TRhizo-localAdaptation

Microbiome Analyses

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Contents

Load Packages & Data

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

## Packages for analyses
library(broom)
library(car)
library(easystats)
library(emmeans)
library(phyloseq)
library(tidyamplicons)
library(vegan)
```

Load Data

```
## Load the data
# Root fitness variables
root.fitness.variable.data <- read_csv(
  "data/localAdaptation-microbiome_data-root.csv",
  show_col_types = FALSE
) %>%
  select(UID, Population, Microbiome:Fixing_Nodule_Density)

# Root microbiome data
root.microbiome.sample.data <- read_csv(
  "data/localAdaptation-microbiome_data-root.csv",
  show_col_types = FALSE
) %>%
  select(Sequence_ID, UID, Population, Microbiome, Nitrogen)

# Soil local adaptation BLUPs
# Aboveground biomass
aboveground.biomass.uncleaned.BLUPs <- read_rds(
  file = "data/aboveground_biomass_uncleaned_BLUPs.rds"
)
# Belowground biomass
belowground.biomass.uncleaned.BLUPs <- read_rds(
  file = "data/belowground_biomass_uncleaned_BLUPs.rds"
)
# Nodule density
nodule.density.uncleaned.BLUPs <- read_rds(
  file = "data/nodule_density_uncleaned_BLUPs.rds"
)
# Fixing nodule density
fixing.nodule.density.uncleaned.BLUPs <- read_rds(
  file = "data/fixing_nodule_density_uncleaned_BLUPs.rds"
)

# Soil microbiome data
soil.microbiome.sample.data <- read_csv(
  "data/localAdaptation-microbiome_data-soil.csv",
  show_col_types = FALSE
) %>%
  select(Sequence_ID, Population, Inoculant_Type)

# ASV data
root.ASV.table <- read_rds("data_analysis/8-microbiome_analyses/root_ASV_abundance_table.rds")
soil.ASV.table <- read_rds("data_analysis/8-microbiome_analyses/soil_ASV_abundance_table.rds")

# Taxonomy tables
root.taxonomy.table <- read_rds("data_analysis/8-microbiome_analyses/root_ASV_taxonomy_table.rds")
soil.taxonomy.table <- read_rds("data_analysis/8-microbiome_analyses/soil_ASV_taxonomy_table.rds")

## Add re-coded Microbiome variable to calculate global nonlocal effects
# Fitness variables
root.fitness.variable.data$Microbiome_Global <- (
  if_else(root.fitness.variable.data$Microbiome == "Local", "Local", "Nonlocal_Global")
```

```
)

# Root microbiome data
root.microbiome.sample.data$Microbiome_Global <- (
  if_else(root.microbiome.sample.data$Microbiome == "Local", "Local", "Nonlocal_Global", "NULL")
)

## Set function to calculate relative abundances
relative_abundance <- function(x) {
  x / sum(x)
}
```

phyloseq & tidyamplicons Processing

Root

```
## Set sample metadata
# Sample and treatment identifiers
root.sample.metadata <- root.microbiome.sample.data %>%
  select(UID, Population, Microbiome, Nitrogen)
# Set row names for phyloseq processing
rownames(root.sample.metadata) <- root.microbiome.sample.data$Sequence_ID

## Set phyloseq components
# ASV
root.ASV.table.phyloseq <- otu_table(root.ASV.table, taxa_are_rows = FALSE)
# Taxonomy
root.taxonomy.table.phyloseq <- tax_table(root.taxonomy.table)
# Sample data
root.sample.data.phyloseq <- sample_data(root.sample.metadata)
rownames(root.sample.data.phyloseq) <- root.microbiome.sample.data$Sequence_ID

## Set the reference phyloseq object
root.phyloseq.reference <- phyloseq(
  otu_table(root.ASV.table.phyloseq, taxa_are_rows = FALSE),
  sample_data(root.sample.data.phyloseq),
  tax_table(root.taxonomy.table.phyloseq)
) %>%
  subset_taxa(Kingdom == "Bacteria")

## Get the number of reads per sample
# Set sample metadata for correct merging
root.sample.reads.metadata <- root.sample.metadata %>%
  rownames_to_column(var = "Sequence_ID")
# Get the number of reads and merge with sample data
root.sample.reads <- sample_sums(root.phyloseq.reference) %>%
  as.data.frame() %>%
  rownames_to_column(var = "Sequence_ID") %>%
  rename(Root_Sample_Reads = 2) %>%
  as_tibble() %>%
  full_join(root.sample.reads.metadata, by = "Sequence_ID")

## Convert the phyloseq reference to tidyamplicons
root.tidyamplicon.base.microbiome.data <- as_tidyamplicons(root.phyloseq.reference)
```

Soil

```
## Set sample metadata
# Sample and treatment identifiers
soil.sample.metadata <- soil.microbiome.sample.data %>%
  select(Population, Inoculant_Type)
# Set row names for phyloseq processing
rownames(soil.sample.metadata) <- soil.microbiome.sample.data$Sequence_ID

## Set phyloseq components
# ASV
soil.ASV.table.phyloseq <- otu_table(soil.ASV.table, taxa_are_rows = FALSE)
# Taxonomy
soil.taxonomy.table.phyloseq <- tax_table(soil.taxonomy.table)
# Sample data
soil.sample.data.phyloseq <- sample_data(soil.sample.metadata)
rownames(soil.sample.data.phyloseq) <- soil.microbiome.sample.data$Sequence_ID

## Set the reference phyloseq object
soil.phyloseq.reference <- phyloseq(
  otu_table(soil.ASV.table.phyloseq, taxa_are_rows = FALSE),
  sample_data(soil.sample.data.phyloseq),
  tax_table(soil.taxonomy.table.phyloseq)
)

## Get the number of reads per sample
# Set sample metadata for correct merging
soil.sample.reads.metadata <- soil.sample.metadata %>%
  rownames_to_column(var = "Sequence_ID")
# Get the number of reads and merge with sample data
soil.sample.reads <- sample_sums(soil.phyloseq.reference) %>%
  as.data.frame() %>%
  rownames_to_column(var = "Sequence_ID") %>%
  rename(Soil_Sample_Reads = 2) %>%
  as_tibble() %>%
  full_join(soil.sample.reads.metadata, by = "Sequence_ID")

## Convert the phyloseq reference to tidyamplicons
soil.tidyamplicon.base.microbiome.data <- as_tidyamplicons(soil.phyloseq.reference)
```

Fitness & Rhizobia Abundances

Data Management

Note: summed abundances and relative abundances across all ASVs identified to Rhizobium in the sample

```
## Add relative abundances
root.tidyamplicon.base.microbiome.data <- add_rel_abundance(root.tidyamplicon.base.microbiome.data)

## Root abundances
root.abundances <- abundances(root.tidyamplicon.base.microbiome.data)

## Root taxa
root.taxa <- taxa(root.tidyamplicon.base.microbiome.data)

## Set tibble of abundances of only Rhizobium
root.fitness.by.rhizobium.data <- root.abundances %>%
  full_join(root.taxa, by = "taxon_id") %>%
  full_join(root.tidyamplicon.base.microbiome.data$samples, by = "sample_id") %>%
  filter(genus == "Rhizobium") %>%
  select(UID, sample, Population:Nitrogen, genus, abundance, rel_abundance) %>%
  rename(Sequence_ID = sample, Genus = genus, Abundance = abundance, Relative_Abundance = rel_abundance
  group_by(Population, Microbiome, Nitrogen) %>%
  summarise(
    Summed_Abundance = sum(Abundance),
    Summed_Relative_Abundance = sum(Relative_Abundance),
    .groups = "keep"
  ) %>%
  full_join(
    select(root.fitness.variable.data, Population:Microbiome_Global),
    by = c("Population", "Microbiome", "Nitrogen")
  ) %>%
  full_join(
    root.sample.reads,
    by = c("Population", "Microbiome", "Nitrogen")
  ) %>%
  select(
    Sequence_ID, UID, Population:Nitrogen, Root_Sample_Reads, Summed_Abundance:Microbiome_Global
  )

