although we found no evidence elsewhere for a correlation between aboveground woody and soil C accumulation, SEMs allow us to directly test whether the influence of species richness on soil C accumulation is

mediated by aboveground woody C.

We select six variables:

- species richness (exogenous)
- percent of planted trees associated with AM fungi (exogenous)
- percent of planted trees that are conifers (exogenous)
- aboveground woody C (endogenous)
- macroaggregates (endogenous)
- soil C accumulation (endogenous)

The first step is to drop rows with NAs in any of these variables, then z-standardize all of them so that regression coefficients in the SEM are standardized:

```
carbon_seq_sub<-carbon_seq[-which(is.na(carbon_seq$soilC) | is.na(carbon_seq$macro250)),]  
  
carbon_seq_standard<-carbon_seq_sub  
standard_cols<-c("species_richness","woodyC","soilC",  
                 "macro250","percentAM","percentCon")  
carbon_seq_standard[,standard_cols]<-scale(carbon_seq_standard[,standard_cols])
```

As explained in the main text and Appendix I, we proposed an initial model based on *a priori* hypotheses and earlier data analyses. The structure of this model is as follows:

```
localfit_orig_model<-psem(  
  lmer(woodyC~species_richness+percentCon+(1|block),data=carbon_seq_standard),  
  lmer(macro250~percentAM+percentCon+woodyC+(1|block),data=carbon_seq_standard),  
  lmer(soilC~species_richness+percentAM+percentCon+woodyC+macro250+(1|block),  
       data=carbon_seq_standard)  
)  
  
summary(localfit_orig_model)  
  
##  |  
##  
##  ## Structural Equation Model of localfit_orig_model  
##  
##  ## Call:  
##    woodyC ~ species_richness + percentCon  
##    macro250 ~ percentAM + percentCon + woodyC  
##    soilC ~ species_richness + percentAM + percentCon + woodyC + macro250  
##  
##    AIC  
##    457.856  
##  
##  ---  
##  Tests of directed separation:  
##  
##              Independ.Claim Test.Type      DF Crit.Value P.Value  
##  macro250 ~ species_richness + ...     coef 58.0493   -0.1347  0.8933  
##          woodyC ~ percentAM + ...     coef 59.0416   -3.9468  0.0002 ***  
##  
##  --  
##  Global goodness-of-fit:  
##  
##  Chi-Squared = 6.883 with P-value = 0.032 and on 2 degrees of freedom
```

```

## Fisher's C = 17.132 with P-value = 0.002 and on 4 degrees of freedom
##
## ---
## Coefficients:
##
##    Response      Predictor Estimate Std.Error      DF Crit.Value P.Value
##    woodyC species_richness  0.1463   0.0599 60.1193     2.4410 0.0176
##    woodyC      percentCon  0.8564   0.0600 60.1829    14.2796 0.0000
##    macro250     percentAM -0.1520   0.0852 59.0404    -1.7827 0.0798
##    macro250     percentCon -0.0673   0.1684 59.1552    -0.3996 0.6909
##    macro250        woodyC -0.2397   0.1748 59.2409    -1.3710 0.1756
##    soilC species_richness  0.2884   0.1252 59.0000     2.3031 0.0248
##    soilC      percentAM  0.2972   0.1324 59.0000     2.2439 0.0286
##    soilC      percentCon -0.0085   0.2725 59.0000    -0.0313 0.9751
##    soilC        woodyC  0.0767   0.2740 59.0000     0.2799 0.7806
##    soilC      macro250  0.1330   0.1249 59.0000     1.0648 0.2913
##
##    Std.Estimate
##    0.1463   *
##    0.8564 ***
##    -0.1520
##    -0.0673
##    -0.2397
##    0.2884   *
##    0.2972   *
##    -0.0085
##    0.0767
##    0.1330
##
##    Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05
##
## ---
## Individual R-squared:
##
##    Response method Marginal Conditional
##    woodyC   none    0.75      0.77
##    macro250  none    0.07      0.73
##    soilC    none    0.16      0.16

```

By running `check_models()` on each subcomponent of the model, it becomes clear that percent conifer and aboveground woody C have moderately high VIF due to their high correlation with each other. We removed aboveground woody C from the model for macroaggregates and percent conifer from the model for soil C accumulation, although we caution that in any case it is a challenge to attribute anything definitely to one or the other variable.

