

# TRhizo-localAdaptation

## Microbiome x Nitrogen Analyses

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## Load Packages & Data

```
## Load the tidyverse
library(tidyverse)

## Packages for analysis
library(broom)
library(easystats)
library(emmeans)
library(lme4)
library(lmerTest)

## Read in data
biomass.data <- read_rds(file = "data/cleaned_biomass_data.rds")
nodule.data <- read_rds(file = "data/cleaned_nodule_data.rds")

## Read in workspace
# load("data_analysis/2-microbiome_x_nitrogen-analyses/M_x_N-analyses-workspace.RData")
```

## Aboveground Biomass $M \times N$ Model

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

## Check Model Assumptions

```
## Check model convergence  
check_convergence(aboveground.biomass.MN.LMM)  
# Model converged  
  
## Check for boundary singularity  
check_singularity(aboveground.biomass.MN.LMM)  
# No singularity  
  
## Visual assessment of model diagnostics  
check_model(aboveground.biomass.MN.LMM)  
# Visual check = assumptions met  
  
## Check normality of fixed effects (Shapiro-Wilk test)  
check_normality(aboveground.biomass.MN.LMM, 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, 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)  
# Statistical evidence for heteroscedasticity (P < 0.001)  
  
## Check for homogeneity of variance (Bartlett test)  
check_homogeneity(aboveground.biomass.MN.LMM)  
# Variances differ between groups (P < 0.001)  
  
## Check for outliers  
check_outliers(aboveground.biomass.MN.LMM)  
# No outliers detected
```

## Marginal & Conditional R<sup>2</sup>

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(aboveground.biomass.MN.LMM)
# Marginal = 0.233
# Conditional = 0.397
```

## ANOVA

```
## Fit an ANOVA with Type III sums-of-squares
aboveground.biomass.MN.LMM.anova <- anova(
  object = aboveground.biomass.MN.LMM,
  type = "III",
  ddf = "Kenward-Roger"
)
```

Table 1: ANOVA table for the Aboveground Biomass M x N Model.

	Sums-of-Squares	Mean-Square	Num_df	Den_df	F	P-value
Microbiome	0.143	0.071	2	54.911	3.243	0.047
Nitrogen	4.148	4.148	1	27.790	188.399	0.000
Microbiome:Nitrogen	0.021	0.010	2	742.517	0.470	0.625

## Partial $\eta^2$ for Fixed Effects

Table 2: Table of the effect sizes for the fixed effects in the Aboveground Biomass M x N Model.

Term	Partial eta-squared	CI	CI_low	CI_high
Microbiome	0.106	0.95	0.001	1
Nitrogen	0.871	0.95	0.790	1
Microbiome:Nitrogen	0.001	0.95	0.000	1

## ranova

```
## Fit a ranova
aboveground.biomass.MN.LMM.ranova <- ranova(
  aboveground.biomass.MN.LMM,
  reduce.terms = FALSE
)
```

Table 3: Summary of the ranova for the Aboveground Biomass M x N Model.

	n_parameters	log_likelihood	AIC	LRT	df	P-value
<none>	11	344.817	-667.634	NA	NA	NA
(1   Microbiome:Population)	10	342.341	-664.683	4.951	1	0.026
(1   Nitrogen:Population)	10	342.867	-665.733	3.901	1	0.048
(1   Population)	10	339.662	-659.324	10.311	1	0.001
(1   Block)	10	343.030	-666.059	3.575	1	0.059

## ICC for Random Effects

Table 4: Table of the effect sizes for the random effects in the Aboveground Biomass M x N Model.

Term	ICC
Microbiome:Population	0.040
Nitrogen:Population	0.035
Population	0.126
Block	0.011

## Contrasts

