

Appendices: R code

Appendices for “Dryland rock detention structures increase herbaceous vegetation cover and stabilize shrub cover over 10 years but do not directly affect soil fertility” by Ossanna et al. (2023). Included are code and analysis used to make figures and full model output.

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Appendices:

- Appendix A: Temporal vegetation trends, Figs 2 & S4
- Appendix B: Coefficient of variation, Figs 3 & S5
- Appendix C: Soil fertility, Figs S6-S8
- Appendix D: Structural equation modeling, models 1-4

Package versions:

- R version 4.3.1
- tidyverse 2.0.0
 - dplyr 1.1.2
 - stringr 1.5.0
 - ggplot 3.4.3
 - tidyr 3.2.1
- agricolae 1.3-6
- plotrix 3.8-2
- ggpubr 0.6.0
- rstatix 0.7.2
- scales 1.2.1
- metagenomeSeq 1.42.0, Biobase 2.60.0, BiocGenerics 0.46.0
- vegan 2.6-4
- dada2 1.28.0, ShortRead 1.58.0, Biostrings 2.68.1 used for DADA2 pipeline (code not included here)
- lavaan 0.6-16

Appendix A: Temporal vegetation trends

Setup

```
library(tidyverse)
library(agricolae)
library(plotrix)
library(ggpubr)
library(rstatix)

# Load data -----

notree.all <- read.csv("data/Herb-and-shrub-cover_2012-2021.csv")
herb.all <- read.csv("data/Herb-cover_2012-2021.csv")
shrub.all <- read.csv("data/Shrub-cover_2012-2021.csv")
invasive.all <- read.csv("data/Invasive-cover_2012-2021.csv")
plant.all <- read.csv("data/Species-cover_2012-2021.csv")
per.div <- read.csv("data/Perennial-plant-diversity_2012-2021.csv")
```

```
# Functions -----

# Convert columns to factor or date as needed
convert.cols <- function(x) {
  x$year.xaxis <- as.Date(x$year.xaxis)

  group.cols <- c("Sample", "Year", "Treatment")

  x[group.cols] <- lapply(x[group.cols], factor)

  return(x)
}
```

```
# Data wrangling -----

notree.all <- convert.cols(notree.all)
herb.all <- convert.cols(herb.all)
shrub.all <- convert.cols(shrub.all)
invasive.all <- convert.cols(invasive.all)
per.div <- convert.cols(per.div)
```

Fig 2a: Grass, forb & shrub cover

```
# Grass, forb, and shrub cover (notree) -----

# Find averages by year
notree.avg <- notree.all %>%
  group_by(Treatment, Year, year.xaxis) %>%
  summarise(mean = mean(Cover),
            SD = sd(Cover),
```

```

SE = std.error(Cover),
.groups = "keep")

# One-way ANOVA for Control
summary(aov(Cover ~ Year, data = filter(notree.all, Treatment == "Control")) # p = 4.3e-06

```

```

##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5   7815  1563.0    7.12 4.3e-06 ***
## Residuals    177  38854   219.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

notree.ctrl <- notree.all |>
  filter(Treatment == "Control")
anova.notree.ctrl <- aov(notree.ctrl$Cover ~ notree.ctrl$Year)
hsd.notree.ctrl <- HSD.test(anova.notree.ctrl, trt = "notree.ctrl$Year")
hsd.notree.ctrl$groups

```

```

##      notree.ctrl$Cover groups
## 2021         42.41935      a
## 2012         31.73194     ab
## 2014         31.58750     ab
## 2018         27.71774      b
## 2013         27.71250      b
## 2015         20.89315      b

```

```

# One-way ANOVA for Treated
summary(aov(Cover ~ Year, data = filter(notree.all, Treatment == "Treated"))) # p = 0.00304

```

```

##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5   7804  1560.7    3.739 0.00304 **
## Residuals    178  74291   417.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

notree.trt <- notree.all |>
  filter(Treatment == "Treated")
anova.notree.trt <- aov(notree.trt$Cover ~ notree.trt$Year)
hsd.notree.trt <- HSD.test(anova.notree.trt, trt = "notree.trt$Year")
hsd.notree.trt$groups

```

```

##      notree.trt$Cover groups
## 2018         41.69960      a
## 2021         37.47581     ab
## 2014         31.40927     abc
## 2015         30.33669     abc
## 2012         26.72446     bc
## 2013         21.80029      c

```

```

# Plot with one-way ANOVA letters
notree.ctrl.letters <- hsd.notree.ctrl$groups
notree.ctrl.letters <- notree.ctrl.letters |>
  mutate(Year = rownames(notree.ctrl.letters)) |>
  arrange(Year)
notree.trt.letters <- hsd.notree.trt$groups
notree.trt.letters <- notree.trt.letters |>
  mutate(Year = rownames(notree.trt.letters)) |>
  arrange(Year)

letters.notree <- data.frame(x = rep(notree.avg$year.xaxis[1:6], 2),
  y = rep(49, 12),
  label = c(notree.ctrl.letters$groups,
    notree.trt.letters$groups),
  Treatment = c(rep("Control", 6),
    rep("Treated", 6)))
ptext.notree <- data.frame(x = rep(as.Date("2019-09-01"), 2),
  y = c(22, 22),
  label = c("ANOVA, p < 0.001", "ANOVA, p = 0.003"),
  Treatment = c("Control", "Treated"))

notree.plot <- ggplot(notree.avg, aes(x = year.xaxis, y = mean,
  group = Treatment,
  color = Treatment)) +

  geom_line() +
  geom_point() +
  geom_pointrange(aes(ymin = mean - SE, ymax = mean + SE)) +
  facet_wrap(~Treatment) +
  xlab(NULL) +
  ylab("Cover (%)") +
  ggtitle("Vegetation cover, 2012-2021") +
  scale_color_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  theme(legend.position = "none") +
  geom_text(data = letters.notree,
    mapping = aes(x = x, y = y, label = label),
    color = "black",
    size = 3.5) +
  geom_text(data = ptext.notree,
    aes(x = x, y = y, label = label),
    color = "gray30",
    size = 2.5) +
  theme(axis.text.x = element_text(color = "black")) +
  theme(plot.margin = margin(t = 0.1, r = 0.1, b = 0.2, l = 0.1, "in"))
notree.plot

```

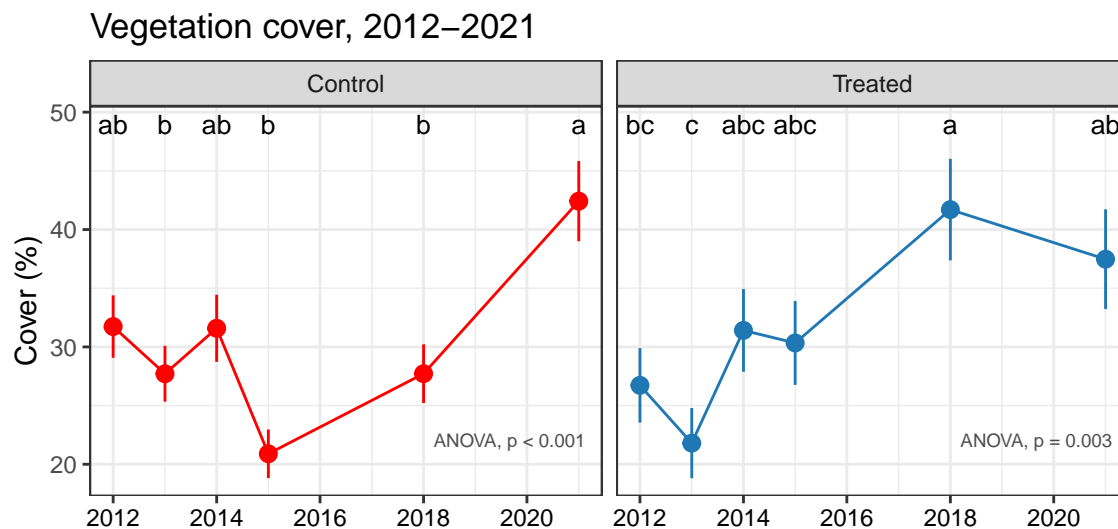


Fig 2b: Herbaceous (grass & forb) cover

```
# Herbaceous cover -----

# Find averages by year
herb.avg <- herb.all %>%
  group_by(Treatment, Year, year.xaxis) %>%
  summarise(mean = mean(Cover),
            SD = sd(Cover),
            SE = std.error(Cover),
            .groups = "keep")

# One-way ANOVA for Control
summary(aov(Cover ~ Year, data = filter(herb.all, Treatment == "Control"))) # 0.00434

##           Df Sum Sq Mean Sq F value Pr(>F)
## Year       5   2821   564.3    3.556 0.00434 **
## Residuals 177  28083   158.7
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

herb.ctrl <- herb.all |>
  filter(Treatment == "Control")
anova.herb.ctrl <- aov(herb.ctrl$Cover ~ herb.ctrl$Year)
hsd.herb.ctrl <- HSD.test(anova.herb.ctrl, trt = "herb.ctrl$Year")
hsd.herb.ctrl$groups

## herb.ctrl$Cover groups
## 2021      26.78629      a
## 2014      22.28333     ab
## 2012      20.03472     ab
## 2018      19.89718     ab
```

```
## 2013      17.41528      b
## 2015      14.21169      b
```

```
# One-way ANOVA for Treated
```

```
summary(aov(Cover ~ Year, data = filter(herb.all, Treatment == "Treated"))) # p = 3.77e-10
```

```
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Year         5    6847   1369.5    12.17 3.77e-10 ***
## Residuals   178   20035    112.6
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
herb.trt <- herb.all |>
  filter(Treatment == "Treated")
anova.herb.trt <- aov(herb.trt$Cover ~ herb.trt$Year)
hsd.herb.trt <- HSD.test(anova.herb.trt, trt = "herb.trt$Year")
hsd.herb.trt$groups
```

```
## herb.trt$Cover groups
## 2018      24.489919      a
## 2021      22.201613      ab
## 2014      15.139113      bc
## 2015      12.368952      cd
## 2012      11.436828      cd
## 2013       6.929598      d
```

```
# Plot with one-way ANOVA letters
```

```
herb.ctrl.letters <- hsd.herb.ctrl$groups
herb.ctrl.letters <- herb.ctrl.letters |>
  mutate(Year = rownames(herb.ctrl.letters)) |>
  arrange(Year)
herb.trt.letters <- hsd.herb.trt$groups
herb.trt.letters <- herb.trt.letters |>
  mutate(Year = rownames(herb.trt.letters)) |>
  arrange(Year)

letters.herb <- data.frame(x = rep(herb.avg$year.xaxis[1:6], 2),
  y = rep(32, 12),
  label = c(herb.ctrl.letters$groups,
    herb.trt.letters$groups),
  Treatment = c(rep("Control", 6),
    rep("Treated", 6)))
ptext.herb <- data.frame(x = rep(as.Date("2019-09-01"), 2),
  y = c(8, 8),
  label = c("ANOVA, p = 0.004", "ANOVA, p < 0.001"),
  Treatment = c("Control", "Treated"))

herb.plot <- ggplot(herb.avg, aes(x = year.xaxis, y = mean,
  group = Treatment,
  color = Treatment)) +
  geom_line() +
  geom_point() +
```

```

geom_pointrange(aes(ymin = mean - SE, ymax = mean + SE)) +
facet_wrap(~Treatment) +
xlab(NULL) +
ylab("Cover (%)") +
ggtitle("Herbaceous cover") +
scale_color_manual(values = c("red", "#1F78B4")) +
theme_bw() +
theme(legend.position = "none") +
geom_text(data = letters.herb,
          mapping = aes(x = x, y = y, label = label),
          color = "black",
          size = 3.5) +
geom_text(data = ptext.herb,
          aes(x = x, y = y, label = label),
          color = "gray30",
          size = 2.5) +
theme(axis.text.x = element_text(color = "black")) +
theme(plot.margin = margin(t = 0.1, r = 0.1, b = 0.2, l = 0.1, "in"))
herb.plot

