

Setup

Load and clean the data

Here i focused just on the control, freshwater, pulse, press treatments in October 2016.

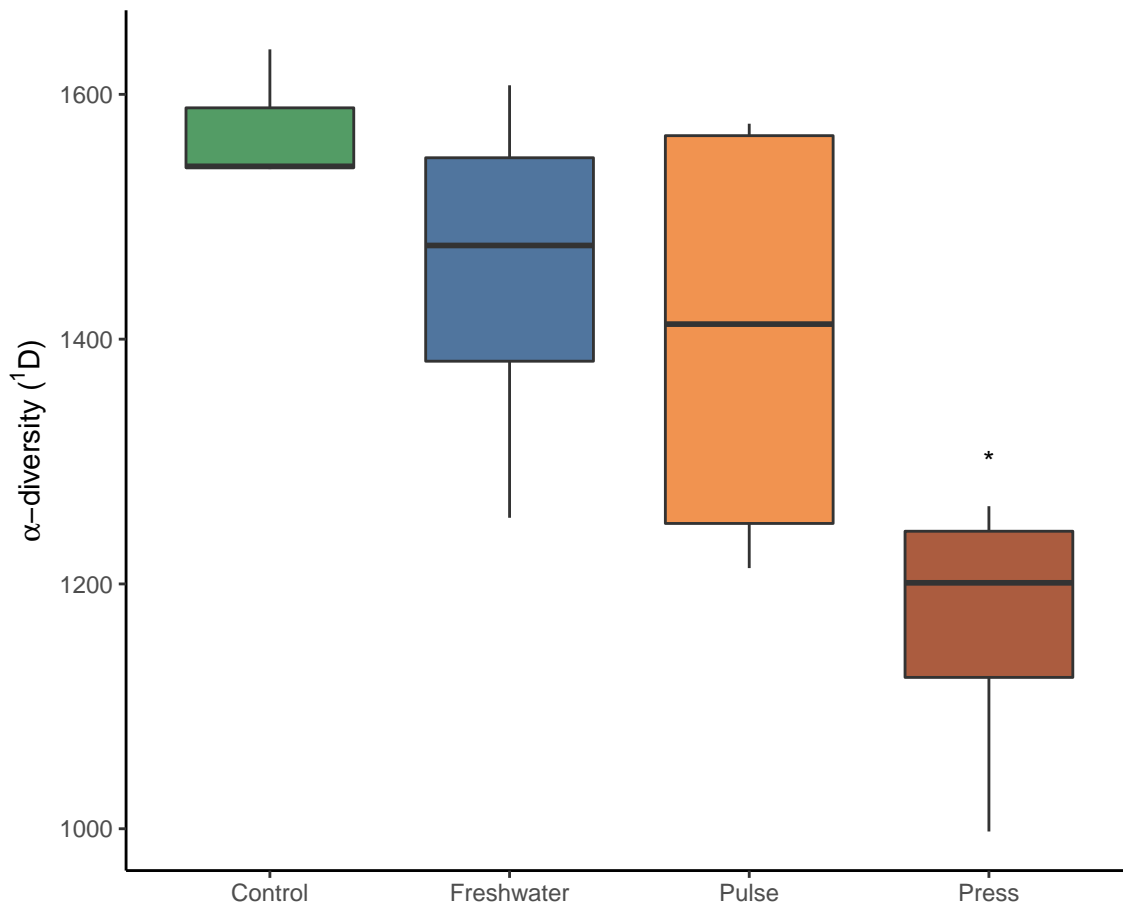
Results

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

For taxa to persist in a treatment, they must be able to grow locally or immigrate from elsewhere. Here, we compare an estimate of local (alpha) diversity for the total DNA community within each treatment.

```
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/alpha.png", width = 4, height = 3, units = "in", dpi = 500)
```



```
alpha.by.treat <- data.frame(richness = otus.rich, diversity = otus.alpha) %>% rownames_to_column("sample")
  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 317586   105862    5.051 0.0193 *
## Residuals   11 230562    20960
## ---
## 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 -118.65511 -451.4353  214.12509 0.7120727
## Pulse-Control      -168.90813 -501.6883  163.87206 0.4549141
## Press-Control      -406.51098 -739.2912 -73.73078 0.0164026
## Pulse-Freshwater   -50.25303 -358.3476  257.84157 0.9595129
## Press-Freshwater   -287.85587 -595.9505   20.23872 0.0693367
## Press-Pulse        -237.60284 -545.6974   70.49175 0.1521425
```

Here we notice that the total community has consistently higher diversity overall. We also see that freshwater additions and pulse saltwater disturbances support lower alpha-diversity than the control, but press saltwater disturbances show a stronger reduction in diversity. ANOVA indicates there are significant differences between the treatments. Tukey's post hoc test suggests there are significant differences between press and freshwater treatments, and between press and control treatments.