```

aboveground.biomass.M.emmeans <- emmeans(
  aboveground.biomass.MN.LMM,
  specs = pairwise ~ Microbiome,
  weights = "cells",
  adjust = "none"
)

aboveground.biomass.N.emmeans <- emmeans(
  aboveground.biomass.MN.LMM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

aboveground.biomass.MxN.emmeans <- emmeans(
  aboveground.biomass.MN.LMM,
  specs = pairwise ~ Microbiome | Nitrogen,
  weights = "cells",
  adjust = "none"
)

```

Table 5: Post-hoc comparisons of the main effect of Microbiome in the Aboveground Biomass ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome	Nonlocal_R - Nonlocal_U	0	0.017	0.015	54.215	1.130	0.264
Microbiome	Nonlocal_R - Local	0	-0.020	0.015	55.428	-1.302	0.198
Microbiome	Nonlocal_U - Local	0	-0.038	0.015	55.073	-2.431	0.018

Table 6: Post-hoc comparisons of the main effect of Nitrogen in the Aboveground Biomass ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-0.181	0.013	27.777	-13.695	0

Table 7: Post-hoc comparisons of the interaction between Microbiome and Nitrogen in the Aboveground Biomass ANOVA.

Factor_1	Factor_2	Contrast	Null Value	Estimate	SE	df	t	P
Ambient_N	Microbiome	Nonlocal_R - Nonlocal_U	0	0.013	0.02	146.488	0.662	0.509
Ambient_N	Microbiome	Nonlocal_R - Local	0	-0.014	0.02	148.599	-0.712	0.478
Ambient_N	Microbiome	Nonlocal_U - Local	0	-0.027	0.02	150.022	-1.363	0.175
N_Addition	Microbiome	Nonlocal_R - Nonlocal_U	0	0.030	0.02	143.909	1.489	0.139
N_Addition	Microbiome	Nonlocal_R - Local	0	-0.022	0.02	148.399	-1.065	0.288
N_Addition	Microbiome	Nonlocal_U - Local	0	-0.051	0.02	144.436	-2.574	0.011

## Contrasts (Effect Sizes)

Table 8: Effect sizes for the contrasts by Microbiome in the Aboveground Biomass ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	0.117	0.104	54.215	-0.091	0.326
(Nonlocal_R - Local)	-0.136	0.105	55.428	-0.345	0.073
(Nonlocal_U - Local)	-0.253	0.104	55.073	-0.463	-0.044

Table 9: Effect sizes for the contrasts by Nitrogen in the Aboveground Biomass ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-1.222	0.094	27.777	-1.415	-1.029

Table 10: Effect sizes for the contrasts by Microbiome and Nitrogen in the Aboveground Biomass ANOVA.

Contrast	Nitrogen	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	Ambient_N	0.089	0.134	146.488	-0.176	0.354
(Nonlocal_R - Local)	Ambient_N	-0.096	0.135	148.599	-0.362	0.170
(Nonlocal_U - Local)	Ambient_N	-0.184	0.135	150.022	-0.452	0.083
(Nonlocal_R - Nonlocal_U)	N_Addition	0.201	0.135	143.909	-0.066	0.467
(Nonlocal_R - Local)	N_Addition	-0.145	0.136	148.399	-0.414	0.124
(Nonlocal_U - Local)	N_Addition	-0.346	0.135	144.436	-0.612	-0.080



## Belowground Biomass $M \times N$ Model

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

### Check Model Assumptions

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

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

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

```
## Check normality of fixed effects (Shapiro-Wilk test)  
check_normality(belowground.biomass.MN.LMM, 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, effects = "random")  
# P = Good  
# B = Good
```

```
## Check for non-constant variance of residuals (Breusch-Pagan test)  
check_heteroscedasticity(belowground.biomass.MN.LMM)  
# Statistical evidence for heteroscedasticity ( $P < 0.001$ )
```

```
## Check for homogeneity of variance (Bartlett test)  
check_homogeneity(belowground.biomass.MN.LMM)  
# Variances differ between groups ( $P < 0.001$ )
```

```
## Check for outliers  
check_outliers(belowground.biomass.MN.LMM)  
# No outliers detected
```

## Marginal & Conditional R<sup>2</sup>

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(belowground.biomass.MN.LMM)
# Marginal = 0.292
# Conditional = 0.376
```

## ANOVA

```
## Fit an ANOVA with Type III sums-of-squares
belowground.biomass.MN.LMM.anova <- anova(
  object = belowground.biomass.MN.LMM,
  type = "III",
  ddf = "Kenward-Roger"
)
```

Table 11: ANOVA table for the Belowground Biomass M x N Model.

