Differential effects of press versus pulse seawater intrusion on microbial communities of a tidal freshwater marsh

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Setup

```
library("tidyverse")
library("vegan")
library("viridis")
library("lubridate")
library("pander")
library("ggrepel")
library("ggtext")
library("cowplot")
library("RVAideMemoire")

theme_set(theme_classic() +
            theme(strip.background = element_blank(),
                  panel.grid.minor = element_blank()))
my.palette <- c("#29843F", "#255386", "#EE7925", "#97340F")
```

Load and clean the data

```
# read in experimental design and change classes and sample names
design <- read_csv("data/design.csv",
   col_types = cols(
     sample_ID = col_character(),
     date = col_date(format = "%m-%Y"),
     treatment = col_factor(levels = c("C", "CS", "F", "PU", "PR"), ordered = T),
     replicate = col_integer())) %>%
  mutate(sample_ID = paste0("s", gsub("\\_", "", sample_ID))) %>%
  arrange(date)
design <- rbind(
  cbind(design, molecule = "DNA"),
  cbind(design, molecule = "RNA")
)
design <- design %>%
  mutate(sample_ID = ifelse(
    molecule == "DNA",
    sample_ID,
    paste0(sample_ID, "_cDNA")))

otus <- read_tsv("data/otu.tsv") %>% select(-taxonomy) %>% t()
```

```
colnames(otus) <- paste0("otu", (otus[1, ]))
otus <- otus[-1,]
head(otus[,1:10])
```

```
##                otu0  otu1 otu2 otu3 otu4 otu5 otu6 otu7  otu8 otu9
## 10_15_C3       6766  2523 2702 3391 2465  975 1336 1157  2692 1656
## 10_15_C3_cDNA 20672 12586 7564 4514 4729 2635 4109 4368 11747 4176
## 10_15_C4       5101  2487 2478 3797 2534 1321 1104 1884  1880 1262
## 10_15_C4_cDNA  9264  6145 3748 3233 3290 2008 1946 3740  4246 2081
## 10_15_C5       2596  1637 2048 5005 1720 1453 2993 1927  1252 1600
## 10_15_C5_cDNA  7777  8289 5486 6732 3642 4192 9127 6692  4319 4162
```

```
tax <- read_tsv("data/tax.tsv",
                col_names = F) %>%
  select(X1, X3) %>%
  rename(OTU = X1, taxonomy = X3)

# create site by species matrices
rna <- otus[(rownames(otus) %>% endsWith("_cDNA")),]
dna <- otus[!(rownames(otus) %>% endsWith("_cDNA")),]

# make rownames of otutable match sample names
rnames <- gsub("7_1", "07_1", rownames(otus)) # make dates two digits
rnames <- gsub("3_1", "03_1", rnames)
rnames <- gsub("\\_", "", rnames) # remove underscores
rnames <- gsub("cDNA", "_cDNA", rnames)
rnames <- paste0("s",rnames)
rownames(otus) <- rnames


# update design object to reflect the samples
design <- inner_join(tibble(sample_ID = rownames(otus)), design) %>%
  filter(molecule == "DNA") %>%
  filter(treatment %in% c("C", "F", "PU", "PR")) %>%
  filter(month(date, label = T) %in% c("Oct")) %>%
  filter(year(date) %in% c(2016)) # subset just to sequential fall samples

# reorder the otu table to match design
otus <- otus[match(design$sample_ID, rownames(otus)),]
all.equal.character(rownames(otus), design$sample_ID) # check that order is correct
```

```
## [1] TRUE
```

