TRhizo-localAdaptation

${\bf Microbiome} \ {\bf x} \ {\bf Nitrogen} \ {\bf Global} \ {\bf Analyses} \ ({\bf Alternate})$

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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")</pre>
nodule.data <- read_rds(file = "data/cleaned_nodule_data.rds")</pre>
## Recode to Microbiome Global to test for global effects
# Biomass data
biomass.data$Microbiome_Global<- if_else(</pre>
  biomass.data$Microbiome == "Local", "Local", "Nonlocal_Global"
# Nodule data
nodule.data$Microbiome_Global<- if_else(</pre>
  nodule.data$Microbiome == "Local", "Local", "Nonlocal_Global"
## Read in workspace
\#\ load("data\_analysis/2-microbiome\_x\_nitrogen-analyses/M\_x\_N-alternate\_global\_analyses-workspace.RData"
```

Aboveground Biomass $M \times N$ Model

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

Check Model Assumptions

```
## Check model convergence
check_convergence(aboveground.biomass.MN.global.LMM)
# Model converged
## Check for boundary singularity
check_singularity(aboveground.biomass.MN.global.LMM)
# No singularity
## Visual assessment of model diagnostics
check_model(aboveground.biomass.MN.global.LMM)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(aboveground.biomass.MN.global.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.global.LMM, effects = "random")
# M:P = Good
\# N:P = Non-normality detected (P = 0.006)
\# M:N:P = Non-normality detected (P = 0.002)
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(aboveground.biomass.MN.global.LMM)
\# Statistical evidence for heteroscedasticity (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(aboveground.biomass.MN.global.LMM)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(aboveground.biomass.MN.global.LMM)
# 2 outliers detected
```

Marginal & Conditional \mathbb{R}^2

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(aboveground.biomass.MN.global.LMM)
# Marginal = 0.230
# Conditional = 0.394
```

ANOVA

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

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_Global | 0.090 | 0.090 | 1 | 28.468 | 4.054 | 0.054 |
| Nitrogen | 3.855 | 3.855 | 1 | 32.443 | 173.774 | 0.000 |
| $Microbiome_Global:Nitrogen$ | 0.012 | 0.012 | 1 | 31.409 | 0.542 | 0.467 |

Partial η^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_Global | 0.125 | 0.95 | 0.000 | 1 |
| Nitrogen | 0.843 | 0.95 | 0.754 | 1 |
| ${\bf Microbiome_Global:Nitrogen}$ | 0.017 | 0.95 | 0.000 | 1 |

ranova

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

Table 3: Summary of the ranova for the Above ground Biomass M ${\bf x}$ N Model.

| | n _parameters | \log _likelihood | AIC | LRT | df | P-value |
|---------------------------------------------|-----------------|--------------------|----------|-------|----|---------|
| <none></none> | 10 | 349.677 | -679.353 | NA | NA | NA |
| $(1 \mid Microbiome_Global:Population)$ | 9 | 347.906 | -677.811 | 3.542 | 1 | 0.060 |
| (1 Nitrogen:Population) | 9 | 348.333 | -678.665 | 2.688 | 1 | 0.101 |
| (1 Microbiome_Global:Nitrogen:Population) | 9 | 349.677 | -681.353 | 0.000 | 1 | 1.000 |
| (1 Population) | 9 | 345.743 | -673.486 | 7.867 | 1 | 0.005 |
| (1 Block) | 9 | 347.586 | -677.171 | 4.182 | 1 | 0.041 |

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_Global:Nitrogen:Population | 0.000 |
| Nitrogen:Population | 0.037 |
| Microbiome_Global:Population | 0.044 |
| Population | 0.120 |
| Block | 0.012 |