	Sums-of-Squares	Mean-Square	Num_df	Den_df	F	P-value
Microbiome	0.078	0.039	2	802.275	3.338	0.036
Nitrogen	4.494	4.494	1	801.735	384.570	0.000
Microbiome:Nitrogen	0.007	0.003	2	805.884	0.292	0.747

## Partial $\eta^2$ for Fixed Effects

Table 12: Table of the effect sizes for the fixed effects in the Belowground Biomass M x N Model.

Term	Partial eta-squared	CI	CI_low	CI_high
Microbiome	0.008	0.95	0.000	1
Nitrogen	0.324	0.95	0.283	1
Microbiome:Nitrogen	0.001	0.95	0.000	1

## ranova

```
## Fit a ranova
belowground.biomass.MN.LMM.ranova <- ranova(
  belowground.biomass.MN.LMM,
  reduce.terms = FALSE
)
```

Table 13: Summary of the ranova for the Belowground Biomass M x N Model.

	n_parameters	log_likelihood	AIC	LRT	df	P-value
<none>	9	632.621	-1247.241	NA	NA	NA
(1   Population)	8	609.501	-1203.002	46.239	1	0.000
(1   Block)	8	630.225	-1244.450	4.791	1	0.029

## ICC for Random Effects

Table 14: Table of the effect sizes for the random effects in the Belowground Biomass M x N Model.

Term	ICC
Population	0.104
Block	0.015

## Contrasts

```
belowground.biomass.M.emmeans <- emmeans(
  belowground.biomass.MN.LMM,
  specs = pairwise ~ Microbiome,
  weights = "cells",
  adjust = "none"
)

belowground.biomass.N.emmeans <- emmeans(
  belowground.biomass.MN.LMM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

belowground.biomass.MxN.emmeans <- emmeans(
  belowground.biomass.MN.LMM,
  specs = pairwise ~ Microbiome | Nitrogen,
  weights = "cells",
  adjust = "none"
)
```

Table 15: Post-hoc comparisons of the main effect of Microbiome in the Belowground Biomass ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome	Nonlocal_R - Nonlocal_U	0	0.017	0.009	802.686	1.898	0.058
Microbiome	Nonlocal_R - Local	0	-0.001	0.009	800.969	-0.138	0.890
Microbiome	Nonlocal_U - Local	0	-0.019	0.009	803.199	-2.027	0.043

Table 16: Post-hoc comparisons of the main effect of Nitrogen in the Belowground Biomass ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-0.147	0.007	801.661	-19.578	0

Table 17: Post-hoc comparisons of the interaction between Microbiome and Nitrogen in the Belowground Biomass ANOVA.

Factor_1	Factor_2	Contrast	Null Value	Estimate	SE	df	t	P
Ambient_N	Microbiome	Nonlocal_R - Nonlocal_U	0	0.016	0.013	802.681	1.219	0.223
Ambient_N	Microbiome	Nonlocal_R - Local	0	-0.006	0.013	802.625	-0.475	0.635
Ambient_N	Microbiome	Nonlocal_U - Local	0	-0.022	0.013	801.563	-1.679	0.094
N_Addition	Microbiome	Nonlocal_R - Nonlocal_U	0	0.026	0.013	807.462	1.990	0.047
N_Addition	Microbiome	Nonlocal_R - Local	0	0.007	0.013	805.198	0.568	0.570
N_Addition	Microbiome	Nonlocal_U - Local	0	-0.018	0.013	805.819	-1.420	0.156