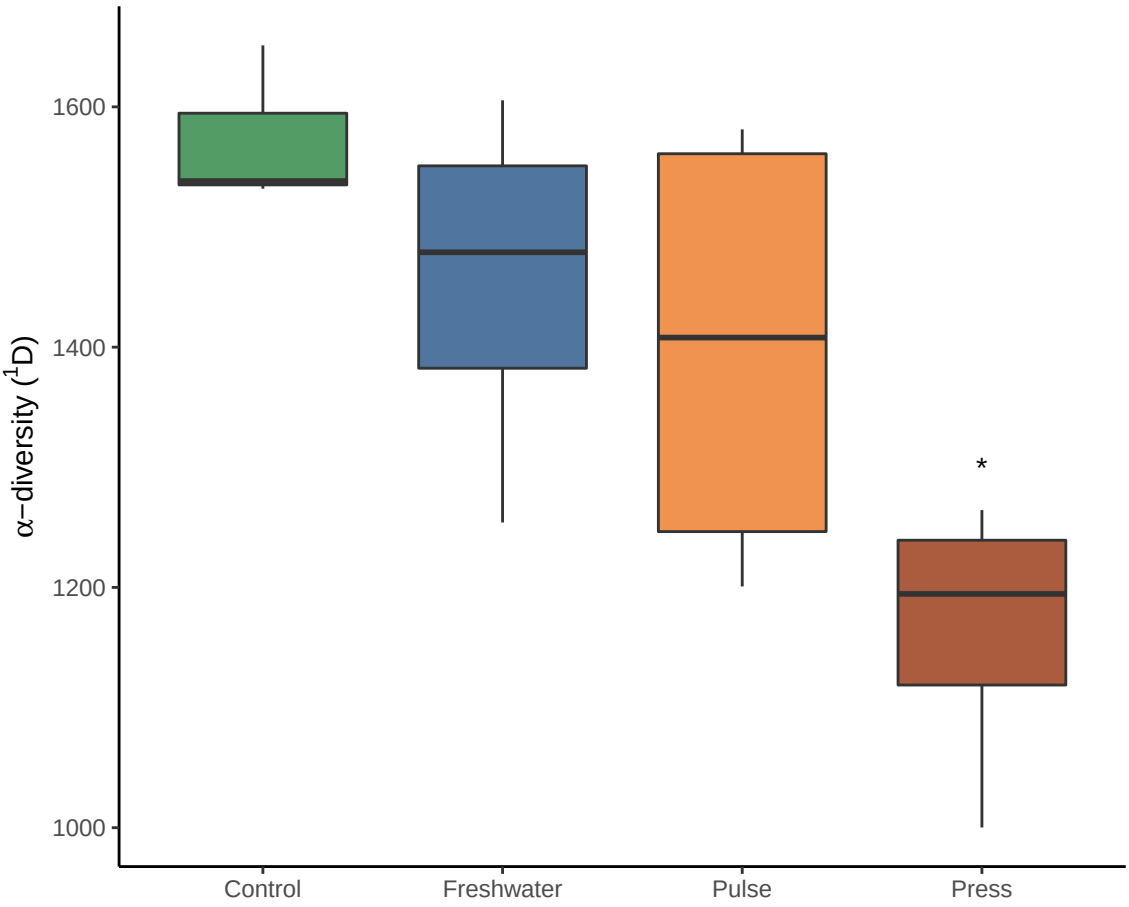
```
# coverage per sample and rarefy to lowest coverage
coverage <- rowSums(otus)
otus <- otus[which(coverage > 10000),]
otus <- rrarefy(otus,  min(coverage))

# remove empty columns
otus <- otus[,-which(colSums(otus) == 0)]

# read environmenal data
env <- read_csv("data/env_data.csv")
env$date <- as_date(paste0(env$Year,'-',env$Month,'-01'))
colnames(env)[4:5] <- c("treatment", "replicate")
```

```r
env <- inner_join(design, env)

levels(design$treatment) <- c("Control", "Control with sides",
                              "Freshwater", "Pulse", "Press")
```

Results

How does the diversity of a local community change under our different experimental treatments?

```r
otus.rich <- rowSums(decostand(otus, method = "pa"))
otus.alpha <- exp(diversity(otus, "shannon"))

data.frame(richness = otus.rich, diversity = otus.alpha) %>%
  rownames_to_column("sample_ID") %>%
  left_join(design) %>%
  group_by(date, treatment, molecule) %>%
  ggplot(aes(x = treatment, y = diversity, fill = treatment)) +
  # geom_point(alpha = 0.5) +
  geom_boxplot(width = .7, position = position_dodge(), alpha = .8) +
  annotate("text", x = "Press", y = 1300, label = "*", size = 4) +
  labs(x = "", y = expression(paste(alpha,"-diversity ("^1,"D)"))) +
  scale_fill_manual(values = my.palette) +
  theme(legend.position = "none") +
  ggsave("figures/Fig1.png", width = 4, height = 3, units = "in", dpi = 500) +
  ggsave("figures/Fig1.pdf", width = 4, height = 3, units = "in")
```




```
alpha.by.treat <- data.frame(richness = otus.rich, diversity = otus.alpha) %>%
  rownames_to_column("sample_ID") %>%
  left_join(design)
alpha.mod <- aov(diversity ~ treatment, data = alpha.by.treat)
summary(alpha.mod)
```

```
##             Df Sum Sq Mean Sq F value Pr(>F)
## treatment    3 323309  107770   5.027 0.0196 *
## Residuals   11 235821   21438
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
(TukeyHSD(alpha.mod))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = diversity ~ treatment, data = alpha.by.treat)
##
## $treatment
##                           diff       lwr       upr     p adj
## Freshwater-Control -119.39601 -455.9501 217.15808 0.7151170
## Pulse-Control      -174.26366 -510.8177 162.29043 0.4387888
## Press-Control      -410.31581 -746.8699 -73.76172 0.0166008
## Pulse-Freshwater    -54.86765 -366.4562 256.72089 0.9499729
## Press-Freshwater   -290.91980 -602.5083  20.66874 0.0695577
```