Contrasts

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

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

aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
  aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans <- emmeans(
   aboveground.biomass.MxN.emmeans(
   aboveground.biomass.MxN.emmeans(
   aboveground.biomass.MxN.emmeans(
   aboveground.biomass.MxN.emmeans(
   aboveground.biomass.MxN.em
```

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

| Term | Contrast | Null Value | Estimate | SE | df | t | P |
|-------------------|-------------------------|------------|----------|-------|--------|-------|-------|
| Microbiome_Global | Local - Nonlocal_Global | 0 | 0.029 | 0.014 | 28.467 | 2.027 | 0.052 |

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

| Term | Contrast | Null Value | Estimate | SE | df | t | Р |
|----------|----------------------------|------------|----------|-------|--------|---------|---|
| Nitrogen | $Ambient_N - N_Addition$ | 0 | -0.181 | 0.013 | 27.838 | -13.515 | 0 |

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

| Factor_1 | Factor_2 | Contrast | Null Value | Estimate | SE | df | t | Р |
|------------|----------------------|-------------------------|------------|----------|-------|--------|-------|-------|
| Ambient_N | $Microbiome_Global$ | Local - Nonlocal_Global | 0 | 0.021 | 0.018 | 54.694 | 1.149 | 0.255 |
| N_Addition | $Microbiome_Global$ | Local - Nonlocal_Global | 0 | 0.037 | 0.018 | 53.866 | 2.045 | 0.046 |

Contrasts (Effect Sizes)

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|---------------------------|-----------|-------|--------|----------|----------|
| (Local - Nonlocal_Global) | 0.197 | 0.097 | 28.467 | -0.002 | 0.396 |

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|--------------------------|-----------|-------|--------|----------|----------|
| (Ambient_N - N_Addition) | -1.215 | 0.095 | 27.838 | -1.409 | -1.021 |

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

| Contrast | Nitrogen | Cohen's d | SE | df | CI Lower | CI Upper |
|------------------------------|---------------|-----------|-------|--------|----------|----------|
| (Local - Nonlocal_Global) | Ambient_N | 0.141 | 0.122 | 54.694 | -0.105 | 0.386 |
| $(Local - Nonlocal_Global)$ | $N_Addition$ | 0.250 | 0.123 | 53.866 | 0.005 | 0.496 |

Belowground Biomass $M \times N$ Model

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

Check Model Assumptions

No outliers detected

```
## Check model convergence
check_convergence(belowground.biomass.MN.global.LMM)
# Model converged
## Check for boundary singularity
check_singularity(belowground.biomass.MN.global.LMM)
# No singularity
## Visual assessment of model diagnostics
check_model(belowground.biomass.MN.global.LMM)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(belowground.biomass.MN.global.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.global.LMM, effects = "random")
\# P = Good
#B = Good
## Check for non-constant variance of residuals (Breusch-Pagan test)
check_heteroscedasticity(belowground.biomass.MN.global.LMM)
# Statistical evidence for heteroscedasticity (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(belowground.biomass.MN.global.LMM)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(belowground.biomass.MN.global.LMM)
```

Marginal & Conditional R²

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(belowground.biomass.MN.global.LMM)
# Marginal = 0.288
# Conditional = 0.373
```

ANOVA

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

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

| | Sums-of-Squares | Mean-Square | Num_df | $\mathrm{Den}_{-}\mathrm{df}$ | \mathbf{F} | P-value |
|----------------------------|-----------------|-------------|-----------|-------------------------------|--------------|---------|
| Microbiome_Global | 0.018 | 0.018 | 1 | 803.909 | 1.520 | 0.218 |
| Nitrogen | 3.860 | 3.860 | 1 | 805.166 | 329.021 | 0.000 |
| Microbiome_Global:Nitrogen | 0.003 | 0.003 | 1 | 806.873 | 0.255 | 0.614 |

Partial η^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_Global | 0.002 | 0.95 | 0.000 | 1 |
| Nitrogen | 0.290 | 0.95 | 0.249 | 1 |
| $Microbiome_Global:Nitrogen$ | 0.000 | 0.95 | 0.000 | 1 |

ranova

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

Table 13: Summary of the ranova for the Below ground Biomass M ${\bf x}$ N Model.

