# TRhizo-localAdaptation

## Microbiome x Nitrogen Model Fitting

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## Load Packages & Data

```
## Load the tidyverse
library(tidyverse)
## Packages for analysis
library(AICcmodavg)
library(broom)
library(easystats)
library(lmerTest)
## Read in data
raw.biomass.data <- read_csv(</pre>
 "data/localAdaptation-experiment_data-biomass.csv",
  col_types = c("ifiifffinn"),
 show_col_types = FALSE
raw.nodule.data <- read_csv(</pre>
  "data/localAdaptation-experiment_data-nodules.csv",
 col_types = c("ifiifffiniinnn"),
  show_col_types = FALSE
## Read in workspace
\#load("data\_analysis/M\_x\_N-model\_fitting-workspace.RData")
```

## Data Management

```
## Biomass data
biomass.data <- raw.biomass.data %>%
select(
    UID, Population, Microbiome, Nitrogen, Block,
    Aboveground_Biomass, Belowground_Biomass
) %>%
slice(-322) %>% # Remove P22, Local, N Addition [outlier, no replicates]
na.omit()

## Nodule data
nodule.data <- raw.nodule.data %>%
select(
    UID, Population, Microbiome, Nitrogen, Block,
    Nodule_Density, Percent_Fixing_Nodules, Fixing_Nodule_Density
) %>%
na.omit()
```

```
## Biomass data
write_rds(biomass.data, file = "data/cleaned_biomass_data.rds")

## Nodule data
write_rds(nodule.data, file = "data/cleaned_nodule_data.rds")
```

## **Aboveground Biomass**

## Aboveground Biomass M x N Model 1

```
aboveground.biomass.MN.LMM.1 <- lmer(
    sqrt(Aboveground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Microbiome:Population)
    + (1 | Nitrogen:Population)
    + (1 | Microbiome:Nitrogen:Population)
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Aboveground Biomass M x N Model 1 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.1)
# Model converged

## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check_model(aboveground.biomass.MN.LMM.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.LMM.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(aboveground.biomass.MN.LMM.1, effects = "random")
# M:P = Good
\# N:P = Non-normality detected (P = 0.006)
\# M:N:P = Non-normality detected (P < 0.001)
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(aboveground.biomass.MN.LMM.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.LMM.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
```

check\_outliers(aboveground.biomass.MN.LMM.1)
# 1 outlier detected

## Aboveground Biomass M x N Model 2

#### Check Aboveground Biomass M x N Model 2 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.2)
# Model failed to converge

## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.2)
# No singularity
```

```
## Visual assessment of model diagnostics
check model(aboveground.biomass.MN.LMM.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.LMM.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(aboveground.biomass.MN.LMM.2, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check heteroscedasticity(aboveground.biomass.MN.LMM.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.LMM.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(aboveground.biomass.MN.LMM.2)
# 4 outliers detected
```

## Aboveground Biomass M x N Model 3

```
aboveground.biomass.MN.LMM.3 <- lmer(
    sqrt(Aboveground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Aboveground Biomass M x N Model 3 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.3)
# Model converged

## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.3)
# No singularity

## Visual assessment of model diagnostics
check_model(aboveground.biomass.MN.LMM.3)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
```

## Aboveground Biomass M x N Model Comparisons

Table 1: AIC table comparing candidate Above ground Biomass M x N Models 1-3.

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
1	$MN\_Model\_1$	12	-666.381	0.000	1.000	0.837	345.379	0.837
3	$MN\_Model\_3$	9	-663.109	3.272	0.195	0.163	340.663	1.000
2	$MN\_Model\_2$	46	-614.756	51.625	0.000	0.000	356.104	1.000

### Aboveground Biomass M x N Model 1 Subsets

#### Aboveground Biomass M x N Model 1.1

```
## Remove (1 | Microbiome:Nitrogen:Population)
aboveground.biomass.MN.LMM.1.1 <- lmer(
    sqrt(Aboveground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Microbiome:Population)
    + (1 | Nitrogen:Population)
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Aboveground Biomass M x N Model 1.1 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.1.1)
# Model converged

## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.1.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check model(aboveground.biomass.MN.LMM.1.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.LMM.1.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(aboveground.biomass.MN.LMM.1.1, effects = "random")
# M:P = Good
\# N:P = Non-normality detected (P = 0.006)
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(aboveground.biomass.MN.LMM.1.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.LMM.1.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(aboveground.biomass.MN.LMM.1.1)
# No outliers detected
```

#### Aboveground Biomass M x N Model 1.2

```
## Remove (1 | Microbiome:Population) and (1 | Microbiome:Nitrogen:Population)
aboveground.biomass.MN.LMM.1.2 <- lmer(
    sqrt(Aboveground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Nitrogen:Population)
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Above ground Biomass M x N Model 1.2 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.1.2)
# Model converged
## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.1.2)
# No singularity
## Visual assessment of model diagnostics
check_model(aboveground.biomass.MN.LMM.1.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.LMM.1.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(aboveground.biomass.MN.LMM.1.2, effects = "random")
\# N:P = Non-normality detected (P = 0.007)
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(aboveground.biomass.MN.LMM.1.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.LMM.1.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(aboveground.biomass.MN.LMM.1.2)
# No outliers detected
```

#### Aboveground Biomass M x N Model 1.3

# No outliers detected

```
## Remove (1 | Nitrogen:Population) and (1 | Microbiome:Nitrogen:Population)
aboveground.biomass.MN.LMM.1.3 <- lmer(
    sqrt(Aboveground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Microbiome:Population)
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Aboveground Biomass M x N Model 1.3 Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.LMM.1.3)
# Model converged
## Check for boundary singularity
check_singularity(aboveground.biomass.MN.LMM.1.3)
# No singularity
## Visual assessment of model diagnostics
check_model(aboveground.biomass.MN.LMM.1.3)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.LMM.1.3, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(aboveground.biomass.MN.LMM.1.3, effects = "random")
# M:P = Good
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(aboveground.biomass.MN.LMM.1.3)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.LMM.1.3)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(aboveground.biomass.MN.LMM.1.3)
```

## Aboveground Biomass M x N Comparisons-Focal Models & Subsets

Table 2: AIC table comparing candidate Above ground Biomass M x N Model 3, Model 1, and Model 1 subsets (Above ground Biomass M x N Models 1.1-1.3).

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
3	$MN\_Model\_1.1$	11	-667.315	0.000	1.000	0.420	344.817	0.420
2	$MN\_Model\_1$	12	-666.381	0.934	0.627	0.263	345.379	0.683
5	$MN\_Model\_1.3$	10	-665.468	1.847	0.397	0.167	342.867	0.850
4	$MN\_Model\_1.2$	10	-664.417	2.898	0.235	0.099	342.341	0.949
1	$MN\_Model\_3$	9	-663.109	4.206	0.122	0.051	340.663	1.000

## **Belowground Biomass**

### Belowground Biomass M x N Model 1

```
belowground.biomass.MN.LMM.1 <- lmer(
    sqrt(Belowground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Microbiome:Population)
    + (1 | Nitrogen:Population)
    + (1 | Microbiome:Nitrogen:Population)
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Belowground Biomass M x N Model 1 Assumptions

```
## Check model convergence
check_convergence(belowground.biomass.MN.LMM.1)
# Model converged

## Check for boundary singularity
check_singularity(belowground.biomass.MN.LMM.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check_model(belowground.biomass.MN.LMM.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(belowground.biomass.MN.LMM.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(belowground.biomass.MN.LMM.1, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(belowground.biomass.MN.LMM.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(belowground.biomass.MN.LMM.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
```

check\_outliers(belowground.biomass.MN.LMM.1)
# No outliers detected

## Belowground Biomass M x N Model 2

#### Check Belowground Biomass M x N Model 2 Assumptions

```
## Check model convergence
check_convergence(belowground.biomass.MN.LMM.2)
# Model failed to converge

## Check for boundary singularity
check_singularity(belowground.biomass.MN.LMM.2)
# Singularity
```

```
## Visual assessment of model diagnostics
check model(belowground.biomass.MN.LMM.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(belowground.biomass.MN.LMM.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(belowground.biomass.MN.LMM.2, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check heteroscedasticity(belowground.biomass.MN.LMM.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(belowground.biomass.MN.LMM.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(belowground.biomass.MN.LMM.2)
# No outliers detected
```