## Contrasts (Effect Sizes)

Table 18: Effect sizes for the constrasts by Microbiome in the Belowground Biomass ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	0.160	0.085	802.686	-0.006	0.327
(Nonlocal_R - Local)	-0.012	0.085	800.969	-0.179	0.155
(Nonlocal_U - Local)	-0.172	0.085	803.199	-0.339	-0.005

Table 19: Effect sizes for the constrasts by Nitrogen in the Belowground Biomass ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-1.356	0.077	801.661	-1.506	-1.205

Table 20: Effect sizes for the constrasts by Microbiome and Nitrogen in the Belowground Biomass ANOVA.

Contrast	Nitrogen	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	Ambient_N	0.146	0.120	802.681	-0.089	0.381
(Nonlocal_R - Local)	Ambient_N	-0.057	0.120	802.625	-0.293	0.179
(Nonlocal_U - Local)	Ambient_N	-0.203	0.121	801.563	-0.440	0.035
(Nonlocal_R - Nonlocal_U)	N_Addition	0.239	0.120	807.462	0.003	0.474
(Nonlocal_R - Local)	N_Addition	0.069	0.121	805.198	-0.169	0.307
(Nonlocal_U - Local)	N_Addition	-0.170	0.120	805.819	-0.404	0.065

## Nodule Density $M \times N$ Model

```
nodule.density.MN.LMM <- 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  
)
```

## Check Model Assumptions

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

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

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

```
## Check normality of fixed effects (Shapiro-Wilk test)  
check_normality(nodule.density.MN.LMM, 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, 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)  
# Statistical evidence for heteroscedasticity ( $P < 0.001$ )
```

```
## Check for homogeneity of variance (Bartlett test)  
check_homogeneity(nodule.density.MN.LMM)  
# Variances differ between groups ( $P < 0.001$ )
```

```
## Check for outliers  
check_outliers(nodule.density.MN.LMM)  
# 1 outlier detected
```

## Marginal & Conditional R<sup>2</sup>

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(nodule.density.MN.LMM)
# Marginal = 0.061
# Conditional = 0.261
```

## ANOVA

```
## Fit an ANOVA with Type III sums-of-squares
nodule.density.MN.LMM.anova <- anova(
  object = nodule.density.MN.LMM,
  type = "III",
  ddf = "Kenward-Roger"
)
```

Table 21: ANOVA table for the Nodule Density M x N Model.

	Sums-of-Squares	Mean-Square	Num_df	Den_df	F	P-value
Microbiome	0.712	0.356	2	56.305	9.257	0.000
Nitrogen	0.512	0.512	1	27.932	13.328	0.001
Microbiome:Nitrogen	0.081	0.041	2	55.584	1.057	0.354

## Partial $\eta^2$ for Fixed Effects

Table 22: Table of the effect sizes for the fixed effects in the Nodule Density M x N Model.

Term	Partial eta-squared	CI	CI_low	CI_high
Microbiome	0.247	0.95	0.089	1
Nitrogen	0.323	0.95	0.103	1
Microbiome:Nitrogen	0.037	0.95	0.000	1

## ranova

```
## Fit a ranova
nodule.density.MN.LMM.ranova <- ranova(
  nodule.density.MN.LMM,
  reduce.terms = FALSE
)
```

Table 23: Summary of the ranova for the Nodule Density M x N Model.

	n_parameters	log_likelihood	AIC	LRT	df	P-value
<none>	12	95.421	-166.842	NA	NA	NA
(1   Microbiome:Population)	11	94.614	-167.228	1.614	1	0.204
(1   Nitrogen:Population)	11	95.411	-168.822	0.019	1	0.889
(1   Microbiome:Nitrogen:Population)	11	92.233	-162.466	6.376	1	0.012
(1   Population)	11	94.472	-166.943	1.899	1	0.168
(1   Block)	11	95.226	-168.452	0.390	1	0.533

## ICC for Random Effects

Table 24: Table of the effect sizes for the random effects in the Nodule Density M x N Model.

Term	ICC
Microbiome:Nitrogen:Population	0.105
Microbiome:Population	0.053
Nitrogen:Population	0.004
Population	0.047
Block	0.003



## Contrasts