| | n _parameters | \log _likelihood | AIC | LRT | df | P-value |
|------------------|-----------------|--------------------|-----------|--------|----|---------|
| <none></none> | 7 | 636.749 | -1259.497 | NA | NA | NA |
| (1 Population) | 6 | 613.515 | -1215.031 | 46.467 | 1 | 0.000 |
| (1 Block) | 6 | 634.422 | -1256.843 | 4.654 | 1 | 0.031 |

ICC for Random Effects

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

| Term | ICC |
|---------------------|------------------|
| Population Block | $0.105 \\ 0.014$ |

Contrasts

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

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

belowground.biomass.MXN.emmeans <- emmeans(
  belowground.biomass.MXN.global.LMM,
  specs = pairwise ~ Microbiome_Global| Nitrogen,
  weights = "cells",
  adjust = "none"
)</pre>
```

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

| Term | Contrast | Null Value | Estimate | SE | df | t | P |
|-------------------|-------------------------|------------|----------|-------|---------|-------|-------|
| Microbiome_Global | Local - Nonlocal_Global | 0 | 0.01 | 0.008 | 803.923 | 1.249 | 0.212 |

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

| Term | Contrast | Null Value | Estimate | SE | df | t | Р |
|----------|------------------------|------------|----------|-------|---------|---------|---|
| Nitrogen | Ambient_N - N_Addition | 0 | -0.147 | 0.008 | 803.673 | -19.536 | 0 |

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

| Factor_1 | Factor_2 | Contrast | Null Value | Estimate | SE | df | t | P |
|------------|-------------------|-------------------------|------------|----------|-------|---------|-------|-------|
| Ambient_N | Microbiome_Global | Local - Nonlocal_Global | 0 | 0.014 | 0.011 | 803.928 | 1.228 | 0.220 |
| N_Addition | Microbiome_Global | Local - Nonlocal_Global | 0 | 0.006 | 0.011 | 807.074 | 0.514 | 0.608 |

Contrasts (Effect Sizes)

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|---------------------------|-----------|-------|---------|----------|----------|
| (Local - Nonlocal_Global) | 0.092 | 0.074 | 803.923 | -0.053 | 0.237 |

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) | -1.353 | 0.077 | 803.673 | -1.503 | -1.202 |

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

| Contrast | Nitrogen | Cohen's d | SE | df | CI Lower | CI Upper |
|------------------------------|---------------|-----------|-------|---------|----------|----------|
| (Local - Nonlocal_Global) | $Ambient_N$ | 0.128 | 0.105 | 803.928 | -0.077 | 0.334 |
| $(Local - Nonlocal_Global)$ | $N_Addition$ | 0.054 | 0.104 | 807.074 | -0.151 | 0.259 |

Nodule Density $M \times N$ Model

Check Model Assumptions

```
## Check model convergence
check convergence(nodule.density.MN.global.LMM)
# Model converged
## Check for boundary singularity
check_singularity(nodule.density.MN.global.LMM)
# Singularity
## Visual assessment of model diagnostics
check_model(nodule.density.MN.global.LMM)
# Visual check = assumptions met
## Check normality of fixed effects (Shapiro-Wilk test)
check_normality(nodule.density.MN.global.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.global.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.global.LMM)
# Statistical evidence for heteroscedasticity (P < 0.001)
## Check for homogeneity of variance (Bartlett test)
check_homogeneity(nodule.density.MN.global.LMM)
# Variances differ between groups (P < 0.001)
## Check for outliers
check_outliers(nodule.density.MN.global.LMM)
# 6 outliers detected
```

Marginal & Conditional R²

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(nodule.density.MN.global.LMM)
# Marginal = 0.055
# Conditional = 0.263
```