Two other things are apparent. One is that the block random effect for soil C reaches a singular fit, so we remove it. The other is that the tests of directed separation show conditional dependence between percent AM and woody C. Since percent AM is exogenous, we add it as a predictor of woody C. We arrive at the following model:

```

localfit_full_model<-psem(
  lmer(woodyC~species_richness+percentCon+percentAM+(1|block),data=carbon_seq_standard),
  lmer(macro250~percentAM+percentCon+(1|block),data=carbon_seq_standard),
  lm(soilC~species_richness+percentAM+woodyC+macro250,
    data=carbon_seq_standard)
)

```

```

summary(localfit_full_model)

## |
## 
## Structural Equation Model of localfit_full_model
## 
## Call:
##   woodyC ~ species_richness + percentCon + percentAM
##   macro250 ~ percentAM + percentCon
##   soilC ~ species_richness + percentAM + woodyC + macro250
## 
##   AIC
##   430.913
## 
## ---
## Tests of directed separation:
## 
##           Independ.Claim Test.Type      DF Crit.Value P.Value
##   macro250 ~ species_richness + ...    coef 59.0081 -0.5527  0.5825
##   soilC ~ percentCon + ...    coef 59.0000 -0.0313  0.9751
##   macro250 ~ woodyC + ...    coef 58.2852 -1.2467  0.2175
## 
## --
## Global goodness-of-fit:
## 
## Chi-Squared = NA with P-value = NA and on 3 degrees of freedom
## Fisher's C = 4.182 with P-value = 0.652 and on 6 degrees of freedom
## 
## ---
## Coefficients:
## 
##   Response Predictor Estimate Std.Error      DF Crit.Value P.Value
##   woodyC species_richness  0.1361  0.0539 59.0976  2.5268  0.0142
##   woodyC percentCon     0.8635  0.0539 59.1531 16.0316  0.0000
##   woodyC percentAM     -0.2125  0.0538 59.0416 -3.9468  0.0002
##   macro250 percentAM     -0.0996  0.0769 60.0035 -1.2954  0.2001
##   macro250 percentCon    -0.2729  0.0770 60.0133 -3.5439  0.0008
##   soilC species_richness  0.2896  0.1190 60.0000  2.4339  0.0179
##   soilC percentAM       0.2956  0.1212 60.0000  2.4380  0.0177
##   soilC woodyC          0.0690  0.1217 60.0000  0.5671  0.5728
##   soilC macro250         0.1340  0.1198 60.0000  1.1183  0.2679
##   Std.Estimate
##   0.1361 *
##   0.8635 ***
##   -0.2125 ***
##   -0.0996
##   -0.2729 ***
##   0.2896 *
##   0.2956 *
##   0.0690
##   0.1340
## 
##   Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05

```

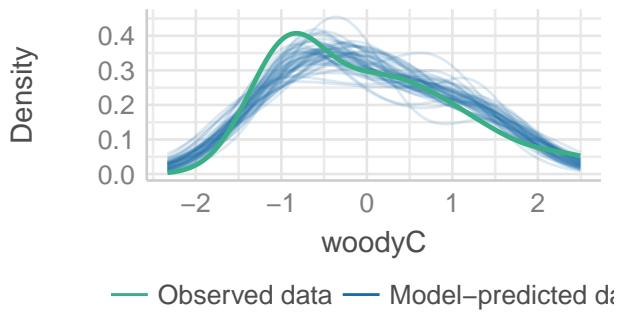
```
##  
## ---  
## Individual R-squared:  
##  
##   Response method Marginal Conditional  
##   woodyC    none      0.79      0.82  
##   macro250   none      0.07      0.71  
##   soilC     none      0.17      NA
```

As the Fisher's C > 0.05 shows, this model fits better than the one above. The in-depth interpretation of this model is in the main text. We can check each of the submodels of the full SEM to make sure that it means standard assumptions.

```
check_model(lmer(woodyC~species_richness+percentCon+percentAM+(1|block), data=carbon_seq_standard))
```