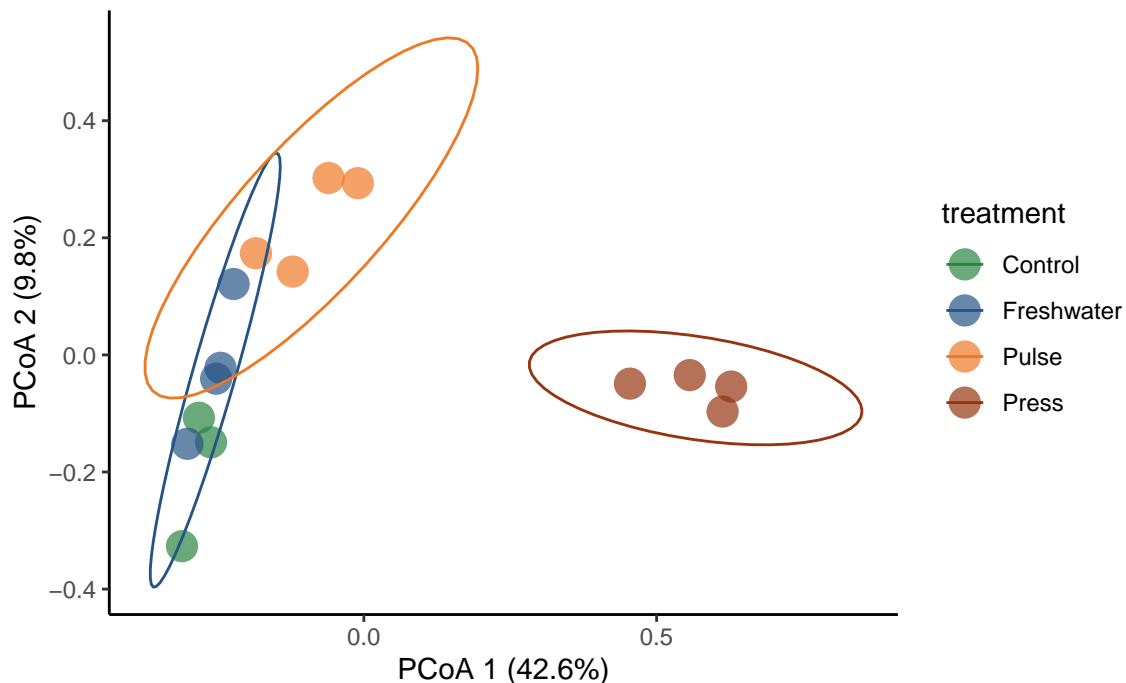
How does saltwater addition change community structure?

Here, we want to know about the diversity among sites (beta diversity).

```
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).
```



```
library(RVAideMemoire)
pairwise.perm <- pairwise.perm.manova(resp = vegdist(otus.hel, method = "euclidean"), fact = design$treatment)
```

```
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	2.368	0.7892	4.427	0.5469	0.001
Residuals	11	1.961	0.1783	NA	0.4531	NA
Total	14	4.329	NA	NA	1	NA

From this analysis, it seems that there is a gradient from freshwater, to pulse, to press treatments, with press treatments having the strongest effect on community structure. A PERMANOVA shows that treatment is a significant predictor of differences in community structure, explaining approx. 54% of the differences.

Redundancy Analysis

We can take this a step further by constraining our ordination by environmental variables to see which variables in particular are associated with differences in community structure. We'll perform a Redundancy Analysis (RDA).