ANOVA

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

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

| | Sums-of-Squares | Mean-Square | Num_df | $\mathrm{Den}_{-}\mathrm{df}$ | \mathbf{F} | P-value |
|----------------------------|-----------------|-------------|-----------|-------------------------------|--------------|---------|
| Microbiome_Global | 0.694 | 0.694 | 1 | 31.390 | 18.108 | 0.000 |
| Nitrogen | 0.322 | 0.322 | 1 | 35.652 | 8.400 | 0.006 |
| Microbiome_Global:Nitrogen | 0.043 | 0.043 | 1 | 89.952 | 1.111 | 0.295 |

Partial η^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_Global | 0.366 | 0.95 | 0.151 | 1 |
| Nitrogen | 0.191 | 0.95 | 0.035 | 1 |
| $Microbiome_Global:Nitrogen$ | 0.012 | 0.95 | 0.000 | 1 |

ranova

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

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

| | n _parameters | \log _likelihood | AIC | LRT | df | P-value |
|--------------------------------------|-----------------|--------------------|----------|--------|----|---------|
| <none></none> | 10 | 97.331 | -174.663 | NA | NA | NA |
| (1 Microbiome_Global:Population) | 9 | 97.331 | -176.663 | 0.000 | 1 | 1.000 |
| (1 Nitrogen:Population) | 9 | 97.331 | -176.663 | 0.000 | 1 | 1.000 |
| (1 Microbiome:Nitrogen:Population) | 9 | 84.203 | -150.406 | 26.256 | 1 | 0.000 |
| (1 Population) | 9 | 94.936 | -171.872 | 4.791 | 1 | 0.029 |
| $(1 \mid Block)$ | 9 | 96.955 | -175.909 | 0.753 | 1 | 0.385 |

ICC for Random Effects

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

| Term | ICC |
|--------------------------------|-------|
| Microbiome:Population | 0.000 |
| Nitrogen:Population | 0.000 |
| Microbiome:Nitrogen:Population | 0.158 |
| Population | 0.058 |
| Block | 0.004 |

Contrasts

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

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

nodule.density.MxN.emmeans <- emmeans(
    nodule.density.mxN.global.LMM,
    specs = pairwise ~ Microbiome_Global| Nitrogen,
    weights = "cells",
    adjust = "none"
)</pre>
```

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

| Term | Contrast | Null Value | Estimate | SE | df | t | Р |
|-------------------|-------------------------|------------|----------|------|-------|-------|---|
| Microbiome_Global | Local - Nonlocal_Global | 0 | 0.087 | 0.02 | 31.39 | 4.252 | 0 |

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

| Term | Contrast | Null Value | Estimate | SE | df | t | Р |
|----------|------------------------|------------|----------|-------|--------|-------|-------|
| Nitrogen | Ambient_N - N_Addition | 0 | 0.066 | 0.019 | 28.085 | 3.452 | 0.002 |

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

| $Factor_1$ | Factor_2 | Contrast | Null Value | Estimate | SE | df | t | P |
|-------------|----------------------|-------------------------|------------|----------|-------|--------|-------|-------|
| Ambient_N | $Microbiome_Global$ | Local - Nonlocal_Global | 0 | 0.065 | 0.029 | 88.672 | 2.263 | 0.026 |
| N_Addition | $Microbiome_Global$ | Local - Nonlocal_Global | 0 | 0.108 | 0.029 | 87.121 | 3.754 | 0.000 |

Contrasts (Effect Sizes)

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|---------------------------|-----------|-------|-------|----------|----------|
| (Local - Nonlocal_Global) | 0.442 | 0.104 | 31.39 | 0.229 | 0.655 |

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) | 0.338 | 0.098 | 28.085 | 0.137 | 0.539 |

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

| Contrast | Nitrogen | Cohen's d | SE | df | CI Lower | CI Upper |
|------------------------------|---------------|-----------|-------|--------|----------|----------|
| (Local - Nonlocal_Global) | $Ambient_N$ | 0.333 | 0.147 | 88.672 | 0.040 | 0.625 |
| $(Local - Nonlocal_Global)$ | $N_Addition$ | 0.552 | 0.148 | 87.121 | 0.258 | 0.845 |

Fixing Nodule Density $M \times N$ Model

Check Model Assumptions

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

Marginal & Conditional \mathbb{R}^2