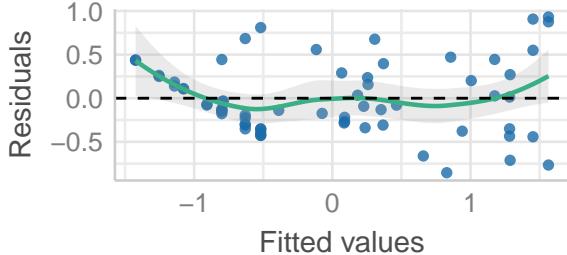
Posterior Predictive Check

Model-predicted lines should resemble observed data



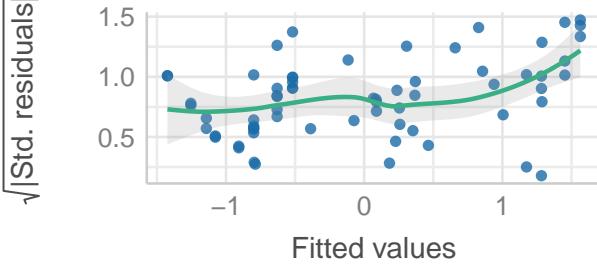
Linearity

Reference line should be flat and horizontal



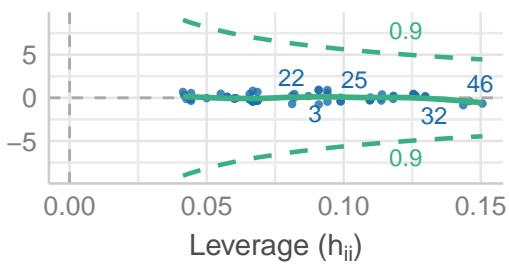
Homogeneity of Variance

Reference line should be flat and horizontal



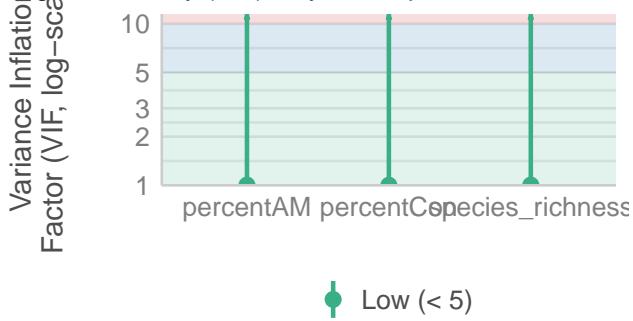
Influential Observations

Points should be inside the contour lines



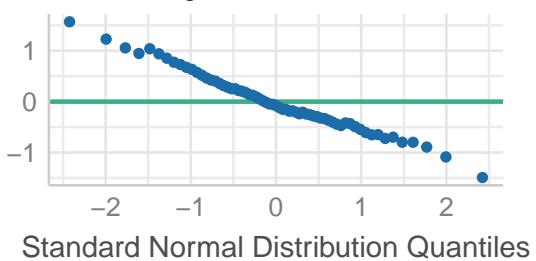
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



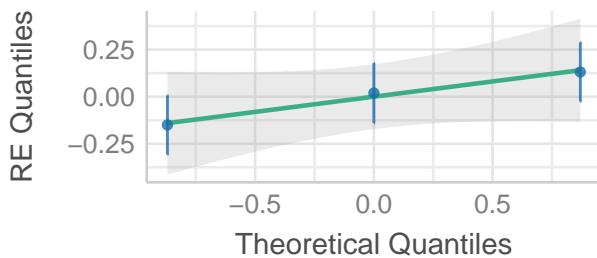
Normality of Residuals

Dots should fall along the line



Normality of Random Effects (block)

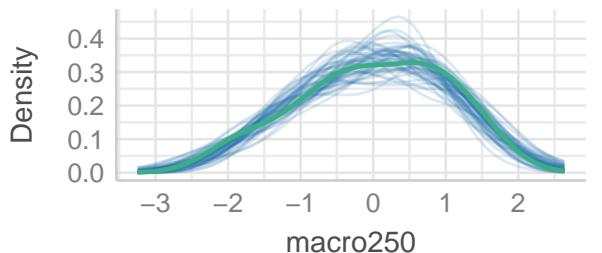
Dots should be plotted along the line



```
check_model(lmer(macro250 ~ percentAM + percentCon + (1 | block), data=carbon_seq_standard))
```

Posterior Predictive Check

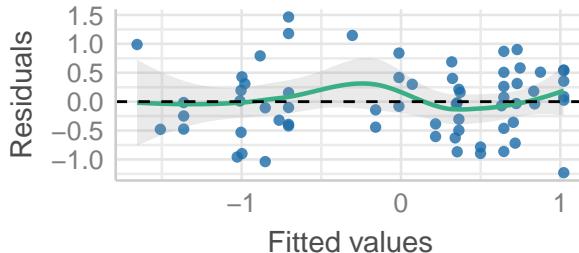
Model-predicted lines should resemble observed data