```

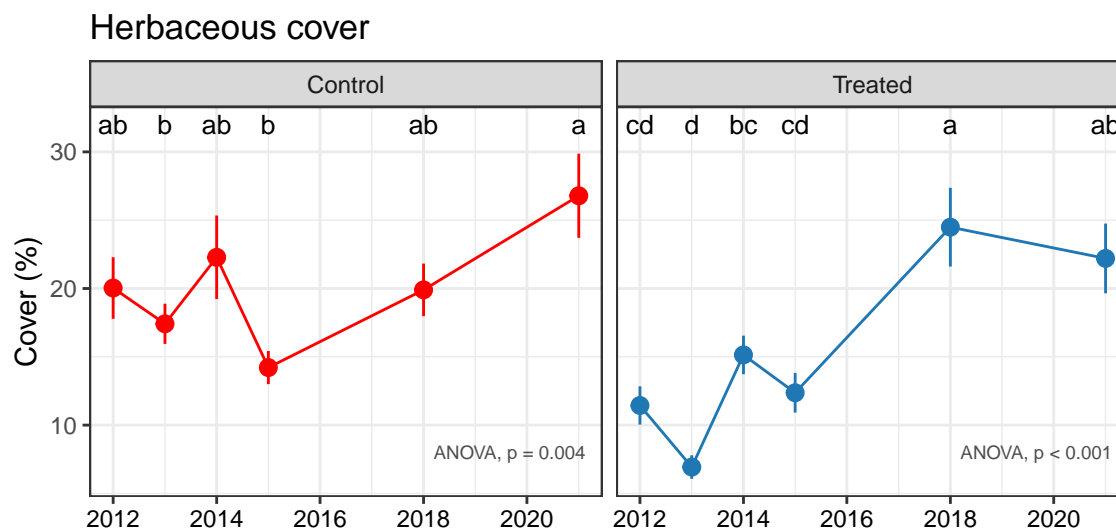


Fig 2c: Shrub cover

```

# Shrub cover -----

# Find averages by year
shrub.avg <- shrub.all %>%
  group_by(Treatment, Year, year.xaxis) %>%
  summarise(mean = mean(Cover),
            SD = sd(Cover),
            SE = std.error(Cover),
            .groups = "keep")

```

```
# One-way ANOVA for Control
summary(aov(Cover ~ Year, data = filter(shrub.all, Treatment == "Control"))) # p = 0.0112
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5    1566    313.1   3.062 0.0112 *
## Residuals    177    18100    102.3
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
shrub.ctrl <- shrub.all |>
  filter(Treatment == "Control")
anova.shrub.ctrl <- aov(shrub.ctrl$Cover ~ shrub.ctrl$Year)
hsd.shrub.ctrl <- HSD.test(anova.shrub.ctrl, trt = "shrub.ctrl$Year")
hsd.shrub.ctrl$groups
```

```
##      shrub.ctrl$Cover groups
## 2021      15.633065      a
## 2012      11.697222     ab
## 2013      10.297222     ab
## 2014       9.304167     ab
## 2018       7.820565      b
## 2015       6.681452      b
```

```
# One-way ANOVA for Treated
summary(aov(Cover ~ Year, data = filter(shrub.all, Treatment == "Treated"))) # NS, p = 0.982
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5     232     46.4   0.141 0.982
## Residuals    178   58465     328.5
```

```
# Plot with one-way ANOVA letters
shrub.ctrl.letters <- hsd.shrub.ctrl$groups
shrub.ctrl.letters <- shrub.ctrl.letters |>
  mutate(Year = rownames(shrub.ctrl.letters)) |>
  arrange(Year)

letters.shrub <- data.frame(x = shrub.avg$year.xaxis[1:6],
  y = rep(21, 6),
  label = shrub.ctrl.letters$groups,
  Treatment = rep("Control", 6))
ptext.shrub <- data.frame(x = rep(as.Date("2019-09-01"), 2),
  y = c(6.5, 6.5),
  label = c("ANOVA, p = 0.011", "ANOVA, p = 0.982"),
  Treatment = c("Control", "Treated"))

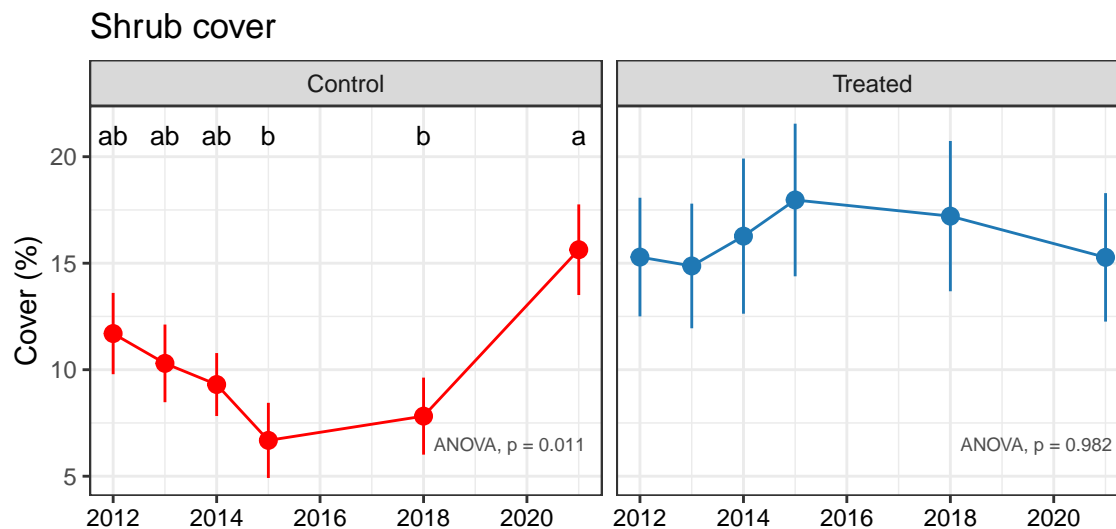
shrub.plot <- ggplot(shrub.avg, aes(x = year.xaxis, y = mean,
  group = Treatment,
  color = Treatment)) +
  geom_line() +
  geom_point() +
  geom_pointrange(aes(ymin = mean - SE, ymax = mean + SE)) +
```



```

facet_wrap(~Treatment) +
xlab(NULL) +
ylab("Cover (%)") +
ggtitle("Shrub cover") +
scale_color_manual(values = c("red", "#1F78B4")) +
theme_bw() +
theme(legend.position = "none") +
geom_text(data = letters.shrub,
          mapping = aes(x = x, y = y, label = label),
          color = "black",
          size = 3.5) +
geom_text(data = ptext.shrub,
          aes(x = x, y = y, label = label),
          color = "gray30",
          size = 2.5) +
theme(axis.text.x = element_text(color = "black")) +
theme(plot.margin = margin(t = 0.1, r = 0.1, b = 0.2, l = 0.1, "in"))
shrub.plot

```



Combine plots for Fig 2

```

# Combine notree, herb & shrub -----
tiff("figures/2023-09_publish-figures/temporal-ANOVA_notree-herb-shrub.tiff", units = "in", height = 8,
ggarrange(notree.plot, herb.plot, shrub.plot,
          ncol = 1, nrow = 3,
          labels = c("(A)", "(B)", "(C)"))
dev.off()

```

Supp Fig 4a: Perennial plant species richness

```
# Perennial plant richness -----

# Find averages by year
rich.avg <- per.div %>%
  group_by(Treatment, Year, year.xaxis) %>%
  summarise(mean = mean(rich),
            SD = sd(rich),
            SE = std.error(rich),
            .groups = "keep")

# One-way ANOVA for Control
summary(aov(rich ~ Year, data = filter(per.div, Treatment == "Control"))) # 0.00881

##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5    92.9  18.587    3.189 0.00881 **
## Residuals    177 1031.7    5.829
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

rich.ctrl <- per.div |>
  filter(Treatment == "Control")
anova.rich.ctrl <- aov(rich.ctrl$rich ~ rich.ctrl$Year)
hsd.rich.ctrl <- HSD.test(anova.rich.ctrl, trt = "rich.ctrl$Year")
hsd.rich.ctrl$groups

##      rich.ctrl$rich groups
## 2012      9.866667      a
## 2013      9.133333     ab
## 2018      8.645161     ab
## 2015      8.612903     ab
## 2014      8.200000     ab
## 2021      7.580645      b

# One-way ANOVA for Treated
summary(aov(rich ~ Year, data = filter(per.div, Treatment == "Treated"))) # p = 0.0516

##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5     76  15.207    2.247 0.0516 .
## Residuals    178   1204    6.766
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Plot with one-way ANOVA letters
rich.ctrl.letters <- hsd.rich.ctrl$groups
rich.ctrl.letters <- rich.ctrl.letters |>
  mutate(Year = rownames(rich.ctrl.letters)) |>
  arrange(Year)

letters.rich <- data.frame(x = rich.avg$year.xaxis[1:6],
```