```

nodule.density.M.emmeans <- emmeans(
  nodule.density.MN.LMM,
  specs = pairwise ~ Microbiome,
  weights = "cells",
  adjust = "none"
)

nodule.density.N.emmeans <- emmeans(
  nodule.density.MN.LMM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

nodule.density.MxN.emmeans <- emmeans(
  nodule.density.MN.LMM,
  specs = pairwise ~ Microbiome | Nitrogen,
  weights = "cells",
  adjust = "none"
)

```

Table 25: Post-hoc comparisons of the main effect of Microbiome in the Nodule Density ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome	Nonlocal_R - Nonlocal_U	0	-0.042	0.025	55.837	-1.680	0.098
Microbiome	Nonlocal_R - Local	0	-0.108	0.025	56.705	-4.249	0.000
Microbiome	Nonlocal_U - Local	0	-0.065	0.025	56.353	-2.577	0.013

Table 26: Post-hoc comparisons of the main effect of Nitrogen in the Nodule Density ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	0.065	0.018	27.932	3.62	0.001

Table 27: Post-hoc comparisons of the interaction between Microbiome and Nitrogen in the Nodule Density ANOVA.

Factor_1	Factor_2	Contrast	Null Value	Estimate	SE	df	t	P
Ambient_N	Microbiome	Nonlocal_R - Nonlocal_U	0	-0.064	0.033	108.526	-1.933	0.056
Ambient_N	Microbiome	Nonlocal_R - Local	0	-0.097	0.033	109.820	-2.933	0.004
Ambient_N	Microbiome	Nonlocal_U - Local	0	-0.033	0.033	110.709	-0.999	0.320
N_Addition	Microbiome	Nonlocal_R - Nonlocal_U	0	-0.025	0.033	106.782	-0.745	0.458
N_Addition	Microbiome	Nonlocal_R - Local	0	-0.120	0.033	109.184	-3.578	0.001
N_Addition	Microbiome	Nonlocal_U - Local	0	-0.095	0.033	107.236	-2.870	0.005

## Contrasts (Effect Sizes)

Table 28: Effect sizes for the constrasts by Microbiome in the Nodule Density ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	-0.217	0.129	55.837	-0.475	0.042
(Nonlocal_R - Local)	-0.549	0.130	56.705	-0.809	-0.289
(Nonlocal_U - Local)	-0.332	0.129	56.353	-0.591	-0.073

Table 29: Effect sizes for the constrasts by Nitrogen in the Nodule Density ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	0.331	0.092	27.932	0.143	0.52

Table 30: Effect sizes for the constrasts by Microbiome and Nitrogen in the Nodule Density ANOVA.

Contrast	Nitrogen	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	Ambient_N	-0.326	0.169	108.526	-0.660	0.009
(Nonlocal_R - Local)	Ambient_N	-0.495	0.169	109.820	-0.830	-0.160
(Nonlocal_U - Local)	Ambient_N	-0.169	0.170	110.709	-0.506	0.167
(Nonlocal_R - Nonlocal_U)	N_Addition	-0.126	0.170	106.782	-0.463	0.210
(Nonlocal_R - Local)	N_Addition	-0.610	0.171	109.184	-0.950	-0.271
(Nonlocal_U - Local)	N_Addition	-0.484	0.169	107.236	-0.819	-0.149

## Fixing Nodule Density $M \times N$ Model

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

## Check Model Assumptions

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

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

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

```
## Check normality of fixed effects (Shapiro-Wilk test)  
check_normality(fixing.nodule.density.MN.LMM, 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, 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)  
# Statistical evidence for heteroscedasticity ( $P < 0.001$ )
```

```
## Check for homogeneity of variance (Bartlett test)  
check_homogeneity(fixing.nodule.density.MN.LMM)  
# Variances differ between groups ( $P < 0.001$ )
```