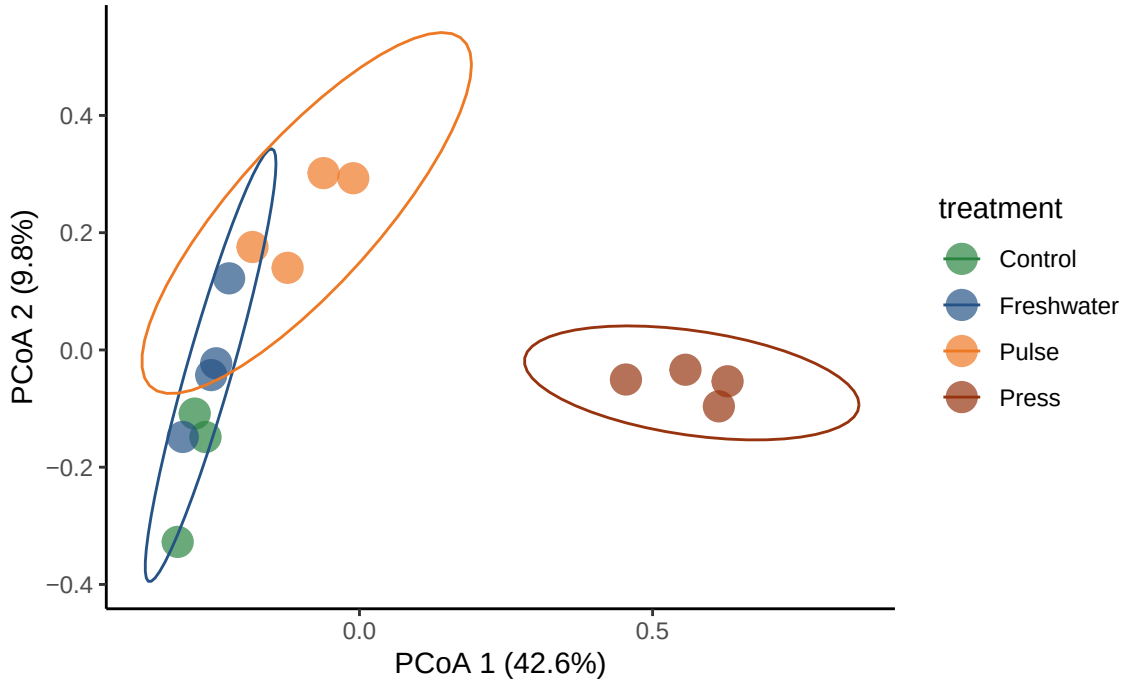
```
## Press-Pulse        -236.05215 -547.6407  75.53639 0.1620493
```

How does saltwater addition change community structure?

```
otus.hel <- decostand(otus, method = "hellinger")
otus.dist <- vegdist(otus.hel, method = "euclidean")
otus.pcoa <- cmdscale(otus.dist, eig = T)

# explained variance
explained <- round(100*eigenvals(otus.pcoa)[c(1,2)]/sum(eigenvals(otus.pcoa)),1)
as.data.frame(scores(otus.pcoa)) %>%
  rownames_to_column("sample_ID") %>%
  left_join(design) %>%
  ggplot(aes(x = Dim1, y = Dim2, color = treatment)) +
  geom_point(size = 5, alpha = 0.7) +
  coord_fixed() +
  scale_color_manual(values = my.palette) +
  labs(x = paste0("PCoA 1 (", explained[1],"%)"),
       y = paste0("PCoA 2 (", explained[2],"%)")) +
  stat_ellipse() +
  ggsave("figures/ordination.png", width = 5, height = 5, units = "in", dpi = 500)
```

```
## Warning: Removed 1 row(s) containing missing values (geom_path).
```

```
## Warning: Removed 1 row(s) containing missing values (geom_path).
```




```
# PERMANOVA
perma.otus <- adonis(otus.hel ~ treatment, method = "euclidean", data = design)
pander(perma.otus$aov.tab)
```