## Belowground Biomass M x N Model 3

```
belowground.biomass.MN.LMM.3 <- lmer(
    sqrt(Belowground_Biomass) ~ Microbiome * Nitrogen
    + (1 | Population)
    + (1 | Block),
    data = biomass.data,
    REML = TRUE
)</pre>
```

#### Check Belowground Biomass M x N Model 3 Assumptions

```
## Check model convergence
check_convergence(belowground.biomass.MN.LMM.3)
# Model converged

## Check for boundary singularity
check_singularity(belowground.biomass.MN.LMM.3)
# No singularity

## Visual assessment of model diagnostics
check_model(belowground.biomass.MN.LMM.3)
# Visual check = assumptions met

## Check normality of fixed effects (Shapiro-Wilk test)
```

#### Below ground Biomass M x N Model Comparisons

Table 3: AIC table comparing candidate Below ground Biomass M x N Models 1-3.

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
3	$MN\_Model\_3$	9	-1247.024	0.000	1.000	0.937	632.621	0.937
1	$MN\_Model\_1$	12	-1241.625	5.399	0.067	0.063	633.001	1.000
2	$MN\_Model\_2$	46	-1183.050	63.974	0.000	0.000	640.251	1.000

## **Nodule Density**

## Nodule Density M x N Model 1

```
nodule.density.MN.LMM.1 <- lmer(
  log(Nodule_Density + 1) ~ Microbiome * Nitrogen
  + (1 | Microbiome:Population)
  + (1 | Nitrogen:Population)
  + (1 | Microbiome:Nitrogen:Population)
  + (1 | Population)
  + (1 | Block),
  data = nodule.data,
  REML = TRUE
)</pre>
```

#### Check Nodule Density M x N Model 1 Assumptions

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.1)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check_model(nodule.density.MN.LMM.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.LMM.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(nodule.density.MN.LMM.1, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(nodule.density.MN.LMM.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.LMM.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
```

check\_outliers(nodule.density.MN.LMM.1)
# 2 outliers detected

## Nodule Density M x N Model 2

#### Check Nodule Density M x N Model 2 Assumptions

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.2)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.2)
# Singularity
```

```
## Visual assessment of model diagnostics
check model(nodule.density.MN.LMM.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.LMM.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(nodule.density.MN.LMM.2, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check heteroscedasticity(nodule.density.MN.LMM.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.LMM.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(nodule.density.MN.LMM.2)
# 4 outliers detected
```

## Nodule Density M x N Model 3

#### Check Nodule Density M x N Model 3 Assumptions

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.3)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.3)
# No singularity

## Visual assessment of model diagnostics
check_model(nodule.density.MN.LMM.3)
# Visual check = assumptions met
```

```
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.LMM.3, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(nodule.density.MN.LMM.3, effects = "random")
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(nodule.density.MN.LMM.3)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.LMM.3)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(nodule.density.MN.LMM.3)
# No outliers detected
```

## Nodule Density M x N Model Comparisons

Table 4: AIC table comparing candidate Nodule Density M x N Models 1-3.

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
1	$MN\_Model\_1$	12	-166.465	0.000	1	1	95.421	1
3	$MN\_Model\_3$	9	-145.500	20.965	0	0	81.858	1
2	$MN\_Model\_2$	46	-123.138	43.326	0	0	110.292	1

## Nodule Density M x N Model 1 Subsets

#### Nodule Density M x N Model 1.1