```
## Calculate the marginal and conditional R-squared
MuMIn::r.squaredGLMM(fixing.nodule.density.MN.global.LMM)
# Marginal = 0.041
# Conditional = 0.162
```

ANOVA

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

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

| | Sums-of-Squares | Mean-Square | Num_df | Den_df | \mathbf{F} | P-value |
|--------------------------------------------|-----------------|-------------|-----------|-----------------------------|--------------|---------|
| Microbiome_Global | 0.444 | 0.444 | 1 | 29.102 | 25.754 | 0.000 |
| Nitrogen | 0.112 | 0.112 | 1 | 32.602 | 6.524 | 0.016 |
| ${\bf Microbiome_Global:} {\bf Nitrogen}$ | 0.012 | 0.012 | 1 | 769.770 | 0.672 | 0.413 |

Partial η^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_Global | 0.469 | 0.95 | 0.244 | 1 |
| Nitrogen | 0.167 | 0.95 | 0.019 | 1 |
| ${\bf Microbiome_Global:Nitrogen}$ | 0.001 | 0.95 | 0.000 | 1 |

ranova

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

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></none> | 9 | 469.499 | -920.999 | NA | NA | NA |
| (1 Microbiome_Global:Population) | 8 | 469.298 | -922.596 | 0.403 | 1 | 0.526 |
| (1 Nitrogen:Population) | 8 | 467.268 | -918.535 | 4.463 | 1 | 0.035 |
| (1 Population) | 8 | 468.582 | -921.165 | 1.834 | 1 | 0.176 |
| (1 Block) | 8 | 461.985 | -907.969 | 15.030 | 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_Global:Population | 0.012 |
| Nitrogen:Population | 0.042 |
| Population | 0.039 |
| Block | 0.032 |

Contrasts

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

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

fixing.nodule.density.MxN.emmeans <- emmeans(
    fixing.nodule.density.MxN.emmeans (
    specs = pairwise ~ Microbiome_Global | Nitrogen,
    weights = "cells",
    adjust = "none"
)</pre>
```

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

| Term | Contrast | Null Value | Estimate | SE | df | t | Р |
|-------------------|-------------------------|------------|----------|-------|--------|-------|---|
| Microbiome_Global | Local - Nonlocal_Global | 0 | 0.053 | 0.011 | 29.103 | 5.063 | 0 |

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 | Р |
|----------|------------------------|------------|----------|-------|--------|-------|-------|
| Nitrogen | Ambient_N - N_Addition | 0 | 0.029 | 0.012 | 27.912 | 2.413 | 0.023 |

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

| Factor_1 | Factor_2 | Contrast | Null Value | Estimate | SE | df | t | P |
|------------|----------------------|-------------------------|------------|----------|-------|--------|-------|-------|
| Ambient_N | $Microbiome_Global$ | Local - Nonlocal_Global | 0 | 0.062 | 0.014 | 96.076 | 4.281 | 0.000 |
| N_Addition | Microbiome_Global | Local - Nonlocal_Global | 0 | 0.046 | 0.014 | 95.137 | 3.181 | 0.002 |

Contrasts (Effect Sizes)

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|---------------------------|-----------|-------|--------|----------|----------|
| (Local - Nonlocal_Global) | 0.407 | 0.081 | 29.103 | 0.242 | 0.573 |

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

| Contrast | Cohen's d | SE | df | CI Lower | CI Upper |
|--------------------------|-----------|------|--------|----------|----------|
| (Ambient_N - N_Addition) | 0.218 | 0.09 | 27.912 | 0.033 | 0.403 |