Table 1: Permutation: free

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
treatment	3	2.369	0.7898	4.43	0.5472	0.001
Residuals	11	1.961	0.1783	NA	0.4528	NA
Total	14	4.33	NA	NA	1	NA

```
pairwise.perm <- pairwise.perm.manova(resp = vegdist(otus.hel, method = "euclidean"),
                                      fact = design$treatment, nperm = 9999,
                                      R2 = TRUE, F = TRUE)
pairwise.perm$p.value
```

```
##               Control Freshwater  Pulse
## Freshwater 0.08571429         NA     NA
## Pulse      0.03780000     0.0378     NA
## Press      0.03780000     0.0378 0.0378
```

```
pairwise.perm$F.value
```

```
##             Control Freshwater    Pulse
## Freshwater 1.249765         NA       NA
## Pulse      2.440048   1.854390       NA
## Press      6.856599   7.396801 5.663425
```

```
pairwise.perm$R2.value
```

```
##              Control Freshwater     Pulse
## Freshwater 0.1999699         NA        NA
## Pulse      0.3279613  0.2360960        NA
## Press      0.5782939  0.5521319 0.4855714
```

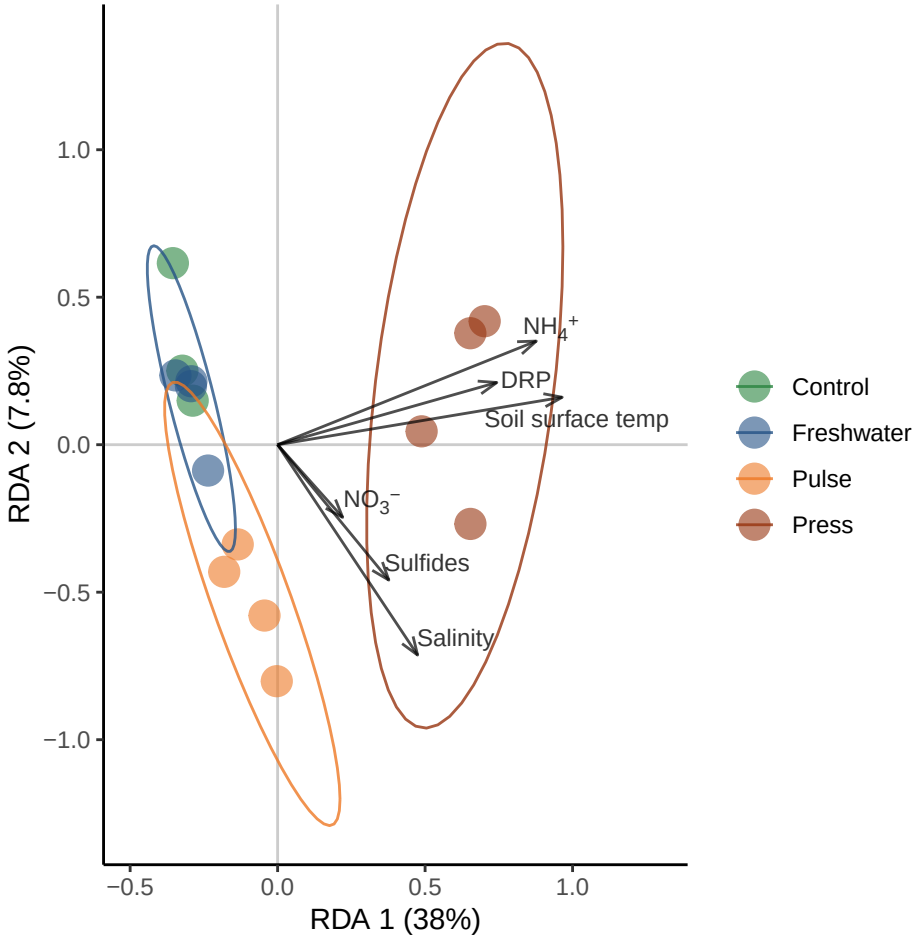
Quantitative predictors with Redundancy Analysis

```
env_vars <- env %>% select(DRP, NH4, NO2_3, Sulfides, Salinity, Soil_surface_temp)

rda.out <- rda(otus.hel ~ ., data = as.data.frame(scale(env_vars)))
# anova(rda.out)
# output:
# Model: rda(formula = otus.hel ~ DRP + NH4 + NO2_3 +
#       Sulfides + Salinity + Soil_surface_temp, data = as.data.frame(scale(env_vars)))
#          Df Variance      F Pr(>F)
# Model     6  0.18684 2.0298  0.004 **
# Residual  8  0.12273
# ---

# anova(rda.out, by = "axis")
# output:   Df Variance   F     Pr(>F)
# RDA1      1 0.117692 7.6717  0.003 **
# RDA2      1 0.024181 1.5762  0.492
# RDA3      1 0.012699 0.8278  1.000
# RDA4      1 0.011460 0.7470  1.000
# RDA5      1 0.010688 0.6967  0.980
# RDA6      1 0.010116 0.6594  0.835
# Residual  8 0.122729
```

```
# anova(rda.out, by = "terms")
# output:
#                   Df Variance      F Pr(>F)
# DRP                1 0.070641 4.6047  0.003 **
# NH4                1 0.041443 2.7014  0.016 *
# NO2_3              1 0.013999 0.9125  0.416
# Sulfides           1 0.016840 1.0977  0.297
# Salinity           1 0.025217 1.6438  0.099 .
# Soil_surface_temp  1 0.018696 1.2187  0.256
# Residual           8 0.122729

env.vecs <- as.data.frame(rda.out$CCA$biplot[,c(1,2)])
