Setup

Load and clean the data

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

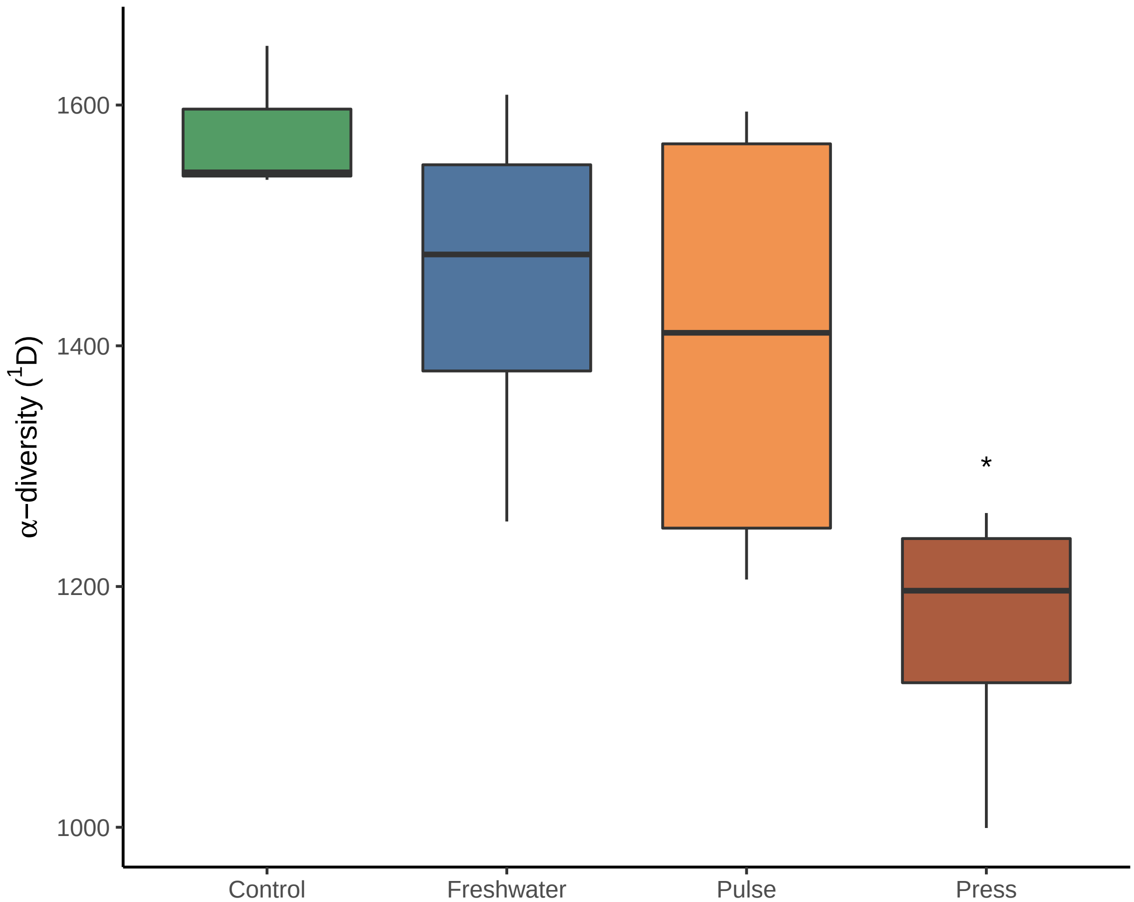
Results

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)
```




```r
alpha.by.treat <- data.frame(richness = otus.rich, diversity = otus.alpha) %>% rownames_to_column("sampl
  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 327434  109145   5.014 0.0198 *
## Residuals   11 239470   21770
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