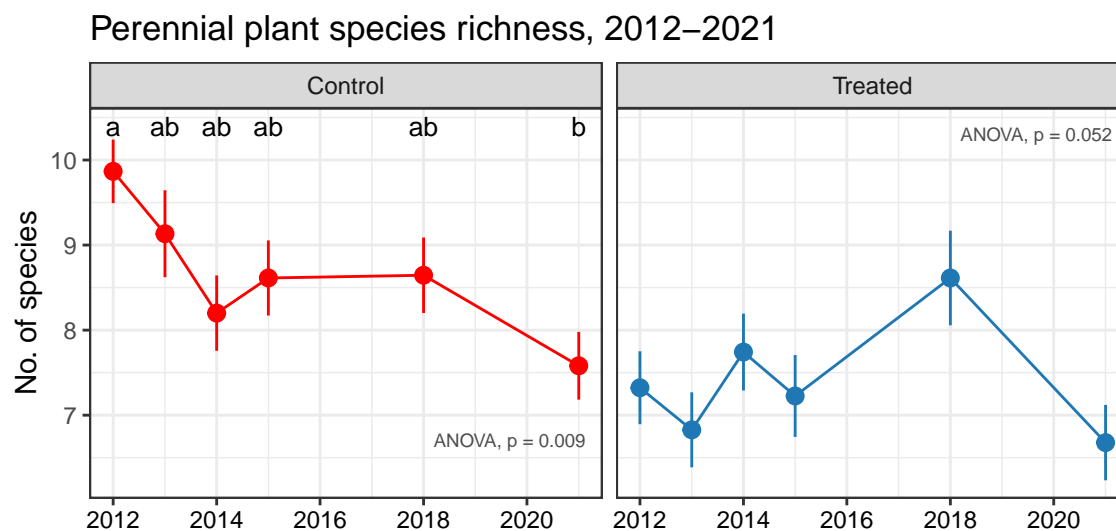
```

y = rep(10.4, 6),
label = rich.ctrl.letters$groups,
Treatment = rep("Control", 6))
ptext.rich <- data.frame(x = rep(as.Date("2019-09-01"), 2),
y = c(6.7, 10.3),
label = c("ANOVA, p = 0.009", "ANOVA, p = 0.052"),
Treatment = c("Control", "Treated"))

rich.plot <- ggplot(rich.avg, aes(x = year.xaxis, y = mean,
group = Treatment,
color = Treatment)) +

geom_line() +
geom_point() +
geom_pointrange(aes(ymin = mean - SE, ymax = mean + SE)) +
facet_wrap(~Treatment) +
xlab(NULL) +
ylab("No. of species") +
ggtitle("Perennial plant species richness, 2012-2021") +
scale_color_manual(values = c("red", "#1F78B4")) +
theme_bw() +
theme(legend.position = "none") +
geom_text(data = letters.rich,
mapping = aes(x = x, y = y, label = label),
color = "black",
size = 3.5) +
geom_text(data = ptext.rich,
aes(x = x, y = y, label = label),
color = "gray30",
size = 2.5) +
theme(axis.text.x = element_text(color = "black")) +
theme(plot.margin = margin(0.1, 0.1, 0.2, 0.1, "in"))
rich.plot

```



Supp Fig 4b: Perennial plant diversity

```
# Perennial plant diversity (Shannon) -----

# Find averages by year
shan.avg <- per.div %>%
  group_by(Treatment, Year, year.xaxis) %>%
  summarise(mean = mean(shan),
            SD = sd(shan),
            SE = std.error(shan),
            .groups = "keep")

# One-way ANOVA for Control
summary(aov(shan ~ Year, data = filter(per.div, Treatment == "Control"))) # p = 0.934

##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5   0.185  0.03702    0.26  0.934
## Residuals    177  25.197  0.14235

# One-way ANOVA for Treated
summary(aov(shan ~ Year, data = filter(per.div, Treatment == "Treated"))) # p = 0.725

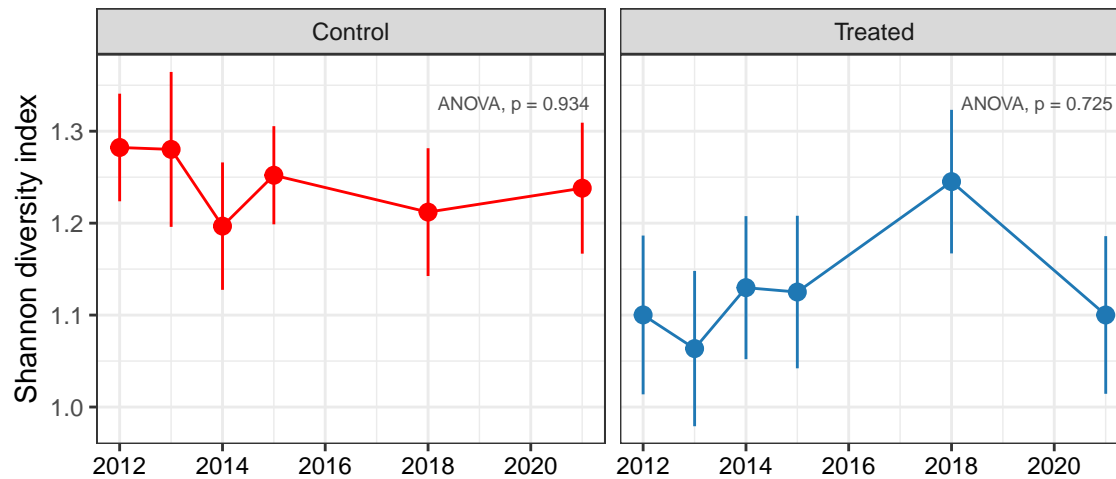
##              Df Sum Sq Mean Sq F value Pr(>F)
## Year          5    0.59  0.1189    0.568  0.725
## Residuals    178  37.27  0.2094

# Plot with one-way ANOVA
ptext.shan <- data.frame(x = rep(as.Date("2019-09-01"), 2),
                        y = c(1.33, 1.33),
                        label = c("ANOVA, p = 0.934", "ANOVA, p = 0.725"),
                        Treatment = c("Control", "Treated"))

shan.plot <- ggplot(shan.avg, aes(x = year.xaxis, y = mean,
                                group = Treatment,
                                color = Treatment)) +

  geom_line() +
  geom_point() +
  geom_pointrange(aes(ymin = mean - SE, ymax = mean + SE)) +
  facet_wrap(~Treatment) +
  xlab(NULL) +
  ylab("Shannon diversity index") +
  ggtitle("Perennial plant diversity") +
  scale_color_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "black")) +
  geom_text(data = ptext.shan,
            aes(x = x, y = y, label = label),
            color = "gray30",
            size = 2.5) +
  theme(plot.margin = margin(0.1, 0.1, 0.2, 0.1, "in"))
shan.plot
```

Perennial plant diversity



Combine plots for Supp Fig 4

```
# Combine richness & Shannon -----

tiff("figures/2023-09_publish-figures/temporal-ANOVA_richness-Shannon.tiff", units = "in", height = 5.5)
ggarrange( rich.plot, shan.plot,
  ncol = 1, nrow = 2,
  labels = c("(A)", "(B)"))

dev.off()
```

Invasive cover and most common species

```
# Average cover by year
invasive.all %>%
  select(-year.axis) |>
  group_by(Treatment, Year) %>%
  summarise(mean = mean(Cover),
    SE = std.error(Cover),
    .groups = "keep") |>
  mutate_if(is.numeric, round, digits = 2)

## 'mutate_if()' ignored the following grouping variables:
## * Columns 'Treatment', 'Year'

## # A tibble: 12 x 4
## # Groups:   Treatment, Year [12]
##   Treatment Year   mean   SE
##   <fct>      <fct> <dbl> <dbl>
## 1 Control   2012    3.34  0.6
```

```
## 2 Control 2013 2.58 0.66
## 3 Control 2014 2.46 0.98
## 4 Control 2015 2.96 0.69
## 5 Control 2018 5.1 1.17
## 6 Control 2021 7.38 1.23
## 7 Treated 2012 3.29 0.84
## 8 Treated 2013 2.18 0.51
## 9 Treated 2014 3.58 0.8
## 10 Treated 2015 5.1 1.08
## 11 Treated 2018 5.17 1.23
## 12 Treated 2021 4.79 0.85
```

```
# Most common species in Control
plant.all |>
  filter(Native == "Invasive",
         Treatment == "Control") |>
  group_by(Common) |>
  summarise(mean = mean(Cover)) |>
  arrange(desc(mean))
```

```
## # A tibble: 4 x 2
##   Common          mean
##   <chr>          <dbl>
## 1 Lehmann lovegrass 4.49
## 2 Stinkgrass       0.833
## 3 Spreading fantails 0.769
## 4 African lovegrass 0.125
```

```
# Most common species in Treated
plant.all |>
  filter(Native == "Invasive",
         Treatment == "Treated") |>
  group_by(Common) |>
  summarise(mean = mean(Cover)) |>
  arrange(desc(mean))
```

```
## # A tibble: 8 x 2
##   Common          mean
##   <chr>          <dbl>
## 1 Lehmann lovegrass 4.75
## 2 Buffelgrass      3.41
## 3 Boer lovegrass   0.625
## 4 African lovegrass 0.531
## 5 Spreading fantails 0.367
## 6 Barnyard         0.225
## 7 Rose Natal grass 0.125
## 8 Stinkgrass       0.125
```

Appendix B: Coefficient of variation

Setup

```
library(tidyverse)
library(car)
library(scales)
library(ggpubr)
```

```
# Load data -----

notree.all <- read_csv("data/Herb-and-shrub-cover_2012-2021.csv")
herb.all <- read_csv("data/Herb-cover_2012-2021.csv")
shrub.all <- read_csv("data/Shrub-cover_2012-2021.csv")
per.div <- read_csv("data/Perennial-plant-diversity_2012-2021.csv")
```