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

| Contrast | Nitrogen | Cohen's d | SE | df | CI Lower | CI Upper |
|------------------------------|---------------|-----------|------|--------|----------|----------|
| (Local - Nonlocal_Global) | $Ambient_N$ | 0.469 | 0.11 | 96.076 | 0.25 | 0.688 |
| $(Local - Nonlocal_Global)$ | $N_Addition$ | 0.347 | 0.11 | 95.137 | 0.13 | 0.565 |

Supplementary: Estimated Marginal Means

Aboveground Biomass $M \times N$ Model

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

| Microbiome_Global | Estimate | SE | df | t | Р |
|--------------------|----------|-------|--------|--------|---|
| Local | 0.335 | 0.017 | 49.138 | 19.443 | 0 |
| $Nonlocal_Global$ | 0.306 | 0.016 | 36.111 | 19.208 | 0 |

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

| Nitrogen | Estimate | SE | df | t | Р |
|---------------|----------|-------|--------|--------|---|
| Ambient_N | 0.224 | 0.016 | 40.118 | 13.770 | 0 |
| $N_Addition$ | 0.405 | 0.016 | 40.151 | 24.834 | 0 |

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

| ${\bf Microbiome_Global}$ | Nitrogen | Estimate | SE | df | t | Р |
|----------------------------|---------------|----------|-------|--------|--------|---|
| Local | $Ambient_N$ | 0.239 | 0.020 | 83.457 | 11.967 | 0 |
| Nonlocal_Global | $Ambient_N$ | 0.218 | 0.018 | 51.690 | 12.349 | 0 |
| Local | $N_Addition$ | 0.430 | 0.020 | 82.762 | 21.582 | 0 |
| Nonlocal_Global | $N_Addition$ | 0.393 | 0.018 | 51.756 | 22.278 | 0 |

Belowground Biomass $M \times N$ Model

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

| Microbiome_Global | Estimate | SE | df | t | Р |
|--------------------|----------|-------|--------|--------|---|
| Local | 0.276 | 0.011 | 43.229 | 26.172 | 0 |
| $Nonlocal_Global$ | 0.267 | 0.009 | 27.914 | 28.154 | 0 |

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

| Nitrogen | Estimate | SE | df | t | Р |
|------------|----------|------|--------|--------|---|
| Ambient_N | 0.196 | 0.01 | 32.576 | 19.962 | 0 |
| N_Addition | 0.343 | 0.01 | 32.556 | 34.839 | 0 |

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

| Microbiome_Global | Nitrogen | Estimate | SE | df | t | Р |
|-------------------|---------------|----------|-------|--------|--------|---|
| Local | $Ambient_N$ | 0.206 | 0.012 | 81.971 | 16.526 | 0 |
| Nonlocal_Global | $Ambient_N$ | 0.192 | 0.011 | 42.377 | 18.251 | 0 |
| Local | $N_Addition$ | 0.347 | 0.012 | 81.212 | 27.877 | 0 |
| Nonlocal_Global | $N_Addition$ | 0.341 | 0.011 | 42.376 | 32.421 | 0 |

Nodule Density $M \times N$ Model

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

| Microbiome_Global | Estimate | SE | df | t | Р |
|--------------------|----------|-------|--------|--------|---|
| Local | 0.389 | 0.020 | 68.286 | 19.532 | 0 |
| $Nonlocal_Global$ | 0.303 | 0.016 | 30.201 | 18.818 | 0 |

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

| Nitrogen | Estimate | SE | df | \mathbf{t} | Р |
|---------------|----------|-------|--------|--------------|---|
| Ambient_N | 0.364 | 0.017 | 41.516 | 20.900 | 0 |
| $N_Addition$ | 0.298 | 0.017 | 41.579 | 17.059 | 0 |