## Export data for figures
write_rds(
  root.fitness.by.rhizobium.data,
  file = "data/fitness_by_rhizobia_data.rds"
)
```

Aboveground Biomass

Fit the Linear Models

```
## Fit the aboveground biomass by rhizobium abundance linear model
aboveground.biomass.by.rhizobium.LM <- lm(
  log(Aboveground_Biomass) ~ Summed_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)

## Fit the aboveground biomass by rhizobium relative abundance linear model
aboveground.biomass.by.rhizobium.RA.LM <- lm(
  log(Aboveground_Biomass) ~ Summed_Relative_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(aboveground.biomass.by.rhizobium.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(aboveground.biomass.by.rhizobium.LM)
# Normality of residuals (P = 0.163)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(aboveground.biomass.by.rhizobium.LM)
# No statistical evidence for heteroscedasticity (P = 0.446)

## Check for outliers
check_outliers(aboveground.biomass.by.rhizobium.LM)
# 1 outlier detected
```

```
## Visual assessment of model diagnostics
check_model(aboveground.biomass.by.rhizobium.RA.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(aboveground.biomass.by.rhizobium.RA.LM)
# Normality of residuals (P = 0.204)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(aboveground.biomass.by.rhizobium.RA.LM)
# No statistical evidence for heteroscedasticity (P = 0.480)

## Check for outliers
check_outliers(aboveground.biomass.by.rhizobium.RA.LM)
# No outliers detected
```

ANOVAs

```
## Fit ANOVAs with Type III sums-of-squares
# Aboveground biomass by rhizobium abundance linear model
aboveground.biomass.by.rhizobium.LM.ANOVA <- Anova(
  mod = aboveground.biomass.by.rhizobium.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)

# Aboveground biomass by rhizobium relative abundance linear model
aboveground.biomass.by.rhizobium.RA.LM.ANOVA <- Anova(
  mod = aboveground.biomass.by.rhizobium.RA.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 1: ANOVA table for the aboveground biomass by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	61.158	1	48.480	0.000
Summed_Abundance	0.666	1	0.528	0.470
Nitrogen	6.043	1	4.790	0.033
Root_Sample_Reads	2.674	1	2.120	0.151
Summed_Abundance:Nitrogen	0.178	1	0.141	0.709
Residuals	68.121	54	NA	NA

Table 2: ANOVA table for the aboveground biomass by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	37.586	1	29.616	0.000
Summed_Relative_Abundance	0.583	1	0.460	0.501
Nitrogen	4.301	1	3.389	0.071
Root_Sample_Reads	1.640	1	1.292	0.261
Summed_Relative_Abundance:Nitrogen	0.407	1	0.321	0.574
Residuals	68.532	54	NA	NA

Effect Sizes

Table 3: Table of effect sizes for the terms in the aboveground biomass by rhizobium abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Abundance	0.010	0.95	0.000	1
Nitrogen	0.081	0.95	0.004	1
Root_Sample_Reads	0.038	0.95	0.000	1
Summed_Abundance:Nitrogen	0.003	0.95	0.000	1

Table 4: Table of effect sizes for the terms in the aboveground biomass by rhizobium relative abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Relative_Abundance	0.008	0.95	0	1
Nitrogen	0.059	0.95	0	1
Root_Sample_Reads	0.023	0.95	0	1
Summed_Relative_Abundance:Nitrogen	0.006	0.95	0	1

Belowground Biomass

```
## Fit the belowground biomass by rhizobium abundance linear model
belowground.biomass.by.rhizobium.LM <- lm(
  log(Belowground_Biomass) ~ Summed_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)

## Fit the belowground biomass by rhizobium relative abundance linear model
belowground.biomass.by.rhizobium.RA.LM <- lm(
  log(Belowground_Biomass) ~ Summed_Relative_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(belowground.biomass.by.rhizobium.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(belowground.biomass.by.rhizobium.LM)
# Normality of residuals (P = 0.487)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(belowground.biomass.by.rhizobium.LM)
# No statistical evidence for heteroscedasticity (P = 0.061)

## Check for outliers
check_outliers(belowground.biomass.by.rhizobium.LM)
# No outliers detected
```

```
## Visual assessment of model diagnostics
check_model(belowground.biomass.by.rhizobium.RA.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(belowground.biomass.by.rhizobium.RA.LM)
# Normality of residuals (P = 0.450)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(belowground.biomass.by.rhizobium.RA.LM)
# Statistical evidence for heteroscedasticity (P = 0.045)

## Check for outliers
check_outliers(belowground.biomass.by.rhizobium.RA.LM)
# No outliers detected
```

ANOVAs

```
## Fit ANOVAs with Type III sums-of-squares
# Aboveground biomass by rhizobium abundance linear model
belowground.biomass.by.rhizobium.LM.ANOVA <- Anova(
  mod = belowground.biomass.by.rhizobium.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)

# Aboveground biomass by rhizobium relative abundance linear model
belowground.biomass.by.rhizobium.RA.LM.ANOVA <- Anova(
  mod = belowground.biomass.by.rhizobium.RA.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 5: ANOVA table for the belowground biomass by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	89.248	1	114.430	0.000
Summed_Abundance	0.007	1	0.009	0.924
Nitrogen	8.909	1	11.423	0.001
Root_Sample_Reads	4.573	1	5.864	0.019
Summed_Abundance:Nitrogen	0.109	1	0.140	0.709
Residuals	42.116	54	NA	NA

Table 6: ANOVA table for the belowground biomass by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	63.191	1	81.844	0.000
Summed_Relative_Abundance	0.209	1	0.271	0.605
Nitrogen	9.707	1	12.572	0.001
Root_Sample_Reads	4.496	1	5.823	0.019
Summed_Relative_Abundance:Nitrogen	0.461	1	0.598	0.443
Residuals	41.693	54	NA	NA

Effect Sizes

Table 7: Table of effect sizes for the terms in the belowground biomass by rhizobium abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Abundance	0.000	0.95	0.000	1
Nitrogen	0.175	0.95	0.047	1
Root_Sample_Reads	0.098	0.95	0.009	1
Summed_Abundance:Nitrogen	0.003	0.95	0.000	1

Table 8: Table of effect sizes for the terms in the belowground biomass by rhizobium relative abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Relative_Abundance	0.005	0.95	0.000	1
Nitrogen	0.189	0.95	0.056	1
Root_Sample_Reads	0.097	0.95	0.009	1
Summed_Relative_Abundance:Nitrogen	0.011	0.95	0.000	1

Nodule Density

```
## Fit the nodule density by rhizobium abundance linear model
nodule.density.by.rhizobium.LM <- lm(
  log(Nodule_Density + 1) ~ Summed_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)

## Fit the nodule density by rhizobium relative abundance linear model
nodule.density.by.rhizobium.RA.LM <- lm(
  log(Nodule_Density + 1) ~ Summed_Relative_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(nodule.density.by.rhizobium.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(nodule.density.by.rhizobium.LM)
# Normality of residuals (P = 0.161)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(nodule.density.by.rhizobium.LM)
# No statistical evidence for heteroscedasticity (P = 0.213)

## Check for outliers
check_outliers(nodule.density.by.rhizobium.LM)
# No outliers detected
```

```
## Visual assessment of model diagnostics
check_model(nodule.density.by.rhizobium.RA.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(nodule.density.by.rhizobium.RA.LM)
# Non-normality of residuals detected (P = 0.042)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(nodule.density.by.rhizobium.RA.LM)
# No statistical evidence for heteroscedasticity (P = 0.127)

## Check for outliers
check_outliers(nodule.density.by.rhizobium.RA.LM)
# No outliers detected
```

ANOVAs

```
## Fit ANOVAs with Type III sums-of-squares
# Aboveground biomass by rhizobium abundance linear model
nodule.density.by.rhizobium.LM.ANOVA <- Anova(
  mod = nodule.density.by.rhizobium.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)