```
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 = a
#           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)

fit <- envfit(rda.out, env_vars, permutations = 999, display = "lc")
fit
```

```
##
## ***VECTORS
##
##           RDA1      RDA2      r2 Pr(>r)
## DRP          0.96252  0.27120 0.5965 0.013 *
## NH4          0.92843  0.37151 0.8919 0.001 ***
## NO2_3        0.66254 -0.74903 0.1106 0.488
## Sulfides     0.63240 -0.77464 0.3552 0.075 .
## Salinity     0.55287 -0.83327 0.7349 0.003 **
## Soil_surface_temp 0.98632  0.16482 0.9572 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

```
#plot(rda.out, type = "p")
#plot(fit, col = "red")
vif.cca(rda.out)
```

```
##           DRP           NH4           NO2_3           Sulfides
##      3.420886      4.700772      5.232420      10.353256
##      Salinity Soil_surface_temp
##      3.736271      7.161344
```

```
env.vecs$labels <- c(
  "DRP",
  "NH<sub>4</sub><sup>+</sup>",
  "NO<sub>3</sub><sup>-</sup>",
  "Sulfides",
  "Salinity",
  "Soil surface temp"
)
as.data.frame(scores(rda.out)$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/rda.png", width = 7, height = 5, units = "in", dpi = 500)

```

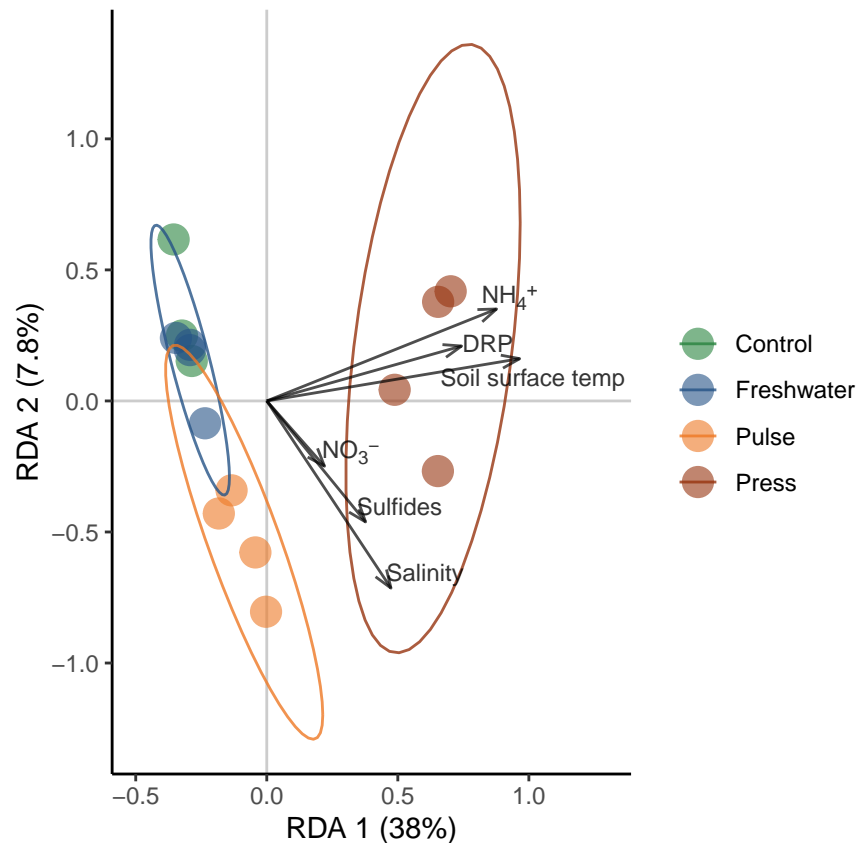
```
## Joining, by = "sample_ID"
```

```
## 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).
```



So we've confirmed our treatments worked and appear to be associated with the differences in composition. Also, pulse treatments appear to be driving composition based on nitrogen, sulfides, and salinity as well, with pulse treatments also showing significant effects of NH₄, DRP, and soil surface temp that distinguish them from the pulse treatments.