(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 -123.45854 -462.6065 215.68947 0.6993543
## Pulse-Control      -171.55981 -510.7078 167.58820 0.4576421
## Press-Control      -413.65077 -752.7988 -74.50276 0.0165584
## Pulse-Freshwater    -48.10127 -362.0913 265.88878 0.9660177
## Press-Freshwater   -290.19223 -604.1823  23.79782 0.0728847
## Press-Pulse        -242.09096 -556.0810  71.89909 0.1522742
```

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).

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```

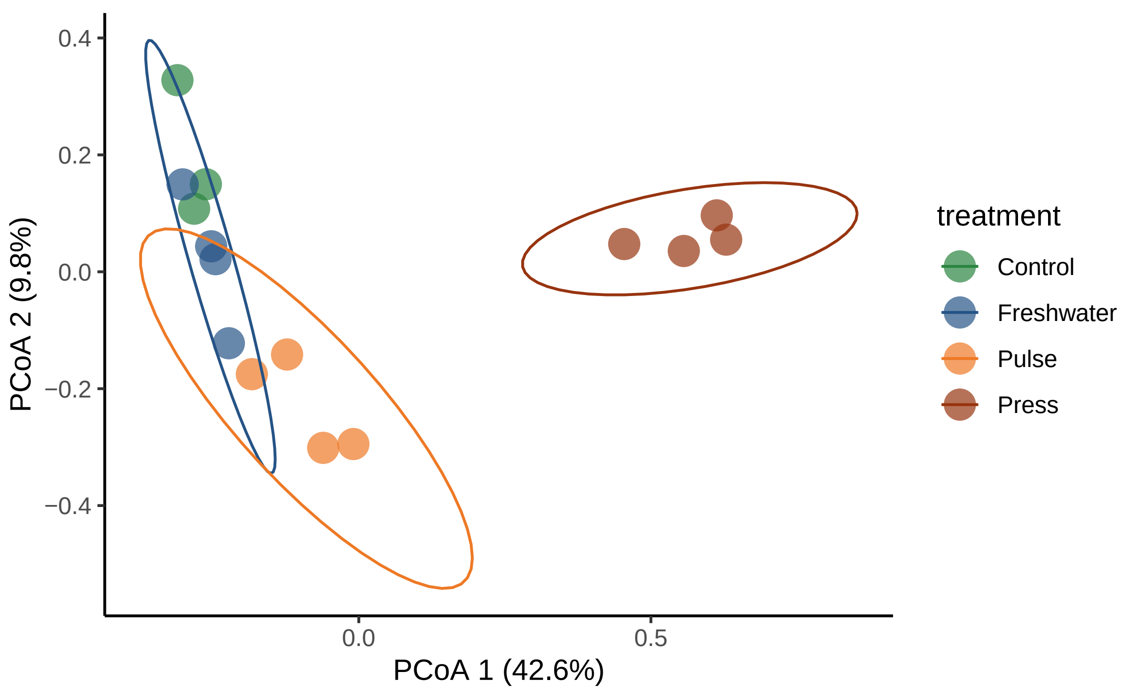