Fig 3a: CV of shrub cover

```
# Shrub cover -----

# Find CV for each sample over time
shrub.sample <- shrub.all |>
  group_by(Sample, Treatment) |>
  summarise(CV = sd(Cover) / mean(Cover),
            .groups = "keep") # NaNs produced because some have 0 cover and can't divide by 0

# Replace NaNs with 0
shrub.sample[1, 3] <- 0
shrub.sample[5, 3] <- 0
shrub.sample[8, 3] <- 0

# Compare means
wilcox.test(filter(shrub.sample, Treatment == "Treated")$CV,
            filter(shrub.sample, Treatment == "Control")$CV,
            exact = FALSE) # p = 0.01429

##
## Wilcoxon rank sum test with continuity correction
##
## data: filter(shrub.sample, Treatment == "Treated")$CV and filter(shrub.sample, Treatment == "Control")$CV
## W = 306, p-value = 0.01429
## alternative hypothesis: true location shift is not equal to 0

# Plot
letters.shrub <- data.frame(x = c(1, 2),
                           y = c(2.5, 2.5),
                           label = c("a", "b"))
```

```

shrub.plot.cv <- shrub.sample |>
  ggplot(aes(x = Treatment, y = CV)) +
  geom_boxplot(alpha = 0.3,
    outlier.shape = NA,
    aes(fill = Treatment)) +
  geom_jitter(size = 1,
    alpha = 0.8,
    aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Shrub cover",
    x = NULL,
    y = "Coefficient of variation") +
  theme_bw() +
  theme(legend.position = "none") +
  scale_y_continuous(labels = percent) +
  theme(axis.text.x = element_text(color = "black")) +
  geom_text(aes(x = 0.95, y = 2.75, label = "Mann-Whitney, \np = 0.014"),
    color = "gray30",
    size = 2.5) +
  geom_text(data = letters.shrub,
    aes(x = x, y = y, label = label),
    color = "black") +
  theme(plot.margin = margin(0.1, 0, 0.1, 0.1, "in")) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
    width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
shrub.plot.cv

```

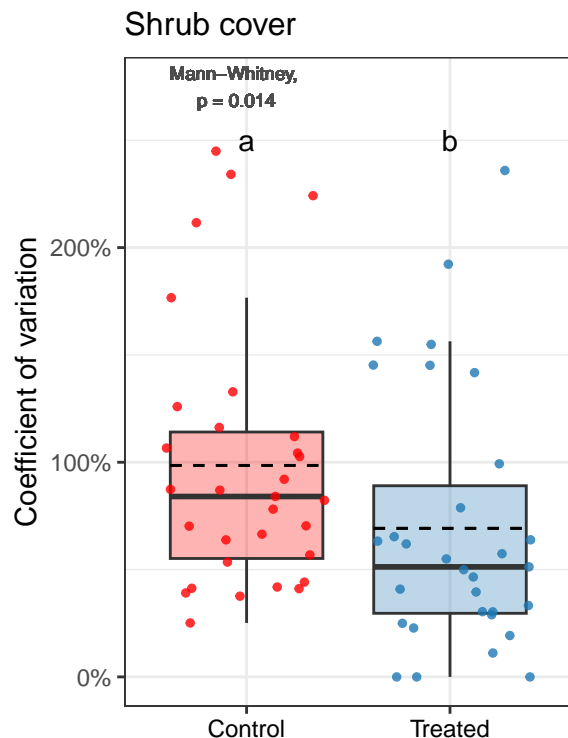


Fig 3b: CV of herbaceous cover

```
# Herb cover -----

# Find CV for each sample over time
herb.sample <- herb.all |>
  group_by(Sample, Treatment) |>
  summarise(CV = sd(Cover) / mean(Cover),
            .groups = "keep")

# Compare means
wilcox.test(filter(herb.sample, Treatment == "Treated")$CV,
            filter(herb.sample, Treatment == "Control")$CV) # NS, p = 0.148

##
## Wilcoxon rank sum exact test
##
## data: filter(herb.sample, Treatment == "Treated")$CV and filter(herb.sample, Treatment == "Control")$CV
## W = 584, p-value = 0.1479
## alternative hypothesis: true location shift is not equal to 0

# Plot
herb.plot.cv <- herb.sample |>
  ggplot(aes(x = Treatment, y = CV)) +
  geom_boxplot(alpha = 0.3,
               outlier.shape = NA,
               aes(fill = Treatment)) +
  geom_jitter(size = 1,
              alpha = 0.8,
              aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Herbaceous cover",
       x = NULL,
       y = NULL) +
  theme_bw() +
  theme(legend.position = "none") +
  scale_y_continuous(labels = percent) +
  theme(axis.text.x = element_text(color = "black")) +
  geom_text(aes(x = 0.95, y = 1.18, label = "Mann-Whitney, \np = 0.122"),
            color = "gray30",
            size = 2.5) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.2, "in")) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
              width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
herb.plot.cv
```

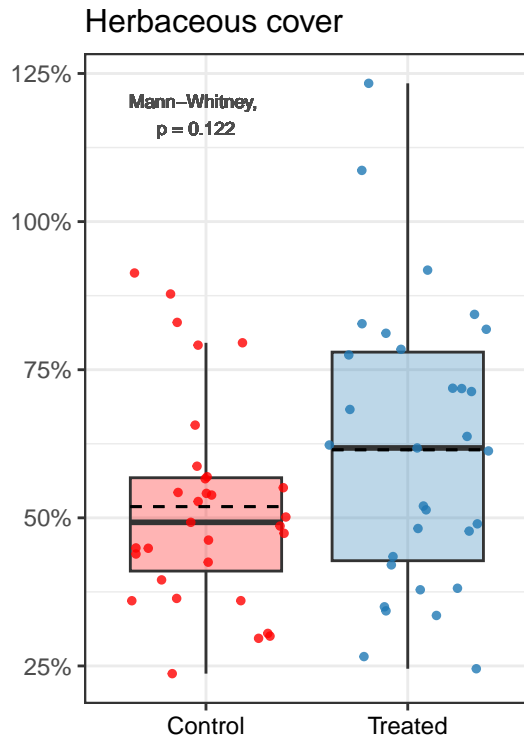


Fig 3c: CV of overall veg cover

```
# Notree cover -----

# Find CV for each sample over time
notree.sample <- notree.all |>
  group_by(Sample, Treatment) |>
  summarise(CV = sd(Cover) / mean(Cover),
    .groups = "keep")

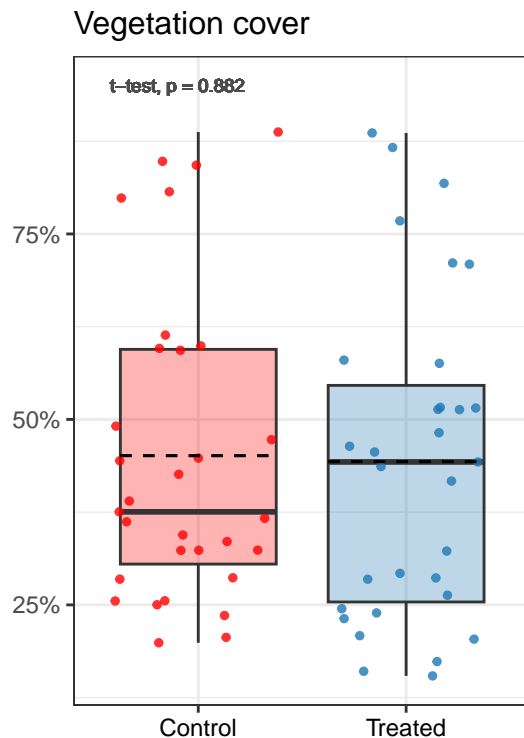
# Compare means
t.test(filter(notree.sample, Treatment == "Treated")$CV,
  filter(notree.sample, Treatment == "Control")$CV) # NS, p = 0.882
```

```
##
## Welch Two Sample t-test
##
## data: filter(notree.sample, Treatment == "Treated")$CV and filter(notree.sample, Treatment == "Control")$CV
## t = -0.14868, df = 59.846, p-value = 0.8823
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -0.11574119 0.09972676
## sample estimates:
## mean of x mean of y
## 0.4430859 0.4510931
```

```

# Plot
notree.plot.cv <- notree.sample |>
  ggplot(aes(x = Treatment, y = CV)) +
  geom_boxplot(alpha = 0.3,
    outlier.shape = NA,
    aes(fill = Treatment)) +
  geom_jitter(size = 1,
    alpha = 0.8,
    aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Vegetation cover",
    x = NULL,
    y = NULL) +
  theme_bw() +
  theme(legend.position = "none") +
  scale_y_continuous(labels = percent) +
  theme(axis.text.x = element_text(color = "black")) +
  geom_text(aes(x = 0.9, y = 0.95, label = "t-test, p = 0.882"),
    color = "gray30",
    size = 2.5) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.2, "in")) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
    width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
notree.plot.cv

```



Combine plots for Fig 3

```
# Combine notree, herb, shrub -----

tiff("figures/2023-09_publish-figures/CV_shrub-herb-notree.tiff", units = "in", height = 4, width = 7,
ggarrange(shrub.plot.cv, herb.plot.cv, notree.plot.cv,
          ncol = 3, nrow = 1,
          labels = c("(A)", "(B)", "(C)"))

dev.off()
```