# Aboveground biomass by rhizobium relative abundance linear model
nodule.density.by.rhizobium.RA.LM.ANOVA <- Anova(
  mod = nodule.density.by.rhizobium.RA.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 9: ANOVA table for the nodule density by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	0.760	1	21.193	0.000
Summed_Abundance	0.027	1	0.741	0.393
Nitrogen	0.053	1	1.488	0.228
Root_Sample_Reads	0.043	1	1.197	0.279
Summed_Abundance:Nitrogen	0.000	1	0.012	0.913
Residuals	1.937	54	NA	NA

Table 10: ANOVA table for the nodule density by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	0.244	1	6.977	0.011
Summed_Relative_Abundance	0.118	1	3.388	0.071
Nitrogen	0.001	1	0.016	0.901
Root_Sample_Reads	0.003	1	0.089	0.767
Summed_Relative_Abundance:Nitrogen	0.051	1	1.449	0.234
Residuals	1.886	54	NA	NA

Effect Sizes

Table 11: Table of effect sizes for the terms in the nodule density by rhizobium abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Abundance	0.014	0.95	0	1
Nitrogen	0.027	0.95	0	1
Root_Sample_Reads	0.022	0.95	0	1
Summed_Abundance:Nitrogen	0.000	0.95	0	1

Table 12: Table of effect sizes for the terms in the nodule density by rhizobium relative abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Relative_Abundance	0.059	0.95	0	1
Nitrogen	0.000	0.95	0	1
Root_Sample_Reads	0.002	0.95	0	1
Summed_Relative_Abundance:Nitrogen	0.026	0.95	0	1

Fixing Nodule Density

```
## Fit the fixing nodule density by rhizobium abundance linear model
fixing.nodule.density.by.rhizobium.LM <- lm(
  log(Fixing_Nodule_Density + 1) ~ Summed_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)

## Fit the fixing nodule density by rhizobium relative abundance linear model
fixing.nodule.density.by.rhizobium.RA.LM <- lm(
  log(Fixing_Nodule_Density + 1) ~ Summed_Relative_Abundance * Nitrogen + Root_Sample_Reads,
  data = root.fitness.by.rhizobium.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.by.rhizobium.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(fixing.nodule.density.by.rhizobium.LM)
# Non-normality of residuals detected (P < 0.001)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(fixing.nodule.density.by.rhizobium.LM)
# No statistical evidence for heteroscedasticity (P = 0.210)

## Check for outliers
check_outliers(fixing.nodule.density.by.rhizobium.LM)
# No outliers detected
```

```
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.by.rhizobium.RA.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(fixing.nodule.density.by.rhizobium.RA.LM)
# Non-normality of residuals detected (P < 0.001)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(fixing.nodule.density.by.rhizobium.RA.LM)
# No statistical evidence for heteroscedasticity (P = 0.574)

## Check for outliers
check_outliers(fixing.nodule.density.by.rhizobium.RA.LM)
# No outliers detected
```

ANOVAs

```
## Fit ANOVAs with Type III sums-of-squares
# Aboveground biomass by rhizobium abundance linear model
fixing.nodule.density.by.rhizobium.LM.ANOVA <- Anova(
  mod = fixing.nodule.density.by.rhizobium.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)

# Aboveground biomass by rhizobium relative abundance linear model
fixing.nodule.density.by.rhizobium.RA.LM.ANOVA <- Anova(
  mod = fixing.nodule.density.by.rhizobium.RA.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 13: ANOVA table for the fixing nodule density by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	0.212	1	10.253	0.002
Summed_Abundance	0.013	1	0.644	0.426
Nitrogen	0.004	1	0.195	0.661
Root_Sample_Reads	0.043	1	2.072	0.156
Summed_Abundance:Nitrogen	0.007	1	0.343	0.560
Residuals	1.115	54	NA	NA

Table 14: ANOVA table for the fixing nodule density by rhizobium abundance and nitrogen treatment, with the number of reads as a covariate.

	Sums-of-Squares	df	F	P-value
(Intercept)	0.069	1	3.447	0.069
Summed_Relative_Abundance	0.049	1	2.447	0.124
Nitrogen	0.000	1	0.011	0.918
Root_Sample_Reads	0.021	1	1.037	0.313
Summed_Relative_Abundance:Nitrogen	0.031	1	1.563	0.217
Residuals	1.073	54	NA	NA

Effect Sizes

Table 15: Table of effect sizes for the terms in the fixing nodule density by rhizobium abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Abundance	0.012	0.95	0	1
Nitrogen	0.004	0.95	0	1
Root_Sample_Reads	0.037	0.95	0	1
Summed_Abundance:Nitrogen	0.006	0.95	0	1

Table 16: Table of effect sizes for the terms in the fixing nodule density by rhizobium relative abundance model.

Term	Partial eta-squared	CI	CI Low	CI High
Summed_Relative_Abundance	0.043	0.95	0	1
Nitrogen	0.000	0.95	0	1
Root_Sample_Reads	0.019	0.95	0	1
Summed_Relative_Abundance:Nitrogen	0.028	0.95	0	1

Fitness by Microbiome Dissimilarity

Data Management

```
## Set the soil abundance matrix
soil.base.abundance.matrix <- abundances_matrix(
  soil.tidyamplicon.base.microbiome.data,
  value = abundance,
  sample_name = sample,
  taxon_name = taxon
)

## Set the sample IDs
soil.abundance.matrix.sample.IDs <- rownames(soil.base.abundance.matrix) %>%
  as_tibble()

## Convert the abundance matrix to a tibble and add the sample IDs
soil.abundance.matrix <- soil.base.abundance.matrix %>%
  as_tibble() %>%
    bind_cols(soil.abundance.matrix.sample.IDs) %>%
  rename(Sequence_ID = value) %>%
    drop_na()

## Merge sample data with the abundance matrix
soil.community.composition.data <- soil.sample.metadata %>%
  rownames_to_column(var = "Sequence_ID") %>%
  left_join(soil.abundance.matrix, by = "Sequence_ID") %>%
  left_join(soil.sample.reads %>%select(Sequence_ID, Soil_Sample_Reads), by = "Sequence_ID") %>%
    filter(Inoculant_Type == "Local" | Inoculant_Type == "Rural" | Inoculant_Type == "Urban") %>%
    filter(Population != "P25")
```

Calculate Fitness Responses (LA Indices)

```
## Aboveground biomass
aboveground.biomass.LA.global.data <- aboveground.biomass.uncleaned.BLUPs %>%
  select(Population, Microbiome_Global, Estimate) %>%
  group_by(Population, Microbiome_Global) %>%
  summarise(AG_Biomass = mean(Estimate), .groups = "keep") %>%
  pivot_wider(names_from = Microbiome_Global, values_from = AG_Biomass) %>%
  mutate(AG_Biomass_LA_Global = Local - Nonlocal_Global) %>%
  ungroup() %>%
    select(1, 4)

## Belowground biomass
belowground.biomass.LA.global.data <- belowground.biomass.uncleaned.BLUPs %>%
  select(Population, Microbiome_Global, Estimate) %>%
  group_by(Population, Microbiome_Global) %>%
  summarise(BG_Biomass = mean(Estimate), .groups = "keep") %>%
  pivot_wider(names_from = Microbiome_Global, values_from = BG_Biomass) %>%
  mutate(BG_Biomass_LA_Global = Local - Nonlocal_Global) %>%
  ungroup() %>%
    select(1, 4)

## Nodule density
nodule.density.LA.global.data <- nodule.density.uncleaned.BLUPs %>%
  select(Population, Microbiome_Global, Estimate) %>%
  group_by(Population, Microbiome_Global) %>%
  summarise(Nod_Density = mean(Estimate), .groups = "keep") %>%
  pivot_wider(names_from = Microbiome_Global, values_from = Nod_Density) %>%
  mutate(Nod_Density_LA_Global = Local - Nonlocal_Global) %>%
  ungroup() %>%
    select(1, 4)

## Fixing nodule density
fixing.nodule.density.LA.global.data <- fixing.nodule.density.uncleaned.BLUPs %>%
  select(Population, Microbiome_Global, Estimate) %>%
  group_by(Population, Microbiome_Global) %>%
  summarise(Fix_Nod_Density = mean(Estimate), .groups = "keep") %>%
  pivot_wider(names_from = Microbiome_Global, values_from = Fix_Nod_Density) %>%
  mutate(Fix_Nod_Density_LA_Global = Local - Nonlocal_Global) %>%
  ungroup() %>%
    select(1, 4)