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

| Microbiome_Global | Nitrogen | Estimate | SE | df | t | Р |
|--------------------|---------------|----------|-------|---------|--------|---|
| Local | $Ambient_N$ | 0.408 | 0.026 | 134.888 | 15.696 | 0 |
| Nonlocal_Global | $Ambient_N$ | 0.343 | 0.020 | 62.361 | 17.292 | 0 |
| Local | N_Addition | 0.371 | 0.026 | 132.079 | 14.303 | 0 |
| $Nonlocal_Global$ | $N_Addition$ | 0.263 | 0.020 | 62.752 | 13.158 | 0 |

Fixing Nodule Density $M \times N$ Model

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

| Microbiome_Global | Estimate | SE | df | t | Р |
|--------------------|----------|-------|--------|--------|---|
| Local | 0.178 | 0.014 | 27.173 | 13.154 | 0 |
| $Nonlocal_Global$ | 0.124 | 0.012 | 18.445 | 10.140 | 0 |

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

| Nitrogen | Estimate | SE | df | \mathbf{t} | Р |
|------------|----------|-------|--------|--------------|---|
| Ambient_N | 0.156 | 0.013 | 23.574 | 11.931 | 0 |
| N_Addition | 0.128 | 0.013 | 23.531 | 9.749 | 0 |

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

| Microbiome_Global | Nitrogen | Estimate | SE | df | t | Р |
|-------------------|---------------|----------|-------|--------|--------|---|
| Local | $Ambient_N$ | 0.198 | 0.016 | 53.412 | 12.223 | 0 |
| Nonlocal_Global | $Ambient_N$ | 0.136 | 0.014 | 30.084 | 9.738 | 0 |
| Local | $N_Addition$ | 0.158 | 0.016 | 52.626 | 9.819 | 0 |
| Nonlocal_Global | $N_Addition$ | 0.113 | 0.014 | 30.127 | 8.050 | 0 |

Export Data

```
\hbox{\it \#\# Export the above ground biomass $LMM$}
write_rds(
 aboveground.biomass.MN.global.LMM,
 file = "data/aboveground_biomass_MN_global_LMM.rds"
## Export the belowground biomass LMM
write_rds(
 belowground.biomass.MN.global.LMM,
 file = "data/belowground_biomass_MN_global_LMM.rds"
## Export the nodule density LMM
write_rds(
 nodule.density.MN.global.LMM,
 file = "data/nodule_density_MN_global_LMM.rds"
## Export the fixing nodule density LMM
write_rds(
 fixing.nodule.density.MN.global.LMM,
  file = "data/fixing_nodule_density_MN_global_LMM.rds"
```

R Session Information

Table 53: Packages required for data management and analysis.

| Package | Loaded Version | Date |
|-----------------------------------------------------------|---------------------------------------------|--------------------------------------------------------------------|
| bayestestR broom correlation datawizard dplyr | 0.13.1 1.0.5 0.8.4 0.8.0 1.1.2 | 2023-04-07 2023-06-09 2023-04-06 2023-06-16 2023-04-20 |
| easystats effectsize emmeans forcats ggplot2 | 0.6.0 0.8.3 1.8.7 1.0.0 3.4.2 | 2022-11-29 2023-01-28 2023-06-23 2023-01-29 2023-04-03 |
| insight kableExtra knitr lme4 lmerTest | 0.19.3 1.3.4 1.43 1.1-34 3.1-3 | 2023-06-29 2021-02-20 2023-05-25 2023-07-04 2020-10-23 |
| lubridate Matrix modelbased parameters performance | 1.9.2 1.6-0 0.8.6 0.21.1 0.10.4 | 2023-02-10 2023-07-08 2023-01-13 2023-05-26 2023-06-02 |
| purrr readr report see stringr | 1.0.1 2.1.4 0.5.7 0.8.0 1.5.0 | 2023-01-10 2023-02-10 2023-03-22 2023-06-05 2022-12-02 |
| tibble tidyr tidyverse | 3.2.1 1.3.0 2.0.0 | 2023-03-20 2023-01-24 2023-02-22 |