scale.vecs <- 1
explained <- round(100*eigenvals(rda.out)[c(1,2)]/sum(eigenvals(rda.out)),1)

# create vector labels
env.vecs$labels <- c(
  "DRP",
  "NH<sub>4</sub><sup>+</sup>",
  "NO<sub>3</sub><sup>-</sup>",
  "Sulfides",
  "Salinity",
  "Soil surface temp"
)

# plot constrained ordination
as.data.frame(scores(rda.out)$sites) %>%
  rownames_to_column("sample_ID") %>%
  left_join(design) %>%
  ggplot(aes(x = RDA1, y = RDA2, color = treatment)) +
  geom_hline(aes(yintercept = 0), alpha = 0.2) +
  geom_vline(aes(xintercept = 0), alpha = 0.2) +
  geom_point(size = 5, alpha = 0.6) +
  stat_ellipse(alpha = 0.8) +
  coord_fixed() +
  scale_color_manual("", values = my.palette) +
  scale_x_continuous(limits = c(-.5, 1.3)) +
  labs(x = paste0("RDA 1 (", explained[1],"%)"),
       y = paste0("RDA 2 (", explained[2],"%)")) +
  geom_segment(data = env.vecs, size = .5,
               aes(x = 0, y = 0,
                   xend = scale.vecs*RDA1,
                   yend = scale.vecs*RDA2),
               alpha = .7, color = "black",
               arrow = arrow(angle = 20,
                             length = unit(.1, "inches"),
                             type = "open")) +
  geom_richtext(alpha = 0.8, data = env.vecs, size = 3,
                  aes(x = (scale.vecs)*RDA1 + .05,
                      y = (scale.vecs)*RDA2 + .05,
                      label = labels),
                  color = "black",
                #label.padding = grid::unit(rep(0,4), "pt"),
```

```
                label.color = NA, fill = NA,
                nudge_y = c(-.05,-.01,0,0,0,-.13),
                nudge_x = c(0.05,0,0.05,0.08,0.08,0)) +
  ggsave("figures/Fig2.png", width = 7, height = 5, units = "in", dpi = 500) +
  ggsave("figures/Fig2.pdf", width = 7, height = 5, units = "in")
```

```
## Joining, by = "sample_ID"
## Too few points to calculate an ellipse
## Warning: Removed 1 row(s) containing missing values (geom_path).
## Too few points to calculate an ellipse
## Warning: Removed 1 row(s) containing missing values (geom_path).
## Too few points to calculate an ellipse
## Warning: Removed 1 row(s) containing missing values (geom_path).
```