## Combine into a single dataframe
soil.fitness.variable.data <- aboveground.biomass.LA.global.data %>%
    full_join(belowground.biomass.LA.global.data, by = "Population") %>%
    full_join(nodule.density.LA.global.data, by = "Population") %>%
    full_join(fixing.nodule.density.LA.global.data, by = "Population") %>%
    filter(Population != "P25")
```

Calculate Microbiome Dissimilarities

```
## Set data for analyses
# Local microbiome
local.community.composition.data <- soil.community.composition.data %>%
    filter(Inoculant_Type == "Local")
# Nonlocal microbiome
nonlocal.community.composition.data <- soil.community.composition.data %>%
    filter(Inoculant_Type != "Local")
```

```
## Set the function
soil_microbiome_BC_dissimilarity_function <- function(local_df, nonlocal_df) {

    ## Set empty dataframe for results
    soil.BC.dissimilarity.data <- data.frame("BC_Dissimilarity" = numeric(length = 29))

    ## Set the nonlocal (rural, urban, or rural + urban) microbiome
  nonlocal.microbiome <- nonlocal_df %>%
   replace(is.na(.), 0) %>%
   summarise(across(everything(), mean)) %>%
   select(-c(Inoculant_Type:Population, Sequence_ID))

  # Remove any columns with 0
  nonlocal.microbiome <- nonlocal.microbiome[, colSums(nonlocal.microbiome != 0) > 0]

  ## Set to X
  for (x in 1:29) {

    ## Set the local microbiome
    local.microbiome <- local_df[x, 4:14301] %>%
        replace(is.na(.), 0)
    # Remove any columns with 0
    local.microbiome <- local.microbiome[, colSums(local.microbiome != 0) > 0]

    ## Combine local and nonlocal into single abundance matrix
    community.matrix <- bind_rows(local.microbiome, nonlocal.microbiome) %>%
        replace(is.na(.), 0)

    ## Calculate pairwise BC dissimilarity distance
    local.vs.nonlocal.BC.distance <- vegdist(
        community.matrix,
        method = "bray"
    )

    ## Export the pairwise BC distance
    soil.BC.dissimilarity.data[x, ] <- local.vs.nonlocal.BC.distance
  }

    ## Set vector of pairwise BC distance
    pairwise.BC.data <- as_tibble(soil.BC.dissimilarity.data)

}
```

```r
## Local vs nonlocal dissimilarity
local.vs.nonlocal.dissimilarity <- soil_microbiome_BC_dissimilarity_function(
  local_df = local.community.composition.data,
  nonlocal_df = nonlocal.community.composition.data
) %>%
  mutate(Population = local.community.composition.data$Population) %>%
  type_convert(col_types = c("nf"))

## Combine fitness and dissimilarity data
fitness.by.soil.dissimarility.data <- soil.fitness.variable.data %>%
  full_join(local.vs.nonlocal.dissimilarity, by = "Population") %>%
  left_join(soil.sample.reads, by = "Population") %>%
  select(
    Population, AG_Biomass_LA_Global:Fix_Nod_Density_LA_Global,
    BC_Dissimilarity, Soil_Sample_Reads
  )

## Export data for figures
write_rds(
    fitness.by.soil.dissimarility.data,
    file = "data/fitness_by_soil_dissimarility_data.rds"
)
```

Aboveground Biomass

Fit the Linear Model

```
## Fit the aboveground biomass by microbiome dissimilarity model
aboveground.biomass.by.BC.dissimilarity.LM <- lm(
  AG_Biomass_LA_Global ~ BC_Dissimilarity + Soil_Sample_Reads,
  data = fitness.by.soil.dissimarility.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(aboveground.biomass.by.BC.dissimilarity.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(aboveground.biomass.by.BC.dissimilarity.LM)
# Non-normality of residuals detected (P < 0.001)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(aboveground.biomass.by.BC.dissimilarity.LM)
# Good

## Check for outliers
check_outliers(aboveground.biomass.by.BC.dissimilarity.LM)
# No outliers detected
```

Model Summary

Table 17: Summary of the aboveground biomass local adaptation global index by microbiome dissimilarity, with the number of reads as a covariate.

Term	Estimate	SE	t	P-value
(Intercept)	0.106	0.130	0.816	0.422
BC_Dissimilarity	-0.115	0.139	-0.833	0.412
Soil_Sample_Reads	0.000	0.000	-0.311	0.758

Effect Sizes

Table 18: Table of effect sizes for the terms in the aboveground biomass local adaptation global index by microbiome dissimilarity model. Adjusted R-squared < 0.001

Term	Standardize Slope	CI	CI Low	CI High
(Intercept)	0.000	0.95	-0.390	0.390
BC_Dissimilarity	-0.232	0.95	-0.805	0.341
Soil_Sample_Reads	-0.087	0.95	-0.660	0.486

Belowground Biomass

Fit the Linear Model

```
## Fit the belowground biomass by microbiome dissimilarity model
belowground.biomass.by.BC.dissimilarity.LM <- lm(
  BG_Biomass_LA_Global ~ BC_Dissimilarity + Soil_Sample_Reads,
  data = fitness.by.soil.dissimarility.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(belowground.biomass.by.BC.dissimilarity.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(belowground.biomass.by.BC.dissimilarity.LM)
# Good

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(belowground.biomass.by.BC.dissimilarity.LM)
# Good

## Check for outliers
check_outliers(belowground.biomass.by.BC.dissimilarity.LM)
# No outliers detected
```

Model Summary

Table 19: Summary of the belowground biomass local adaptation global index by microbiome dissimilarity, with the number of reads as a covariate.

Term	Estimate	SE	t	P-value
(Intercept)	0.034	0.051	0.658	0.517
BC_Dissimilarity	-0.036	0.055	-0.662	0.514
Soil_Sample_Reads	0.000	0.000	-0.401	0.692

Effect Sizes

Table 20: Table of effect sizes for the terms in the belowground biomass local adaptation global index by microbiome dissimilarity model. Adjusted R-squared < 0.001

Term	Standardize Slope	CI	CI Low	CI High
(Intercept)	0.000	0.95	-0.393	0.393
BC_Dissimilarity	-0.186	0.95	-0.763	0.391
Soil_Sample_Reads	-0.113	0.95	-0.690	0.465

Nodule Density

Fit the Linear Model

```
## Fit the nodule density by microbiome dissimilarity model
nodule.density.by.BC.dissimilarity.LM <- lm(
  Nod_Density_LA_Global ~ BC_Dissimilarity + Soil_Sample_Reads,
  data = fitness.by.soil.dissimarility.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(nodule.density.by.BC.dissimilarity.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(nodule.density.by.BC.dissimilarity.LM)
# Good

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(nodule.density.by.BC.dissimilarity.LM)
# Good

## Check for outliers
check_outliers(nodule.density.by.BC.dissimilarity.LM)
# No outliers detected
```

Model Summary

Table 21: Summary of the nodule density local adaptation global index by microbiome dissimilarity, with the number of reads as a covariate.

Term	Estimate	SE	t	P-value
(Intercept)	-0.201	0.289	-0.696	0.493
BC_Dissimilarity	0.204	0.309	0.662	0.514
Soil_Sample_Reads	0.000	0.000	1.032	0.312

Effect Sizes

Table 22: Table of effect sizes for the terms in the nodule density local adaptation global index by microbiome dissimilarity model. Adjusted R-squared < 0.001

Term	Standardize Slope	CI	CI Low	CI High
(Intercept)	0.000	0.95	-0.388	0.388
BC_Dissimilarity	0.184	0.95	-0.387	0.755
Soil_Sample_Reads	0.287	0.95	-0.284	0.857

Fixing Nodule Density

Fit the Linear Models