```
## Remove (1 | Microbiome:Nitrogen:Population)
nodule.density.MN.LMM.1.1 <- lmer(
   log(Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Microbiome:Population)
        + (1 | Nitrogen:Population)
        + (1 | Population)
        + (1 | Block),
        data = nodule.data,
        REML = TRUE
)</pre>
```

#### Check Nodule Density M x N Model 1.1 Assumptions

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.1.1)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.1.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check model(nodule.density.MN.LMM.1.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.LMM.1.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(nodule.density.MN.LMM.1.1, effects = "random")
# M:P = Good
# N:P = Good
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(nodule.density.MN.LMM.1.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.LMM.1.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(nodule.density.MN.LMM.1.1)
# 1 outlier detected
```

#### Nodule Density M x N Model 1.2

```
## Remove (1 | Microbiome:Population) and (1 | Microbiome:Nitrogen:Population)
nodule.density.MN.LMM.1.2 <- lmer(
    log(Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Nitrogen:Population)
        + (1 | Population)
        + (1 | Block),
        data = nodule.data,
        REML = TRUE
)</pre>
```

#### Check Nodule Density M x N Model 1.2 Assumptions

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.1.2)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.1.2)
# No singularity

## Visual assessment of model diagnostics
check_model(nodule.density.MN.LMM.1.2)
# Visual check = assumptions met

## Check normality of fixed effects (Shapiro-Wilk test)
check normality(nodule.density.MN.LMM.1.2) effects = "fixed")
```

```
check_normality(nodule.density.MN.LMM.1.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(nodule.density.MN.LMM.1.2, effects = "random")
# N:P = Good
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(nodule.density.MN.LMM.1.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.LMM.1.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(nodule.density.MN.LMM.1.2)
# No outliers detected
```

#### Nodule Density M x N Model 1.3

```
## Remove (1 | Nitrogen:Population) and (1 | Microbiome:Nitrogen:Population)
nodule.density.MN.LMM.1.3 <- lmer(
    log(Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Microbiome:Population)
        + (1 | Population)
        + (1 | Block),
        data = nodule.data,
        REML = TRUE
)</pre>
```

#### Check Nodule Density M x N Model 1.3 Assumptions

check\_homogeneity(nodule.density.MN.LMM.1.3)
# Variances differ between groups (P < 0.001)</pre>

check\_outliers(nodule.density.MN.LMM.1.3)

## Check for outliers

```
## Check model convergence
check_convergence(nodule.density.MN.LMM.1.3)
# Model converged

## Check for boundary singularity
check_singularity(nodule.density.MN.LMM.1.3)
# No singularity

## Visual assessment of model diagnostics
check_model(nodule.density.MN.LMM.1.3)
# Visual check = assumptions met

## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.LMM.1.3, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)</pre>
```

## Nodule Density M x N Comparisons-Focal Models & Subsets

Table 5: AIC table comparing candidate Nodule Density M x N Model 3, Model 1, and Model 1 subsets (Nodule Density M x N Models 1.1-1.3).

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
2	$MN\_Model\_1$	12	-166.465	0.000	1.000	0.864	95.421	0.864
3	$MN\_Model\_1.1$	11	-162.147	4.318	0.115	0.100	92.233	0.964
5	$MN\_Model\_1.3$	10	-160.110	6.355	0.042	0.036	90.187	1.000
4	$MN\_Model\_1.2$	10	-146.583	19.882	0.000	0.000	83.424	1.000
1	$MN\_Model\_3$	9	-145.500	20.965	0.000	0.000	81.858	1.000

## Fixing Nodule Density

## Fixing Nodule Density M x N Model 1

#### Check Fixing Nodule Density M x N Model 1 Assumptions

```
## Check model convergence
check_convergence(fixing.nodule.density.MN.LMM.1)
# Model converged