Taxonomic analyses

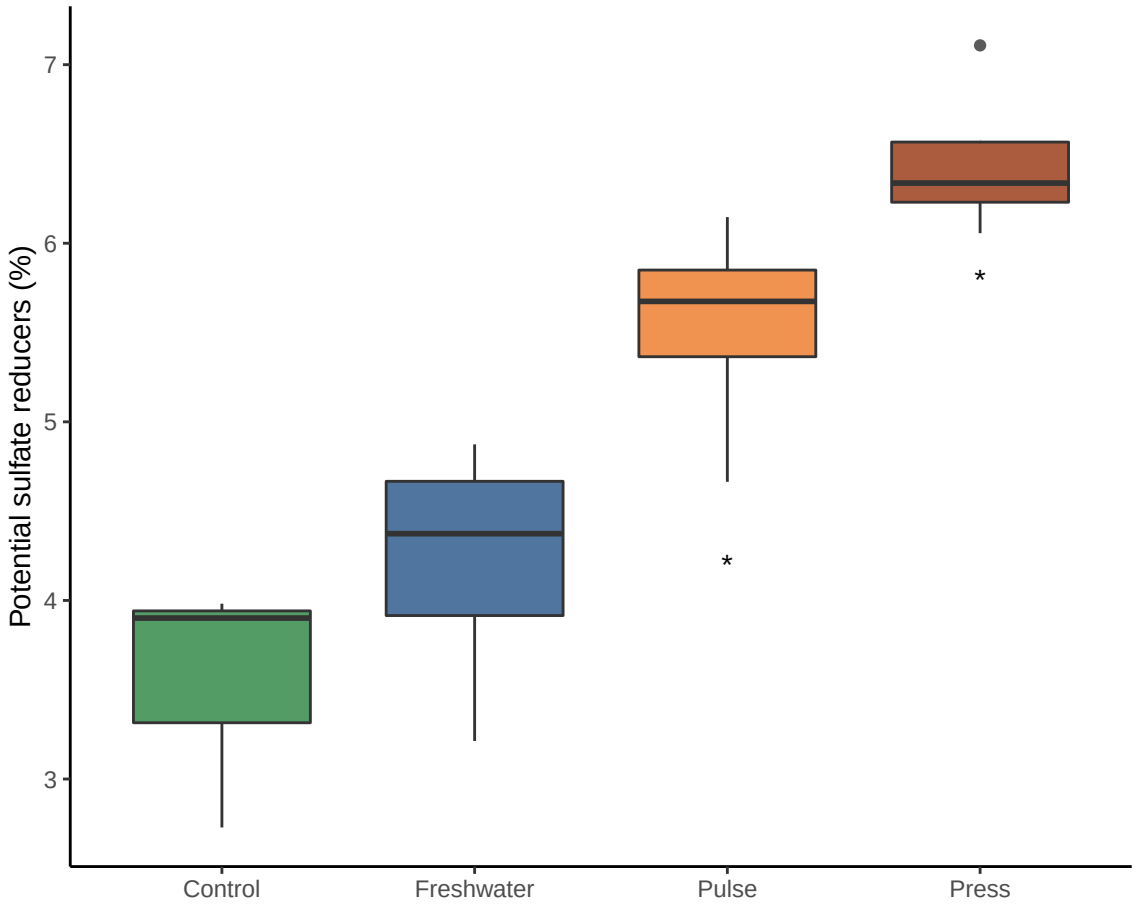
```
tax.expand <- tax %>% separate(taxonomy, into = c("domain", "phylum",
                                                  "class", "order",
                                                  "family", "genus",
                                                  "species"), ",")
```

```
## Warning: Expected 7 pieces. Missing pieces filled with `NA` in 36880 rows [1, 2,
## 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

```
# separate putative sulfate reducers
desulf.tax <- tax.expand %>%
  group_by(domain, phylum, class, order, family, genus, species) %>%
  filter(stringr::str_detect(order, "sulf")|stringr::str_detect(order, "Sulf"))
#unique(desulf.tax$OTU)
desulf.cols <- which(colnames(otus) %in% paste0("otu",desulf.tax$OTU))
otus.rel <- decostand(otus, method = "total")