— Observed data — Model-predicted data

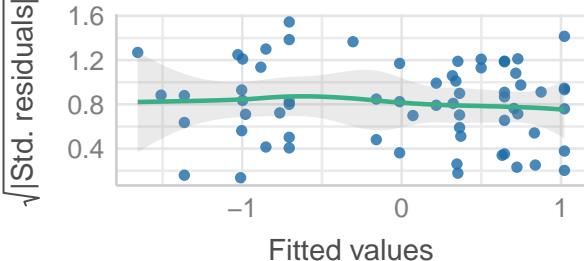
Linearity

Reference line should be flat and horizontal



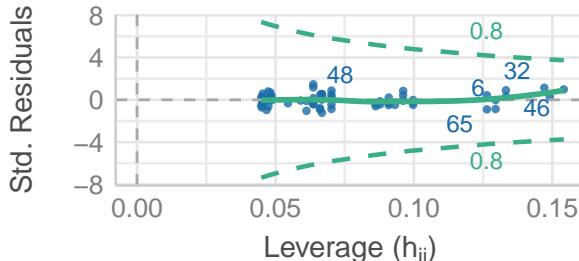
Homogeneity of Variance

Reference line should be flat and horizontal



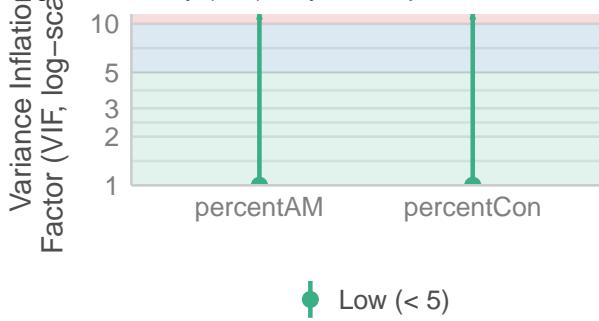
Influential Observations

Points should be inside the contour lines



Collinearity

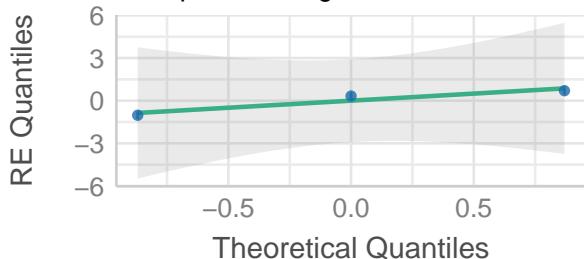
High collinearity (VIF) may inflate parameter uncertainty



● Low (< 5)

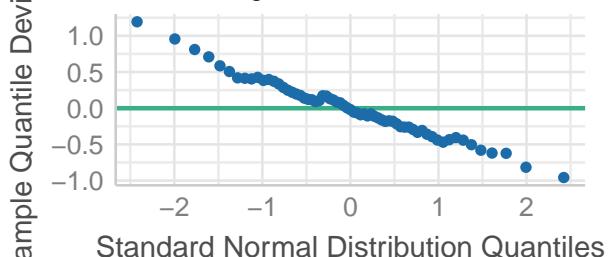
Normality of Random Effects (block)

Dots should be plotted along the line



Normality of Residuals

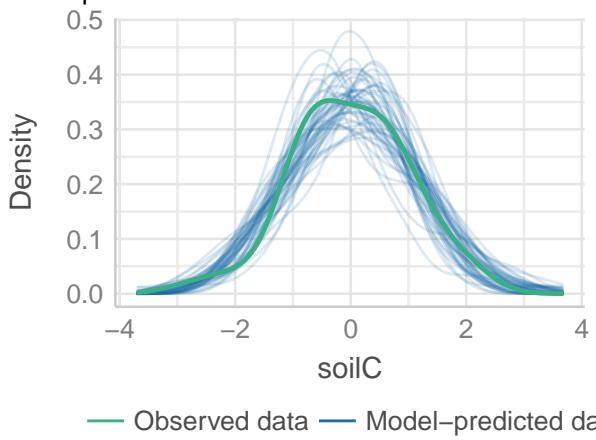
Dots should fall along the line



```
check_model(lm(soilC~species_richness+percentAM+woodyC+macro250,data=carbon_seq_standard))
```