Supp Fig 5a: CV of perennial richness

```
# Richness -----

# Find CV for each sample over time
rich.sample <- per.div |>
  group_by(Sample, Treatment) |>
  summarise(CV = sd(rich) / mean(rich),
            .groups = "keep")

# Compare means
wilcox.test(filter(rich.sample, Treatment == "Treated")$CV,
             filter(rich.sample, Treatment == "Control")$CV) # NS, p = 0.093

## Warning in wilcox.test.default(filter(rich.sample, Treatment == "Treated")$CV,
## : cannot compute exact p-value with ties

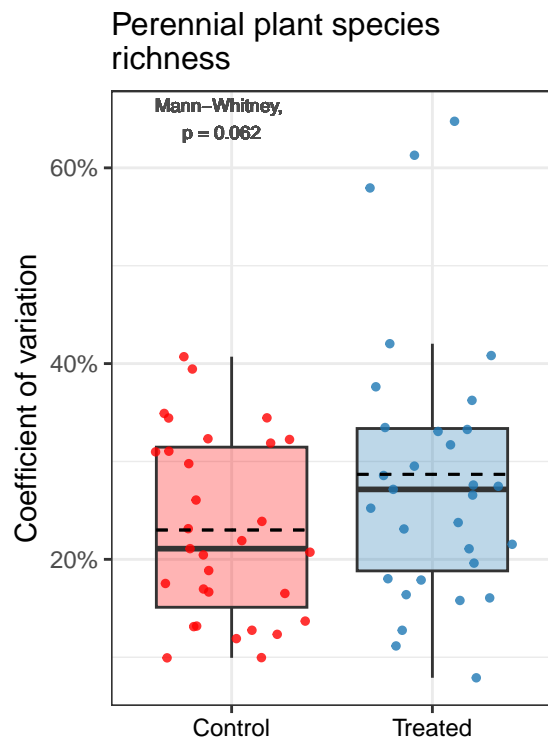
##
## Wilcoxon rank sum test with continuity correction
##
## data: filter(rich.sample, Treatment == "Treated")$CV and filter(rich.sample, Treatment == "Control")$CV
## W = 586.5, p-value = 0.1375
## alternative hypothesis: true location shift is not equal to 0

# Plot
rich.plot.cv <- rich.sample |>
  ggplot(aes(x = Treatment, y = CV)) +
  geom_boxplot(alpha = 0.3,
              outlier.shape = NA,
              aes(fill = Treatment)) +
  geom_jitter(size = 1,
              alpha = 0.8,
              aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Perennial plant species richness",
       x = NULL,
       y = "Coefficient of variation") +
  theme_bw() +
```

```

theme(legend.position = "none") +
scale_y_continuous(labels = percent) +
theme(axis.text.x = element_text(color = "black")) +
geom_text(aes(x = 0.95, y = 0.65, label = "Mann-Whitney, \np = 0.062"),
  color = "gray30",
  size = 2.5) +
theme(plot.margin = margin(0.1, 0.1, 0.1, 0.1, "in")) +
stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
  width = 0.75, linetype = "dashed") +
theme(plot.title = element_text(size = 12))
rich.plot.cv

```



Supp Fig 5b: CV of perennial diversity

```

# Shannon -----

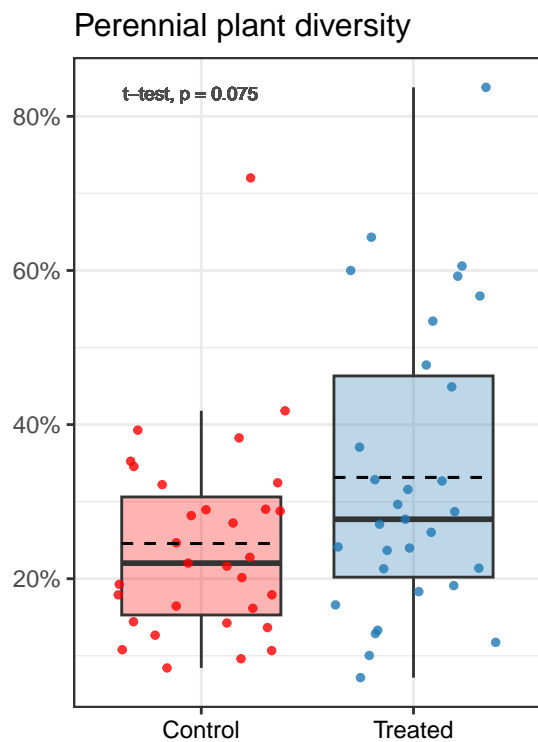
# Find CV for each sample over time
shan.sample <- per.div |>
  group_by(Sample, Treatment) |>
  summarise(CV = sd(shan) / mean(shan),
    .groups = "keep")

# Compare
t.test(filter(shan.sample, Treatment == "Treated")$CV,
  filter(shan.sample, Treatment == "Control")$CV) # NS, p = 0.075

```

```
##
## Welch Two Sample t-test
##
## data: filter(shan.sample, Treatment == "Treated")$CV and filter(shan.sample, Treatment == "Control")
## t = 2.0794, df = 52.755, p-value = 0.04246
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## 0.00303293 0.16871186
## sample estimates:
## mean of x mean of y
## 0.3315000 0.2456276
```

```
# Plot
shan.plot.cv <- shan.sample |>
  ggplot(aes(x = Treatment, y = CV)) +
  geom_boxplot(alpha = 0.3,
               outlier.shape = NA,
               aes(fill = Treatment)) +
  geom_jitter(size = 1,
              alpha = 0.8,
              aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Perennial plant diversity",
       x = NULL,
       y = NULL) +
  theme_bw() +
  theme(legend.position = "none") +
  scale_y_continuous(labels = percent) +
  theme(axis.text.x = element_text(color = "black")) +
  geom_text(aes(x = 0.95, y = 0.83, label = "t-test, p = 0.075"),
            color = "gray30",
            size = 2.5) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.15, "in")) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
              width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
shan.plot.cv
```



Combine plots for Supp Fig 5

```
# Combine richness & Shannon -----

# Supplemental figure
tiff("figures/2023-09_publish-figures/CV_rich-shan.tiff", units = "in", height = 4, width = 5.5, res = 300)
ggarrange(rich.plot.cv, shan.plot.cv,
  ncol = 2, nrow = 1,
  labels = c("(A)", "(B)"))

dev.off()
```

Appendix C: Soil fertility

Setup

```
library(tidyverse)
library(ggpubr)
library(metagenomeSeq)
library(vegan)
```

```
# Load data -----
```

```
barc.asv <- read.table("data/bac-arc_clean_asv.txt", sep = "\t",
                      header = T, row.names = 1)
fungi.asv <- read.table("data/fungi_clean_asv.txt",
                      sep = "\t", header = T, row.names = 1)
meta <- read.csv("data/sequencing_metadata.csv")

dat.2021 <- read.csv("data/Veg-soil-elev_2021.csv")
```

```
# 16S -----
```

```
# Normalization
```

```
barc.MR <- newMRexperiment(t(barc.asv))
p <- cumNormStat(barc.MR)
```

```
## Default value being used.
```

```
barc.MR <- cumNorm(barc.MR, p = p)
barc.norm <- t(MRcounts(barc.MR, norm = T, log = F))
```

```
# Richness and Shannon
```

```
meta$barc.richness <- specnumber(barc.norm)
meta$barc.shannon <- diversity(barc.norm, index = "shannon")
```

```
# Bray-Curtis distance
```

```
barc.dist <- vegdist(barc.norm, method = "bray")
```

```
# ITS -----
```

```
# Normalization
```

```
fungi.MR <- newMRexperiment(t(fungi.asv))
p <- cumNormStat(fungi.MR)
```

```
## Default value being used.
```

```
fungi.MR <- cumNorm(fungi.MR, p = p)
fungi.norm <- t(MRcounts(fungi.MR, norm = T, log = F))
```

```
# Richness and Shannon
```



```
meta$fungi.richness <- specnumber(fungi.norm)
meta$fungi.shannon <- diversity(fungi.norm, index = "shannon")

# Bray-Curtis distance
fungi.dist <- vegdist(fungi.norm, method = "bray")
```

Fig 4a: Bacteria & archaea NMDS ordination

Note that PERMANOVA model results (adonis2) will vary slightly each time and will not exactly match values published in the paper.

```
# NMDS ordination
barc.nmds <- metaMDS(barcdist, k = 2)

## Run 0 stress 0.1684425
## Run 1 stress 0.1688409
## ... Procrustes: rmse 0.02151677 max resid 0.09447774
## Run 2 stress 0.1709022
## Run 3 stress 0.1753505
## Run 4 stress 0.16937
## Run 5 stress 0.1680364
## ... New best solution
## ... Procrustes: rmse 0.03985045 max resid 0.2926766
## Run 6 stress 0.1837181
## Run 7 stress 0.1705619
## Run 8 stress 0.1684443
## ... Procrustes: rmse 0.03979688 max resid 0.2925489
## Run 9 stress 0.1859954
## Run 10 stress 0.1771314
## Run 11 stress 0.1679538
## ... New best solution
## ... Procrustes: rmse 0.01218716 max resid 0.0742584
## Run 12 stress 0.1902025
## Run 13 stress 0.169861
## Run 14 stress 0.1838188
## Run 15 stress 0.1829934
## Run 16 stress 0.1742163
## Run 17 stress 0.1786032
## Run 18 stress 0.180892
## Run 19 stress 0.1691579
## Run 20 stress 0.1680367
## ... Procrustes: rmse 0.0121855 max resid 0.07407049
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      20: stress ratio > sratmax

barc.nmds$stress

## [1] 0.1679538
```

```

meta$barc.NMDS1 <- barc.nmds$points[ , 1]
meta$barc.NMDS2 <- barc.nmds$points[ , 2]

# PERMANOVA
adonis2(barc.dist ~ meta$Treatment)

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = barc.dist ~ meta$Treatment)
##           Df SumOfSqs      R2      F Pr(>F)
## meta$Treatment  1   0.2559 0.02881 1.7799  0.029 *
## Residual       60   8.6275 0.97119
## Total          61   8.8835 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Plot (using dat.2021 for same NMDS values presented in paper):
barc.nmds.plot.21 <- dat.2021 %>%
  ggplot(aes(x = barc.NMDS1, y = barc.NMDS2, color = Treatment, shape = Treatment)) +
  geom_point() +
  scale_color_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  labs(x = "Axis 1",
       y = "Axis 2",
       title = "Bacteria & archaea",
       color = "Treatment",
       shape = "Treatment") +
  theme(legend.position = "bottom") +
  theme(plot.margin = margin(0.1, 0.2, 0.1, 0.1, "in")) +
  theme(legend.title = element_blank()) +
  geom_text(aes(x = 0.2, y = -0.55, label = "PERMANOVA, p = 0.029"),
            size = 2.5, color = "gray30") +
  geom_text(aes(x = 0.25, y = -0.65, label = "Stress = 0.168"),
            size = 2.5, color = "gray30") +
  theme(plot.title = element_text(size = 12))
barc.nmds.plot.21