# plot
sulf.plot <- as.data.frame(rowSums(otus.rel[,desulf.cols])) %>%
  rownames_to_column(var = "sample_ID") %>%
  rename(sulf_percent = "rowSums(otus.rel[, desulf.cols])") %>%
  # gather(-sample_ID, key = otu, value = abundance) %>%
  left_join(design) %>%
  group_by(date, treatment, molecule) %>%
  ggplot(aes(x = treatment, y = sulf_percent*100, fill = treatment)) +
  #geom_jitter(alpha = 0.25, show.legend = F) +
  geom_boxplot(alpha = .8, width = .7) +
  #scale_y_log10() +
  annotate("text", x = "Press", y = 5.8, label = "*", size = 4) +
  annotate("text", x = "Pulse", y = 4.2, label = "*", size = 4) +
  scale_fill_manual(values = my.palette) +
  theme(legend.position = "none") +
  labs(y = "Potential sulfate reducers (%)", x = "") +
  ggsave("figures/Fig3a.png", width = 4, height = 3, units = "in", dpi = 500) +
  ggsave("figures/Fig3a.pdf", width = 4, height = 3, units = "in")
```

```
## Joining, by = "sample_ID"
```

```
sulf.plot
```




```
sulf.data <- as.data.frame(rowSums(otus.rel[,desulf.cols])) %>%
  rownames_to_column(var = "sample_ID") %>%
  rename(sulf_percent = "rowSums(otus.rel[, desulf.cols])") %>%
  # gather(-sample_ID, key = otu, value = abundance) %>%
  left_join(design)
```

```
## Joining, by = "sample_ID"
```

```
summary(aov(sulf_percent ~ treatment, data = sulf.data))
```

```
##             Df    Sum Sq  Mean Sq F value   Pr(>F)
## treatment    3 0.0018599 6.20e-04   15.59 0.000282 ***
## Residuals   11 0.0004373 3.98e-05
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
TukeyHSD(aov(sulf_percent ~ treatment, data = sulf.data))
```

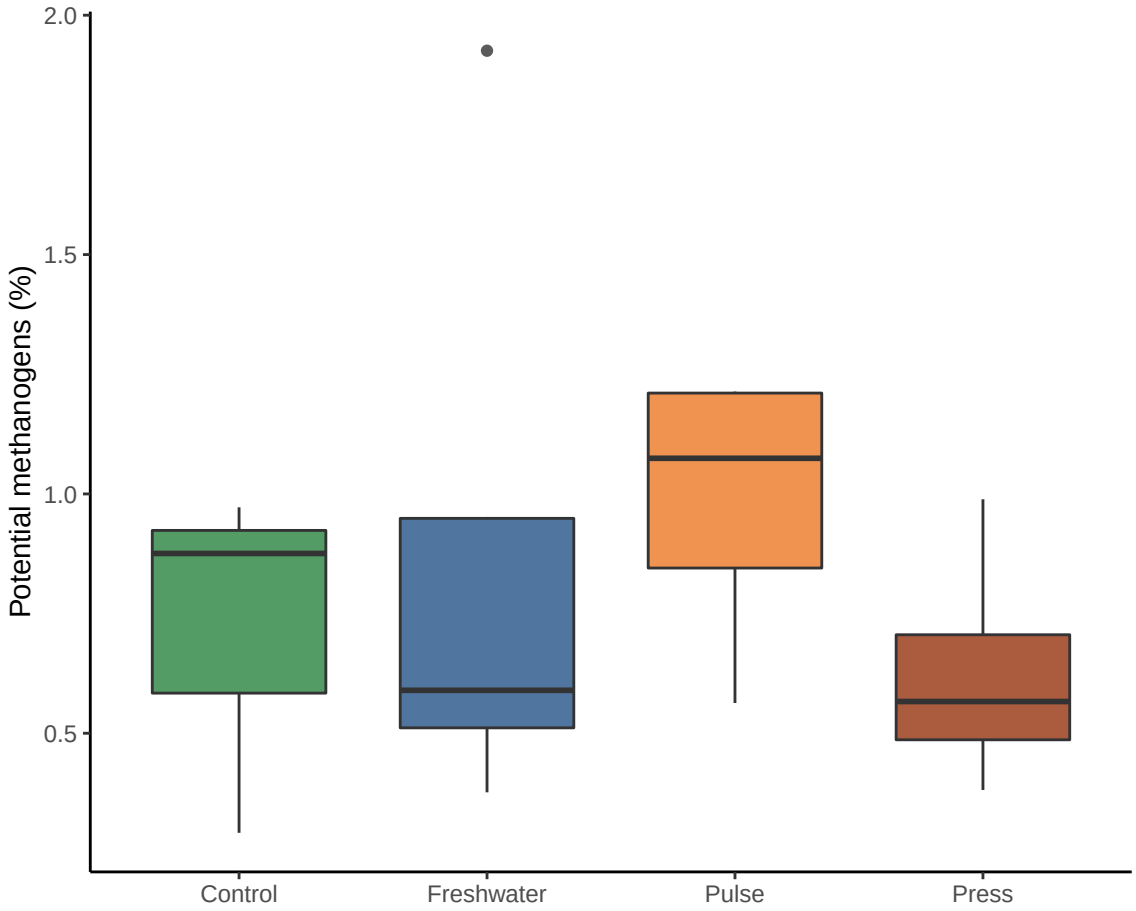
```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = sulf_percent ~ treatment, data = sulf.data)
##
## $treatment
##                          diff           lwr        upr     p adj
## Freshwater-Control 0.006713602 -0.0077797765 0.02120698 0.5278834
## Pulse-Control      0.020027253  0.0055338744 0.03452063 0.0073892
```