```
## Check for outliers  
check_outliers(fixing.nodule.density.MN.LMM)  
# 1 outlier detected
```

## Marginal & Conditional R<sup>2</sup>

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(fixing.nodule.density.MN.LMM)
# Marginal = 0.043
# Conditional = 0.198
```

## ANOVA

```
## Fit an ANOVA with Type III sums-of-squares
fixing.nodule.density.MN.LMM.anova <- anova(
  object = fixing.nodule.density.MN.LMM,
  type = "III",
  ddf = "Kenward-Roger"
)
```

Table 31: ANOVA table for the Fixing Nodule Density M x N Model.

	Sums-of-Squares	Mean-Square	Num_df	Den_df	F	P-value
Microbiome	0.332	0.166	2	55.390	10.067	0.000
Nitrogen	0.092	0.092	1	27.929	5.595	0.025
Microbiome:Nitrogen	0.015	0.007	2	741.058	0.442	0.643

## Partial $\eta^2$ for Fixed Effects

Table 32: Table of the effect sizes for the fixed effects in the Fixing Nodule Density M x N Model.

Term	Partial eta-squared	CI	CI_low	CI_high
Microbiome	0.267	0.95	0.103	1
Nitrogen	0.167	0.95	0.013	1
Microbiome:Nitrogen	0.001	0.95	0.000	1

## ranova

```
## Fit a ranova
fixing.nodule.density.MN.LMM.ranova <- ranova(
  fixing.nodule.density.MN.LMM,
  reduce.terms = FALSE
)
```

Table 33: Summary of the ranova for the Fixing Nodule Density M x N Model.

	n_parameters	log_likelihood	AIC	LRT	df	P-value
<none>	11	468.444	-914.888	NA	NA	NA
(1   Microbiome:Population)	10	464.364	-908.729	8.159	1	0.004
(1   Nitrogen:Population)	10	465.844	-911.687	5.201	1	0.023
(1   Population)	10	468.051	-916.101	0.787	1	0.375
(1   Block)	10	461.688	-903.377	13.511	1	0.000

## ICC for Random Effects

Table 34: Table of the effect sizes for the random effects in the Fixing Nodule Density M x N Model.

Term	ICC
Microbiome:Population	0.062
Nitrogen:Population	0.045
Population	0.026
Block	0.029

## Contrasts

```
fixing.nodule.density.M.emmeans <- emmeans(
  fixing.nodule.density.MN.LMM,
  specs = pairwise ~ Microbiome,
  weights = "cells",
  adjust = "none"
)

fixing.nodule.density.N.emmeans <- emmeans(
  fixing.nodule.density.MN.LMM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

fixing.nodule.density.MxN.emmeans <- emmeans(
  fixing.nodule.density.MN.LMM,
  specs = pairwise ~ Microbiome | Nitrogen,
  weights = "cells",
  adjust = "none"
)
```

Table 35: Post-hoc comparisons of the main effect of Microbiome in the Fixing Nodule Density ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome	Nonlocal_R - Nonlocal_U	0	-0.017	0.014	54.768	-1.173	0.246
Microbiome	Nonlocal_R - Local	0	-0.062	0.014	55.901	-4.323	0.000
Microbiome	Nonlocal_U - Local	0	-0.045	0.014	55.478	-3.161	0.003

Table 36: Post-hoc comparisons of the main effect of Nitrogen in the Fixing Nodule Density ANOVA.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	0.028	0.012	27.918	2.328	0.027

Table 37: Post-hoc comparisons of the interaction between Microbiome and Nitrogen in the Fixing Nodule Density ANOVA.