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, ...].
```

```
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)
```

```
##      [1]      0      1     19     25     36     39     66     87    116    149    160    184
##     [13]    220    226    275    279    288    297    319    353    356    377    378    379
##     [25]    401    403    441    463    499    500    520    562    576    599    609    641
##     [37]    644    646    754    772    804    819    843    883    943    959   1030   1050
##     [49]   1086   1098   1143   1188   1257   1263   1268   1276   1327   1337   1350   1427
##     [61]   1498   1505   1529   1536   1575   1592   1610   1628   1633   1772   1777   1873
##     [73]   1997   2029   2048   2072   2082   2117   2140   2173   2333   2399   2426   2548
##     [85]   2571   2606   2702   2735   2736   2782   2825   2867   2870   2925   2979   2997
##     [97]   3024   3027   3057   3078   3111   3114   3122   3163   3200   3240   3250   3299
##    [109]   3335   3428   3432   3535   3541   3567   3635   3651   3711   3735   3761   3861
##    [121]   3871   4093   4100   4140   4145   4186   4291   4321   4364   4401   4422   4433
##    [133]   4477   4572   4631   4665   4778   4865   4929   5091   5096   5208   5257   5376
##    [145]   5423   5477   5662   5776   5842   5854   5875   5905   5964   5982   5987   6057
##    [157]   6196   6350   6373   6506   6535   6612   6644   6791   6807   6813   6897   6915
##    [169]   6997   7070   7406   7408   7418   7421   7523   7626   7644   7684   7745   7773
##    [181]   7922   8051   8161   8185   8200   8205   8233   8254   8256   8297   8325   8506
##    [193]   8515   8622   8806   8827   9233   9243   9526   9562   9609   9783   9845   9856
##    [205]   9918  10034  10056  10095  10174  10211  10373  10386  10459  10471  10576  10681
##    [217]  10711  10799  10808  10817  10875  10914  10941  11113  11116  11239  11270  11292
##    [229]  11476  11585  11675  11741  11760  11767  11814  11826  11959  12208  12229  12282
##    [241]  12423  12740  12808  12868  12922  13183  13198  13251  13470  13562  13659  13758
##    [253]  13820  13907  13947  14367  14630  14650  14690  14788  14826  15030  15180  15250
##    [265]  15277  15351  15635  15647  15879  15925  15989  15992  16028  16037  16083  16140
##    [277]  16348  16357  16445  16456  16586  16765  16775  16926  17061  17100  17260  17566
##    [289]  17724  17787  17793  17980  18027  18032  18290  18480  18515  18517  18549  18616
##    [301]  18736  18777  18853  19015  19367  19854  19888  20013  20099  20163  20313  20363
##    [313]  20489  20633  20655  20795  21004  21091  21230  21237  21281  21282  21344  21384
##    [325]  21500  22071  22182  22299  22329  22491  22728  22831  22960  23054  23056  23082
##    [337]  23125  23132  23186  23612  23638  23662  24084  24192  24245  24998  25136  25151
##    [349]  25154  25461  25578  25969  26054  26508  26802  26836  26873  26883  26952  27081
##    [361]  27403  27444  27492  27706  27801  28406  28524  28934  29143  29159  29212  29262
##    [373]  29636  29970  30125  30341  30379  30462  30775  31326  31656  31892  31916  32009
##    [385]  32142  32180  32748  32754  32832  32920  33439  33572  33809  34330  34441  34442
##    [397]  34481  34641  34818  34857  35209  35285  35326  35536  35770  36083  36255  36331
```

```
desulf.cols <- which(colnames(otus) %in% paste0("otu", desulf.tax$OTU))
```

```
otus.rel <- decostand(otus, method = "total")
```

```
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/sulfate-reduction.png", width = 4, height = 3, units = "in", dpi = 500)

## Joining, by = "sample_ID"
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 0.0018960 0.000632   16.65 0.000211 ***
## Residuals  11 0.0004176 0.000038
## ---
## 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 0.007142192 -7.021052e-03 0.02130544 0.4601657
## Pulse-Control      0.020342289  6.179046e-03 0.03450553 0.0056592
## Press-Control      0.029669357  1.550611e-02 0.04383260 0.0002880
## Pulse-Freshwater   0.013200098  8.748193e-05 0.02631271 0.0483578
## Press-Freshwater   0.022527165  9.414549e-03 0.03563978 0.0014914
## Press-Pulse        0.009327068 -3.785548e-03 0.02243968 0.1999570

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)

## [1] 68 121 209 268 286 295 497 758 827 876 979 1149
## [13] 1982 2103 2309 2424 2459 2904 2928 2944 3079 3722 3882 3923
## [25] 4227 4379 4500 4616 5384 5797 6665 6712 7184 7254 8390 11093
## [37] 11885 13468 14010 15042 15237 16186 17222 19094 19186 19870 22033 22354
## [49] 25908 27302 29102 30503 31126 34123 34155