```
## Press-Control      0.029223298  0.0147299192 0.04371668 0.0004002
## Pulse-Freshwater   0.013313651 -0.0001046102 0.02673191 0.0519878
## Press-Freshwater   0.022509696  0.0090914347 0.03592796 0.0017966
## Press-Pulse        0.009196045 -0.0042222161 0.02261431 0.2243040
```

```
# separate putative methanogens
methan.tax <- tax.expand %>%
  group_by(domain, phylum, class, order, family, genus, species) %>%
  filter(domain == "d:Archaea") %>%
  filter(stringr::str_detect(order, "Meth") | stringr::str_detect(genus, "Meth"))
#unique(methan.tax$OTU)
methan.cols <- which(colnames(otus) %in% paste0("otu",methan.tax$OTU))
methan.plot <- as.data.frame(rowSums(otus.rel[,methan.cols])) %>%
  rownames_to_column(var = "sample_ID") %>%
  rename(methan_percent = "rowSums(otus.rel[, methan.cols])") %>%
  # gather(-sample_ID, key = otu, value = abundance) %>%
  left_join(design) %>%
  group_by(date, treatment, molecule) %>%
  ggplot(aes(x = treatment, y = methan_percent*100, fill = treatment)) +
  #geom_jitter(alpha = 0.25, show.legend = F) +
  geom_boxplot(alpha = .8, width = 0.7) +
  scale_fill_manual(values = my.palette) +
  theme(legend.position = "none") +
  #scale_y_log10() +
  labs(y = "Potential methanogens (%)", x = "") +
  ggsave("figures/Fig3b.png", width = 4, height = 3, units = "in", dpi = 500) +
  ggsave("figures/Fig3b.pdf", width = 4, height = 3, units = "in")
```

```
## Joining, by = "sample_ID"
```

```
methan.plot
```




```
methan.dat <- as.data.frame(rowSums(otus.rel[,methan.cols])) %>%
  rownames_to_column(var = "sample_ID") %>%
  rename(methan_percent = "rowSums(otus.rel[, methan.cols])") %>%
  # gather(-sample_ID, key = otu, value = abundance) %>%
  left_join(design)
```

```
## Joining, by = "sample_ID"
```

```
summary(aov(methan_percent ~ treatment, data = methan.dat))
```

```
##             Df    Sum Sq   Mean Sq F value Pr(>F)
## treatment    3 2.954e-05 9.848e-06   0.476  0.705
## Residuals   11 2.273e-04 2.067e-05
```

```
TukeyHSD(aov(methan_percent ~ treatment, data = methan.dat))
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = methan_percent ~ treatment, data = methan.dat)
##
## $treatment
##                             diff          lwr         upr     p adj
## Freshwater-Control  0.0015728544 -0.008877024 0.012022733 0.9676605
## Pulse-Control       0.0026821798 -0.007767698 0.013132058 0.8652156
## Press-Control      -0.0008746492 -0.011324527 0.009575229 0.9940594
## Pulse-Freshwater    0.0011093253 -0.008565382 0.010784032 0.9851204
```

```
## Press-Freshwater   -0.0024475036 -0.012122211 0.007227204 0.8699996
## Press-Pulse        -0.0035568289 -0.013231536 0.006117878 0.6931620
```

```
# plot combined
plot_grid(sulf.plot, methan.plot, labels = c("a.", "b."), label_fontface = "plain") +
  ggsave("figures/Fig3.png", width = 8, height = 3.2, units = "in", dpi = 500) +
  ggsave("figures/Fig3.pdf", width = 8, height = 3.2, units = "in")
```