Factor_1	Factor_2	Contrast	Null Value	Estimate	SE	df	t	P
Ambient_N	Microbiome	Nonlocal_R - Nonlocal_U	0	-0.022	0.018	134.344	-1.237	0.218
Ambient_N	Microbiome	Nonlocal_R - Local	0	-0.073	0.018	136.472	-4.035	0.000
Ambient_N	Microbiome	Nonlocal_U - Local	0	-0.050	0.018	137.757	-2.787	0.006
N_Addition	Microbiome	Nonlocal_R - Nonlocal_U	0	-0.012	0.018	132.939	-0.685	0.494
N_Addition	Microbiome	Nonlocal_R - Local	0	-0.052	0.018	137.268	-2.854	0.005
N_Addition	Microbiome	Nonlocal_U - Local	0	-0.040	0.018	133.012	-2.204	0.029

## Contrasts (Effect Sizes)

Table 38: Effect sizes for the contrasts by Microbiome in the Fixing Nodule Density ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	-0.130	0.111	54.768	-0.353	0.092
(Nonlocal_R - Local)	-0.482	0.112	55.901	-0.707	-0.257
(Nonlocal_U - Local)	-0.352	0.112	55.478	-0.575	-0.128

Table 39: Effect sizes for the contrasts by Nitrogen in the Fixing Nodule Density ANOVA.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	0.215	0.092	27.918	0.025	0.404

Table 40: Effect sizes for the contrasts by Microbiome and Nitrogen in the Fixing Nodule Density ANOVA.

Contrast	Nitrogen	Cohen's d	SE	df	CI Lower	CI Upper
(Nonlocal_R - Nonlocal_U)	Ambient_N	-0.173	0.140	134.344	-0.449	0.104
(Nonlocal_R - Local)	Ambient_N	-0.565	0.141	136.472	-0.844	-0.287
(Nonlocal_U - Local)	Ambient_N	-0.393	0.141	137.757	-0.672	-0.113
(Nonlocal_R - Nonlocal_U)	N_Addition	-0.096	0.141	132.939	-0.374	0.182
(Nonlocal_R - Local)	N_Addition	-0.404	0.142	137.268	-0.684	-0.123
(Nonlocal_U - Local)	N_Addition	-0.308	0.140	133.012	-0.584	-0.031

## Supplementary: Estimated Marginal Means

### Aboveground Biomass $M \times N$ Model

Table 41: Estimated marginal means of the main effect of Microbiome in the Aboveground Biomass ANOVA.

Microbiome	Estimate	SE	df	t	P
Nonlocal_R	0.315	0.017	50.425	18.472	0
Nonlocal_U	0.297	0.017	49.844	17.500	0
Local	0.335	0.017	50.833	19.619	0

Table 42: Estimated marginal means of the main effect of Nitrogen in the Aboveground Biomass ANOVA.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.224	0.016	39.721	14.095	0
N_Addition	0.406	0.016	39.796	25.433	0

Table 43: Estimated marginal means of the interaction between Microbiome and Nitrogen in the Aboveground Biomass ANOVA.

Microbiome	Nitrogen	Estimate	SE	df	t	P
Nonlocal_R	Ambient_N	0.224	0.02	84.262	11.471	0
Nonlocal_U	Ambient_N	0.211	0.02	86.401	10.714	0
Local	Ambient_N	0.238	0.02	87.501	12.068	0
Nonlocal_R	N_Addition	0.409	0.02	87.719	20.598	0
Nonlocal_U	N_Addition	0.379	0.02	82.967	19.444	0
Local	N_Addition	0.431	0.02	86.776	21.783	0



## Belowground Biomass $M \times N$ Model

Table 44: Estimated marginal means of the main effect of Microbiome in the Belowground Biomass ANOVA.

Microbiome	Estimate	SE	df	t	P
Nonlocal_R	0.275	0.011	42.353	26.162	0
Nonlocal_U	0.258	0.010	41.768	24.603	0
Local	0.277	0.011	42.869	26.208	0

Table 45: Estimated marginal means of the main effect of Nitrogen in the Belowground Biomass ANOVA.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.196	0.01	32.322	19.985	0
N_Addition	0.343	0.01	32.303	34.879	0

Table 46: Estimated marginal means of the interaction between Microbiome and Nitrogen in the Belowground Biomass ANOVA.