## Check for boundary singularity
check_singularity(fixing.nodule.density.MN.LMM.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.MN.LMM.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(fixing.nodule.density.MN.LMM.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(fixing.nodule.density.MN.LMM.1, effects = "random")
# M:P = Good
# N:P = Good
\# M:N:P = Non-normality detected (P < 0.001)
\# P = Non-normality detected (P < 0.001)
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(fixing.nodule.density.MN.LMM.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(fixing.nodule.density.MN.LMM.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
```

check\_outliers(fixing.nodule.density.MN.LMM.1)
# 1 outlier detected

## Fixing Nodule Density M x N Model 2

#### Check Fixing Nodule Density M x N Model 2 Assumptions

```
## Check model convergence
check_convergence(fixing.nodule.density.MN.LMM.2)
# Model failed to converge

## Check for boundary singularity
check_singularity(fixing.nodule.density.MN.LMM.2)
# Singularity
```

```
## Visual assessment of model diagnostics
check model(fixing.nodule.density.MN.LMM.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(fixing.nodule.density.MN.LMM.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(fixing.nodule.density.MN.LMM.2, effects = "random")
# M:P = Good
# N:P = Good
# M:N:P = Good
#P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check heteroscedasticity(fixing.nodule.density.MN.LMM.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(fixing.nodule.density.MN.LMM.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(fixing.nodule.density.MN.LMM.2)
# 2 outliers detected
```

## Fixing Nodule Density M x N Model 3

```
fixing.nodule.density.MN.LMM.3 <- lmer(
  log(Fixing_Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Population)
        + (1 | Block),
    data = nodule.data,
    REML = TRUE
)</pre>
```

#### Check Fixing Nodule Density M x N Model 3 Assumptions

## Fixing Nodule Density M x N Model Comparisons

Table 6: AIC table comparing candidate Fixing Nodule Density M x N Models 1-3.

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
1	$MN\_Model\_1$	12	-913.042	0.000	1.000	0.974	468.710	0.974
3	$MN\_Model\_3$	9	-905.830	7.213	0.027	0.026	462.023	1.000
2	$MN\_Model\_2$	46	-856.870	56.173	0.000	0.000	477.158	1.000

### Fixing Nodule Density M x N Model 1 Subsets

#### Fixing Nodule Density M x N Model 1.1

```
## Remove (1 | Microbiome:Nitrogen:Population)
fixing.nodule.density.MN.LMM.1.1 <- lmer(
    log(Fixing_Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Microbiome:Population)
        + (1 | Nitrogen:Population)
        + (1 | Block),
        data = nodule.data,
        REML = TRUE
)</pre>
```

#### Check Fixing Nodule Density M x N Model 1.1 Assumptions

```
## Check model convergence
check_convergence(fixing.nodule.density.MN.LMM.1.1)
# Model converged

## Check for boundary singularity
check_singularity(fixing.nodule.density.MN.LMM.1.1)
# No singularity
```

```
## Visual assessment of model diagnostics
check model(fixing.nodule.density.MN.LMM.1.1)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(fixing.nodule.density.MN.LMM.1.1, effects = "fixed")
\# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(fixing.nodule.density.MN.LMM.1.1, effects = "random")
# M:P = Good
# N:P = Good
\# P = Non-normality detected (P < 0.001)
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(fixing.nodule.density.MN.LMM.1.1)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(fixing.nodule.density.MN.LMM.1.1)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(fixing.nodule.density.MN.LMM.1.1)
# 1 outlier detected
```

#### Fixing Nodule Density M x N Model 1.2

# 1 outlier detected

```
## Remove (1 | Microbiome:Population) and (1 | Microbiome:Nitrogen:Population)
fixing.nodule.density.MN.LMM.1.2 <- lmer(
    log(Fixing_Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Nitrogen:Population)
        + (1 | Population)
        + (1 | Block),
    data = nodule.data,
    REML = TRUE
)</pre>
```

#### Check Fixing Nodule Density M x N Model 1.2 Assumptions