```

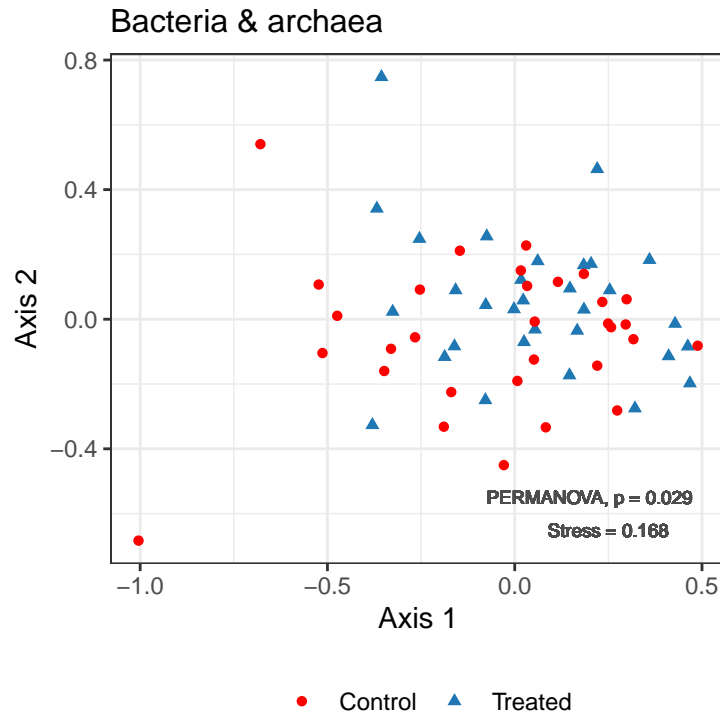


Fig 4b: Fungi NMDS ordination

Note that PERMANOVA model results (`adonis2`) will vary slightly each time and will not exactly match values published in the paper.

```
# NMDS ordination
fungi.nmfs <- metaMDS(fungi.dist, k = 2)

## Run 0 stress 0.2461785
## Run 1 stress 0.2501643
## Run 2 stress 0.2481007
## Run 3 stress 0.2418595
## ... New best solution
## ... Procrustes: rmse 0.1094014 max resid 0.3661558
## Run 4 stress 0.2426668
## Run 5 stress 0.2472044
## Run 6 stress 0.2432081
## Run 7 stress 0.2453265
## Run 8 stress 0.2582324
## Run 9 stress 0.2425263
## Run 10 stress 0.2402668
## ... New best solution
## ... Procrustes: rmse 0.07886488 max resid 0.4603727
## Run 11 stress 0.2415877
## Run 12 stress 0.2372747
## ... New best solution
## ... Procrustes: rmse 0.07424125 max resid 0.3981365
## Run 13 stress 0.24211
```

```
## Run 14 stress 0.2510688
## Run 15 stress 0.241698
## Run 16 stress 0.2579116
## Run 17 stress 0.2395923
## Run 18 stress 0.2413674
## Run 19 stress 0.2521348
## Run 20 stress 0.2420784
## *** Best solution was not repeated -- monoMDS stopping criteria:
##      3: no. of iterations >= maxit
##     17: stress ratio > sratmax
```

```
fungi.nm.ds$stress
```

```
## [1] 0.2372747
```

```
meta$fungi.NMDS1 <- fungi.nm.ds$points[, 1]
meta$fungi.NMDS2 <- fungi.nm.ds$points[, 2]
```

```
# PERMANOVA
```

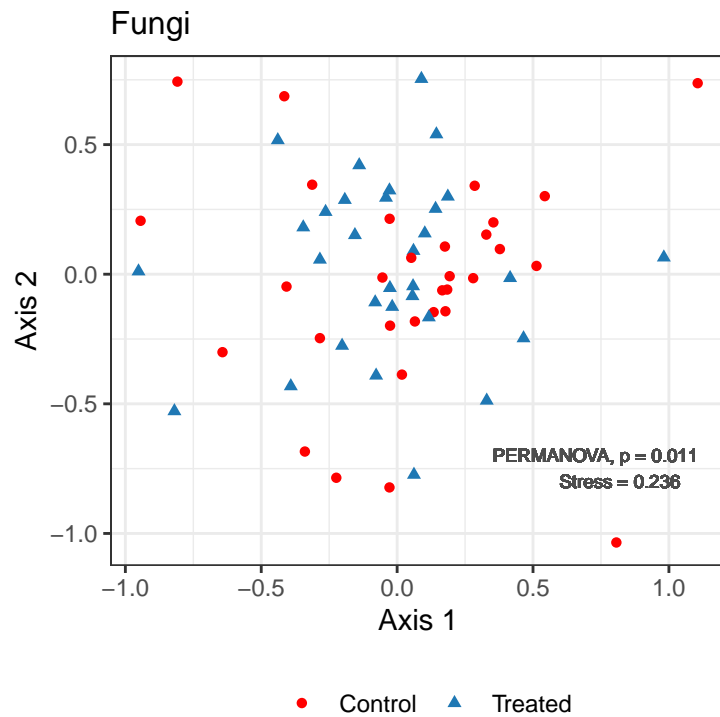
```
adonis2(fungi.dist ~ meta$Treatment)
```

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = fungi.dist ~ meta$Treatment)
##              Df SumOfSqs      R2      F Pr(>F)
## meta$Treatment  1   0.4511 0.02371 1.457  0.007 **
## Residual       60  18.5783 0.97629
## Total          61  19.0294 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Plot (using dat.2021 for same NMDS values presented in paper):
```

```
fungi.nm.ds.plot.21 <- dat.2021 %>%
  ggplot(aes(x = fungi.NMDS1, y = fungi.NMDS2, color = Treatment, shape = Treatment)) +
  geom_point() +
  scale_color_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  labs(x = "Axis 1",
       y = "Axis 2",
       title = "Fungi",
       color = "Treatment",
       shape = "Treatment") +
  theme(legend.position = "bottom") +
  theme(legend.title = element_blank()) +
  theme(plot.margin = margin(0.1, 0.2, 0.1, 0.1, "in")) +
  geom_text(aes(x = 0.73, y = -0.7, label = "PERMANOVA, p = 0.011"),
            size = 2.5, color = "gray30") +
  geom_text(aes(x = 0.82, y = -0.8, label = "Stress = 0.236"),
            size = 2.5, color = "gray30") +
```

```
theme(plot.title = element_text(size = 12))
fungi.nmds.plot.21
```



Combine plots for Fig 4

```
# Combine NMDS -----

tiff("figures/2023-09_publish-figures/Soil-NMDS.tiff", height = 4, width = 7, units = "in", res = 1000)
ggarrange(barc.nmds.plot.21, fungi.nmds.plot.21,
  nrow = 1, ncol = 2,
  labels = c("(A)", "(B)"),
  common.legend = TRUE, legend = "bottom")
dev.off()
```

Supp Fig 6a: Total soil nitrogen

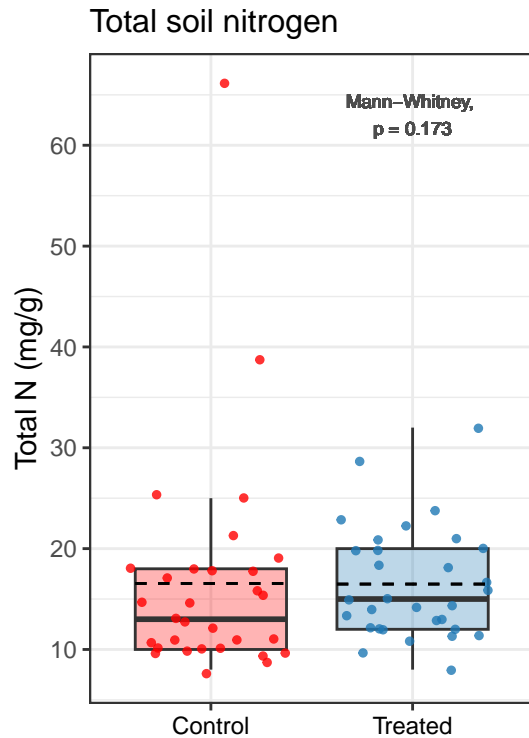
```
# Total N -----

# Mann-Whitney
wilcox.test(filter(dat.2021, Treatment == "Control")$TN_ppt,
  filter(dat.2021, Treatment == "Treated")$TN_ppt,
  exact = FALSE) # p-value = 0.1731
```

```
##
```

```
## Wilcoxon rank sum test with continuity correction
##
## data: filter(dat.2021, Treatment == "Control")$TN_ppt and filter(dat.2021, Treatment == "Treated")$
## W = 383.5, p-value = 0.1731
## alternative hypothesis: true location shift is not equal to 0
```

```
# Plot
tn.plot.21 <- dat.2021 |>
  ggplot(aes(x = Treatment, y = TN_ppt)) +
  geom_boxplot(alpha = 0.3,
    outlier.shape = NA,
    aes(fill = Treatment)) +
  geom_jitter(size = 1,
    alpha = 0.8,
    aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Total soil nitrogen",
    x = NULL,
    y = "Total N (mg/g)") +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "#000000")) +
  theme(plot.margin = margin(0.1, 0.2, 0.1, 0.1, "in")) +
  geom_text(aes(x = 2, y = 63, label = "Mann-Whitney, \np = 0.173"),
    color = "gray30",
    size = 2.5) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
    width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
tn.plot.21
```



Supp Fig 6b: Total soil carbon

```
# Total C -----
```

```
# Mann-Whitney
```

```
wilcox.test(filter(dat.2021, Treatment == "Control")$TC_ppt,  
            filter(dat.2021, Treatment == "Treated")$TC_ppt,  
            exact = FALSE) # p-value = 0.2397
```

```
##
```

```
## Wilcoxon rank sum test with continuity correction
```

```
##
```

```
## data: filter(dat.2021, Treatment == "Control")$TC_ppt and filter(dat.2021, Treatment == "Treated")$
```