```
perma.otus <- adonis(otus.hel ~ treatment, method = "euclidean", data = design)
pander(perma.otus$aov.tab)
```

Table 1: Permutation: free

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
treatment	3	2.372	0.7905	4.431	0.5472	0.001
Residuals	11	1.962	0.1784	NA	0.4528	NA
Total	14	4.334	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)))

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)

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 = env.vecs$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"

## Warning: Use of `env.vecs$labels` is discouraged. Use `labels` instead.

## Too few points to calculate an ellipse

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

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## Too few points to calculate an ellipse

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```
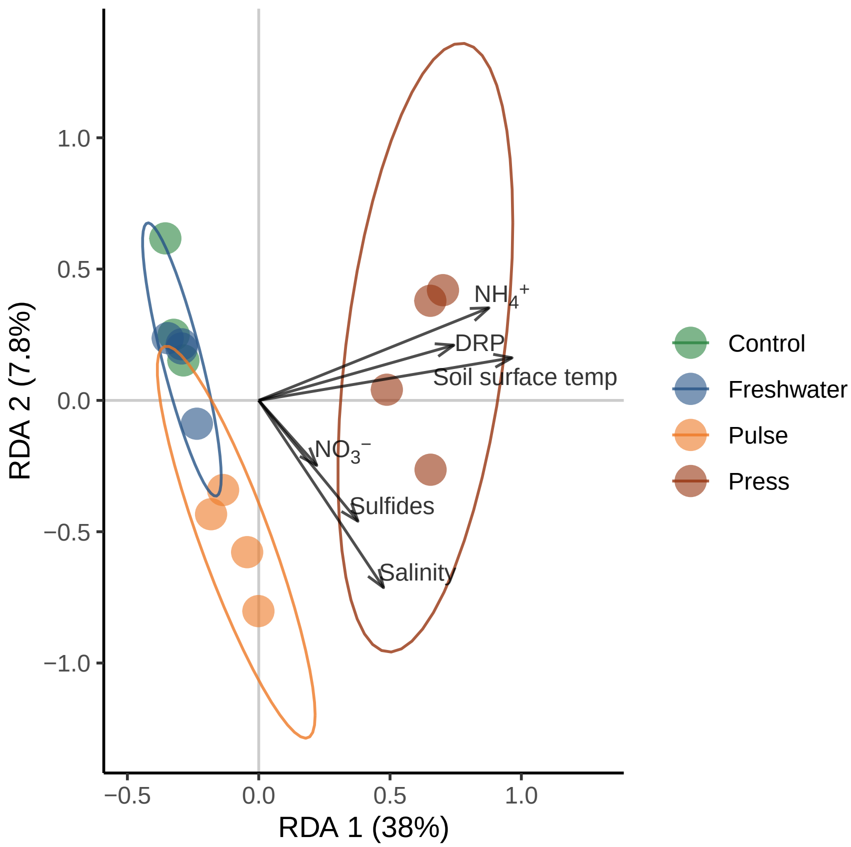




```
envfit(rda.out, env_vars)
```

```
##
## ***VECTORS
```

```
##
##                         RDA1     RDA2     r2 Pr(>r)
## DRP                  0.96332  0.26837 0.5382  0.015 *
## NH4                  0.92893  0.37025 0.8050  0.001 ***
## NO2_3                0.66435 -0.74742 0.1007  0.543
## Sulfides             0.63373 -0.77355 0.3238  0.090 .
## Salinity             0.55484 -0.83196 0.6710  0.002 **
## Soil_surface_temp    0.98702  0.16059 0.8646  0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Permutation: free
## Number of permutations: 999
```

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", "genu
```

```
## 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
```

```r
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 = "Percent potential sulfate reducers", x = "") +
  ggsave("figures/sulfate-reduction.png", width = 4, height = 3, units = "in", dpi = 500)
```

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

```r
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"
```

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

```
##             Df    Sum Sq   Mean Sq F value   Pr(>F)
## treatment    3 0.0018495 0.0006165   15.56 0.000285 ***
## Residuals   11 0.0004358 0.0000396
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
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 0.006926150 -0.0075422445 0.02139454 0.5019006
## Pulse-Control      0.020049381  0.0055809866 0.03451778 0.0072483
```

```
## Press-Control      0.029245426  0.0147770315 0.04371382 0.0003918
## Pulse-Freshwater   0.013123231 -0.0002718991 0.02651836 0.0553369
## Press-Freshwater   0.022319276  0.0089241458 0.03571441 0.0018934
## Press-Pulse        0.009196045 -0.0041990854 0.02259118 0.2231442
```

```
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 = "Percent 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))
```