```
## Fit the fixing Nodule Density by microbiome dissimilarity model
fixing.nodule.density.by.BC.dissimilarity.LM <- lm(
  Fix_Nod_Density_LA_Global ~ BC_Dissimilarity + Soil_Sample_Reads,
  data = fitness.by.soil.dissimarility.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(fixing.nodule.density.by.BC.dissimilarity.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(fixing.nodule.density.by.BC.dissimilarity.LM)
# Good

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(fixing.nodule.density.by.BC.dissimilarity.LM)
# Good

## Check for outliers
check_outliers(fixing.nodule.density.by.BC.dissimilarity.LM)
# No outliers detected
```

Model Summary

Table 23: Summary of the fixing nodule density local adaptation global index by microbiome dissimilarity, with the number of reads as a covariate.

Term	Estimate	SE	t	P-value
(Intercept)	-0.038	0.063	-0.610	0.547
BC_Dissimilarity	0.040	0.067	0.598	0.555
Soil_Sample_Reads	0.000	0.000	0.638	0.529

Effect Sizes

Table 24: Table of effect sizes for the terms in the fixing nodule density local adaptation global index by microbiome dissimilarity model. Adjusted R-squared < 0.001

Term	Standardize Slope	CI	CI Low	CI High
(Intercept)	0.000	0.95	-0.393	0.393
BC_Dissimilarity	0.168	0.95	-0.409	0.746
Soil_Sample_Reads	0.179	0.95	-0.398	0.757

Rhizobium Abundance by Microbiome & Nitrogen

Fit the (Generalized) Linear Models

```
## Fit the rhizobium abundance by microbiome and nitrogen model
rhizobium.abundance.GLM <- glm(
  Summed_Abundance ~ Microbiome_Global * Nitrogen,
  data = root.fitness.by.rhizobium.data,
  family = poisson(link = "log")
)

## Fit the rhizobium relative abundance by microbiome and nitrogen model
rhizobium.relative.abundance.LM <- lm(
  Summed_Relative_Abundance ~ Microbiome_Global * Nitrogen,
  data = root.fitness.by.rhizobium.data
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(rhizobium.abundance.GLM)
# Overdispersion
# Residuals could be improved

## Check for overdispersion
check_overdispersion(rhizobium.abundance.GLM)
# Overdispersion detected
```

```
## Visual assessment of model diagnostics
check_model(rhizobium.relative.abundance.LM)
# Visual check = assumptions met

## Check normality of predictors (Shapiro-Wilk test)
check_normality(rhizobium.relative.abundance.LM)
# Non-normality of residuals detected (P < 0.001)

## Check for non-constant variance of residuals (i.e., heteroscedasticity)
check_heteroscedasticity(rhizobium.relative.abundance.LM)
# Statistical evidence for heteroscedasticity (P < 0.001)

## Check for outliers
check_outliers(rhizobium.relative.abundance.LM)
# 1 outlier detected
```

ANOVAs

```
## Fit ANOVAs with Type III sums-of-squares
# Rhizobium abundance by microbiome and nitrogen model
rhizobium.abundance.GLM.ANOVA <- Anova(
  mod = rhizobium.abundance.GLM,
  type = "III",
  test.statistic = "Wald",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)

# Rhizobium relative abundance by microbiome and nitrogen model
rhizobium.relative.abundance.LM.ANOVA <- Anova(
  mod = rhizobium.relative.abundance.LM,
  type = "III",
  test.statistic = "F",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 25: ANOVA table for rhizobium abundance by microbiome, nitrogen, and the interaction.

	df	chi-squared	P-value
(Intercept)	1	1155839.252	0.00
Microbiome_Global	1	0.769	0.38
Nitrogen	1	39.060	0.00
Microbiome_Global:Nitrogen	1	373.176	0.00

Table 26: ANOVA table for rhizobium relative abundance by microbiome, nitrogen, and the interaction.

	Sums-of-Squares	df	F	P-value
(Intercept)	0.013	1	5.783	0.020
Microbiome_Global	0.000	1	0.034	0.854
Nitrogen	0.003	1	1.097	0.299
Microbiome_Global:Nitrogen	0.001	1	0.367	0.547
Residuals	0.127	55	NA	NA

Effect Sizes

Table 27: Table of effect sizes (Cohen's w) for the terms in the rhizobium abundance by microbiome and nitrogen model.

Term	Cohens_w
Intercept	138.795
Microbiome	0.113
Nitrogen	0.807
Microbiome x Nitrogen	2.494

Table 28: Table of effect sizes (partial eta-squared) for the terms in the rhizobium relative abundance by microbiome and nitrogen model.

Term	Partial eta-squared	CI	CI Low	CI High
Microbiome_Global	0.001	0.95	0	1
Nitrogen	0.020	0.95	0	1
Microbiome_Global:Nitrogen	0.007	0.95	0	1

Community Composition by Microbiome & Nitrogen

Data Management

Note: summed abundances and relative abundances across all ASVs identified to Rhizobium in the sample

```
## Convert abundance to relative abundance
root.phyloseq.reference.relativized <- transform_sample_counts(
  root.phyloseq.reference,
  relative_abundance
)
```

NMDS Ordination

```
## Calculate Bray-Curtis Distance
root.BC.distance.matrix <- distance(
  root.phyloseq.reference.relativized,
  method = "bray"
)

## Set tibble with scores and predictor variables
root.BC.distance.data <- scores(root.BC.distance.matrix) %>%
  as.data.frame() %>%
  rownames_as_column(var = "Sequence_ID") %>%
  full_join(root.microbiome.sample.data, by = "Sequence_ID") %>%
  select(Sequence_ID, Population:Microbiome_Global, LA1:LA9)

## Export NMDS data for figures
write_rds(
  root.BC.distance.data,
  file = "data/root_BC_distance_data.rds"
)
```

```
## NMDS Ordination
root.BC.NMDS <- ordinate(
  root.phyloseq.reference.relativized,
  method = "NMDS",
  distance = "bray",
  k = 3
)

## Export NMDS data for figures
write_rds(
  root.BC.NMDS,
  file = "data/root_BC_NMDS.rds"
)
```

PERMANOVAs

```
## PERMANOVA by microbiome, nitrogen, and the interaction
root.community.composition.PERMANOVA <- adonis2(
  root.BC.distance.matrix ~ Microbiome * Nitrogen,
  data = root.BC.distance.data,
  permutations = 10000
)

## PERMANOVA by microbiome (global), nitrogen, and the interaction
root.community.composition.global.PERMANOVA <- adonis2(
  root.BC.distance.matrix ~ Microbiome_Global * Nitrogen,
  data = root.BC.distance.data,
  permutations = 10000
)
```

Table 29: Summary of the PERMANOVA comparing root composition by microbiome, nitrogen, and the two-way interaction.

Term	df	Sums-of-Squares	R2	F	P-value
Microbiome	2	0.549	0.029	0.853	0.815
Nitrogen	1	0.297	0.016	0.924	0.561
Microbiome:Nitrogen	2	0.803	0.042	1.248	0.082
Residual	54	17.385	0.913	NA	NA
Total	59	19.034	1.000	NA	NA