```
## W = 396.5, p-value = 0.2397
```

```
## alternative hypothesis: true location shift is not equal to 0
```

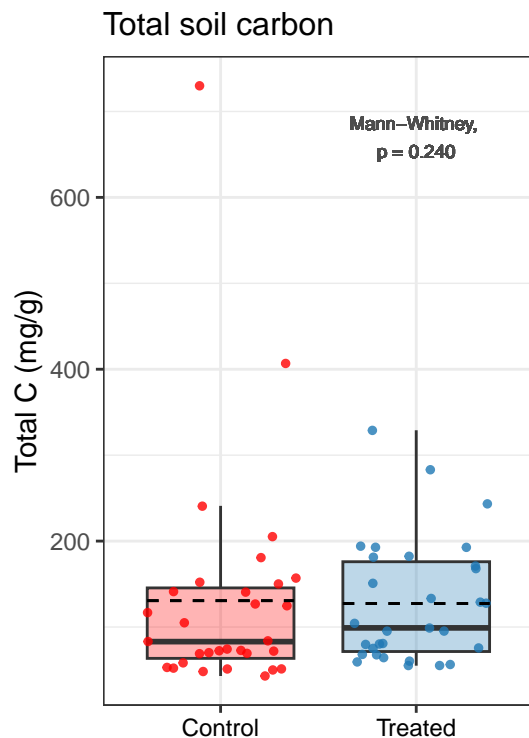
```
# Plot
```

```
tc.plot.21 <- dat.2021 |>  
  ggplot(aes(x = Treatment, y = TC_ppt)) +  
  geom_boxplot(alpha = 0.3,  
              outlier.shape = NA,  
              aes(fill = Treatment)) +  
  geom_jitter(size = 1,  
             alpha = 0.8,  
             aes(color = Treatment)) +  
  scale_color_manual(values = c("red", "#1F78B4")) +
```

```

scale_fill_manual(values = c("red", "#1F78B4")) +
labs(title = "Total soil carbon",
      x = NULL,
      y = "Total C (mg/g)") +
theme_bw() +
theme(legend.position = "none") +
theme(axis.text.x = element_text(color = "#000000")) +
theme(plot.margin = margin(0.1, 0.2, 0.1, 0.1, "in")) +
geom_text(aes(x = 2, y = 670, label = "Mann-Whitney, \np = 0.240"),
          color = "gray30",
          size = 2.5) +
stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
            width = 0.75, linetype = "dashed") +
theme(plot.title = element_text(size = 12))
tc.plot.21

```



Supp Fig 6c: Soil organic matter

```

# Organic matter -----
# Mann-Whitney
wilcox.test(filter(dat.2021, Treatment == "Control")$OM_perc,
            filter(dat.2021, Treatment == "Treated")$OM_perc) # p-value = 0.4332

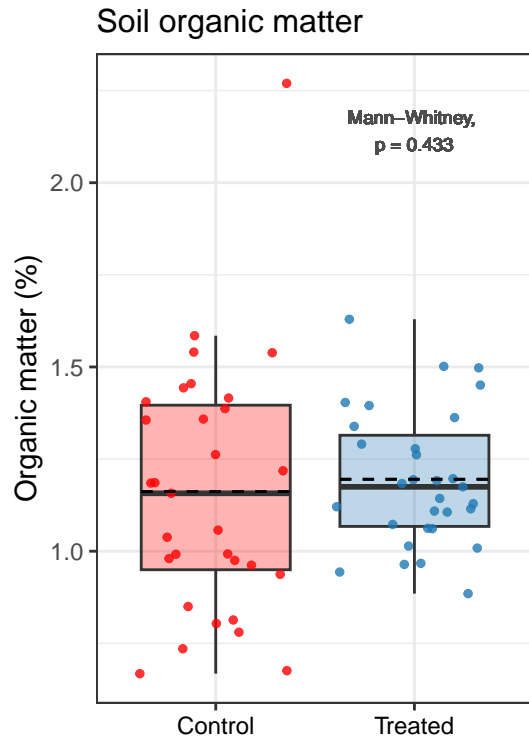
##
## Wilcoxon rank sum exact test

```



```
##
## data: filter(dat.2021, Treatment == "Control")$OM_perc and filter(dat.2021, Treatment == "Treated")
## W = 424, p-value = 0.4332
## alternative hypothesis: true location shift is not equal to 0
```

```
# Plot
om.plot.21 <- dat.2021 |>
  ggplot(aes(x = Treatment, y = OM_perc)) +
  geom_boxplot(alpha = 0.3,
    outlier.shape = NA,
    aes(fill = Treatment)) +
  geom_jitter(size = 1,
    alpha = 0.8,
    aes(color = Treatment)) +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  labs(title = "Soil organic matter",
    x = NULL,
    y = "Organic matter (%)") +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "#000000")) +
  theme(plot.margin = margin(0.1, 0.2, 0.1, 0.1, "in")) +
  geom_text(aes(x = 2, y = 2.14, label = "Mann-Whitney, \np = 0.433"),
    color = "gray30",
    size = 2.5) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
    width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
om.plot.21
```



Combine plots for Supp Fig 6

```
# Combine soil chem -----

# TN, TC, OM
tiff("figures/2023-09_publish-figures/Soil-chem.tiff", units = "in", height = 4, width = 7, res = 300)
ggarrange(tn.plot.21, tc.plot.21, om.plot.21,
          ncol = 3, nrow = 1,
          labels = c("(A)", "(B)", "(C)"))

dev.off()
```

Supp Fig 7a: Bacterial & archaeal richness

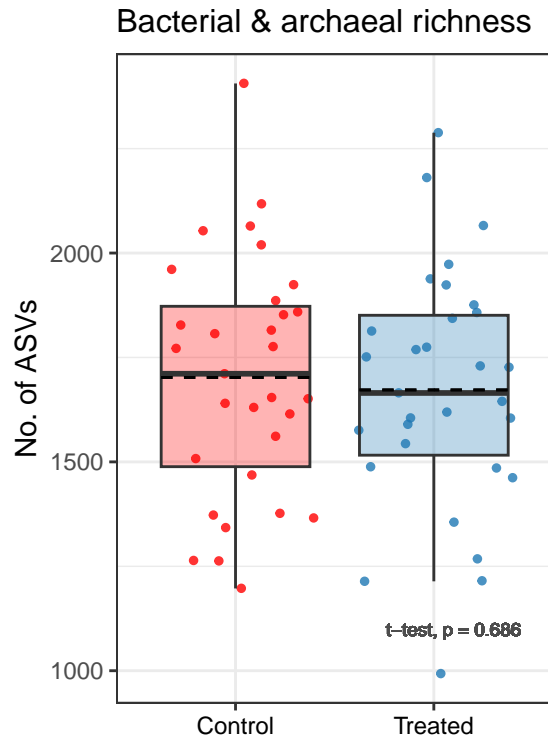
```
# Barc richness -----

# T-test
t.test(filter(dat.2021, Treatment == "Control")$barc.richness,
       filter(dat.2021, Treatment == "Treated")$barc.richness) # NS, p = 0.686
```

```
##
## Welch Two Sample t-test
##
## data: filter(dat.2021, Treatment == "Control")$barc.richness and filter(dat.2021, Treatment == "Treated")$barc.richness
```

```
## t = 0.40607, df = 59.999, p-value = 0.6861
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -116.6407 176.0601
## sample estimates:
## mean of x mean of y
## 1702.065 1672.355
```

```
# Plot
barc.rich.plot.21 <- dat.2021 |>
  ggplot(aes(Treatment, barc.richness)) +
  geom_jitter(aes(color = Treatment),
              alpha = 0.8,
              size = 1) +
  geom_boxplot(aes(fill = Treatment),
               alpha = 0.3,
               outlier.shape = NA) +
  xlab(NULL) +
  ylab("No. of ASVs") +
  ggtitle("Bacterial & archaeal richness") +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "#000000")) +
  geom_text(aes(x = 2.1, y = 1100, label = "t-test, p = 0.686"),
            color = "gray30",
            size = 2.5) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.1, "in")) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
              width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 11.5))
barc.rich.plot.21
```



Supp Fig 7b: Fungal richness

```
# Fungi richness -----
# T-test
t.test(filter(dat.2021, Treatment == "Control")$fungi.richness,
        filter(dat.2021, Treatment == "Treated")$fungi.richness) # NS,  $p = 0.938$ 
```

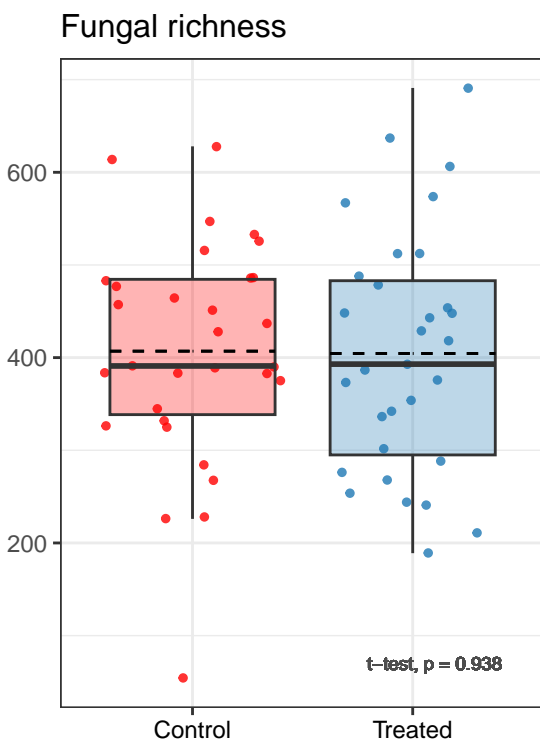
```
##
## Welch Two Sample t-test
##
## data: filter(dat.2021, Treatment == "Control")$fungi.richness and filter(dat.2021, Treatment == "Treated")$fungi.richness
## t = 0.078, df = 59.653, p-value = 0.9381
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -61.22215 66.18989
## sample estimates:
## mean of x mean of y
## 406.9677 404.4839
```

```
# Plot
fungi.rich.plot.21 <- dat.2021 %>%
  ggplot(aes(Treatment, fungi.richness)) +
  geom_jitter(aes(color = Treatment),
              alpha = 0.8,
              size = 1) +
```

```

geom_boxplot(aes(fill = Treatment),
             alpha = 0.3,
             outlier.shape = NA) +
xlab(NULL) +
ylab(NULL) +
ggtitle("Fungal richness") +
scale_color_manual(values = c("red", "#1F78B4")) +
scale_fill_manual(values = c("red", "#1F78B4")) +
theme_bw() +
theme(legend.position = "none") +
theme(axis.text.x = element_text(color = "#000000")) +
geom_text(aes(x = 2.1, y = 70, label = "t-test, p = 0.938"),
          color = "gray30",
          size = 2.5) +
theme(plot.margin = margin(0.1, 0.1, 0.1, 0.1, "in")) +
stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
            width = 0.75, linetype = "dashed") +
theme(plot.title = element_text(size = 12))
fungi.rich.plot.21

```



Combine plots for Supp Fig 7