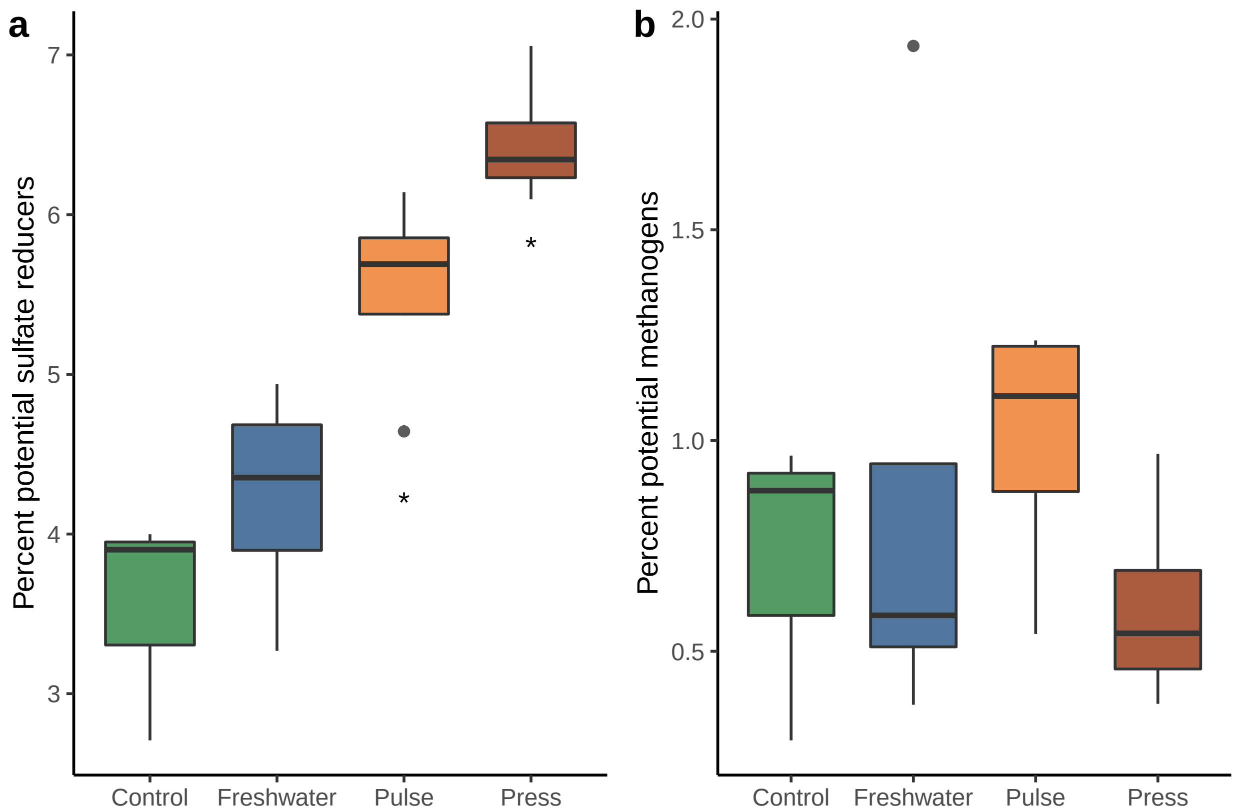
```
##             Df    Sum Sq   Mean Sq F value Pr(>F)
## treatment    3 3.475e-05 1.158e-05   0.546  0.661
## Residuals   11 2.334e-04 2.122e-05
```

```
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  0.001586248 -0.009001644 0.012174139 0.9680798
```

```
## Pulse-Control       0.002859788 -0.007728103 0.013447680 0.8471680
## Press-Control      -0.001041194 -0.011629085 0.009546698 0.9904743
## Pulse-Freshwater    0.001273540 -0.008528942 0.011076023 0.9786929
## Press-Freshwater   -0.002627441 -0.012429924 0.007175041 0.8500023
## Press-Pulse        -0.003900982 -0.013703465 0.005901501 0.6406687
```

```r
plot_grid(sulf.plot, methan.plot, labels = "auto") +
  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.