```
## Check model convergence
check_convergence(fixing.nodule.density.MN.LMM.1.2)
# Model converged
## Check for boundary singularity
check_singularity(fixing.nodule.density.MN.LMM.1.2)
# No singularity
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.MN.LMM.1.2)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(fixing.nodule.density.MN.LMM.1.2, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(fixing.nodule.density.MN.LMM.1.2, effects = "random")
# N:P = Good
\# P = Non-normality detected (P < 0.001)
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(fixing.nodule.density.MN.LMM.1.2)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(fixing.nodule.density.MN.LMM.1.2)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(fixing.nodule.density.MN.LMM.1.2)
```

#### Fixing Nodule Density M x N Model 1.3

```
## Remove (1 | Nitrogen:Population) and (1 | Microbiome:Nitrogen:Population)
fixing.nodule.density.MN.LMM.1.3 <- lmer(
   log(Fixing_Nodule_Density + 1) ~ Microbiome * Nitrogen
        + (1 | Microbiome:Population)
        + (1 | Population)
        + (1 | Block),
        data = nodule.data,
        REML = TRUE
)</pre>
```

#### Check Fixing Nodule Density M x N Model 1.3 Assumptions

```
## Check model convergence
check_convergence(fixing.nodule.density.MN.LMM.1.3)
# Model converged
## Check for boundary singularity
check_singularity(fixing.nodule.density.MN.LMM.1.3)
# No singularity
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.MN.LMM.1.3)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(fixing.nodule.density.MN.LMM.1.3, effects = "fixed")
# Suggests statistical non-normality of residuals (P < 0.001)
# Mixed-effects models are robust to this assumption
## Check normality of random effects
check_normality(fixing.nodule.density.MN.LMM.1.3, effects = "random")
# M:P = Good
\# P = Non-normality detected (P < 0.001)
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(fixing.nodule.density.MN.LMM.1.3)
# Heteroscedasticity (non-constant error variance) detected (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(fixing.nodule.density.MN.LMM.1.3)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(fixing.nodule.density.MN.LMM.1.3)
# 1 outlier detected
```

## Fixing Nodule Density M x N Comparisons-Focal Models & Subsets

Table 7: AIC table comparing candidate Fixing Nodule Density M x N Model 3, Model 1, and Model 1 subsets (Fixing Nodule Density M x N Models 1.1-1.3).

	Model	K	AICc	Delta_AICc	Model_Likelihood	AICc_Weight	Residual_LL	Summed_Weight
3	$MN\_Model\_1.1$	11	-914.570	0.000	1.000	0.577	468.444	0.577
2	$MN\_Model\_1$	12	-913.042	1.527	0.466	0.269	468.710	0.846
5	$MN\_Model\_1.3$	10	-911.422	3.147	0.207	0.120	465.844	0.965
4	$MN\_Model\_1.2$	10	-908.464	6.106	0.047	0.027	464.364	0.993
1	$MN\_Model\_3$	9	-905.830	8.740	0.013	0.007	462.023	1.000

## R Session Information

Table 8: Packages required for data management and analysis.

Package	Loaded Version	Date
AICcmodavg	2.3-2	2023-03-20
bayestestR	0.13.1	2023-04-07
broom	1.0.5	2023-06-09
correlation	0.8.4	2023-04-06
datawizard	0.8.0	2023-06-16
dplyr	1.1.2	2023-04-20
easystats	0.6.0	2022-11-29
effectsize	0.8.3	2023-01-28
forcats	1.0.0	2023-01-29
ggplot2	3.4.2	2023-04-03
insight	0.19.3	2023-06-29
kableExtra	1.3.4	2021-02-20
knitr	1.43	2023-05-25
lme4	1.1-34	2023-07-04
lmerTest	3.1-3	2020-10-23
lubridate	1.9.2	2023-02-10
Matrix	1.6-0	2023-07-08
modelbased	0.8.6	2023-01-13
parameters	0.21.1	2023-05-26
performance	0.10.4	2023-06-02
purrr	1.0.1	2023-01-10
readr	2.1.4	2023-02-10
report	0.5.7	2023-03-22
see	0.8.0	2023-06-05
stringr	1.5.0	2022-12-02
tibble	3.2.1	2023-03-20
tidyr	1.3.0	2023-01-24
tidyverse	2.0.0	2023-02-22