Microbiome	Nitrogen	Estimate	SE	df	t	P
Nonlocal_R	Ambient_N	0.200	0.012	77.457	16.262	0
Nonlocal_U	Ambient_N	0.184	0.012	79.925	14.845	0
Local	Ambient_N	0.206	0.012	81.218	16.554	0
Nonlocal_R	N_Addition	0.354	0.012	81.288	28.405	0
Nonlocal_U	N_Addition	0.329	0.012	75.905	26.889	0
Local	N_Addition	0.347	0.012	80.475	27.921	0

## Nodule Density $M \times N$ Model

Table 47: Estimated marginal means of the main effect of Microbiome in the Nodule Density ANOVA.

Microbiome	Estimate	SE	df	t	P
Nonlocal_R	0.281	0.02	67.451	13.754	0
Nonlocal_U	0.324	0.02	66.331	15.886	0
Local	0.389	0.02	67.905	19.024	0

Table 48: Estimated marginal means of the main effect of Nitrogen in the Nodule Density ANOVA.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.364	0.017	40.146	21.579	0
N_Addition	0.299	0.017	40.189	17.676	0

Table 49: Estimated marginal means of the interaction between Microbiome and Nitrogen in the Nodule Density ANOVA.

Microbiome	Nitrogen	Estimate	SE	df	t	P
Nonlocal_R	Ambient_N	0.311	0.025	128.458	12.278	0
Nonlocal_U	Ambient_N	0.375	0.026	131.381	14.662	0
Local	Ambient_N	0.408	0.026	133.578	15.915	0
Nonlocal_R	N_Addition	0.251	0.026	131.202	9.689	0
Nonlocal_U	N_Addition	0.275	0.025	124.751	10.872	0
Local	N_Addition	0.370	0.026	130.742	14.505	0

## Fixing Nodule Density $M \times N$ Model

Table 50: Estimated marginal means of the main effect of Microbiome in the Fixing Nodule Density ANOVA.

Microbiome	Estimate	SE	df	t	P
Nonlocal_R	0.116	0.014	34.618	8.236	0
Nonlocal_U	0.133	0.014	34.181	9.455	0
Local	0.178	0.014	34.763	12.626	0

Table 51: Estimated marginal means of the main effect of Nitrogen in the Fixing Nodule Density ANOVA.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.156	0.013	24.851	12.132	0
N_Addition	0.128	0.013	24.856	9.977	0

Table 52: Estimated marginal means of the interaction between Microbiome and Nitrogen in the Fixing Nodule Density ANOVA.

Microbiome	Nitrogen	Estimate	SE	df	t	P
Nonlocal_R	Ambient_N	0.125	0.016	61.714	7.599	0
Nonlocal_U	Ambient_N	0.147	0.017	63.433	8.878	0
Local	Ambient_N	0.197	0.017	64.297	11.886	0
Nonlocal_R	N_Addition	0.107	0.017	64.784	6.402	0
Nonlocal_U	N_Addition	0.119	0.016	60.819	7.278	0
Local	N_Addition	0.159	0.017	63.338	9.591	0

## Export Data

```
## Export the aboveground biomass LMM
write_rds(
  aboveground.biomass.MN.LMM,
  file = "data/aboveground_biomass_MN_LMM.rds"
)

## Export the belowground biomass LMM
write_rds(
  belowground.biomass.MN.LMM,
  file = "data/belowground_biomass_MN_LMM.rds"
)

## Export the nodule density LMM
write_rds(
  nodule.density.MN.LMM,
  file = "data/nodule_density_MN_LMM.rds"
)

## Export the fixing nodule density LMM
write_rds(
  fixing.nodule.density.MN.LMM,
  file = "data/fixing_nodule_density_MN_LMM.rds"
)
```

## R Session Information

Table 53: Packages required for data management and analysis.

Package	Loaded Version	Date
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
emmeans	1.8.7	2023-06-23
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