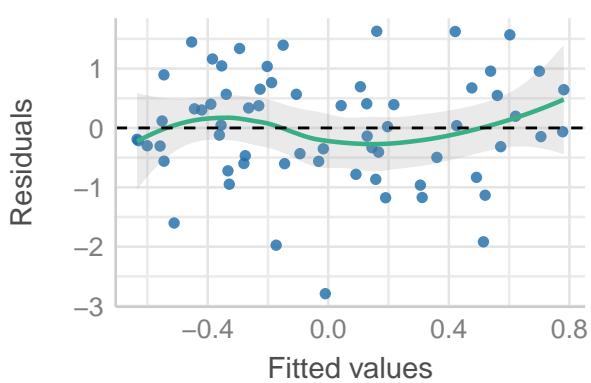
Posterior Predictive Check

Model-predicted lines should resemble observed data



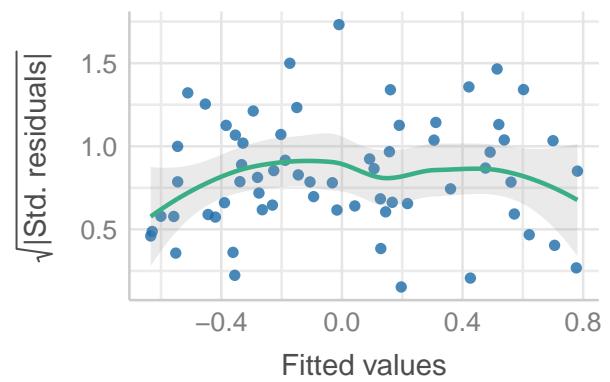
Linearity

Reference line should be flat and horizontal



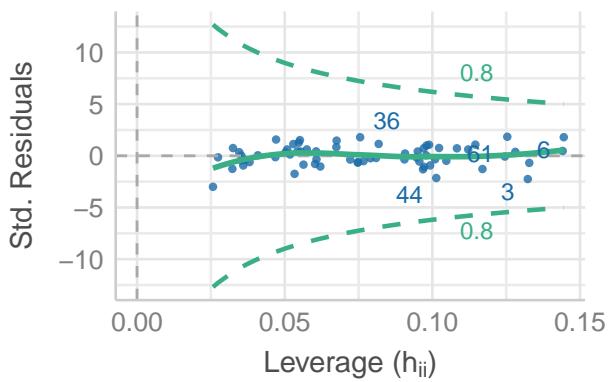
Homogeneity of Variance

Reference line should be flat and horizontal



Influential Observations

Points should be inside the contour lines



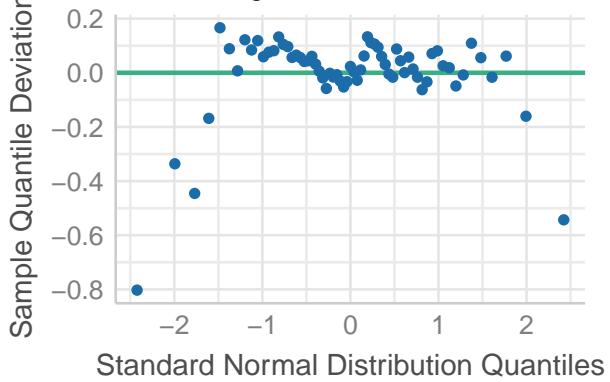
Collinearity

High collinearity (VIF) may inflate parameter uncertainty



Normality of Residuals

Dots should fall along the line



● Low (< 5)

Each of these plots looks reasonably good, barring violations of the normality of residuals assumption, which shouldn't be too concerning. There is no particular need to fit a more minimal model that only retains significant terms, but if we did, it would look like this:

```

localfit_min_model<-psem(
  lmer(woodyC~species_richness+percentCon+percentAM+(1|block), data=carbon_seq_standard),
  lmer(macro250~percentCon+(1|block), data=carbon_seq_standard),
  lm(soilC~species_richness+percentAM,
    data=carbon_seq_standard)
)

```

References

- Grossman, J. J., Cavender-Bares, J., Hobbie, S. E., Reich, P. B., & Montgomery, R. A. (2017). Species richness and traits predict overyielding in stem growth in an early-successional tree diversity experiment. *Ecology*, 98(10), 2601–2614.
- Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91(1), 299–305.
- Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412(6842), 72–76.
- Niinemets, Ü., & Valladares, F. (2006). Tolerance to Shade, Drought, and Waterlogging of Temperate Northern Hemisphere Trees and Shrubs. *Ecological Monographs*, 76(4), 521–547.