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/methanogens.png", width = 4, height = 3, units = "in", dpi = 500)

## Joining, by = "sample_ID"
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))

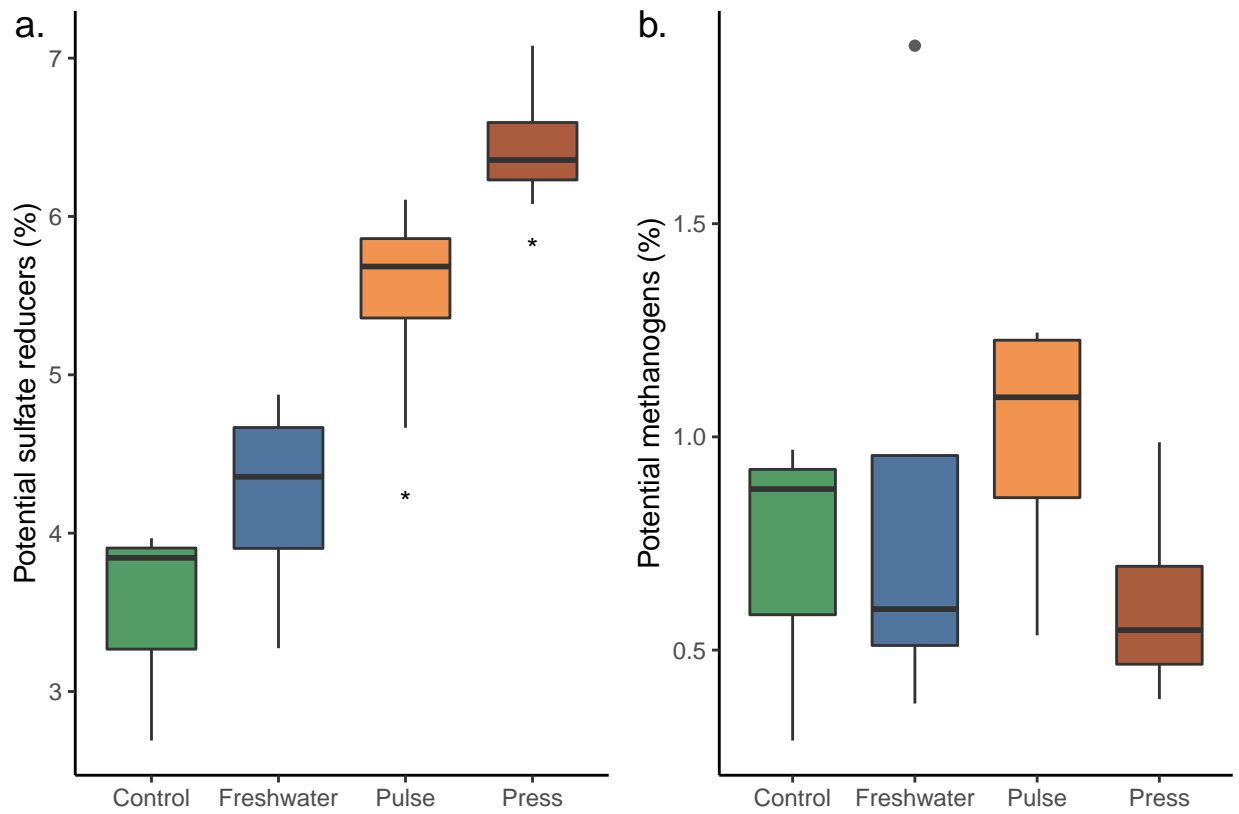
##           Df      Sum Sq   Mean Sq F value Pr(>F)
## treatment   3 3.243e-05  1.081e-05   0.516  0.679
## Residuals  11 2.302e-04  2.093e-05

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  0.0015920711 -0.008923662  0.012107804  0.9671234
## Pulse-Control       0.0027939858 -0.007721747  0.013309719  0.8531955
## Press-Control      -0.0009532628 -0.011468996  0.009562470  0.9924918
## Pulse-Freshwater    0.0012019147 -0.008533762  0.010937592  0.9815918
## Press-Freshwater   -0.0025453338 -0.012281011  0.007190343  0.8589148
## Press-Pulse        -0.0037472485 -0.013482926  0.005988429  0.6633797

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

```



From this analysis, it looks like press treatments significantly affect sulfate reducers, but less so methanogens.