```

# Combine soil richness -----
tiff("figures/2023-09_publish-figures/Soil-richness.tiff", units = "in", height = 4, width = 5.5, res =
ggarrange(barc.rich.plot.21, fungi.rich.plot.21,

```

```

ncol = 2, nrow = 1,
labels = c("(A)", "(B)"))

dev.off()

```

Supp Fig 8a: Chemoheterotrophic bacteria & archaea

```

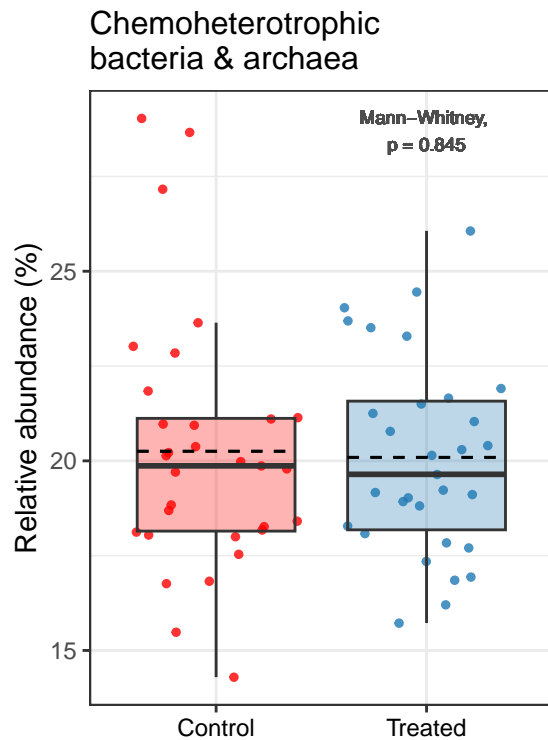
# Chemoheterotrophs -----

# Mann-Whitney
wilcox.test(filter(dat.2021, Treatment == "Control")$chemoheterotrophy_perc,
            filter(dat.2021, Treatment == "Treated")$chemoheterotrophy_perc) # p = 0.8449

##
## Wilcoxon rank sum exact test
##
## data: filter(dat.2021, Treatment == "Control")$chemoheterotrophy_perc and filter(dat.2021, Treatment == "Treated")$chemoheterotrophy_perc
## W = 466, p-value = 0.8449
## alternative hypothesis: true location shift is not equal to 0

# Plot
chemohet.plot.21 <- dat.2021 %>%
  ggplot(aes(Treatment, chemoheterotrophy_perc)) +
  geom_jitter(aes(color = Treatment),
             alpha = 0.8,
             size = 1) +
  geom_boxplot(aes(fill = Treatment),
              alpha = 0.3,
              outlier.shape = NA) +
  xlab(NULL) +
  ylab("Relative abundance (%)") +
  ggtitle("Chemoheterotrophic \nbacteria & archaea") +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "#000000")) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.1, "in")) +
  geom_text(aes(x = 2, y = 28.7, label = "Mann-Whitney, \np = 0.845"),
            color = "gray30",
            size = 2.5) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
              width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
chemohet.plot.21

```



Supp Fig 8b: Nitrogen-cycling bacteria & archaea

```
# N-cyclers -----
# Mann-Whitney
wilcox.test(filter(dat.2021, Treatment == "Control")$n.cycler_perc,
             filter(dat.2021, Treatment == "Treated")$n.cycler_perc) # p-value = 0.5854
```

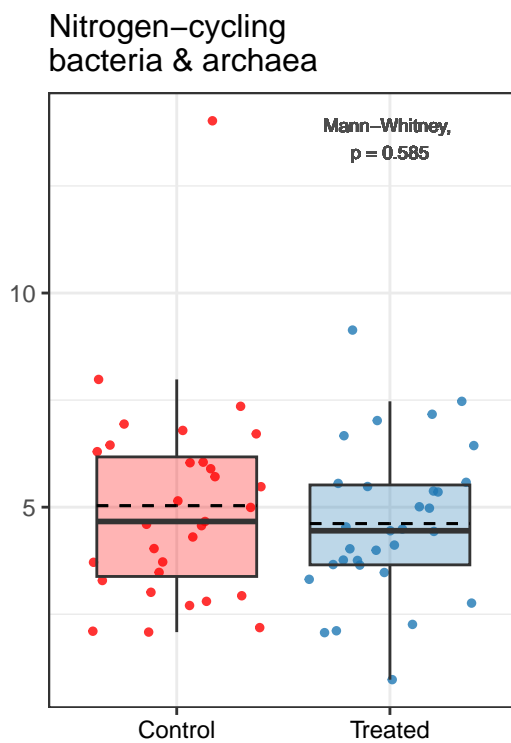
```
##
## Wilcoxon rank sum exact test
##
## data: filter(dat.2021, Treatment == "Control")$n.cycler_perc and filter(dat.2021, Treatment == "Treated")$n.cycler_perc
## W = 520, p-value = 0.5854
## alternative hypothesis: true location shift is not equal to 0
```

```
# Plot
ncycler.plot.21 <- dat.2021 %>%
  ggplot(aes(Treatment, n.cycler_perc)) +
  geom_jitter(aes(color = Treatment),
              alpha = 0.8,
              size = 1) +
  geom_boxplot(aes(fill = Treatment),
               alpha = 0.3,
               outlier.shape = NA) +
  xlab(NULL) +
  ylab(NULL) +
```

```

ggtitle("Nitrogen-cycling \nbacteria & archaea") +
scale_color_manual(values = c("red", "#1F78B4")) +
scale_fill_manual(values = c("red", "#1F78B4")) +
theme_bw() +
theme(legend.position = "none") +
theme(axis.text.x = element_text(color = "#000000")) +
theme(plot.margin = margin(0.1, 0.1, 0.1, 0.25, "in")) +
geom_text(aes(x = 2, y = 13.6, label = "Mann-Whitney, \np = 0.585"),
          color = "gray30",
          size = 2.5) +
stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
            width = 0.75, linetype = "dashed") +
theme(plot.title = element_text(size = 12))
ncycler.plot.21

```



Supp Fig 8c: Saprotrophic fungi

```

# Saprotrophs -----

# T-test
t.test(filter(dat.2021, Treatment == "Control")$saprotroph,
       filter(dat.2021, Treatment == "Treated")$saprotroph) # NS, p = 0.272

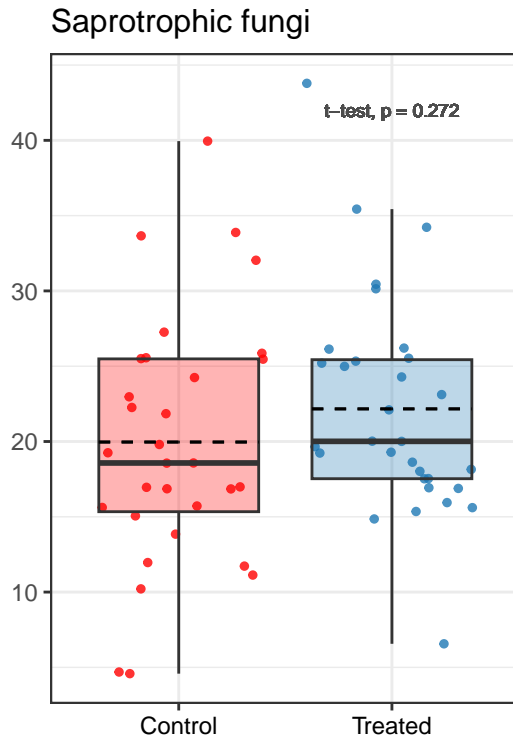
##
## Welch Two Sample t-test
##

```



```
## data: filter(dat.2021, Treatment == "Control")$saprotroph and filter(dat.2021, Treatment == "Treated")$saprotroph
## t = -1.1097, df = 59.068, p-value = 0.2716
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -6.168384 1.767276
## sample estimates:
## mean of x mean of y
## 19.96490 22.16546
```

```
# Plot
sapro.plot.21 <- dat.2021 %>%
  ggplot(aes(Treatment, saprotroph)) +
  geom_jitter(aes(color = Treatment),
    alpha = 0.8,
    size = 1) +
  geom_boxplot(aes(fill = Treatment),
    alpha = 0.3,
    outlier.shape = NA) +
  xlab(NULL) +
  ylab(NULL) +
  ggtitle("Saprotrophic fungi") +
  scale_color_manual(values = c("red", "#1F78B4")) +
  scale_fill_manual(values = c("red", "#1F78B4")) +
  theme_bw() +
  theme(legend.position = "none") +
  theme(axis.text.x = element_text(color = "#000000")) +
  theme(plot.margin = margin(0.1, 0.1, 0.1, 0.25, "in")) +
  geom_text(aes(x = 2, y = 42, label = "t-test, p = 0.272"),
    color = "gray30",
    size = 2.5) +
  stat_summary(fun = mean, geom = "errorbar", aes(ymax = after_stat(y), ymin = after_stat(y)),
    width = 0.75, linetype = "dashed") +
  theme(plot.title = element_text(size = 12))
sapro.plot.21
```



Combine plots for Supp Fig 8

```
# Combine FAPROTAX and FUNGuild -----
tiff("figures/Soil-functional.tiff", units = "in", height = 4, width = 7, res = 300)
ggarrange(chemohet.plot.21, ncycler.plot.21, sapro.plot.21,
  ncol = 3, nrow = 1,
  labels = c("(A)", "(B)", "(C)"))
dev.off()
```

```
## Warning in dev.off(): unable to open TIFF file 'figures/Soil-functional.tiff'
```

```
## pdf
## 2
```

Appendix D: Structural equation modeling

Setup

```
library(lavaan)
library(tidyverse)
```

```
# Load data -----  
dat.2021 <- read.csv("data/Veg-soil-elev_2021.csv")
```

Latent variables

Model 1

Model 2

Model 3

Model 4