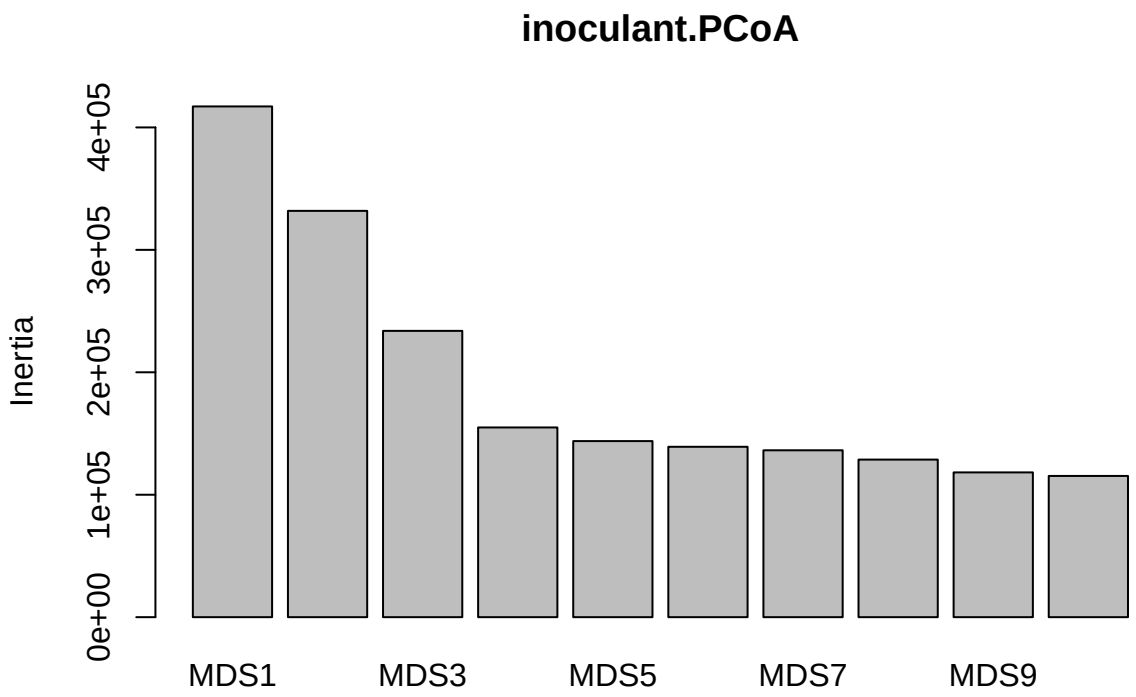
Table 30: Summary of the PERMANOVA comparing root composition by microbiome (local vs. nonlocal global), nitrogen, and the two-way interaction.

Term	df	Sums-of-Squares	R2	F	P-value
Microbiome_Global	1	0.256	0.013	0.793	0.824
Nitrogen	1	0.297	0.016	0.921	0.569
Microbiome_Global:Nitrogen	1	0.394	0.021	1.220	0.162
Residual	56	18.087	0.950	NA	NA
Total	59	19.034	1.000	NA	NA

Inoculant Community Composition

```
## Set the base abundance matrix for the inoculant communities
inoculant.base.abundance.matrix <- abundances_matrix(
  soil.tidyamplicon.base.microbiome.data,
  value = abundance,
  sample_name = sample,
  taxon_name = taxon
)

## Set the sample IDs
inoculant.abundance.matrix.sample.IDs <- rownames(inoculant.base.abundance.matrix) %>%
  as_tibble()

## Convert the abundance matrix to a tibble and add the sample IDs
inoculant.abundance.matrix <- inoculant.base.abundance.matrix %>%
  as_tibble() %>%
  bind_cols(inoculant.abundance.matrix.sample.IDs) %>%
  rename(Sequence_ID = value)

## Inoculant and control data (N Addition and Ambient N)
inoculant.community.composition.data <- soil.sample.metadata %>%
  rownames_to_column(var = "Sequence_ID") %>%
  left_join(inoculant.abundance.matrix, by = "Sequence_ID") %>%
  full_join(soil.sample.reads %>% select(Sequence_ID, Soil_Sample_Reads), by = "Sequence_ID") %>%
  slice(-20) # Remove P25 (missing data)

## Export data for figures
write_rds(
  inoculant.community.composition.data,
  file = "data/inoculant_community_composition_data.rds"
)
```

```
## Conduct a Principal Coordinates Analysis (PCoA)
inoculant.PCoA <- capscale(
  inoculant.abundance.matrix %>% select(-Sequence_ID) ~ 1,
  data = inoculant.abundance.matrix
)

## Check the screeplot
screeplot(inoculant.PCoA)
```




```
# Sharp reduction and plateau in inertia after MDS3

## Get eigenvalue summary
inoculant.PCoA.eigen.values <- eigenvals(inoculant.PCoA) %>%
  summary()
# MDS1-MDS2 explain ~18.9% of the variation in the data
# MDS1 = 10.5%
# MDS2 = 8.4%
# Poor ordination, but caveated with drastic differences in group representation

## Export PCoA for figures
write_rds(
  inoculant.PCoA,
  file = "data/inoculant_PCoA.rds"
)
```

Inoculant Rhizobium Abundances

Data Management

```
## Root abundances
inoculant.abundances <- abundances(soil.tidyamplicon.base.microbiome.data)

## Root taxa
inoculant.taxa <- taxa(soil.tidyamplicon.base.microbiome.data)

## Set tibble of abundances of only Rhizobium
inoculant.rhizobium.abundance.data <- inoculant.abundances %>%
  full_join(inoculant.taxa, by = "taxon_id") %>%
  full_join(soil.tidyamplicon.base.microbiome.data$samples, by = "sample_id") %>%
  filter(genus == "Rhizobium") %>%
  select(Population, Inoculant_Type, abundance) %>%
  rename(Rhizobium_Abundance = abundance) %>%
  group_by(Population, Inoculant_Type) %>%
  summarise(
    Summed_Rhizobium_Abundance = sum(Rhizobium_Abundance),
    .groups = "keep"
  )

## Set tibble of samples with no rhizobia
inoculant.rhizobium.abundance.supplement.data <- tibble(
  Population = c(
    "Ambient_N-1", "N_Addition-1", "N_Addition-2", "N_Addition-3",
    "P1", "P4", "P6", "P8", "P12", "P14", "P16", "P18", "P23", "P30",
    "P34", "P43", "P45", "P48"
  ),
  Inoculant_Type = c("Ambient_N", rep("N_Addition", 3), rep("Local", 14)),
  Summed_Rhizobium_Abundance = c(rep(0, 18))
)

## Bind all inoculant vs. control data into a single tibble
inoculant.rhizobium.abundance.full.data <- bind_rows(
  inoculant.rhizobium.abundance.data,
  inoculant.rhizobium.abundance.supplement.data
)

## Export data for figures
write_rds(
  inoculant.rhizobium.abundance.data,
  file = "data/inoculant_rhizobium_abundance_data.rds"
)
```

Fit the Generalized Linear Model

```
## Fit the rhizobium abundance by inoculant and control
inoculant.rhizobium.abundance.GLM <- glm(
  Summed_Rhizobium_Abundance ~ Inoculant_Type,
  data = inoculant.rhizobium.abundance.full.data,
  family = poisson(link = "log")
)
```

Check Model Assumptions

```
## Visual assessment of model diagnostics
check_model(inoculant.rhizobium.abundance.GLM)
# Residuals could be improved but are tolerable

## Check for overdispersion
check_overdispersion(inoculant.rhizobium.abundance.GLM)
# Overdispersion detected
```

ANOVA

```
## Fit ANOVA with Type III sums-of-squares
inoculant.rhizobium.abundance.GLM.ANOVA <- Anova(
  mod = inoculant.rhizobium.abundance.GLM,
  type = "III",
  test.statistic = "Wald",
  contrasts = list(topic = contr.sum, sys = contr.sum)
)
```

Table 31: ANOVA table for rhizobium abundance by inoculant and control communities.

	df	chi-squared	P-value
(Intercept)	1	4890.397	0
Inoculant_Type	4	617.394	0

Effect Sizes

Table 32: Table of effect sizes (Cohen's w) for the terms in the rhizobium abundance by inoculant communities.

Term	Cohens_w
Intercept	10.425
Inoculant Type	3.704

Supplementary: Fitness by Rhizobium Estimated Marginal Means

Aboveground Biomass

```
## Set the estimated marginal means
# Nitrogen
aboveground.biomass.by.rhizobium.N.emmeans <- emmeans(
  aboveground.biomass.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Nitrogen x Rhizobium
aboveground.biomass.by.rhizobium.NR.emmeans <- emtrends(
  aboveground.biomass.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  var = "Summed_Abundance",
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 33: Estimated marginal means of the main effect of nitrogen in the aboveground biomass by rhizobium model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	-3.067	0.212	54	-14.464	0
N_Addition	-1.929	0.206	54	-9.362	0

Table 34: Estimated marginal trends of the interaction between nitrogen and rhizobium abundance in the aboveground biomass by rhizobium model.

Nitrogen	Rhizobia Trend	SE	df	t	P
Ambient_N	-0.000108	0.000149	54	-0.726815	0.470479
N_Addition	-0.000045	0.000080	54	-0.568100	0.572322

Contrasts

Table 35: Post-hoc comparisons of the main effect of nitrogen in the aboveground biomass by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-1.138	0.296	54	-3.845	0

Table 36: Post-hoc comparisons of the interaction between nitrogen and rhizobium abundance in the aboveground biomass by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-6.2e-05	0.000166	54	-0.375375	0.708852

Contrast Effect Sizes

Table 37: Effect sizes for the constrasts by nitrogen in the aboveground biomass by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-1.013	0.281	54	-1.577	-0.45

Table 38: Effect sizes for the constrasts by nitrogen and rhizobia in the aboveground biomass by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-5.6e-05	0.000148	54	-0.000353	0.000242

Belowground Biomass

```
## Set the estimated marginal means
# Nitrogen
belowground.biomass.by.rhizobium.N.emmeans <- emmeans(
  belowground.biomass.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Nitrogen x Rhizobium
belowground.biomass.by.rhizobium.NR.emmeans <- emtrends(
  belowground.biomass.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  var = "Summed_Abundance",
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 39: Estimated marginal means of the main effect of nitrogen in the belowground biomass by rhizobium model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	-3.331	0.167	54	-19.976	0
N_Addition	-2.229	0.162	54	-13.754	0

Table 40: Estimated marginal trends of the interaction between nitrogen and rhizobium abundance in the belowground biomass by rhizobium model.

Nitrogen	Rhizobia Trend	SE	df	t	P
Ambient_N	1.1e-05	0.000117	54	0.095988	0.923885
N_Addition	-3.8e-05	0.000063	54	-0.600991	0.550360

Contrasts

Table 41: Post-hoc comparisons of the main effect of nitrogen in the belowground biomass by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-1.102	0.233	54	-4.736	0

Table 42: Post-hoc comparisons of the interaction between nitrogen and rhizobium abundance in the belowground biomass by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	4.9e-05	0.000131	54	0.374669	0.709374

Contrast Effect Sizes

Table 43: Effect sizes for the constrasts by nitrogen in the belowground biomass by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-1.248	0.29	54	-1.829	-0.667

Table 44: Effect sizes for the constrasts by nitrogen and rhizobia in the belowground biomass by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	5.6e-05	0.000148	54	-0.000242	0.000353

Nodule Density

```
## Set the estimated marginal means
# Nitrogen
nodule.density.by.rhizobium.N.emmeans <- emmeans(
  nodule.density.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Nitrogen x Rhizobium
nodule.density.by.rhizobium.NR.emmeans <- emtrends(
  nodule.density.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  var = "Summed_Abundance",
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 45: Estimated marginal means of the main effect of nitrogen in the nodule density by rhizobium model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.355	0.036	54	9.915	0
N_Addition	0.268	0.035	54	7.702	0

Table 46: Estimated marginal trends of the interaction between nitrogen and rhizobium abundance in the nodule density by rhizobium model.

Nitrogen	Rhizobia Trend	SE	df	t	P
Ambient_N	2.2e-05	2.5e-05	54	0.860791	0.393159
N_Addition	2.5e-05	1.3e-05	54	1.825838	0.073406

Contrasts

Table 47: Post-hoc comparisons of the main effect of nitrogen in the nodule density by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	0.087	0.05	54	1.741	0.087

Table 48: Post-hoc comparisons of the interaction between nitrogen and rhizobium abundance in the nodule density by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-3e-06	2.8e-05	54	-0.109912	0.912886

Contrast Effect Sizes

Table 49: Effect sizes for the constrasts by nitrogen in the nodule density by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	0.459	0.267	54	-0.077	0.995

Table 50: Effect sizes for the constrasts by nitrogen and rhizobia in the nodule density by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-1.6e-05	0.000148	54	-0.000313	0.000281

Fixing Nodule Density

```
## Set the estimated marginal means
# Nitrogen
fixing.nodule.density.by.rhizobium.N.emmeans <- emmeans(
  fixing.nodule.density.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Nitrogen x Rhizobium
fixing.nodule.density.by.rhizobium.NR.emmeans <- emtrends(
  fixing.nodule.density.by.rhizobium.LM,
  specs = pairwise ~ Nitrogen,
  var = "Summed_Abundance",
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 51: Estimated marginal means of the main effect of nitrogen in the fixing nodule density by rhizobium model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.161	0.027	54	5.951	0
N_Addition	0.108	0.026	54	4.088	0

Table 52: Estimated marginal trends of the interaction between nitrogen and rhizobium abundance in the fixing nodule density by rhizobium model.

Nitrogen	Rhizobia Trend	SE	df	t	P
Ambient_N	1.5e-05	1.9e-05	54	0.802249	0.425926
N_Addition	3.0e-06	1.0e-05	54	0.270412	0.787874

Contrasts

Table 53: Post-hoc comparisons of the main effect of nitrogen in the fixing nodule density by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	0.054	0.038	54	1.418	0.162

Table 54: Post-hoc comparisons of the interaction between nitrogen and rhizobium abundance in the fixing nodule density by rhizobium model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	1.2e-05	2.1e-05	54	0.585846	0.56042

Contrast Effect Sizes

Table 55: Effect sizes for the constrasts by nitrogen in the fixing nodule density by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	0.374	0.266	54	-0.16	0.907

Table 56: Effect sizes for the constrasts by nitrogen and rhizobia in the fixing nodule density by rhizobium model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	8.7e-05	0.000148	54	-0.000211	0.000384

Supplementary: Rhizobium Abundance by Microbiome & Nitrogen Estimated Marginal Means

Rhizobium Abundance

```
## Set the estimated marginal means
# Microbiome
rhizobium.abundance.M.emmeans <- emmeans(
  rhizobium.abundance.GLM,
  specs = pairwise ~ Microbiome_Global,
  weights = "cells",
  adjust = "none"
)

# Nitrogen
rhizobium.abundance.N.emmeans <- emmeans(
  rhizobium.abundance.GLM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Microbiome x Nitrogen
rhizobium.abundance.MN.emmeans <- emmeans(
  rhizobium.abundance.GLM,
  specs = pairwise ~ Nitrogen | Microbiome_Global,
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 57: Estimated marginal means of the main effect of microbiome in the rhizobium abundance by microbiome and nitrogen model.

Microbiome	Estimate	SE	df	t	P
Local	7.632	0.005	Inf	1549.888	0
Nonlocal_Global	7.743	0.003	Inf	2309.742	0

Table 58: Estimated marginal means of the main effect of nitrogen in the rhizobium abundance by microbiome and nitrogen model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	7.596	0.004	Inf	1825.019	0
N_Addition	7.811	0.004	Inf	2119.537	0

Table 59: Estimated marginal means of the interaction between microbiome and rhizobium abundance in the rhizobium abundance by microbiome and nitrogen model.

Nitrogen	Microbiome	Estimate	SE	df	t	P
Ambient_N	Local	7.601152	0.007070	Inf	1075.100	0
N_Addition	Local	7.662703	0.006856	Inf	1117.688	0
Ambient_N	Nonlocal_Global	7.593480	0.005149	Inf	1474.759	0
N_Addition	Nonlocal_Global	7.885480	0.004337	Inf	1818.251	0

Contrasts

Table 60: Post-hoc comparisons of the main effect of microbiome in the rhizobium abundance by microbiome and nitrogen model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome_Global	Local - Nonlocal_Global	0	-0.111	0.006	Inf	-18.683	0

Table 61: Post-hoc comparisons of the main effect of nitrogen in the rhizobium abundance by microbiome and nitrogen model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-0.215	0.006	Inf	-38.691	0

Table 62: Post-hoc comparisons of the interaction between microbiome and nitrogen abundance in the rhizobium abundance by microbiome and nitrogen model.

Microbiome	Term	Contrast	Null Value	Estimate	SE	df	t	P
Local	Nitrogen	Ambient_N - N_Addition	0	-0.062	0.010	Inf	-6.250	0
Nonlocal_Global	Nitrogen	Ambient_N - N_Addition	0	-0.292	0.007	Inf	-43.375	0

Contrast Effect Sizes

Table 63: Effect sizes for the constrasts by microbiome in the rhizobium abundance by microbiome and nitrogen model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Local - Nonlocal_Global)	-0.003	0	Inf	-0.003	-0.002

Table 64: Effect sizes for the constrasts by nitrogen in the rhizobium abundance by microbiome and nitrogen model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-0.005	0.001	Inf	-0.007	-0.004

Table 65: Effect sizes for the constrasts by microbiome and nitrogen and rhizobia in the rhizobium abundance by microbiome and nitrogen model.

Contrast	Microbiome	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	Local	-0.001561	0.000291	Inf	-0.002131	-0.000991
(Ambient_N - N_Addition)	Nonlocal_Global	-0.007406	0.000726	Inf	-0.008830	-0.005982

Rhizobium Relative Abundance

```
## Set the estimated marginal means
# Microbiome
rhizobium.relative.abundance.M.emmeans <- emmeans(
  rhizobium.relative.abundance.LM,
  specs = pairwise ~ Microbiome_Global,
  weights = "cells",
  adjust = "none"
)

# Nitrogen
rhizobium.relative.abundance.N.emmeans <- emmeans(
  rhizobium.relative.abundance.LM,
  specs = pairwise ~ Nitrogen,
  weights = "cells",
  adjust = "none"
)

# Microbiome x Nitrogen
rhizobium.relative.abundance.MN.emmeans <- emmeans(
  rhizobium.relative.abundance.LM,
  specs = pairwise ~ Nitrogen | Microbiome_Global,
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means & Trends

Table 66: Estimated marginal means of the main effect of microbiome in the rhizobium relative abundance by microbiome and nitrogen model.

Microbiome	Estimate	SE	df	t	P
Local	0.048	0.011	55	4.448	0
Nonlocal_Global	0.043	0.008	55	5.632	0

Table 67: Estimated marginal means of the main effect of nitrogen in the rhizobium relative abundance by microbiome and nitrogen model.

Nitrogen	Estimate	SE	df	t	P
Ambient_N	0.039	0.009	55	4.350	0
N_Addition	0.051	0.009	55	5.777	0

Table 68: Estimated marginal means of the interaction between microbiome and rhizobium relative abundance in the rhizobium relative abundance by microbiome and nitrogen model.

Nitrogen	Microbiome	Estimate	SE	df	t	P
Ambient_N	Local	0.036606	0.015223	55	2.404723	0.019578
N_Addition	Local	0.059155	0.015223	55	3.885977	0.000276
Ambient_N	Nonlocal_Global	0.040078	0.011044	55	3.629069	0.000624
N_Addition	Nonlocal_Global	0.046582	0.010764	55	4.327555	0.000064

Contrasts

Table 69: Post-hoc comparisons of the main effect of microbiome in the rhizobium relative abundance by microbiome and nitrogen model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Microbiome_Global	Local - Nonlocal_Global	0	0.004	0.013	55	0.337	0.737

Table 70: Post-hoc comparisons of the main effect of nitrogen in the rhizobium relative abundance by microbiome and nitrogen model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Nitrogen	Ambient_N - N_Addition	0	-0.012	0.013	55	-0.949	0.347

Table 71: Post-hoc comparisons of the interaction between microbiome and nitrogen abundance in the rhizobium relative abundance by microbiome and nitrogen model.

Microbiome	Term	Contrast	Null Value	Estimate	SE	df	t	P
Local	Nitrogen	Ambient_N - N_Addition	0	-0.023	0.022	55	-1.047	0.299
Nonlocal_Global	Nitrogen	Ambient_N - N_Addition	0	-0.007	0.015	55	-0.422	0.675

Contrast Effect Sizes

Table 72: Effect sizes for the constrasts by microbiome in the rhizobium relative abundance by microbiome and nitrogen model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Local - Nonlocal_Global)	0.093	0.275	55	-0.459	0.644

Table 73: Effect sizes for the constrasts by nitrogen in the rhizobium relative abundance by microbiome and nitrogen model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	-0.247	0.261	55	-0.771	0.277

Table 74: Effect sizes for the constrasts by microbiome and nitrogen and rhizobia in the rhizobium relative abundance by microbiome and nitrogen model.

Contrast	Microbiome	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - N_Addition)	Local	-0.468414	0.449438	55	-1.369108	0.432281
(Ambient_N - N_Addition)	Nonlocal_Global	-0.135105	0.320621	55	-0.777643	0.507433

Supplementary: Inoculant Rhizobium Abundances Estimated Marginal Means

```
## Set the estimated marginal means
inoculant.rhizobium.abundance.emmeans <- emmeans(
  inoculant.rhizobium.abundance.GLM,
  specs = pairwise ~ Inoculant_Type,
  weights = "cells",
  adjust = "none"
)
```

Estimated Marginal Means

Table 75: Estimated marginal means by community type in the rhizobium abundance by inoculant and control communities model.

Community Type	Estimate	SE	df	t	P
Ambient_N	4.423	0.063	Inf	69.931	0.000
Local	4.017	0.023	Inf	171.991	0.000
N_Addition	-13.303	270.964	Inf	-0.049	0.961
Rural	4.147	0.063	Inf	65.964	0.000
Urban	5.349	0.049	Inf	109.762	0.000

Contrasts

Table 76: Post-hoc comparisons by community type in the rhizobium abundance by inoculant and control communities model.

Term	Contrast	Null Value	Estimate	SE	df	t	P
Inoculant_Type	Ambient_N - Local	0	0.406	0.067	Inf	6.017	0.000
Inoculant_Type	Ambient_N - N_Addition	0	17.725	270.964	Inf	0.065	0.948
Inoculant_Type	Ambient_N - Rural	0	0.276	0.089	Inf	3.092	0.002
Inoculant_Type	Ambient_N - Urban	0	-0.927	0.080	Inf	-11.605	0.000
Inoculant_Type	Local - N_Addition	0	17.320	270.964	Inf	0.064	0.949
Inoculant_Type	Local - Rural	0	-0.130	0.067	Inf	-1.937	0.053
Inoculant_Type	Local - Urban	0	-1.332	0.054	Inf	-24.651	0.000
Inoculant_Type	N_Addition - Rural	0	-17.450	270.964	Inf	-0.064	0.949
Inoculant_Type	N_Addition - Urban	0	-18.652	270.964	Inf	-0.069	0.945
Inoculant_Type	Rural - Urban	0	-1.202	0.080	Inf	-15.115	0.000

Contrast Effect Sizes

Table 77: Effect sizes for the constrasts by community type in the rhizobium abundance by inoculant and control communities model.

Contrast	Cohen's d	SE	df	CI Lower	CI Upper
(Ambient_N - Local)	0.048	0.010	Inf	0.029	0.066
(Ambient_N - N_Addition)	2.086	31.889	Inf	-60.415	64.587
(Ambient_N - Rural)	0.032	0.011	Inf	0.011	0.054
(Ambient_N - Urban)	-0.109	0.015	Inf	-0.139	-0.079
(Local - N_Addition)	2.038	31.889	Inf	-60.462	64.539
(Local - Rural)	-0.015	0.008	Inf	-0.031	0.001
(Local - Urban)	-0.157	0.019	Inf	-0.193	-0.120
(N_Addition - Rural)	-2.054	31.889	Inf	-64.554	60.447
(N_Addition - Urban)	-2.195	31.889	Inf	-64.696	60.306
(Rural - Urban)	-0.142	0.018	Inf	-0.178	-0.105

R Session Information

Table 78: 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
car	3.1-2	2023-03-30
carData	3.0-5	2022-01-06
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
lattice	0.21-8	2023-04-05
lubridate	1.9.2	2023-02-10
modelbased	0.8.6	2023-01-13
parameters	0.21.1	2023-05-26
performance	0.10.4	2023-06-02
permute	0.9-7	2022-01-27
phyloseq	1.40.0	2022-04-26
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
tidyamplicons	0.2.2	2022-09-10
tidyr	1.3.0	2023-01-24
tidyverse	2.0.0	2023-02-22
vegan	2.6-4	2022-10-11